Université de Montréal

Neural Substrates Mediating the Behavioural Effects of Antipsychotic Medications and Pavlovian Cues: Importance for Maladaptive Processes in Psychiatric Disorders

Par

Alice Servonnet

Département de Neurosciences, Faculté de Médecine

Thèse présentée en vue de l'obtention du grade de Docteure en Philosophie (Ph. D)

en Neurosciences

Université de Montréal

Département de Neurosciences, Faculté de Médecine

Cette thèse intitulée

Neural Substrates Mediating the Behavioural Effects of Antipsychotic Medications and Pavlovian Cues: Importance for Maladaptive Processes in Psychiatric Disorders

Présentée par

Alice Servonnet

A été évaluée par un jury composé des personnes suivantes

Bénédicte Amilhon
Présidente du comité

Anne-Noël Samaha
Directrice de recherche

Paul J. Fletcher
Membre du jury

Mihaela D. Iordanova
Examinatrice externe

Adriana Di Polo
Représentante du doyen

RÉSUMÉ

Les antipsychotiques sont administrés chroniquement pour prévenir de nouveaux épisodes psychotiques dans la schizophrénie. Ces médicaments diminuent l'activité des récepteurs dopaminergiques de type 2. Diminuer chroniquement la transmission dopaminergique induit des compensations pouvant mener à une sensibilisation du système dopaminergique. Cette sensibilisation pourrait diminuer l'efficacité des antipsychotiques et exacerber la psychose. Chez le rat, la sensibilisation dopaminergique induite par les antipsychotiques augmente les effets psychomoteurs et motivationnels des agonistes dopaminergiques.

Le premier objectif de la présente thèse était de caractériser les substrats neuronaux régulant l'expression de la sensibilisation dopaminergique évoquée par les antipsychotiques. Ceci est important afin d'améliorer le traitement à long terme de la schizophrénie. Pour ce faire, des rats ont reçu un traitement cliniquement pertinent à l'antipsychotique halopéridol. Ce traitement sensibilise aux effets psychomoteurs de l'agoniste dopaminergique d-amphétamine. Cet indice comportemental de sensibilisation dopaminergique a été utilisé pour déterminer les contributions spécifiques du système dopaminergique et l'implication des effets centraux de la d-amphétamine. Puisqu'il y a une relation étroite entre le stress et l'activité dopaminergique, les réponses liées au stress ont également été mesurées. Ceci est important, puisque le stress exacerbe la psychose. La présente thèse démontre que les récepteurs dopaminergiques régulent de manière distincte la sensibilisation dopaminergique. En effet, la transmission via les récepteurs de type 2 exacerbe cette sensibilisation, alors que la transmission via les récepteurs de type 1 la tempère. Également, la présente thèse suggère que des processus périphériques sont nécessaires à l'expression de la sensibilisation dopaminergique. De plus, la sensibilisation pourrait augmenter les réponses au stress. En effet, cette sensibilisation est renversée lorsque la synthèse de l'hormone de stress corticostérone est inhibée, en plus d'être associée à certains comportements suggérant un stress augmenté.

Chez le rat, la sensibilisation dopaminergique évoquée par les antipsychotiques potentialise également les effets motivationnels des stimuli conditionnés prédisant des récompenses. Lorsque ces stimuli acquièrent trop de valeur motivationnelle, ils peuvent motiver des comportements pathologiques. Ainsi, une potentialisation de la valeur motivationnelle des stimuli conditionnés

provoquée par les antipsychotiques pourrait avoir des implications importantes dans des processus motivationnels anormaux dans la schizophrénie, tels que la psychose et la forte prévalence de toxicomanie. Ainsi, le deuxième objectif de la présente thèse était d'étudier les mécanismes neurobiologiques régulant les effets comportementaux des stimuli conditionnés, particulièrement le rôle du noyau basolatéral de l'amygdale. Ici, le rôle de ce noyau a été étudié chez des animaux non traités aux antipsychotiques, puisque sa contribution reste incomprise. Ce travail pourrait révéler des mécanismes neurobiologiques potentiellement impliqués dans la sensibilisation dopaminergique évoquée par les antipsychotiques. La présente thèse démontre que l'activation optogénétique de l'amygdale basolatérale potentialise les effets comportementaux des stimuli conditionnés, en augmentant leur valeur motivationnelle et leur capacité à guider le comportement vers des récompenses imminentes. Ainsi, une activité excessive de l'amygdale basolatérale pourrait attribuer trop de pouvoir aux stimuli conditionnés, et ceci pourrait jouer un rôle dans l'état motivationnel anormal provoqué par les antipsychotiques.

La présente thèse identifie de nouveaux mécanismes par lesquels les antipsychotiques et les stimuli conditionnés favorisent des réponses pathologiques.

MOTS-CLÉS: Amygdale basolatérale, antipsychotique, motivation, optogénétique, schizophrénie, sensibilisation dopaminergique, stimuli conditionnés.

ABSTRACT

Schizophrenia requires long-term antipsychotic treatment to prevent psychosis relapse. Antipsychotic drugs temper psychotic symptoms by reducing dopamine D2 receptor-mediated signalling. Chronically decreasing dopamine transmission can produce neuronal compensation leading to supersensitivity to dopamine stimulation. In patients, this dopamine supersensitivity would compromise antipsychotic efficacy and exacerbate psychotic symptoms. In laboratory animals, antipsychotic-evoked dopamine supersensitivity enhances the psychomotor and reward-enhancing effects of dopamine agonists.

The first objective of the present thesis was to characterize the biological substrates mediating the expression of antipsychotic-evoked dopamine supersensitivity, a necessary work for developing better long-term treatment strategies. To do so, rats were chronically exposed to a clinically relevant antipsychotic treatment regimen, using the drug haloperidol. Haloperidol produces dopamine supersensitivity, as indicated by an exaggerated psychomotor response to the dopamine agonist d-amphetamine. This behavioural index of supersensitivity was used to examine the specific contributions of the dopamine system and the central effects of d-amphetamine. Given that there is a close relationship between stress and dopamine activity, it was also determined whether antipsychotic-evoked dopamine supersensitivity alters stress-like responses. This is important to consider because stress is a contributing factor to psychosis relapse. The present thesis first reveals that D1- and D2-mediated transmissions contribute distinctively to the expression of antipsychotic-evoked dopamine supersensitivity, with D2 transmission promoting this supersensitivity and D1 transmission tempering it. The present thesis also provides evidence that peripheral processes play a necessary role in dopamine supersensitivity. Additionally, antipsychotic-evoked dopamine supersensitivity could potentiate stress-like responses. Indeed, the expression of supersensitivity is reversed by inhibition of the synthesis of the stress hormone corticosterone and is linked with some signs of heightened stress-related behaviours.

In rats, antipsychotic-evoked dopamine supersensitivity potentiates the incentive motivational effects of reward-predictive conditioned stimuli. When these stimuli acquire too much motivational value, they motivate maladaptive responses. Hence, the increased motivational value of conditioned stimuli elicited by antipsychotic exposure could be involved in impaired motivational

processes found in schizophrenia, such as psychosis and the greater vulnerability to drug addiction. Thereby, the last goal of the present thesis was to investigate the neurobiological substrates mediating the behavioural effects of reward-predictive stimuli, with a special focus on the role of the basolateral nucleus of the amygdala. This was investigated in antipsychotic-naïve rats because there are important caveats in our current understanding of the functional role of the basolateral amygdala. Such investigation could give novel insights on the neurobiological effects of antipsychotic-evoked dopamine supersensitivity. Here it is shown that optogenetic stimulation of basolateral amygdala neurons potentiates the behavioural effects of conditioned stimuli, by increasing their motivational value and their ability to guide behaviour toward impending rewards. The implication for this is that excessive activity in the basolateral amygdala could attribute too much motivational power to conditioned stimuli, and this could be involved in the abnormal motivational state produced by antipsychotic drugs.

Taken together, the present thesis provides novel mechanisms by which antipsychotic drugs and reward-predictive stimuli promote maladaptive responses.

KEYWORDS: Antipsychotic drugs, basolateral amygdala, conditioned stimuli, dopamine supersensitivity, motivation, optogenetics, schizophrenia.

TABLE OF CONTENTS

FIGURES

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

ANNEX – 1ST REVIEW

ANNEX – 2ND REVIEW

TABLE

CHAPTER II

ABBREVIATIONS

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
cAMP	Cyclic adenosine monophosphate
ChR	Channelrhodopsin
COMT	Catechol-O-methyltransferase
CREB	cAMP response element-binding protein
CS	Conditioned stimuli
DNA	Deoxyribonucleic acid
DAT	Dopamine transporter
DL	Dorsolateral
DOPAC	3,4-dihydroxyphenylacetic acid
$D2^{HIGH}$	Dopamine receptor type 2 in high affinity
$D2_L$	Long isoform of dopamine receptor 2
$D2_S$	Short isoform of dopamine receptor 2
ERK	Extracellular signal-regulated kinase
GABA	Ɣ-aminobutyric acid
GPi	Internal segment of the globus pallidus
GPe	External segment of the globus pallidus
GSK3β	Glycogen synthase kinase-3β
ICSS	Intra-cranial self-stimulation
ITI	Inter-trial interval
L-dopa	L-3,4-dihydroxyphenylalanine
MAP	Mitogen-activated protein
MD	Mediodorsal
MPTP	1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine
NMDA	N-methyl-D-aspartic acid
PKA	Protein kinase A
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
STN	Subthalamic nucleus
VL	Ventrolateral
VM	Ventromedial
VMAT2	Vesicular monoamine transporter 2
VTA	Ventral tegmental area
UCS	Unconditioned stimuli

REMERCIEMENTS

Tout ce travail aurait été impossible sans les personnes suivantes. Tout d'abord, je tiens à remercier ma directrice, Dre Anne-Noël Samaha. Merci de m'avoir accordé votre confiance, d'avoir été aussi présente, de m'avoir aidé à tirer un maximum de mon doctorat et de m'avoir donné beaucoup de liberté dans mon travail. Votre générosité est grandement appréciée. Je tiens également à remercier Dr Pierre-Paul Rompré. Vous avez été mon co-directeur durant la maîtrise, et vous avez continué à être un mentor durant mon doctorat. Merci pour cela, et merci d'avoir toujours été disponible et d'avoir fait part de votre grand savoir. Je tiens également à remercier d'autres collaborateurs importants durant mon doctorat : merci au Dr Giovanni Hernandez qui a implémenté l'optogénétique dans le laboratoire et qui m'a permis d'utiliser cette super technologie (*¡muchas gracias!*), merci au Dr Daniel Lévesque, qui a fait partie de mon comité de parrainage et qui a toujours été disponible à travers les années, merci à la Dre Cynthia El Hage pour avoir collaboré dans un de mes articles, et un grand merci également à tous les stagiaires avec lesquels je m'estime très chanceuse d'avoir travaillé, qui étaient tous aussi excellents les uns que les autres et qui m'ont permis de pousser plus loin mon travail de thèse : Frederik Asselin, Marc-Antoine Charron, Louis-Phillipe Moreau, Casey Bourdeau Caporuscio, Alice Gravel-Chouinard, Mathilde Legrix et Laurence Théroux. Je tiens également à faire un remerciement spécial à des collègues qui ont rendu la vie de laboratoire beaucoup plus intéressante et qui ont été toujours présentes pour moi : Dre Florence Allain, Dre Ellie-Anna Minogianis et Ndeye Aissatou Ndiaye. Merci beaucoup à vous. Finalement, un grand merci à mes proches, tout particulièrement mes parents. Merci à vous de m'avoir encouragé dans cette très longue aventure qu'a été l'université.

CHAPTER I. Introduction

Antipsychotic medications are the only effective drugs to temper psychotic symptoms in individuals with schizophrenia. Psychosis is linked to an increase in the activity of the neurotransmitter dopamine. Antipsychotic drugs produce their therapeutic effects by reducing the actions of dopamine in the brain via an interaction with type 2 dopamine receptors. Schizophrenia is a life-long illness. It requires chronic antipsychotic treatment to prevent new episodes of psychosis. Thereby, it is common practice that schizophrenia patients are exposed to antipsychotic drugs for long periods of time. However, this is not without consequences. Over time, chronically reducing dopamine transmission can produce compensatory changes that result in supersensitivity to dopamine stimulation. This dopamine supersensitivity would reduce the antidopaminergic effects of antipsychotic drugs, leading to treatment failure. Antipsychotic-evoked dopamine supersensitivity would also exacerbate psychotic symptoms. In rats, this supersensitivity reduces the antidopaminergic effects of antipsychotic drugs (resembling treatment tolerance in humans) and potentiates the behavioural effects of dopamine agonists. The first objective of the present thesis was to characterise biological substrates mediating the expression of antipsychotic-evoked dopamine supersensitivity in rats, and to find strategies to reduce the behavioural manifestations of this supersensitivity. Hence, in the Introduction, I first describe the dopamine system, the role of dopamine in schizophrenia and its treatment, and the behavioural and neurochemical manifestations of antipsychotic-evoked dopamine supersensitivity.

Dopamine agonists can enhance the motivational properties of conditioned rewards—*i.e.*, appetitive conditioned stimuli (CS), and this effect is potentiated by antipsychotic-evoked dopamine supersensitivity. CS that acquire too much motivational properties can greatly influence behaviour. Thereby, the increased motivational effects of CS evoked by chronic antipsychotic exposure could be linked to impaired motivation-related processes found in schizophrenia, such as psychotic symptoms and comorbid drug addiction. Because of these important implications, the second objective of the present thesis was to investigate the neurobiological substrates mediating the behavioural effects of CS, with a special focus on the role of the basolateral nucleus of the amygdala. In the present thesis, the role of this nucleus was investigated in the normal brain, that is, in non-antipsychotic-treated rats. This is because so far, there are important caveats in our current understanding of how basolateral amygdala neurons may intensify the motivational properties of appetitive CS. Such investigations could give insights on how CS elicit inappropriate responses following antipsychotic drug exposure producing dopamine supersensitivity, but it could

also give insight on psychiatric illnesses defined by abnormal motivation (*e.g.*, addiction). Hence, here I also present a description of how CS guide behaviour, how these effects are studied in laboratory animals, and what we know of the role of the basolateral amygdala in these processes. Because optogenetic methods were used in the present thesis to manipulate the activity of basolateral amygdala neurons, I last describe this methodological approach.

1. THE DOPAMINE SYSTEM

1.1. The Dopamine Synapse and Dopamine Agents

1.1.1. Dopamine Synthesis and Release

FIG. 1.1A illustrates dopamine synthesis and release and shows examples of dopamine agents that block dopamine storage in vesicles. Dopamine is a catecholamine that is synthetized from the amino acid tyrosine. Tyrosine is converted to l-3,4-dihydroxyphenylalanine (L-dopa) by the enzyme tyrosine hydroxylase (Feldman *et al.*, 1997). L-dopa is then converted to dopamine by the enzyme aromatic L-amino acid decarboxylase. An acute diet low in tyrosine depletes brain levels of dopamine and can be used as a tool to study dopamine functions (Leyton *et al.*, 2004). Neurotransmitter vesicles have great internal concentrations of protons, and these are transported in vesicles by proton pumps (Johnson, 1987). Dopamine is stored in vesicles via the vesicular monoamine transporter 2 (VMAT2), which exchanges cytoplasmic dopamine for vesicular proton (Erickson *et al.*, 1992; Liu *et al.*, 1992; Feldman *et al.*, 1997). Dopamine agents that deplete vesicular content of dopamine include reserpine and amphetamines (*e.g.*, d-amphetamine and methamphetamine). Reserpine and amphetamines both block VMAT2 (Carlsson *et al.*, 1957; Fleckenstein *et al.*, 2007), but amphetamines also reduce the concentration of vesicular protons. Consequently, this decreases dopamine transport into vesicles (Fleckenstein *et al.*, 2007).

1.1.2. Dopamine-mediated Signalling

FIG. 1.1B illustrates dopamine receptors and shows examples of dopamine receptor agonists and antagonists. Dopamine signals through G-protein coupled receptors that are classified into two groups: D1-like and D2-like receptors.

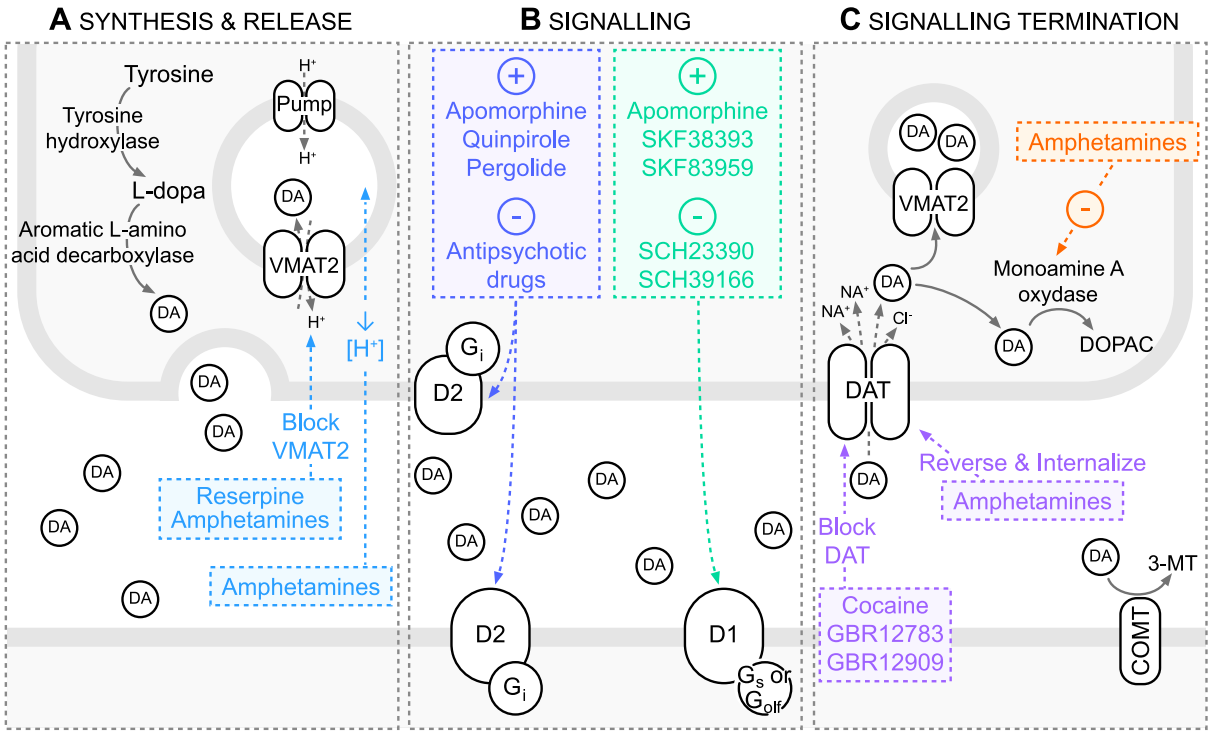

FIG. 1.1 — The dopamine synapse. (***A***) Dopamine synthesis and release. (***B***) Dopamine signals via D1-like and D2-like receptors. (***C***) Termination of dopamine signalling. COMT, catechol-O-methyltransferase; DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; VMAT2, vesicular monoamine transporter 2; 3-MT, 3-methoxytyramine.

There are two known D1-like receptors that are both highly conserved across species. The first cloned was the D1 receptor (Dearry *et al.*, 1990; Zhou *et al.*, 1990), and then the D5 receptor (Sunahara *et al.*, 1991; Tiberi *et al.*, 1991). Dopamine has a 10 times greater affinity for D5 receptors relative to D1 receptors (Sunahara *et al.*, 1991). D1-like receptors have a great affinity for G_s and G_{olf} proteins (Dearry *et al.*, 1990; Sunahara *et al.*, 1991; Zhuang *et al.*, 2000). Hence, D1-like receptor activation stimulates the activity of the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA)-dependent pathway and this pathway regulates gene expression via regulation of the activity of transcription factors such as cAMP response element-binding protein (CREB) and Fos (Robertson *et al.*, 1989; Das *et al.*, 1997). D1 receptors are found in multiple brain regions including the striatum (nucleus accumbens and caudate-putamen), the substantia nigra (SN), the ventral tegmental area (VTA), the prefrontal cortex, the hippocampus, the globus pallidus, the amygdala, the thalamus and the olfactory tubercle (Huang *et al.*, 1992; Levey *et al.*, 1993). The concentration of D5 receptors in the brain is generally lower than the one of D1 receptors (Sunahara *et al.*, 1991; Tiberi *et al.*, 1991). D5 receptors are located in similar

regions to D1 receptors, including the striatum, the SN, the thalamus, the prefrontal cortex and the hippocampus (Khan *et al.*, 2000). D1-like receptor agonists include apomorphine, SKF38393 and SKF83959 (Dearry *et al.*, 1990; Sunahara *et al.*, 1991; Millan *et al.*, 2002; Neumeyer *et al.*, 2003), whereas SCH23390 and SCH39166 are examples of D1-like receptor antagonists (Dearry *et al.*, 1990; McQuade *et al.*, 1991; Sunahara *et al.*, 1991).

D2-like receptors include three receptors that are also highly conserved across species. The D2 receptor was the first cloned (Bunzow *et al.*, 1988), followed by the D3 receptor (Sokoloff *et al.*, 1990) and the D4 receptor (Van Tol *et al.*, 1991). Dopamine has a greater affinity for the D2 receptor relative to D3 and D4 receptors (Sokoloff *et al.*, 1990; Van Tol *et al.*, 1991). There are two isoforms of the D2 receptor, the long isoform ($D2_L$) and the short isoform ($D2_S$) that comprises 29 amino acids less than the long isoform (Dal Toso *et al.*, 1989; Eidne *et al.*, 1989). These isoforms are spatially segregated. $D2_S$ receptors are preferentially located on the presynaptic side, whereas $D2_L$ receptors are preferentially located on the postsynaptic side (Usiello *et al.*, 2000). D2-like receptors have a great affinity for $G_{i/o}$ protein and thereby, they regulate the activity of several intracellular signaling pathways including the cAMP/PKA-, glycogen synthase kinase-3β (GSK3β)/AKT- and mitogen-activated protein (MAP) kinase-dependant pathways (Bonci and Hopf, 2005). Presynaptic D2-like receptors inhibit dopamine release and synthesis, whereas post-synaptic D2-like receptors reduce neuron excitability (Ford, 2014). D2 receptors are generally more expressed than D3 and D4 receptors (Van Tol *et al.*, 1991; Levesque *et al.*, 1992). D2 receptors are located in multiple brain regions including the striatum, the SN, the VTA, the globus pallidus, the olfactory tubercle, the hypothalamus, the habenula and the amygdala (Brock *et al.*, 1992; Levey *et al.*, 1993). In contrast, D3 receptors are located in a limited number of regions, such as the nucleus accumbens, the olfactory bulb, the islands of Calleja and the cerebellum (Levesque *et al.*, 1992). Like D2 receptors, D4 receptors are located in several regions including the striatum, the SN, the VTA, the hippocampus, the amygdala, the olfactory tubercle, the globus pallidus, the hypothalamus and the cerebellum (Defagot *et al.*, 1997; Primus *et al.*, 1997). D2-like receptor agonists include quinpirole, pergolide and apomorphine (Krueger, 1990; Van Tol *et al.*, 1991; Millan *et al.*, 2002). D2-like receptor antagonists include antipsychotic drugs such as haloperidol, sulpiride, raclopride and clozapine (Bunzow *et al.*, 1988; Sokoloff *et al.*, 1990; Van Tol *et al.*, 1991) (see also Section 2.3, page 40).

1.1.3. Termination of Dopamine Signalling

FIG. 1.1C shows how dopamine-mediated signalling is terminated and illustrates examples of how dopamine agents interfere with these processes. Extracellular dopamine can be inactivated by the enzyme catechol-O-methyltransferase (COMT), which metabolises dopamine in 3-methoxytyramine (Axelrod and Tomchick, 1958; Wood *et al.*, 1987). Dopamine can also be recaptured in the terminals by the dopamine transporter (DAT) (Giros *et al.*, 1991; Giros *et al.*, 1992). Dopamine is co-transported with sodium and chloride ions (Krueger, 1990; McElvain and Schenk, 1992). Recaptured dopamine can be recycled by being stocked in vesicles again (Michael *et al.*, 1987) or can be inactivated by the enzyme monoamine oxidase A, which metabolizes dopamine in 3,4-dihydroxyphenylacetic acid (DOPAC) (Rosengren, 1960). Indirect dopamine agonists disrupt the termination of dopamine-mediated signalling and thereby, they extend dopamine actions. For instance, cocaine, GBR12783 and GBR12909 enhance dopamine extracellular concentrations by blocking its reuptake by DAT (Bonnet and Costentin, 1986; Rothman and Baumann, 2003). Amphetamines reduce dopamine reuptake via distinct mechanisms from DAT blockers. Indeed, amphetamines reverse the transport of dopamine so that DAT release dopamine instead of recapturing it, and they also promote DAT internalization (Fleckenstein *et al.*, 2007). Additionally, amphetamines reduce dopamine inactivation in terminals by inhibiting the activity of the monoamine oxidase A (Robinson, 1985). It is noteworthy that the psychostimulant drugs above can exert dopamine-independent effects as well, such as increasing serotonin and noradrenaline transmissions (Rothman and Baumann, 2003).

1.2. Dopamine Pathways

1.2.1. Localisation and Projections of Dopamine Neurons

Dopamine is synthetized in a limited number of neurons, but they send dopaminergic projections to a great number of brain regions. Dopamine neurons are found in the mesencephalon, specifically in the SN pars compacta (SNc), the VTA and midline nuclei (Fallon and Moore, 1978). SNc dopamine neurons project massively to the caudate-putamen, and to a lesser extent to other regions such as the nucleus accumbens, the amygdala, the olfactory tubercle and to the prefrontal cortex, including cingulate, prelimbic, infralimbic and orbitofrontal cortices (Fallon and Moore, 1978; Fuxe *et al.*, 1985; Gerfen *et al.*, 1987). VTA dopamine neurons send projections to these areas as

well, but notably to a lesser extent in the caudate-putamen and to a greater extent in the nucleus accumbens, and send projections to other areas such as the lateral habenula and the hippocampus (Fallon and Moore, 1978; Swanson, 1982; Fuxe *et al.*, 1985; Ikemoto, 2007). Dopamine neurons are also located in a region medial to the VTA and SNc: the midline nuclei. Dopamine neurons of that region project to the nucleus accumbens, the olfactory tubercle, the medial habenula and the septum (Fuxe *et al.*, 1985; Ikemoto, 2007). Lastly, dopamine neurons are also found in the hypothalamus, the dorsal and medial raphe nuclei, the retina and the olfactory bulb (Fuxe *et al.*, 1985; Trulson *et al.*, 1985). Overall, these dopamine pathways are well conserved across species (Bjorklund and Dunnett, 2007). The mesostriatal, mesolimbic and mesocortical dopamine pathways are particularly relevant to the pathophysiology and treatment of schizophrenia. As shown on FIG. 1.2, these dopamine pathways are divided based on their targeted area: *i*) SNc/VTA dopaminergic projections to the caudate-putamen represent the mesostriatal pathway, *ii*) VTA/SNc/midline nuclei dopaminergic projections to the nucleus accumbens, the septum, the olfactory tubercle and the amygdala form the mesolimbic pathway and *iii*) VTA/SNc dopaminergic projections to the prefrontal cortex form the mesocortical dopamine pathway (Bjorklund and Dunnett, 2007). Dopaminergic projections to the nucleus accumbens and caudate-putamen are especially important in psychosis, and thereby they are critical targets of antipsychotic drugs. These dopaminergic projections are found in basal ganglia circuits. Hence, next is a neuroanatomical description of basal ganglia circuits and of how striatal dopamine is integrated in these circuits.

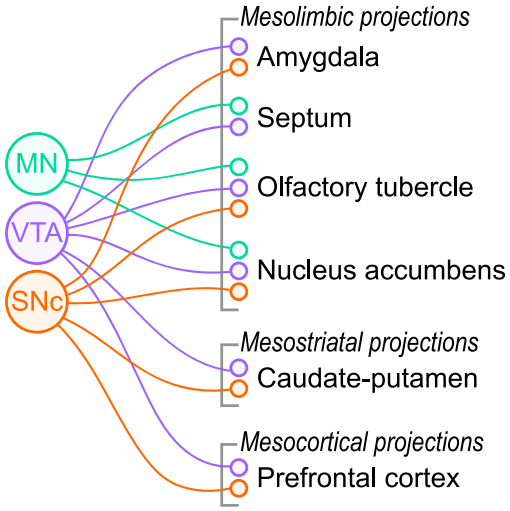

FIG. 1.2 — Mesolimbic, mesostriatal and mesocortical dopaminergic pathways. These dopaminergic pathways originate from dopamine neurons of the midline nuclei (MN), ventral tegmental area (VTA) and substantia nigra pars compacta (SNc).

1.2.2. Striatal Dopamine in Basal Ganglia Circuits

Under the influence of striatal dopamine transmission, basal ganglia circuits regulate a wide variety of functions, including motor performance, execution of goal directed behaviour and associative learning. In summary, multiple basal ganglia circuits exist in parallel with the same pattern of looping pathway:

cortex → basal ganglia → thalamus → cortex (Alexander and Crutcher, 1990). The specific connections of each parallel basal ganglia circuit determine their respective function. The basal ganglia integrates information coming from the cortex, and 'shapes' messages by concurrently inhibiting information and relaying other to the thalamus. The thalamus then directly communicates these shaped messages back to the cortex. Shaping those messages is important, for instance, to execute appropriate actions and to make adequate decisions.

Interestingly, the striatum is the input region of the basal ganglia, meaning that it receives direct projections from the cortex. Striatal neurons are medium spiny neurons that release the neurotransmitter ɣ-aminobutyric acid (GABA) and that generally express either D1 or D2 receptors (Gerfen *et al.*, 1990). In the caudate-putamen, most D1-expressing neurons constitute the *direct* pathway, because they directly project to the output nuclei of the basal ganglia (*i.e.*, the internal segment of the globus pallidus, GPi, and the SN pars reticulata, SNr) (Alexander *et al.*, 1986; Alexander and Crutcher, 1990). Activation of the direct pathway ultimately stimulates cortical activity (see FIG. 1.3 for a more detailed description of the neuroanatomy and functional connectivity of basal ganglia circuits). Most D2-expressing neurons of the caudate-putamen constitute the *indirect* pathway, because they do not project directly to the output nuclei. Instead, D2-expressing neurons influence the activity of the output nuclei indirectly via the external segment of the globus pallidus (GPe) and the subthalamic nucleus (STN). Activation of the indirect pathway ultimately inhibits cortical activity. When dopamine is released within the caudate-putamen, it favours the activity of the direct pathway. Indeed, stimulation of D1 receptors activates the neurons of the direct pathway, whereas stimulation of D2 receptors inhibits the activity of the neurons of the indirect pathway.

In the core subdivision of the nucleus accumbens, D1- and D2-expressing neurons are not preferentially found in the direct and indirect pathways, respectively [note that the following description on D1/D2 receptors is based on findings by Kupchik *et al.* (2015); and neuroanatomy is based on Sesack and Grace (2010)]. As in the caudate-putamen, there is a direct pathway composed of D1-expressing neurons that project directly to the output nuclei (GPi and SNr; see FIG. 1.3). Nucleus accumbens neurons also send projections to dorsolateral (DL) ventral pallidum neurons, and these neurons either form an output or an intermediate nucleus. Indeed, some DL ventral pallidum neurons send direct projections to the mediodorsal (MD) thalamus (these neurons

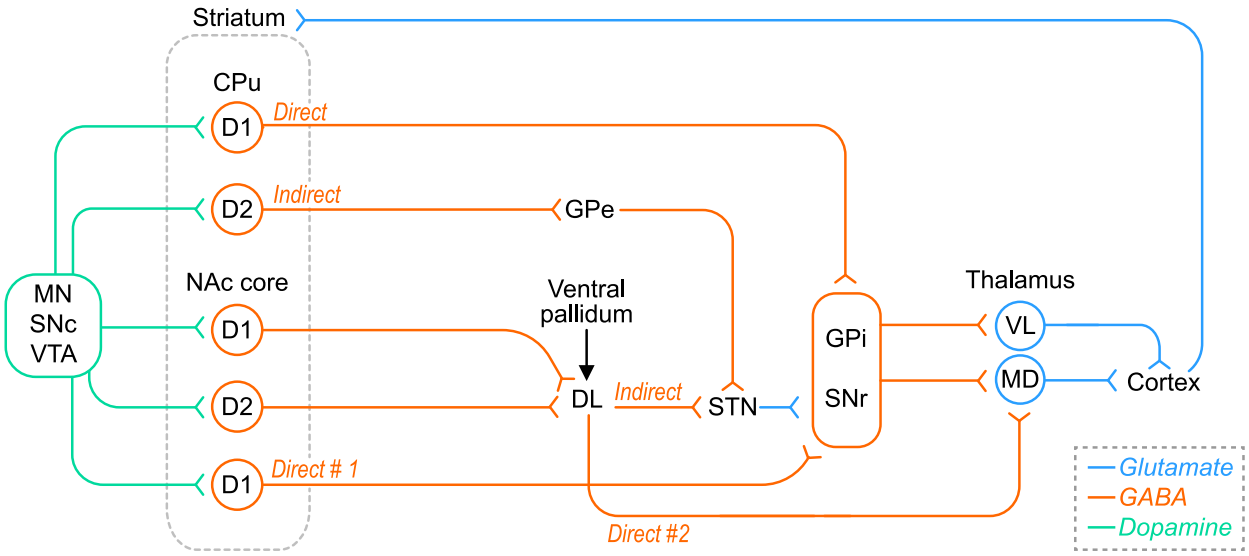

FIG. 1.3 — Basal ganglia circuits.

The caudate-putamen (CPu) as the input region — Activation of the **direct pathway** (D1-expressing neurons) has the following consequences: inhibition of the output nuclei (internal segment of the globus pallidus, GPi, and the substantia nigra pars reticulata, SNr) → disinhibition of the ventrolateral (VL) thalamus → activation of cortical activity. Activation of the **indirect pathway** (D2-expressing neurons) has the following consequences: inhibition of the external segment of the globus pallidus (GPe) → disinhibition of the subthalamic nucleus (STN) → activation of the GPi/SNr → inhibition of the VL thalamus → inhibition of cortical activity.

The nucleus accumbens (NAc) core as the input region — There are two direct pathways. For the **first direct pathway**, activation of D1-expressing neurons has the following consequences: inhibition of GPi/SNr → disinhibition of the mediodorsal (MD) thalamus → activation of cortical activity. For the **second direct pathway**, activation of D1- and D2-expressing neurons has the following consequences: inhibition of the dorsolateral (DL) ventral pallidum → disinhibition of the MD thalamus → activation of cortical activity. Activation of the **indirect pathway** (D1- and D2-expressing neurons) has the following consequences: inhibition of DL ventral pallidum → disinhibition of the STN → activation of GPi/SNr → inhibition of MD thalamus → inhibition of cortical activity.

MN, midline nuclei; SNc, substantia nigra pars compacta; VTA, ventral tegmental area.

---

form an output nucleus), whereas some DL ventral pallidum neurons send projections to the STN (these neurons form an intermediate nucleus). Hence, there is a *second* direct pathway originating in the nucleus accumbens core. This pathway is composed of D1- but also D2-expressing neurons that project to DL ventral pallidum-to-MD thalamus neurons. Regarding the indirect pathway, it is composed of D2- but also D1-expressing neurons that project to DL ventral pallidum-to-STN neurons. Despite the discrepancies between the nucleus accumbens and the caudate-putamen,

activation of the direct and indirect pathways of the nucleus accumbens has similar outcomes because the direct pathway promotes cortical activity, and the indirect pathway inhibits it (see FIG. 1.3). However, while dopamine transmission in the caudate-putamen favors the activation of the direct pathway, dopamine transmission in the nucleus accumbens has mixed effects on the activity of basal ganglia circuits because D1- and D2-expressing neurons are found in both pathways. The nucleus accumbens also has a *shell* subdivision. However, I am not aware of studies that dissected the composition of D1- and D2-expressing neurons in the direct and indirect pathways of this subdivision of the nucleus accumbens.

1.3. Dopamine Functions

1.3.1. Motricity

Dopamine transmission within the striatum regulates the quick and proper execution of planned movements in everyday life. Impairment in striatal dopamine transmission causes an imbalance in the activity of the direct and indirect pathways, and this leads to motor dysfunctions. The role of dopamine transmission in motricity is especially well-characterized for the mesostriatal dopamine pathway. For instance, a reduction of mesostriatal dopamine transmission, as in Parkinson's disease (Greenfield and Bosanquet, 1953; Bernheimer *et al.*, 1973) or after exposure to the neurotoxin 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) (Langston *et al.*, 1983; Javitch and Snyder, 1984), enhances the activity of the indirect pathway (Albin *et al.*, 1989). In accordance with the inhibitory actions of the indirect pathway, Parkinson's disease and MPTP exposure are linked to a reduced capacity to initiate movement, a slower execution of movement and motor blocks during movement execution (Langston *et al.*, 1983; Morris, 2000).

Additionally, psychostimulant drugs stimulate dopamine transmission, which consequently potentiates locomotor activity and produces stereotypy at high dosage (Feldman *et al.*, 1997). Stereotypy refers to an execution of repeated movement with no apparent goal. Dopamine transmission in the striatum regulates psychomotor activity, with the nucleus accumbens preferentially regulating hyperlocomotor activity and the caudate-putamen preferentially regulating stereotypy (Kelly *et al.*, 1975; Kelly and Iversen, 1976; Pijnenburg *et al.*, 1976; French and Vantini, 1984; Kelley *et al.*, 1988; Dalia *et al.*, 1998; Gong *et al.*, 1999). As it will be described later (see Section 2.3.3.3, page 49), antipsychotic drug administration tempers the psychomotor

response to psychostimulants and this inhibitory effect serves as an index of the antidopaminergic effects of these medications.

1.3.2. Motivation

Dopamine plays an important role in regulating incentive motivation, a critical property of natural or drug rewards. The incentive motivational value of rewards confers to them the ability to elicit approach, to be avidly worked for, and to be likely to reinforce subsequent behavioural actions directed toward their obtention (Wise and Rompre, 1989; Robinson and Berridge, 1993; Wise, 2004). Additionally, because rewards have incentive motivational value, they gain incentive salience, which refers to '*the attractiveness of external stimuli, events, places and their mental representations; their ability to capture attention*' (Robinson and Berridge, 1993). In addition to regulating incentive motivation, dopamine modulates the motivational properties of aversive stimuli (*i.e.*, aversive motivation) and thereby, this confers an important role of this neurotransmitter in psychiatric disorders characterized by abnormal motivated responses such as in addiction, depression (Robinson and Berridge, 1993; Salamone and Correa, 2012; Berridge, 2018) and schizophrenia (Section 2.2.1, page 34). The role of dopamine in motivational processes is especially well characterized for incentive motivation, and the following description focuses on this.

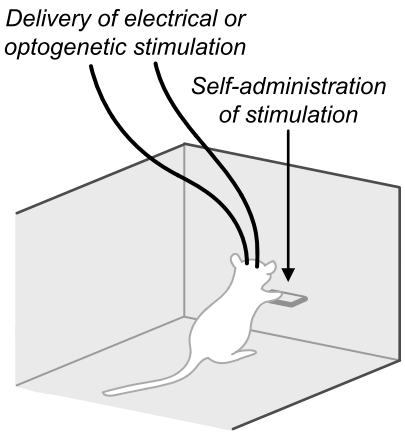

FIG. 1.4 — Intra-cranial self-stimulation (ICSS). In this behavioural paradigm, animals can voluntarily self-administer electrical or optogenetic stimulations into the brain (as exemplified here, lever presses lead to stimulation delivery).

Increasing dopamine transmission alone has reinforcing effects. This important effect of dopamine has been largely studied using protocols of intra-cranial self-stimulation [ICSS; originally designed by Olds and Milner (1954)]. This protocol determines the extent to which brain stimulations reinforce behavioural responses (FIG. 1.4). Electrical stimulation of VTA or SNc neurons supports ICSS, indicating that these stimulations alone are sufficient to produce reinforcing effects (Crow, 1972; Corbett and Wise, 1980; Wise, 1981). Similarly, optogenetic stimulation of VTA or SNc dopamine neuron is reinforcing (Witten *et al.*, 2011; Kim *et al.*, 2012; Rossi *et al.*, 2013; Ilango *et al.*, 2014; Saunders *et al.*, 2018).

Conversely, inhibition of VTA or SNc dopamine neurons produces avoidance (Ilango *et al.*, 2014). Even if VTA (but not SNc) dopamine neurons can co-release glutamate (El Mestikawy *et al.*, 2011), removal of co-released glutamate by a genetic deletion of the vesicular transporter of glutamate 2 in dopamine neurons does not impair the reinforcing properties of optogenetic activation of VTA dopamine neurons (Wang *et al.*, 2017). This indicates that dopamine alone is sufficient to produce reinforcing effects.

Dopamine projections to the striatum are sufficient to produce reinforcing effects on their own, indicating that mesostriatal and mesolimbic projections are critical in regulating motivational processes. Indeed, optogenetic activation of VTA dopamine projections in the nucleus accumbens or of SNc dopamine projections in the dorsal caudate-putamen is sufficient to promote self-stimulation (Steinberg *et al.*, 2014; Saunders *et al.*, 2018). Similarly, animals voluntarily self-administer dopamine agonists directly into the nucleus accumbens or caudate putamen (Carlezon *et al.*, 1995; Ikemoto *et al.*, 1997). Both D1-like and D2-like-mediated signalling in the striatum seem to promote reinforcing effects. Indeed, injection of D1-like and/or D2-like receptor antagonist into the nucleus accumbens reduces optogenetic self-stimulation of VTA dopamine neurons (Steinberg *et al.*, 2014). Hence, by stimulating D1-like-expressing neurons and inhibiting D2-like-expressing neurons, dopamine promotes motivational processes. This is further confirmed by the observation that mice avidly work for optogenetic stimulation of D1- but not D2-expressing neurons in the nucleus accumbens (Cole *et al.*, 2018) and the caudate-putamen (Kravitz *et al.*, 2012; Vicente *et al.*, 2016). Activation of D2-expressing neurons could actually be aversive, as it seems to promote freezing and avoidance (Kravitz *et al.*, 2012; Cole *et al.*, 2018).

As it will be described in Section 3.2 (page 61), appetitive CS can acquire incentive motivational effects on their own, like a primary reward would do. Interestingly, mesostriatal and mesolimbic dopamine transmissions regulate the incentive motivational effects of CS. For instance, infusion of dopamine agonists into the nucleus accumbens or caudate-putamen is sufficient to enhance the incentive motivational value of CS (Taylor and Robbins, 1984; Kelley and Delfs, 1991; White *et al.*, 1991; Chu and Kelley, 1992) [but see (El Hage *et al.*, 2015)]. Additionally, CS evoke greater dopamine release in the nucleus accumbens when animals have attributed incentive motivational value to them, and blocking dopamine transmission prevents CS from acquiring incentive motivational value (Flagel *et al.*, 2011b). Furthermore, combining the presentation of a neutral cue

with optogenetic stimulation of VTA (but not SNc) dopamine neurons is sufficient to imbue motivational value to that cue, even if it is not associated with a primary reward (Saunders *et al.*, 2018). Similarly, pairing optogenetic stimulation of VTA dopamine neurons with a neutral contextual cue is sufficient to attribute motivational salience to that context (Tsai *et al.*, 2009).

1.3.3. Associative Learning

Dopamine-mediated signalling shapes learning processes, and this is well exemplified in studies where animals learn that a cue (CS) predicts an impending reward (Schultz, 1998). When the cue is not an effective predictor yet, VTA and SNc dopamine neurons fire in response to reward delivery but not to the cue. When the cue has acquired predictive value, dopamine neurons shift their response toward the cue. Hence, throughout learning, dopamine neurons fire more and more in response to the cue and not to the reward anymore, as long as the expected reward does not change. When animals' expectation of the reward is not met because the prediction is incorrect (*e.g.*, no reward delivery following cue presentation), dopamine neurons shift their activity. This adaptative response of dopamine neurons is referred to as a *reward prediction error* (Schultz, 1998). For instance, if no reward is delivered, dopamine neurons show a decrease in their activity. Such adaptative responses from dopamine neurons mediate associative learning and help animals to be more efficiently guided by surrounding stimuli. This important role of dopamine in learning is further demonstrated by studies using optogenetic methods. Indeed, optogenetic stimulation of VTA dopamine neurons evokes conditioned responses indicative that reward was above expectancy (Steinberg *et al.*, 2013), whereas optogenetic inhibition of VTA dopamine neurons elicits conditioned responses indicative that the reward was below expectancy (Chang *et al.*, 2016).

1.3.4. Working Memory

The role of dopamine in working memory exemplifies how this neurotransmitter can—to some extent—regulate cognitive deficits in schizophrenia. Working memory is defined by the ability to store for a short period of time an information (initially an external cue), and to retrieve this information that is not readily available anymore (now an internal cue) in order to perform an action or to make a decision (Goldman-Rakic, 1992, 1995). Hence, working memory is guided by internal but not external cues, and differs from learning that requires long-term storage of information (Goldman-Rakic, 1992, 1995). The role of mesocortical dopamine transmission in working

memory has been extensively studied, especially the role of D1-like-mediated signalling in the prefrontal cortex. Dopamine but not serotonin nor noradrenaline denervation in the prefrontal cortex of non-human primates reduces performance on a working memory task (Brozoski *et al.*, 1979). Furthermore, administration of a D1-like but not a D2-like receptor antagonist into the prefrontal cortex impairs working memory (Sawaguchi and Goldman-Rakic, 1991). The effects of mesocortical dopamine transmission on working memory are not linear. Instead, this relationship follows an inverted-U shaped form, where too little or too much dopamine transmission is detrimental for working memory (Cools and D'Esposito, 2011). For instance, among healthy individuals, working memory seems to vary in function of the level of mesocortical dopamine transmission, where individuals with estimated 'balanced' mesocortical dopamine transmission show the best performance in tasks testing working memory (Papenberg *et al.*, 2019). Hence, abnormal dopamine transmission in the prefrontal cortex potentially produces cognitive deficits in disorders such as schizophrenia (see next section).

2. DOPAMINE IN SCHIZOPHRENIA

Schizophrenia is a psychiatric disorder with a 1% prevalence worldwide, affecting 280,000 persons in Canada (Ernest *et al.*, 2017). Schizophrenia is a lifelong illness where people start to experience symptoms usually during late adolescence to early adulthood (Loranger, 1984; Hafner *et al.*, 1993). Symptoms would be the result of an abnormal development of the brain starting as early as during the prenatal period (Lewis and Levitt, 2002). A complex interaction between genetic and environmental factors would contribute to the abnormal development of the brain and the emergence of schizophrenia symptoms. Genetic factors are not sufficient but seem to represent an important contributing factor to the development of schizophrenia. Indeed, studies on monozygotic twins reveal that if a twin has schizophrenia, the other twin has a ~50% risk of having schizophrenia as well (Cardno and Gottesman, 2000). Even if twins are exposed to the same environment, genetic factors are still determinant in schizophrenia. Indeed, a dizygotic twin that has a twin with schizophrenia has a ~15 % risk of having schizophrenia as well, which is a lower risk relative to monozygotic twins (Cardno and Gottesman, 2000). Genetic irregularities that could contribute to schizophrenia include chromosomal abnormalities, as well as specific polymorphisms or mutations of genes including *COMT* (encodes COMT), *DTNBP1* (encodes the protein dysbindin) and *NRG1* (encodes the protein neuregulin 1) (Harrison and Owen, 2003; Owen *et al.*, 2005). These genetic

factors could contribute to abnormal neuronal development because the proteins above are involved in neuronal migration, cellular differentiation and synaptic plasticity (Harrison and Owen, 2003). Environmental factors that are linked to schizophrenia include obstetrical complications (such as maternal infection, pre-eclampsia and asphyxia at birth) and stressors early in life such as emotional, sexual or physical abuse during childhood (Lewis and Levitt, 2002; Read *et al.*, 2005).

Dopamine plays an important role in the symptomology of schizophrenia. Next is a description of schizophrenia symptoms, the contribution of dopamine in these symptoms and how manipulating the dopamine system can improve certain schizophrenia symptoms.

2.1. Schizophrenia Symptoms

Schizophrenia symptoms can be classified in three types: the psychotic symptoms, the negative symptoms, and the cognitive symptoms. Psychotic symptoms represent a group of symptoms that are defined by a loss of contact with reality. Psychotic symptoms include: *i*) hallucinations, that are commonly auditory but can be of any sensory type, *ii*) delusion, where a person has strong beliefs that are unrealistic, such as being tracked down or not having the control over their body, *iii*) disorganised thoughts, where the thought process and the communication of thoughts are illogical and confused, and *iv*) disorganised behaviour, to the point where one's ability to normally function in everyday life is greatly impaired (Liddle, 1987; Mueser and McGurk, 2003). Negative symptoms refer to a group of symptoms that are generally defined by a reduced motivation, pleasure and social capabilities. More specifically, negative symptoms include : *i*) one's interest are diminished and have more difficulty to experience pleasure, *ii*) reduced motivation to initiate and perform activities/tasks, *iii*) social distancing, *iv*) weak communication, because less words are used to communicate and communication is reduced in general, and *v*) reduced facial expression and tonality of verbal communication (Liddle, 1987; Mueser and McGurk, 2003). Lastly, schizophrenia patients can show cognitive impairments, including impaired working memory, attention and concentration, verbal/visual learning and memory, and reasoning and problem solving (Nuechterlein *et al.*, 2004).

At the beginning stages of schizophrenia, negative symptoms are the first to emerge, followed by the cognitive symptoms (Ernest *et al.*, 2017; McCutcheon *et al.*, 2019a). It can then take years before schizophrenia patients experience a first episode of psychosis. The prevalence of

schizophrenia is similar across men and women, but symptoms commonly emerge earlier in men (15-25 years old) than women (20-29 years old) (Loranger, 1984; Hafner *et al.*, 1993). While negative and cognitive symptoms are usually continuous throughout the course of schizophrenia illness, psychotic symptoms are episodic and come by cycles (Bunzow *et al.*, 1988; Yung and McGorry, 1996; McCutcheon *et al.*, 2019a). A cycle is composed of three phases: the prodromal phase, the active phase and the residual phase. The prodromal phase is defined by the gradual emergence of symptoms including social isolation, depressed mood, sleep disturbance, anxiety and great preoccupations (Yung and McGorry, 1996). Commonly, prodromal symptoms evolve to an attenuated form of psychotic symptoms, such as starting to be suspicious of others (*i.e.*, a form of delusion). The prodromal phase can last weeks to years and does not necessarily lead to an episode of psychosis but does in most cases. The active phase represents the psychosis. Then the residual phase follows and is characterized by the same symptoms experienced during the prodromal phase (Ernest *et al.*, 2017). The severity of each type of schizophrenia symptoms differs from one individual to another. Also, within each class of symptoms, some symptoms are more likely to co-occur and others are less likely to be present in a same individual (McCutcheon *et al.*, 2019a). Hence, schizophrenia is a complex and heterogenous psychiatric disorder.

2.2. Roles of Dopamine in Schizophrenia Symptoms

Dopamine dysfunctions play an important role in schizophrenia symptoms, especially psychotic symptoms (Howes and Kapur, 2009). Initially, it was thought that schizophrenia symptoms result from excessive dopamine transmission because antipsychotic drugs produce antidopaminergic effects (Carlsson and Lindqvist, 1963; van Rossum, 1966; Creese *et al.*, 1976; Seeman *et al.*, 1976). However, it has since been recognized that dopamine contributions are more complex. A dominant view is that psychotic symptoms involve excessive subcortical dopamine transmission, whereas negative and cognitive symptoms involve low cortical dopamine transmission (Weinberger, 1987; Davis *et al.*, 1991). Furthermore, excessive subcortical and low cortical dopamine activities would co-exist because they reciprocally interact (Weinberger, 1987; Davis *et al.*, 1991). In other words, if mesocortical dopamine transmission is low, this would consequently increase striatal dopamine activity, or vice versa. These theories are still relevant to this day, but findings in the last decades are more supportive of an important role of dopamine in psychotic symptoms rather than negative and cognitive symptoms. This is discussed next in light of more recent findings that benefited of

more advanced technologies allowing *in vivo* measurement in the brain of schizophrenia patients. Note that a special focus is placed on dopamine here, because of the importance of this system in antipsychotic drug effects. However, dopamine is not the sole contributor to schizophrenia symptoms. For instance, serotonin (Kapur and Remington, 1996) and glutamate (Laruelle *et al.*, 2005) contribute to schizophrenia symptoms as well.

2.2.1. Psychotic Symptoms

Multiple studies strongly suggest that excessive dopamine-mediated signalling in the striatum is involved in psychosis. Stimulation of dopamine transmission using amphetamines (Lieberman *et al.*, 1987) or L-dopa (Seeman, 1987) can produce *de novo* psychotic symptoms in non-schizophrenic individuals, and can also be sufficient to exacerbate psychotic symptoms in individuals with schizophrenia. Furthermore, blockade of dopamine transmission effectively tempers psychotic symptoms (see Section 2.3.2.1, page 43). Hence, dopamine transmission seems both necessary and sufficient to psychotic symptoms. Dopamine transmission in the striatum seems to critically contribute to psychotic symptoms, at least for a majority of patients (see below). Using *in vivo* neuroimaging technology has been helpful to draw this conclusion, especially positron emission tomography, single-photon emission tomography and single photon emission computed tomography. Using these neuroimaging techniques, different aspects of dopamine signalling have been studied *in vivo* in individuals with schizophrenia, including dopamine synthesis and storage, dopamine release, dopamine receptors availability and DAT availability. Selective radioactive dopamine agents serve as radiotracer to estimate these values. Synthesis and storage of dopamine is studied *in vivo* by using radioactive L-dopa, that is converted in radioactive dopamine that accumulates in dopamine terminals (Reith *et al.*, 1994; Lindstrom *et al.*, 1999). Dopamine receptor/transporter availability is estimated with selective radioactive ligands. Dopamine release is determined by measuring the change in D2-like receptor availability following the administration of a dopamine agonist that stimulates dopamine release (such as d-amphetamine) (Laruelle and Abi-Dargham, 1999). Released dopamine competes with the D2-like receptor radioligand and thereby the reduction of D2-like receptor availability serves as an index of dopamine release.

In vivo neuroimaging studies revealed that schizophrenia patients show excessive dopamine transmission in the striatum, and these changes are mostly evident on the presynaptic side. FIG. 1.5 illustrates these important findings. Recent meta-analyses including hundreds of schizophrenia

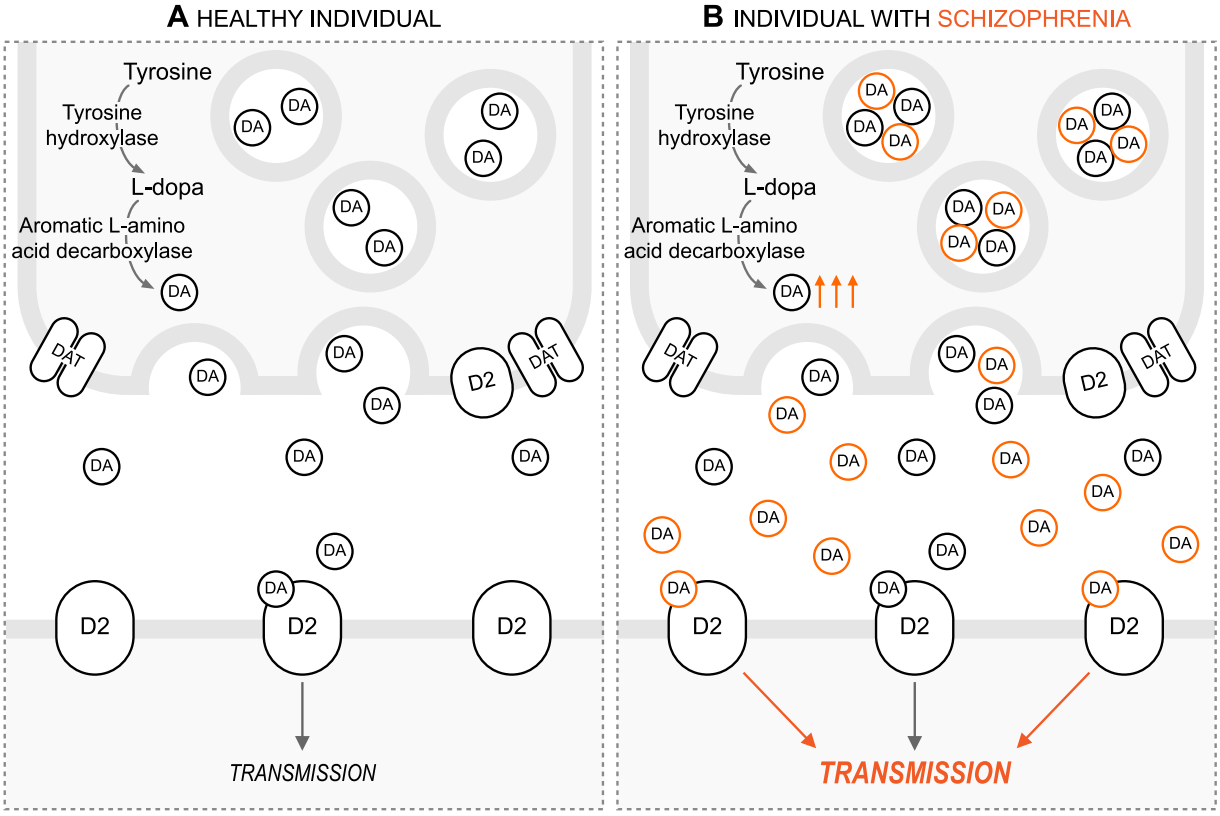

FIG. 1.5 — Excessive striatal dopamine transmission in psychosis: what changes and does not change at the dopamine synapse of individuals with schizophrenia. (***A***) Relative to healthy individuals, (***B***) changes in the dopamine synapse of schizophrenia patients are highlighted in orange. *What changes:* Psychotic symptoms are linked to greater synthesis, storage and release of dopamine. There might be a small increase in the density of D2-like receptors, but this is likely a consequence of antipsychotic medications and not a consequence of schizophrenia *per se*. Also, the proportion of D2-like receptors occupied by dopamine is increased. *What does not change:* Schizophrenia patients do not seem to show alterations in the numbers of D1-like receptors (not illustrated), dopamine transporters and dopamine vesicles.

patients revealed that they have greater striatal levels of dopamine synthesis/storage and dopamine release than healthy individuals (Howes *et al.*, 2012; McCutcheon *et al.*, 2018). This enhancement is most important in both the caudate and putamen relative to the nucleus accumbens (McCutcheon *et al.*, 2018). This effect is independent of medication status, suggesting it is linked to the disorder itself (Howes *et al.*, 2012). Also, these changes do not seem to be a consequence of a greater level of dopamine terminals and dopamine vesicles, because schizophrenia patients and healthy individuals have similar striatal levels of DAT (index of dopamine terminal density) (Howes *et al.*, 2012; Fusar-Poli and Meyer-Lindenberg, 2013) and VMAT2 (index of dopamine vesicle density) (Taylor *et al.*, 2000).

Importantly, the elevation in presynaptic dopaminergic functions has been linked to psychotic symptoms. Schizophrenia patients that are hospitalized due to heavy psychotic symptoms have greater levels of dopamine release in the striatum relative to stabilized patients (Laruelle *et al.*, 1999). As mentioned above, amphetamines can exacerbate psychotic symptoms, and they can also be used as tools to measure *in vivo* dopamine release. Schizophrenia patients showing the largest enhancement in dopamine release in the striatum following amphetamine administration also show the largest amphetamine-induced exacerbation of psychotic symptoms (Laruelle *et al.*, 1999). Schizophrenia itself does not seem linked to elevated striatal levels of D1-like receptors, but schizophrenia patients show an inconsistent small increase in striatal levels of D2-like receptors that is at least partially due to antipsychotic drug exposure (Silvestri *et al.*, 2000; Kestler *et al.*, 2001; Howes *et al.*, 2012). Because dopamine release is more important, dopamine occupies a greater proportion of D2-like receptors in schizophrenia patients relative to healthy individuals, independently of whether patients show greater levels of D2-like receptor availability due to antipsychotic treatment (Abi-Dargham *et al.*, 2000). In that latter study, dopamine occupancy was determined by measuring D2-like receptor availability during two occasions, at baseline and following a treatment that depletes dopamine brain levels (α-methyl-*para*-tyrosine, an inhibitor of tyrosine hydroxylase). Abi-Dargham *et al.* (2000) showed that dopamine depletion ameliorated psychotic symptoms, and that patients showing the greatest dopamine occupancy at baseline have the best amelioration of psychotic symptoms after dopamine depletion. While changes in presynaptic dopaminergic functions have been consistently found across studies, this cannot be generalized to all patients. Indeed, there are patients showing no increase, especially the ones that do not respond to antipsychotic medications (this is further described in Section 2.3.2.1, page 43) (Demjaha *et al.*, 2012). Nonetheless, the findings above strongly suggest that for most patients, psychotic symptoms involve an enhancement of presynaptic dopaminergic functions and consequently, greater dopamine-mediated signalling.

A key question is *how* does excessive dopamine transmission in the striatum contribute to psychotic symptoms? As described previously (Section 1.2.2, page 24), the striatum is part of basal ganglia circuits. Dopamine signalling within the striatum is important in integrating and processing information coming from cortical regions. Hence, an increase in striatal dopamine transmission could lead to an impaired gating of information (Maia and Frank, 2017; McCutcheon *et al.*, 2019b). Surrounding stimuli would elicit abnormally high dopaminergic responses, and this would

contribute to imbalanced perception and thought processes that is characteristic of psychotic symptoms. One particular function of striatal dopamine that could importantly contribute to psychosis is the attribution of motivational salience to stimuli. Outside of the context of schizophrenia, the role of dopamine in incentive motivation and of this dopaminergic function in drug addiction [see for instance Robinson and Berridge (1993)] have led to the idea that this neurotransmitter could also regulate impaired motivational processes in psychosis. Kapur (2003) theorized that impairment in the attribution of incentive salience could especially play an important role during the prodromal stage. Indeed, continued high dopamine signalling would attribute *aberrant* motivational value to irrelevant internal and external stimuli. Hence, patients would give an exaggerated importance to irrelevant surrounding stimuli, which would contribute to delusional thoughts and hallucinations. For example, if an individual with schizophrenia has delusional thoughts involving being pursued, a car that is passing by would normally represent a neutral cue, but excessive dopamine transmission in the striatum would imbue that car with aberrant motivational salience (FIG. 1.6). The motivational salience gained by the car would contribute to the thought that, for instance, it is driven by someone pursuing the individual with schizophrenia. Because the increase in dopamine transmission is persistent in schizophrenia patients, the aberrant salience and motivational effect of these stimuli would persist and escalate. A psychotic episode is then reached when the increasing importance of these thoughts and perceptions significantly impairs the life of patients. Similarly, internal and external stimuli could be imbued with aberrant motivational salience in individuals with clinical high-risk of psychosis as well (Howes *et al.*, 2020).

2.2.2. Negative and Cognitive Symptoms

Cognitive and negative symptoms are hypothesised to be underlined by low mesocortical dopamine transmission (Weinberger, 1987; Davis *et al.*, 1991). If this hypothesis is true, then stimulating dopamine transmission should improve these deficits, and it seems to do so. Schizophrenia patients have been consistently shown to have impaired working memory (Forbes *et al.*, 2009), and this is improved by an acute administration of d-amphetamine (Kirrane *et al.*, 2000; Barch and Carter, 2005). Other cognitive deficits could also be due to low mesocortical dopamine transmission. For instance, schizophrenia patients have impaired performance in tasks engaging diverse cognitive function such as attention, and acute stimulant exposure improves their performance relative to

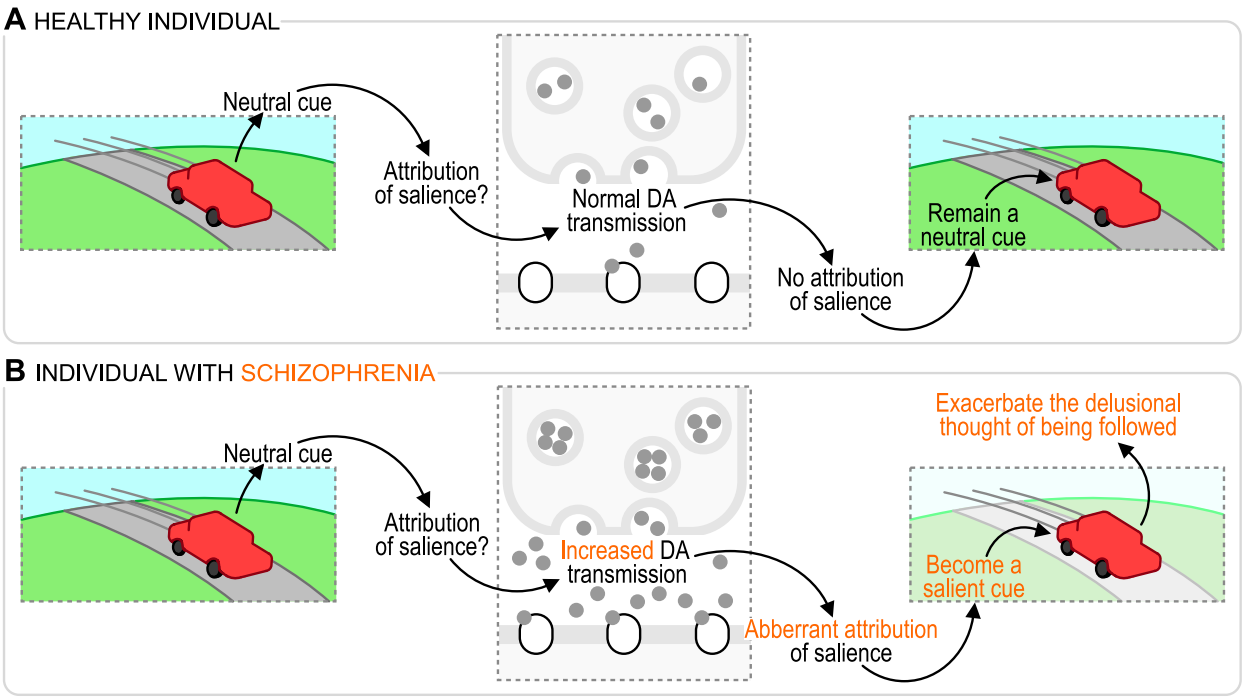

FIG. 1.6 — Sustained, high dopamine transmission would attribute aberrant salience to irrelevant stimuli, and this would promote psychotic symptoms. (***A***) The example illustrated here shows that with normal levels of dopamine transmission in the striatum, irrelevant cues (such as a car) are not imbued with motivational salience and thereby, they remain neutral relative to their motivational effects. (***B***) In individuals with schizophrenia, irrelevant cues can be imbued with motivational salience due to the continuous state of increased dopaminergic transmission in the striatum. Consequently, the aberrant salience of irrelevant cues can exacerbate ongoing psychotic symptoms such as, for example, the delusional thought of being pursued by a car, as illustrated here.

placebo (Daniel *et al.*, 1991; Siegel *et al.*, 1996). However, note that chronic stimulant administration (armodafinil, concomitant with antipsychotic treatment) does not improve cognitive functions of schizophrenia patients (Kane *et al.*, 2010; Bobo *et al.*, 2011). This could be due to the choice of the stimulant, and/or that a chronic regimen loses the beneficial effects of an acute administration. Nonetheless, acute stimulant administration improves cognitive functions in schizophrenia, and this could reflect the involvement of low mesocortical dopamine transmission in the cognitive deficits of schizophrenia patients. Interestingly, stimulating dopamine transmission also improves negative symptoms. Indeed, chronic concomitant administration of a stimulant with antipsychotic drugs improves negative symptoms over time, in stabilized patients showing low psychotic symptoms (Kane *et al.*, 2010; Bobo *et al.*, 2011; Lasser *et al.*, 2013).

While dopamine agonists could somewhat improve cognitive and negative symptoms, evidence that dopamine transmission is low in the prefrontal cortex of individuals with schizophrenia is mostly indirect. Neuronal activity in the prefrontal cortex of schizophrenia patients is lower relative to healthy controls, and this difference is most evident during cognitive tasks (Davidson and Heinrichs, 2003). Interestingly, amphetamine enhances both cognitive functions in schizophrenia patients and neuronal activity in the prefrontal cortex (Daniel *et al.*, 1991). Also, schizophrenia patients have greater levels of D1-like receptor availability in the prefrontal cortex (Abi-Dargham *et al.*, 2002; Abi-Dargham *et al.*, 2012). Abi-Dargham *et al.* (2002) suggested that this up-regulation of D1-like receptor availability is a compensatory change due to lower cortical dopamine release. A study by Slifstein *et al.* (2008) is in line with this idea. In their study, they measured D1-like receptor availability in the prefrontal cortex of healthy individuals. D1-like receptor availability was compared between individuals with estimated high or low cortical dopamine release, based on the COMT polymorphism they express. Individuals expressing the Val/Val polymorphism of COMT are presumed to have lower levels of extracellular dopamine in the prefrontal cortex due to increased COMT activity, whereas individuals expressing the Met/Met polymorphism have presumed high cortical dopamine release due to decreased COMT activity (Chen *et al.*, 2004). Slifstein *et al.* (2008) showed that individuals with presumed high levels of cortical dopamine release have lower D1-like availability in the prefrontal cortex than individuals with presumed lower cortical dopamine release. Hence, schizophrenia patients could have greater levels of D1-like receptors in the prefrontal cortex due to decreased dopamine release (Abi-Dargham *et al.*, 2002; Abi-Dargham *et al.*, 2012). Individuals with schizophrenia that express Val/Val COMT have worst deficit in working memory than patients expressing Met/Met COMT (Goldberg *et al.*, 2003), suggesting that lower levels of dopamine release in the prefrontal cortex are linked to working memory deficits in schizophrenia. However, the COMT polymorphism has not been consistently associated with negative symptoms, with some reports showing that Val/Val COMT is linked to more severe negative symptoms than Met/Met COMT (Wang *et al.*, 2010; Mao *et al.*, 2016), whereas other reports found no correlation (Tovilla-Zarate *et al.*, 2013; Clelland *et al.*, 2016).

Other evidence suggesting that mesocortical dopamine transmission is low come from the hypothesis that in schizophrenia, excessive dopamine transmission in the striatum—a key feature of psychotic symptoms—could consequently reduce mesocortical dopamine transmission, or could

be the consequence of low mesocortical dopamine transmission (Weinberger, 1987; Davis *et al.*, 1991). Recent animal studies support that view. For instance, transgenic mice overexpressing D2 receptors in the striatum show impaired working memory and impaired coherent activity between VTA dopamine and prefrontal cortex neurons during working memory (Duvarci *et al.*, 2018). Also, increased neuronal activity in the prefrontal cortex supresses behaviours mediated by enhanced dopamine transmission in the striatum (Ferenczi *et al.*, 2016). There are some reports suggesting that a negative relationship between subcortical and cortical dopamine transmission exists in schizophrenia patients. For instance, Meyer-Lindenberg *et al.* (2002) showed that schizophrenia patients have lower neuronal activity in the prefrontal cortex during a cognitive task relative to healthy controls, and this decreased activity in the prefrontal cortex negatively correlates with dopamine storage in the striatum (a relationship only found in schizophrenia patients).

2.3. The Pharmacological Treatment of Schizophrenia Symptoms

2.3.1. What Are Antipsychotic Drugs?

Schizophrenia is not curable but pharmacological and psychosocial treatments can be used to control the expression of schizophrenia symptoms (Mueser and McGurk, 2003). Antipsychotic drugs are the only drugs that have been proven successful to temper psychotic symptoms. They are molecules that share the ability to decrease dopamine transmission. The first antipsychotic drug ever used in schizophrenia patients was chlorpromazine in 1952 (Laborit *et al.*, 1952; Ban, 2007). This drug was initially meant to be used as an adjunctive anesthetic, but instead started to be used to treat schizophrenia patients due to its 'calming' effects. Chlorpromazine is the first of many antipsychotic compounds that decrease dopamine transmission via an interaction with D2-like receptors. Shortly after, reserpine was the second antipsychotic drug ever developed and used (Hollister *et al.*, 1955). It is the only antipsychotic drug that is not a D2-like ligand. Instead, as mentioned previously, reserpine decreases dopamine transmission by emptying out dopamine vesicles (Carlsson *et al.*, 1957). However, it was later stopped being prescribed as it has low antipsychotic efficacy and produces multiple side effects such as hypotension and depression (Feldman *et al.*, 1997).

Since the 50's, dozens of D2-like ligands have been developed to treat schizophrenia symptoms. They are classified into two types: typical antipsychotics and atypical antipsychotics. Typical

antipsychotic drugs include chlorpromazine and related chemical compounds (such as fluphenazine, thioridazine and trifluoperazine), as well as haloperidol, sulpiride and pimozide (Katzung, 1992). The first atypical antipsychotic drug ever developed was clozapine in 1966 (Hippius, 1989). The atypical class was termed so because clozapine has antipsychotic effects but does not have the 'typical' motor side effects that all the other antipsychotic drugs had at the time. Indeed, antipsychotic drugs can produce motor dysfunctions including Parkinson-like symptoms [*i.e.*, rigidity and shaking; (Simpson and Angus, 1970)] and tardive dyskinesia [*i.e.*, involuntary movements, mostly orofacial; (Simpson *et al.*, 1979)]. The profile of clozapine was unexpected, because it was thought that the therapeutic effects of antipsychotic drugs are necessarily accompanied with motor dysfunctions (Hippius, 1989). Since then, other atypical drugs with a considered low risk of inducing motor dysfunctions have been developed, including olanzapine, risperidone, quetiapine, aripiprazole, ziprasidone, sertindole and amisulpiride (Shapiro *et al.*, 2003; Spiegel and Fatemi, 2003).

Antipsychotic drugs share the common characteristic to interact with D2-like receptors. They are all D2-like receptor antagonist with the notable exception that aripiprazole is a partial D2-like receptor agonist (Shapiro *et al.*, 2003). Antipsychotic drugs also interact with other receptors than D2-like receptors, such as D1-like receptors, as well as serotoninergic, noradrenergic, cholinergic and histaminergic receptors (FIG. 1.7) (Miyamoto *et al.*, 2005; Richtand *et al.*, 2007). While most antipsychotic drugs interact with numerous receptors, their actions on D2-like receptors seem particularly important in their ability to relieve schizophrenia symptoms, especially psychotic symptoms (see next section). For instance, antipsychotic drugs with greater affinity for D2-like receptors are clinically effective at lower doses than antipsychotic drugs with a lower D2-like receptor affinity. Indeed, there is a positive correlation between the doses at which antipsychotic drugs are therapeutically effective and their affinity for D2-like receptors (Creese *et al.*, 1976; Seeman *et al.*, 1976). More recent findings using similar analysis revealed that this positive correlation is true for D2 receptors, but not for D3 and D4 receptors (Richtand *et al.*, 2007) nor D1-like receptors (Seeman, 1987). Furthermore, the degree of *in vivo* occupancy of striatal D2-like receptors by antipsychotic drugs predicts clinical outcomes. At clinically efficacious doses, antipsychotic drugs typically occupy above ~60-65 % of striatal D2-like receptors (Farde *et al.*, 1989; Wiesel *et al.*, 1990; Farde *et al.*, 1992; Nordstrom *et al.*, 1993; Kapur *et al.*, 2000b). Antipsychotic treatments are less efficient to improve schizophrenia symptoms when antipsychotic

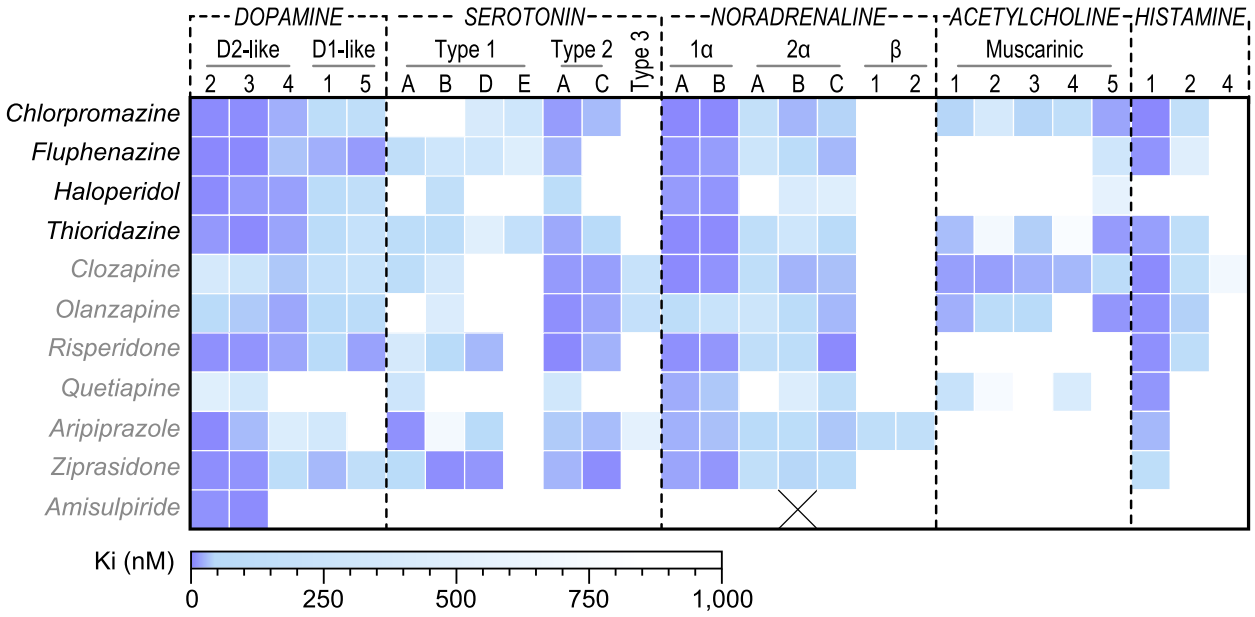

FIG. 1.7 — Visual representation of antipsychotic drug affinity (Ki) for dopamine and non-dopamine receptors. The Ki values come from the Ki database of the Psychoactive Drug Screening Program (PDSP) of the National Institute of Mental Health (NIMH). Values are PDSP certified or the mean Ki values listed in the database if PDSD certified value is not available, as in Richtand *et al*. (2007). The 'X' indicates that no Ki value was available. Each column is associated to a receptor, and each row is associated to an antipsychotic drug. Typical antipsychotic drugs are in black and atypical drugs are in grey.

drug dosage leads to striatal D2-like occupancy below ~60 % (Kapur *et al.*, 2000b). Similarly, the subjective well-being of patients is improved when striatal D2-like occupancy is above ~60 % relative to when D2-like occupancy is below this threshold (de Haan *et al.*, 2003).

Typical and atypical antipsychotic drugs interact distinctively with D2-like receptors. While typical and atypical antipsychotic drugs generally produce therapeutic effects with D2-like occupancy above ~60 %, some atypical antipsychotic drugs can be clinically efficacious at striatal D2-like occupancy well under 60 % (Farde *et al.*, 1989; Wiesel *et al.*, 1990; Farde *et al.*, 1992; Pilowsky *et al.*, 1996; Kapur *et al.*, 2000c). Also, atypical antipsychotic drugs dissociate more rapidly from D2-like receptors than antipsychotic drugs, while both drug classes bind to a similar rate to these receptors (Kapur and Seeman, 2001) [but see (Sahlholm *et al.*, 2016)]. By interacting more 'loosely' with D2-like receptors, atypical antipsychotic drugs are thought to interfere to a lesser extent with physiological dopamine transmission than typical antipsychotic drugs (Kapur and Seeman, 2001).

2.3.2. The Relative Efficacy of Antipsychotic Drugs

2.3.2.1. Psychotic Symptoms

As described earlier, psychotic symptoms involve high dopamine transmission in the striatum, at least for most patients. The antidopaminergic effects of antipsychotic drugs are effective to temper psychotic symptoms relative to placebo, as showed across thousands of schizophrenia patients treated with different type of antipsychotic drugs, whether typical or atypical (Huhn *et al.*, 2019). However, antipsychotic drugs can be ineffective to treat psychotic symptoms in some patients. Possible explanations for this include that antipsychotic drugs promote neuroadaptations leading to dopamine supersensitivity (this is further described in Section 2.4, page 50). Another possibility is that psychotic symptoms involve different mechanisms that vary from patients to patients. Hence, non-dopamine mechanisms could importantly contribute, which would reduce the efficacy of antipsychotic drugs (even though they act on other receptors than dopamine receptors) (Miyamoto *et al.*, 2005; Richtand *et al.*, 2007). Also, for some patients, antipsychotic drugs could be targeting the wrong aspects of the dopamine system. As described earlier (Section 2.2.1, page 34), psychotic symptoms are generally linked to potentiated presynaptic dopaminergic functions, leading to greater dopamine release and consequently greater occupation of dopamine receptors. However, some patients do not show enhanced presynaptic dopaminergic functions (Demjaha *et al.*, 2012). These patients do not respond to antipsychotic drugs, whereas patients that show enhanced presynaptic dopaminergic functions are responsive (Demjaha *et al.*, 2012). Hence, by acting downstream the problem (*i.e.*, enhanced presynaptic dopaminergic functions), antipsychotic efficacy could be limited to a certain population of patients. This raises the question whether antipsychotic drugs are effective because they simply block the downstream effects of dopamine? If so, does this mean that blocking D1-like or D2-like receptors would similarly reduce psychotic symptoms because the downstream effects of dopamine are blocked? The answer is no. Indeed, blockade of D1-like receptors does not improve psychotic symptoms (Den Boer *et al.*, 1995; Karlsson *et al.*, 1995). Hence, even if a majority of patients show enhanced presynaptic dopaminergic functions and that antipsychotic drugs do not directly act on these aspects, the actions of antipsychotic drugs on D2-like mediated signalling are important in their ability to produce therapeutic effects.

Another important question is *why* do antipsychotic drugs improve psychotic symptoms? As described earlier, a continued hyperdopaminergic state would attribute aberrant motivational value to surrounding stimuli, contributing to the importance of delusional thoughts and hallucinations, and eventually this escalates to a psychotic episode (Kapur, 2003). By reducing dopamine transmission, antipsychotic drugs would temper the attribution of aberrant motivational value to stimuli, which would consequently help to not exacerbate delusions and hallucinations. For instance, antipsychotic drugs would allow to not attribute aberrant salience to a neutral car, preventing a worsening of the delusional thought of being pursued (FIG. 1.6, page 38). Hence, antipsychotic drugs do not 'erase' psychotic symptoms, but they would extinguish aberrantly salient stimuli and prevent the attribution of aberrant salience to stimuli (Kapur, 2003; Kapur *et al.*, 2005). Accordingly, from patients' perspective, antipsychotic drugs would help them to feel detached from their psychotic symptoms (Mizrahi *et al.*, 2005).

2.3.2.2. Negative and Cognitive Symptoms

The role of dopamine in negative and cognitive symptoms is unclear but could involve low dopamine transmission. Hence, it does not come as a surprise that antipsychotic drugs are not effective to temper negative and cognitive symptoms. Meta-analyses revealed that cognitive symptoms are generally improved in a moderate manner (if at all) by antipsychotic drugs, to a point where it can be questioned if it is significantly beneficial for patients (Mishara and Goldberg, 2004; Desamericq *et al.*, 2014). Similar conclusions can be made with negative symptoms. Meta-analyses have revealed that antipsychotic treatment can be ineffective, or can moderately improve negative symptoms to an extent that is unlikely impactful for patients (Fusar-Poli *et al.*, 2015; Krause *et al.*, 2018). Furthermore, another meta-analysis revealed that antipsychotic drugs are effective to treat psychotic symptoms in patients with early onset schizophrenia (before 18 years old), but they do not improve their negative symptoms (Harvey *et al.*, 2016).

When improvement of negative symptoms are noted, they could be in fact confounded by other effects of antipsychotic drugs (Fusar-Poli *et al.*, 2015; Krause *et al.*, 2018). Indeed, antipsychotic drugs can improve *secondary* negative symptoms, meaning that some symptoms experienced by patients can be similar to negative symptoms but have another cause such as depression or social deprivation (Kirschner *et al.*, 2017). Furthermore, by reducing psychotic symptoms, antipsychotic drugs can also indirectly improve negative symptoms because psychotic symptoms can be at the

source of negative symptoms (such as social isolation caused by delusions or hallucinations) (Kirschner *et al.*, 2017). Krause *et al.* (2018) showed that only one out of six antipsychotic drugs tested in their study (amisulpiride) improved negative symptoms in schizophrenia patients that are less likely to have secondary negative symptoms, because they have no to little psychotic symptoms. Hence, antipsychotic drugs might not target the neuronal substrates involved in primary negative symptoms, explaining their underwhelming effects. While antipsychotic drugs seem more appropriate to treat psychotic symptoms in most patients, non-dopamine drugs can serve as adjunctive treatment to temper negative and cognitive symptoms, as well as complimentary non-pharmacological approaches such as psychosocial treatments (Mueser and McGurk, 2003; Erhart *et al.*, 2006).

2.3.3. How to Probe Antipsychotic-like Effects in Laboratory Animals?

Animals studies allow to identify new compounds with antipsychotic-like properties, as well as to identify the neurobiological mechanisms involved in antipsychotic efficacy but also in antipsychotic failure. These valuable descriptions are obtained using behavioural paradigms with predictive validity to measure antipsychotic-like effects in laboratory animals. Next is a description of how to adequately mimic antipsychotic treatment in laboratory animals, followed by a description on behavioural paradigms used to estimate antipsychotic-like efficacy.

2.3.3.1. Clinically Representative Treatment Regimen

Using an adequate antipsychotic treatment regimen is the first step to appropriately study antipsychotic-like effects in laboratory animals. This allows to increase the translational value of preclinical studies. Two variables of the treatment regimen have to be carefully considered: the antipsychotic dose and the kinetic of treatment. A way to compare animal and human dosing is to use the proportion of D2-like receptor occupied by antipsychotics in the striatum. This index is a good comparator because, as mentioned previously, all antipsychotic drugs interact with D2-like receptors and this interaction is closely linked to antipsychotic efficacy. Therefore, for a given antipsychotic drug, the dose used in animals should match D2-like occupancy that is linked to therapeutic effects in schizophrenia patients—usually above ~65 %, no more than 80 % to avoid a greater risk of motor dysfunctions as a side effect (Kapur *et al.*, 2003).

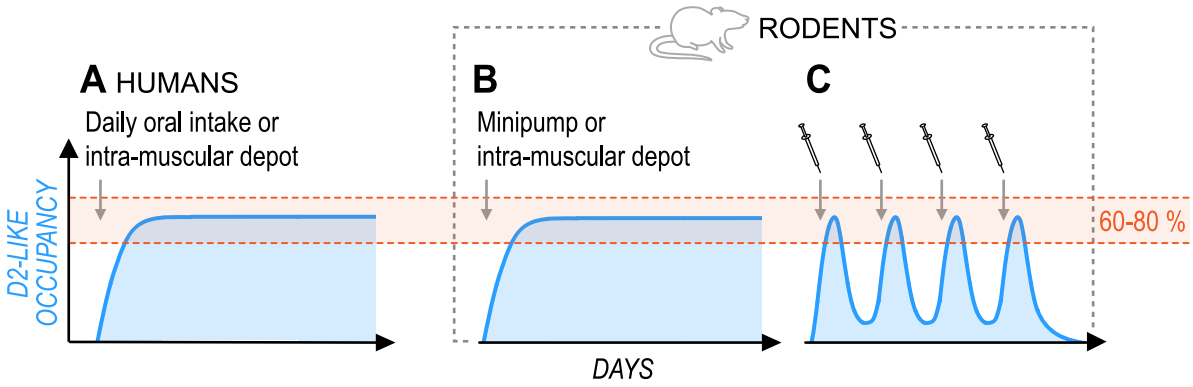

FIG. 1.8 — Occupancy of striatal D2-like receptors in schizophrenia patients and laboratory animals. Both in humans and laboratory animals, antipsychotic drugs produce antipsychotic effects typically when they occupy ~60-80 % of D2-like receptors in the striatum. (***A***) When schizophrenia patients adhere to their treatment, daily oral intake of antipsychotic drugs leads to continuously high D2-like occupancy in the striatum. Intra-muscular depot of a long-acting injectable antipsychotic also achieves sustained and high D2-like occupancy. (***B***) In rodents, administration of antipsychotic drugs via subcutaneous minipumps or via an intra-muscular depot of long-acting injectable antipsychotic achieves continuously high D2-like occupancy. Thus, this mimics the temporal dynamic of antipsychotic treatment in compliant patients. (***C***) Because antipsychotic drugs are quickly metabolised in rodents, daily injections lead to a transiently high occupancy of D2-like receptors. Thus, this treatment regimen does not mimic standard antipsychotic treatment regimen used in the clinic.

The kinetics of treatment should also match what is recommended for treatment in humans. Prescription practices favour that patients are continuously exposed to antipsychotic drugs, leading to continuous high levels of D2-like receptor occupancy above the minimal threshold associated with therapeutic effects. In schizophrenia patients, this is achieved by daily intake of oral antipsychotic drugs or via intra-muscular depot of long-acting injectable antipsychotics every few weeks (FIG. 1.8A) (Farde *et al.*, 1989; Remington *et al.*, 2006; Mamo *et al.*, 2008). To achieve continuous and high levels of D2-like receptor occupancy in rodents, antipsychotic drugs can be administered via an osmotic minipump implanted subcutaneously (that continuously deliver its content) or via an intra-muscular depot of long-acting injectable antipsychotics every few weeks as in humans (FIG. 1.8B) (Kapur *et al.*, 2003; Turrone *et al.*, 2003b). Of note, it is common in preclinical studies to expose rodents chronically to antipsychotic drug via daily injections given through the systemic route. However, this method does not mimic the kinetic of antipsychotic treatment in humans, because rodents metabolise antipsychotic drugs more quickly than humans. Hence, after a single subcutaneous injection, D2-like receptor occupancy is high 1-2 hours after the injection (*i.e.*, above the minimal threshold for therapeutic effects), but very low 24 hours later,

leading to transient, high exposure to antipsychotic drugs (FIG. 1.8C) (Kapur *et al.*, 2000a; Kapur *et al.*, 2003). While this treatment regimen is not modelling standard antipsychotic treatment in humans, it could represent an alternative approach to the chronic management of schizophrenia symptoms, because regular, transient exposure could reduce the incidence of aversive effects over time and still be therapeutically efficacious (this is further described in Section 2.4.3, page 56).

2.3.3.2. Conditioned Avoidance Responding

Conditioned avoidance responding is a common behavioural paradigm used to measure antipsychotic-like effects in animals. The apparatus used to measure this behaviour typically consists of a test chamber with two compartments (FIG. 1.9A). Animals learn that a cue (such as a tone) predicts an electrical shock that is delivered through the floor in the compartment where the animal is located. No electrical shock is given in the adjacent compartment. With repeated training, the cue acquires predictive value and become a CS. Thereby, animals learn to go to the safe side during the CS rather than during the delivery of the electrical shock (Cook and Sepinwall, 1975; Wadenberg and Hicks, 1999). When animals go to the safe side in response to the CS, this is considered a *conditioned avoidance response*. When animals go to the safe side during the electric shock delivery, this is referred to as an *unconditioned escape*. When animals do not escape during the electrical shock, this is referred to as a *failure*. In well-trained animals, antipsychotic treatment reduces conditioned avoidance responding without reducing unconditioned escape from the electrical shock or failure to escape (Cook and Sepinwall, 1975; Wadenberg and Hicks, 1999). By not decreasing the numbers of unconditioned escape and failure to escape, antipsychotic drugs are unlikely to reduce conditioned avoidance responding by generally reducing motor performance (Beninger, 1989; Wadenberg and Hicks, 1999).

Antipsychotic drugs reliably reduce conditioned avoidance responding at clinically representative doses (Wadenberg and Hicks, 1999; Wadenberg *et al.*, 2001). Generally, non-antipsychotic compounds either reduce avoidance and escape or neither of those, but do not reduce avoidance and spare escape like antipsychotic drugs do (Wadenberg and Hicks, 1999). Hence, the selective reduction of conditioned avoidance responding is a well validated predictor of antipsychotic-like effects. As mentioned previously (Section 2.3.2.1, page 43), antipsychotic drugs are thought to reduce the motivational salience of surrounding stimuli, an effect that would contribute to the reduction of psychotic symptoms. Hence, antipsychotic drugs could have similar psychological

effects in the conditioned avoidance responding paradigm—*i.e.*, attenuating the motivational salience of the aversive CS so that it is less powerful to elicit avoidance (Kapur *et al.*, 2005).

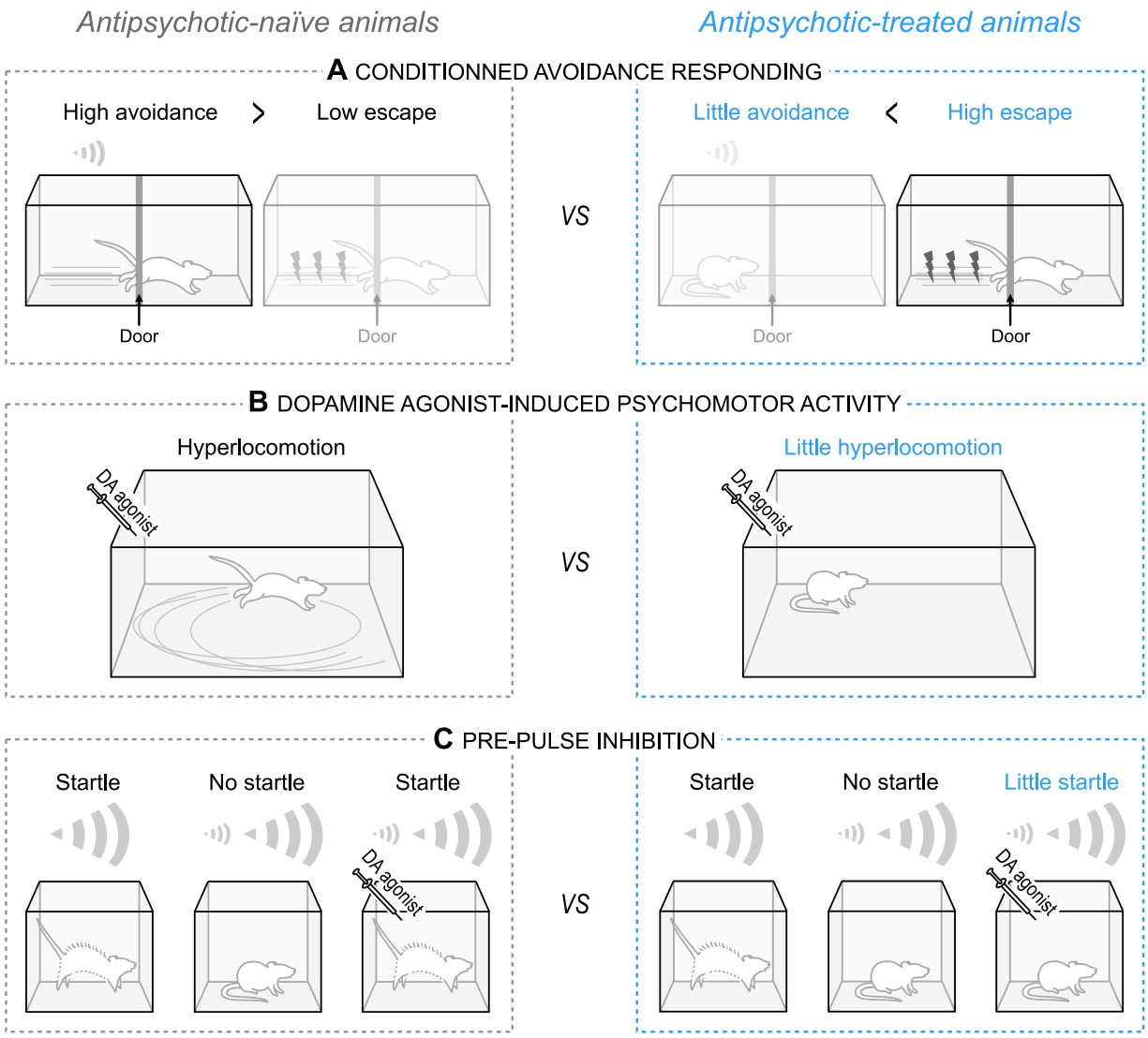

FIG. 1.9 — Behavioural measures of antipsychotic-like effects in laboratory animals. (*A*) In the conditioned avoidance responding paradigm, animals learn that a conditioned stimulus (CS, here a tone) predicts an electric shock. When animals go to the adjacent compartment during CS presentation, this is considered to be an avoidance response, whereas during the electric shock, this is considered to be an escape response. Antipsychotic drugs decrease avoidance but not escape responding. (*B*) Antipsychotic drugs reduce the psychomotor response to dopamine agonists. (*C*) In the pre-pulse inhibition paradigm, a startle pulse does not evoke a startle reflex when preceded by a pre-pulse of low intensity. This effect is termed pre-pulse inhibition. Dopamine agonists inhibit pre-pulse inhibition, and antipsychotic drugs reverse that effect of dopamine agonists.

2.3.3.3. Locomotor Activity

The antidopaminergic effects of antipsychotic drugs can be probed by measuring their influence on the behavioural effects of dopamine agonists. A simple test is to measure the inhibitory effects of antipsychotic drugs on the locomotor activating effects of dopamine agonists (FIG. 1.9B). This suppressive effect of antipsychotics has been demonstrated with psychostimulant drugs including d-amphetamine and apomorphine (Niemegeers and Janssen, 1979; Ljungberg and Ungerstedt, 1985). Importantly, this inhibitory effect of antipsychotic drugs relies on their ability to reduce dopamine transmission in the striatum—a key region in psychosis. Indeed, infusion of antipsychotic drugs into the nucleus accumbens or caudate-putamen is sufficient to reduce psychomotor activity induced by dopamine agonists administered either locally or through the systemic route (Pijnenburg *et al.*, 1975; Ervin *et al.*, 1981; van den Boss *et al.*, 1988; Duvauchelle *et al.*, 1992; Baker *et al.*, 1996; Dalia *et al.*, 1998).

An important issue to consider is that because antipsychotic drugs alone can reduce spontaneous locomotor activity, the ability of these drugs to reduce the stimulating effects of dopamine agonists could be non-specific. Such reduction in basal activity can be caused by the sedative effects of antipsychotics (Spiegel and Fatemi, 2003). Also, in accordance with the role of dopamine in motricity and motivation (Section 1.3, page 27), antipsychotic drugs reduce the ability of animals to perform actions but also their motivation to perform actions (Beninger, 1989). Even if antipsychotic drugs can reduce spontaneous locomotor activity, this effect does not represent a sufficient explanation as to why these medications reduce the locomotor response to dopamine agonists, because the effects of antipsychotic drugs on both type of activity (spontaneous and stimulated) does not necessarily correlate. Indeed, some antipsychotic drugs are much more potent to reduce amphetamine-induced locomotion than spontaneous locomotion, and they can be effective to reduce hyperlocomotor activity without influencing basal activity (Schaefer and Michael, 1984; Arnt, 1995).

2.3.3.4. Pre-pulse Inhibition

Pre-pulse inhibition measures gating of sensory information and recruits basal ganglia circuits (Swerdlow *et al.*, 1992). In this paradigm, the startle reflex is measured following an auditory startle pulse. The amplitude of the startle reflex is compared with a condition where the startle

pulse is shortly preceded by an auditory pulse of a very low intensity that is difficult to detect. This pre-pulse reduces the startle reflex evoked by the startle pulse. This effect is termed pre-pulse inhibition.

Dopaminergic activity influences pre-pulse inhibition, especially D2-like transmission. Psychostimulant drugs and D2-like agonists but not D1-like agonists disturb pre-pulse inhibition, and antipsychotic drugs but not D1-like antagonists restore pre-pulse inhibition (FIG. 1.9C) (Mansbach *et al.*, 1988; Swerdlow *et al.*, 1991; Schwarzkopf *et al.*, 1993; Swerdlow and Geyer, 1993; Wan and Swerdlow, 1993; Caine *et al.*, 1995; Varty and Higgins, 1995). Interestingly, antipsychotic drug dosing that are effective to reverse pre-pulse inhibition in laboratory animals positively correlate with clinically effective doses in schizophrenia patients and with D2-like receptors affinity (Swerdlow *et al.*, 1994). The effects of antipsychotic drugs on pre-pulse inhibition rely on their ability to reduce striatal dopamine transmission, a relevant effect in the context of psychosis. Indeed, infusion of dopamine in the striatum impairs pre-pulse inhibition, and this is reversed by antipsychotic drug administration (Wan and Swerdlow, 1993; Swerdlow *et al.*, 1994).

2.4. Antipsychotic-evoked Dopamine Supersensitivity

It is common practice to favour a continuous exposure to antipsychotic drugs, as it is thought to be the best approach to stabilize patients and to prevent psychosis relapse. Patients can take daily oral antipsychotics, or they can be treated with extended releasing formulation of antipsychotic drugs, ensuring a continuous delivery of antipsychotic drugs for weeks. Additionally, patients can be treated with more than one antipsychotic drug at the time. Like chronic exposure to any drug would do, chronic antipsychotic exposure produces numerous side effects over time that are deleterious for patients (Murray *et al.*, 2016). Indeed, antipsychotic drugs can produce metabolic disorders (*e.g.*, weight gain, type 2 diabetes), endocrine disorders (*e.g.*, hyperprolactinemia), cardiovascular disorders (*e.g.*, hypertension), as well as motoric disturbances (Katzung, 1992; Muench and Hamer, 2010; De Hert *et al.*, 2011). Also, chronically interfering with D2-like transmission can promote neuronal adaptations leading to supersensitivity to dopamine stimulation. We previously reviewed the clinical and preclinical manifestations of antipsychotic-evoked dopamine supersensitivity, as well as contributing factors and neurobiological mechanisms potentially implicated in this long-

term effect of antipsychotic drugs [see Annex, page 249; (Servonnet and Samaha, 2020)]. Below I briefly describe important concepts on antipsychotic-evoked dopamine supersensitivity.

2.4.1. Behavioural Manifestations of Dopamine Supersensitivity

In schizophrenia patients, dopamine supersensitivity would counteract the therapeutic effects of antipsychotic drugs and exacerbate psychotic symptoms (FIG. 1.10A) (Chouinard *et al.*, 1978; Chouinard and Jones, 1980; Chouinard *et al.*, 2017). As described earlier, antipsychotic drugs can effectively treat psychotic symptoms because they have antidopaminergic effects. Hence, dopamine supersensitivity would break through during treatment and counteract the antidopaminergic effects of antipsychotic drugs, leading to a tolerance to these effects. Consequently, patients would be more likely to experience a relapse to psychosis. Additionally, patients could be more prone to relapse because dopamine supersensitivity exacerbates psychotic symptoms *per se*, as they involve high dopamine transmission (Howes and Kapur, 2009). Such exacerbation of psychotic symptoms is most pronounced when antipsychotic dosing is decreased, or treatment is ceased (Chouinard *et al.*, 1978; Chouinard and Jones, 1980). Schizophrenia patients frequently stop their medication because of their aversive effects (Lieberman *et al.*, 2005) and this evidently can cause psychosis relapse. However, psychosis relapse commonly occur even in compliant patients (Rubio *et al.*, 2020), and this could be due to dopamine supersensitivity.

It is unlikely feasible to establish in humans whether antipsychotic drugs cause dopamine supersensitivity over time. However, there are several lines of evidence supporting that antipsychotic drugs can have this effect in humans. This is most evident when relating signs of dopamine supersensitivity with the emergence and expression of tardive dyskinesia. This is a side effect purely produced by antipsychotic drug exposure and could involve dopamine supersensitivity (Casey, 1991; Waln and Jankovic, 2013). Antipsychotic-evoked dopamine supersensitivity and tardive dyskinesia seem to commonly co-occur in schizophrenia patients (Chouinard *et al.*, 1978; Chouinard and Jones, 1980; Fallon and Dursun, 2011; Fallon *et al.*, 2012). In fact, tardive dyskinesia seems to be an important predictor of psychosis relapse in compliant patients, as shown in a meta-analysis including 5,130 patients (Rubio *et al.*, 2020). Hence, both dopamine supersensitivity and tardive dyskinesia could be parallel consequences of neuronal adaptations provoked by chronic antipsychotic drug exposure. Interestingly, dopamine supersensitivity and tardive dyskinesia share common characteristics, and their expression in

schizophrenia patients follows a similar course. They are both produced by long-term exposure to antipsychotic drugs, can persist following antipsychotic treatment cessation, and can be tempered by increased antipsychotic drug exposure, or worsen by decreased antipsychotic drug exposure (Chouinard *et al.*, 1978). Furthermore, a longitudinal study following a large cohort of

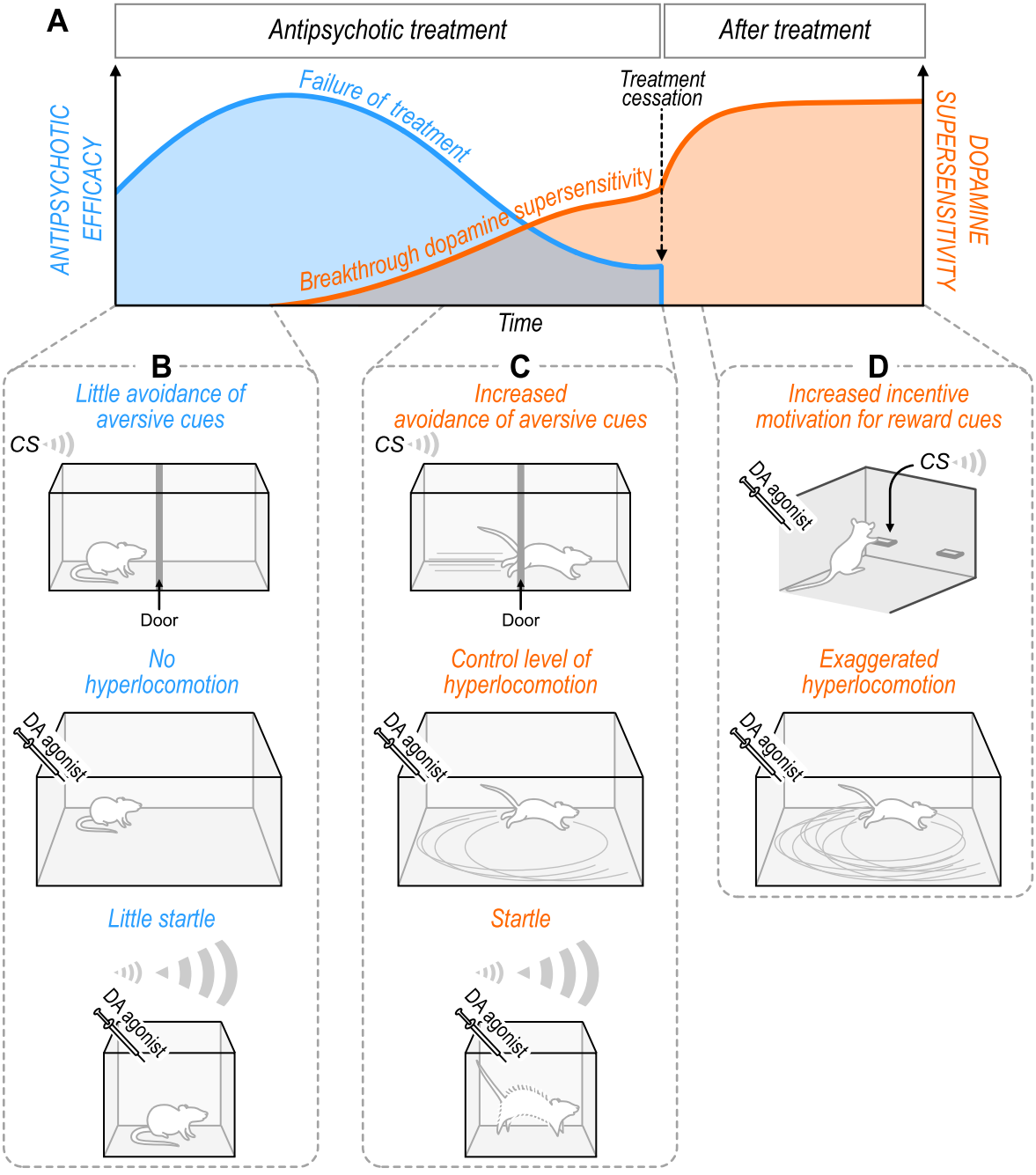

FIG. 1.10 — Antipsychotic-evoked dopamine supersensitivity. (*A*) Early into treatment, antipsychotic drugs have strong antipsychotic-like effects (blue area). Over time, dopamine *(see next page →)*

(FIG. 1.10 →) supersensitivity *breaks through* during ongoing antipsychotic treatment (orange area) and reduces antipsychotic efficacy. The expression of dopamine supersensitivity is even higher after treatment cessation because it is no longer tempered by antipsychotic drugs. (*B*) Early into treatment, antipsychotic drugs produce antipsychotic-like effects, as indicated by a reduction of conditioned avoidance responding, of the locomotor activating effects of dopamine agonists and of the abolition of pre-pulse inhibition induced by dopamine agonists. (*C*) These antipsychotic-like effects are lost over time due to dopamine supersensitivity, and this resembles treatment tolerance in humans. (*D*) After treatment cessation, dopamine supersensitivity potentiates the psychomotor and reward-enhancing effects of dopamine agonist. CS, conditioned stimuli.

---

schizophrenia patients (N = 8,620) over a year showed that the emergence and increased severity of tardive dyskinesia is paralleled by a worsening of schizophrenia symptoms, most likely psychotic symptoms (Tenback *et al.*, 2007). Another important observation supporting that antipsychotic drugs produce dopamine supersensitivity in humans is that treated schizophrenia patients are more sensitive to the psychotogenic effects of amphetamines than untreated patients, as suggested by a meta-analysis (Lieberman *et al.*, 1987).

Preclinical studies support the notion that antipsychotic drugs can produce dopamine supersensitivity over time. FIGS. 1.10B-D summarise behavioural manifestations of antipsychotic-evoked dopamine supersensitivity in laboratory animals. Some aspects of antipsychotic-evoked dopamine supersensitivity observed in humans are mimicked in laboratory animals, including treatment tolerance. As described earlier, antipsychotic-like efficacy is measured in laboratory animals by evaluating the suppressive effects of these drugs on conditioned avoidance responding, on dopamine agonist-induced psychomotor effects and on dopamine agonist-induced disruption of pre-pulse inhibition. Using these indexes, it was shown that antipsychotic drugs are effective to produce antipsychotic-like effects early into treatment (FIG. 1.10B), but these effects are reduced or lost later during the treatment (FIG. 1.10C), resembling treatment tolerance in humans (Asper *et al.*, 1973; MØller Nielsen *et al.*, 1974; Samaha *et al.*, 2007; Samaha *et al.*, 2008; Amato *et al.*, 2018). Like in humans, increasing antipsychotic exposure overcomes the lost of antipsychotic-like effects provoked by dopamine supersensitivity (Samaha *et al.*, 2007). Furthermore, chronic antipsychotic treatment can produce orofacial motor disturbances in rodents, termed vacuous chewing movement, that is related to tardive dyskinesia in humans (Waddington *et al.*, 1983). Antipsychotic treatment regimens that produce dopamine supersensitivity are more prone to produce vacuous chewing movement than antipsychotic treatment regimens that do not produce dopamine supersensitivity over time (Turrone *et al.*, 2003a, 2005).

Dopamine supersensitivity is most evident following treatment cessation because it is no longer tempered by antipsychotic drugs. Notably, rodents with a history of chronic antipsychotic exposure are supersensitive to the psychomotor effects of dopamine agonists (FIG. 1.10D) (Gianutsos *et al.*, 1974; Sayers *et al.*, 1975; Smith and Davis, 1975; Vonvoigtlander *et al.*, 1975; Smith and Davis, 1976; Clow *et al.*, 1979; Montanaro *et al.*, 1982; Rebec *et al.*, 1982; Ericson *et al.*, 1996; Kosten, 1997; Pudiak and Bozarth, 1997; Meng *et al.*, 1998; Samaha *et al.*, 2007). Antipsychotic treated-rodents are supersensitive to other effects of dopamine agonists as well. Indeed, d-amphetamine potentiates the incentive motivational value gained by CS (Robbins *et al.*, 1983), and this d-amphetamine effect is potentiated in rats that received an antipsychotic treatment regimen producing dopamine supersensitivity, but not by a regimen that do not produce this supersensitivity (FIG. 1.10D) (Bedard *et al.*, 2011, 2013; El Hage *et al.*, 2015). This is an important observation in regard to psychotic symptoms, given that it could be favored by an aberrant attribution of motivational salience to surrounding stimuli (Kapur, 2003) (Section 2.2.1, page 34). Also, as described later (Section 3.4, page 66), when CS gain too much motivational value, they can promote inappropriate responses in drug addiction. Thereby, this effect of dopamine supersensitivity could play a role in the high prevalence of drug addiction among individuals with schizophrenia (Samaha, 2014). Indeed, approximately 40% of schizophrenia patients also have a substance use disorder (Kavanagh *et al.*, 2002; Swartz *et al.*, 2006; Hunt *et al.*, 2018), while this illness is affecting 10-20 % of the general population (Anthony *et al.*, 1996; Veldhuizen *et al.*, 2007).

2.4.2. Mechanisms Underlying Dopamine Supersensitivity

The neurobiological mechanisms underlying antipsychotic-evoked dopamine supersensitivity are largely unknown. Antipsychotic-evoked dopamine supersensitivity could be underlined by enhanced D2-like transmission. By chronically occupying D2-like receptors, chronic exposure to antipsychotic drugs up-regulates the striatal levels of these receptors both in humans (Silvestri *et al.*, 2000; Kestler *et al.*, 2001) and laboratory animals (Burt *et al.*, 1977; Fleminger *et al.*, 1983; Severson *et al.*, 1984; MacKenzie and Zigmond, 1985; Wilmot and Szczepanik, 1989; Jiang *et al.*, 1990; Marin and Chase, 1993; Merchant *et al.*, 1994; Huang *et al.*, 1997; Samaha *et al.*, 2007; Samaha *et al.*, 2008; Ginovart and Kapur, 2012; Tadokoro *et al.*, 2012; Oda *et al.*, 2015). In laboratory animals, the link between dopamine supersensitivity and their affinity state for

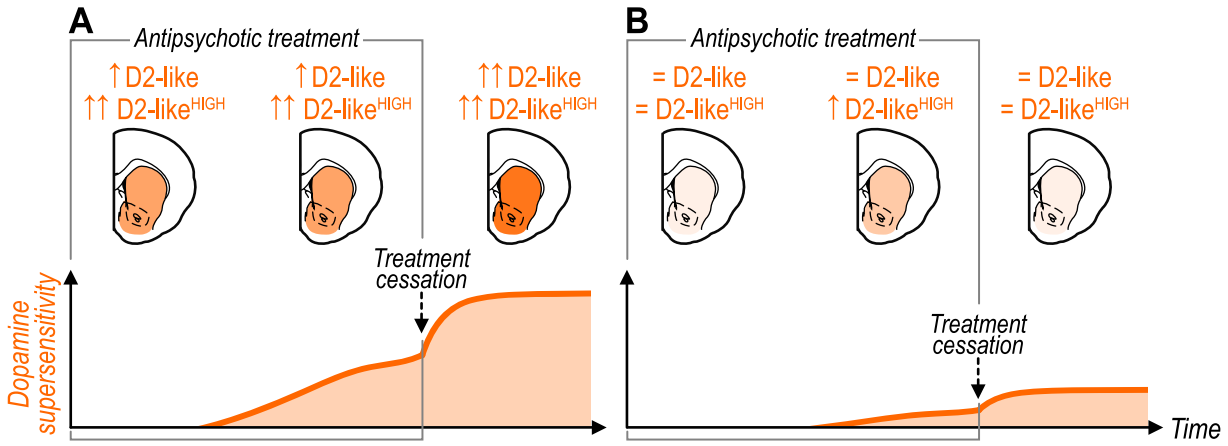

FIG. 1.11 — Antipsychotic-evoked dopamine supersensitivity is paralleled by an increase in the number of D2-like receptors and D2-like receptors in a high affinity state for dopamine (D2-like^{HIGH}) in the striatum. The '=' symbol indicates no change relative to antipsychotic-naïve animals. (***A***) Antipsychotic treatment regimens producing dopamine supersensitivity enhance early on the striatal level of D2-like and D2-like^{HIGH} receptors, even before the emergence of dopamine supersensitivity. After treatment cessation, the enhanced level of D2-like^{HIGH} receptors remains stable, while the number of D2-like receptors is further enhanced. (***B***) Antipsychotic treatment regimens less likely to produce dopamine supersensitivity do not alter the number of D2-like receptors in the striatum but elevate the number of D2-like^{HIGH} receptors late into treatment. Their number returns to control levels after discontinuation of antipsychotic treatment.

dopamine has been directly examined. D2-like receptors are in a high affinity state for dopamine when they are bound to $G_{i/o}$ proteins (D2-like^{HIGH}), but in a low affinity state when they are not bound to these proteins. Studies have shown that dopamine supersensitivity correlates with changes in the density of D2-like/D2-like^{HIGH} receptors. Dopamine-supersensitive rats show elevated D2-like and D2-like^{HIGH} receptor levels in the striatum (Samaha *et al.*, 2007). These alterations are observed before the emergence of behavioural signs of dopamine supersensitivity and persist after treatment cessation only when the antipsychotic treatment regimen produces persistent dopamine supersensitivity (FIG. 1.11A) (Samaha *et al.*, 2007). Furthermore, antipsychotic treatment regimens that do not induce dopamine supersensitivity can also increase the striatal level of D2-like^{HIGH} receptor late into treatment (FIG. 1.11B) (Seeman *et al.*, 2005; Samaha *et al.*, 2008). However, this enhancement is more moderate than the one produced by treatment regimens evoking dopamine supersensitivity, and does not persist after treatment cessation (FIGS. 1.11A versus 1.11B) (Samaha *et al.*, 2007; Samaha *et al.*, 2008). Other than D2-like receptors, antipsychotic-evoked dopamine supersensitivity could involve D1-like-, neurotensin-, serotonin-, noradrenaline- and glutamate-

mediated signalling, as well as alterations in dopamine reuptake, but no clear mechanisms have been identified yet (Servonnet and Samaha, 2020). For instance, repeated injections of dopamine agonists sensitise to the behavioural effects of these drugs over time, and this is accompanied by a greater release of striatal dopamine in response to the agonist (Robinson *et al.*, 1988; Akimoto *et al.*, 1989; Paulson and Robinson, 1995). However, antipsychotic-evoked dopamine supersensitivity does not correlate with a potentiation in dopamine release stimulated by dopamine agonists (Compton and Johnson, 1988; Ichikawa and Meltzer, 1992; Samaha *et al.*, 2007).

So far, very little is known on how antipsychotic-evoked dopamine supersensitivity exacerbates the ability of dopamine agonists to enhance the incentive motivational properties of appetitive CS. Microinfusion of dopamine agonists into the nucleus accumbens enhances the instrumental pursuit of appetitive CS in otherwise neurologically intact animals (Taylor and Robbins, 1984; Kelley and Delfs, 1991), and this effect is amplified in dopamine-supersensitive rats that received repeated cocaine injections (Taylor and Horger, 1999). However, in antipsychotic-treated rats, neuronal transmission within the nucleus accumbens is neither sufficient nor necessary for the sensitised response to appetitive CS (El Hage *et al.*, 2015). There is also no evidence of the implication of the caudate-putamen (El Hage *et al.*, 2015). Hence, extra-striatal regions may regulate the exacerbated motivation for appetitive CS produced by antipsychotic-evoked dopamine supersensitivity.

2.4.3. Can Dopamine Supersensitivity Be Prevented?

While the neurobiological mechanisms underlying antipsychotic-evoked dopamine supersensitivity remain elusive, there have been promising leads on how to decrease the incidence of dopamine supersensitivity evoked by chronic antipsychotic drug exposure. According to animal studies, atypical antipsychotic drugs are less likely to persistently promote dopamine supersensitivity after antipsychotic treatment withdrawal (Samaha *et al.*, 2007; Fukushiro *et al.*, 2008; Carvalho *et al.*, 2009; Tadokoro *et al.*, 2012; Bedard *et al.*, 2013). However, atypical antipsychotic drugs can produce dopamine supersensitivity during ongoing antipsychotic treatment, as indicated by a loss of antipsychotic-like effects (Samaha *et al.*, 2007; Amato *et al.*, 2018). Alternatively, one promising approach that would be less likely to produce dopamine supersensitivity either during ongoing treatment or after treatment cessation is by extending the period in between antipsychotic intake, so that D2-like occupancy is transiently and regularly above 65 % rather than continuously above 65 % with more frequent dosing (*e.g.*, taking an oral

antipsychotic drug every other day instead of everyday). We reviewed extended dosing strategies in humans and laboratory animals [see Annex, page 290; (Servonnet *et al.*, 2020a)]. In stabilised patients, extended dosing strategies represent a safe and effective way to manage schizophrenia symptoms, even if extended intake generally decreases to 50 % antipsychotic drug exposure (McCreadie *et al.*, 1980; Remington *et al.*, 2005; Remington *et al.*, 2011; Takeuchi *et al.*, 2014). Similarly, extending the interval between intra-muscular depot of long-acting injectable antipsychotics can remain effective to treat patients (Nyberg *et al.*, 1995; Carpenter *et al.*, 1999; Uchida and Suzuki, 2014). Extended dosing has also been studied in laboratory animals. As described previously, animals that are given daily systemic administration of antipsychotic drugs are exposed transiently rather than continuously (Section 2.3.3.1, page 45). By allowing predictable and regular periods of physiological dopamine transmission, extended antipsychotic exposure is less likely to produce neuronal adaptations leading to dopamine supersensitivity relative to continuous exposure in rats. Consequently, while continuous antipsychotic treatment becomes ineffective to produce antipsychotic-like effects over time, extended treatment remains effective to produce these effects (Samaha *et al.*, 2008). Also, the persistent effects of dopamine supersensitivity after treatment cessation are mitigated with extended dosing strategies. Indeed, after treatment cessation, animals that were transiently exposed either show a comparable behavioural response to dopamine agonists than antipsychotic-naïve animals, or a reduced response relative to continuously-treated animals (Ericson *et al.*, 1996; Samaha *et al.*, 2008; Bedard *et al.*, 2011; Servonnet *et al.*, 2017).

2.4.4. Could Stress Play a Role in Antipsychotic-evoked Dopamine Supersensitivity?

One aspect that need further investigation is the role of stress in antipsychotic-evoked dopamine supersensitivity. This is important to consider given that stress is a contributing factor to psychosis relapse (McCutcheon *et al.*, 2019a) and that the degree of stress positively correlates with the severity of psychotic symptoms (Naeem *et al.*, 2006). Furthermore, stressors elicit greater release of dopamine in the striatum of schizophrenia patients than of healthy individuals (Mizrahi *et al.*, 2012). Knowing that antipsychotic-evoked dopamine supersensitivity may exacerbate psychotic symptoms in patients, one possible mechanism by which this supersensitivity does so is by enhancing the vulnerability to stressors. In other words, antipsychotic-evoked dopamine

supersensitivity could produce a cross-sensitisation between the effects of stress and dopamine stimulation.

Outside of the context of antipsychotic drugs, there are several lines of evidence supporting that stress and dopamine activity are tightly connected. For instance, stressors are sufficient to enhance the behavioural response to dopamine agonists (Antelman *et al.*, 1980; Robinson *et al.*, 1985; Leyton and Stewart, 1990; Piazza *et al.*, 1990). Similarly, the vulnerability to stressors predicts the vulnerability to the behavioural effects of dopamine agonists. For instance, rats that show a greater locomotor response to a novel environment [a behavioural sign found in chronically stressed animals (Marin *et al.*, 2007)] are supersensitive to the behavioural effects of d-amphetamine, cocaine, apomorphine and GBR12909 (Piazza *et al.*, 1989; Hooks *et al.*, 1991; Hooks *et al.*, 1994). Furthermore, the stress hormones glucocorticoids (mainly corticosterone in rodents, cortisol in humans) are both sufficient and necessary for the behavioural response to dopamine agonists. For instance, administration of corticosterone is sufficient to potentiate the behavioural effects of d-amphetamine (Piazza *et al.*, 1991; Cador *et al.*, 1993). Similarly, when corticosterone plasmatic levels are enhanced by stress, this correlates with a greater psychomotor response to d-amphetamine (Piazza *et al.*, 1991). Removal of adrenal glands (*i.e.*, adrenalectomy) abolishes the plasmatic levels of corticosterone and reduces the psychomotor effects of d-amphetamine, and the latter impairment is rescued by corticosterone replacement therapy (Cador *et al.*, 1993).

In the context of antipsychotic-evoked dopamine supersensitivity, there are some correlational or indirect evidence that dopamine supersensitivity could enhance stress-related effects. For instance, psychosis relapse seems provoked by more minor stressors in schizophrenia patients showing signs of dopamine supersensitivity relative to patients that do not show signs of this supersensitivity (Fallon and Dursun, 2011). Also, antipsychotic drugs reduce the behavioural response to stressors, and when dopamine supersensitivity breaks through during ongoing treatment, antipsychotic drugs lose their ability to reduce that response. Indeed, early into treatment, antipsychotic treatment reduces the locomotor response to acute stress, but this effect is lost late into treatment with the emergence of dopamine supersensitivity (Amato *et al.*, 2018). Similarly, antipsychotic treatment regimen producing dopamine supersensitivity loses over time their ability to reduce conditioned avoidance responding elicited by an aversive CS (Samaha *et al.*, 2007). Hence, with the emergence

of dopamine supersensitivity, animals become more responsive to dopamine stimulation and perhaps to stressors as well, and this should be considered given that stress exacerbates psychosis.

3. IMPORTANCE OF CONDITIONED STIMULI IN BEHAVIOUR

As described in Section 2.4.1 (page 51), antipsychotic-evoked dopamine supersensitivity enhances the motivational properties of reward-predictive cues. This could play a role in abnormal motivational processes in schizophrenia, such as psychotic symptoms (Kapur, 2003) and the greater vulnerability to drug addiction (Samaha, 2014). The neurobiological mechanisms underlying the ability of dopamine supersensitivity to potentiate the incentive effects of conditioned rewards are unclear. There is no evidence of the implication of the striatum (El Hage *et al.*, 2015), even though this region critically regulates the motivational properties of appetitive CS (Taylor and Robbins, 1984; Kelley and Delfs, 1991; White *et al.*, 1991; Chu and Kelley, 1992). We were therefore interested in investigating the potential role of extra-striatal structures, especially the basolateral nucleus of the amygdala. It has been well characterized that the integrity of this nucleus is necessary for CS to evoke motivated responses (this is later characterised in Section 4.2, page 69). However, there are important aspects that are unclear on how the basolateral amygdala regulates the behavioural effects of CS, especially concerning whether this nucleus is sufficient to intensify appetitive conditioning. Thus, one of the goals of the present thesis was to better understand the role of the basolateral amygdala in the behavioural effects of appetitive CS in a normal state (*i.e.*, no prior antipsychotic drug exposure). Such investigations could give important insights on neurobiological mechanisms underlying antipsychotic-evoked dopamine supersensitivity. This work could also have important implications in maladaptive motivated responses found in psychiatric disorders such as drug addiction and depression. Before describing the basolateral amygdala and its role in appetitive conditioning (Section 4), here I first describe the importance of CS in behaviour, how the behavioural effects of CS are studied in laboratory animals, followed by a brief description on how CS influence psychiatric disorders.

3.1. What Are Conditioned Stimuli?

For survival, animals need to avoid life-threatening situations such as predators, and to locate essential rewards such as food, water and mating partners. However, as first described by Ivan Petrovitch Pavlov (Pavlov, 1927), it is necessary to associate surrounding environmental stimuli

(*e.g.*, smell, noise, place) with biologically relevant events to predict their incoming availability and consequently, to make an adequate response (escape or approach). Indeed, animals are more likely to avoid threatening situations by being able to identify an upcoming danger through environmental stimuli (*e.g.*, sensing predator smell and sound), rather than through direct contact with a dangerous situation. As indicated by Pavlov, animals would become extinct if they were avoiding threatening situations when '*the teeth of the foe were in their flesh*' (Pavlov, 1927). Similarly, rewards themselves are usually not readily available but can be localised through environmental stimuli (*e.g.*, smell of food). Such environmental stimuli are initially neutral for animals but become biologically relevant when they become effective predictors of rewards or threats. This process of stimulus-reward/threat association is termed Pavlovian conditioning, named after Pavlov. Hence, through Pavlovian conditioning, environmental stimuli become predictors of impending reward or threat and are therefore termed CS—*conditioned stimuli*. In contrast, stimuli that require no conditioning to be biologically relevant (reward, threat) are termed *unconditioned stimuli* (UCS). On their own, CS can evoke some of the responses evoked by the associated UCS. For instance, a food-predicting CS can elicit salivation and approach (Bindra, 1974), whereas a threat-predicting CS can elicit freezing and an increase in blood pressure (LeDoux, 2000). CS have the ability to internally represent the associated UCS because of the conditioned responses elicited by CS presentation, and/or because the CS allow to internally represent UCS emotional value, and/or its sensory properties (Cardinal *et al.*, 2002).

The study of Pavlovian conditioning has had and still has a broad impact on the neuroscience field. Studying CS-UCS conditioning allowed to unveil neurobiological mechanisms involved, for instance, in learning [*e.g.*, Kamin (1967)], memory [*e.g.*, Nader and Einarsson (2010), Tonegawa *et al.* (2018)], fear and anxiety [*e.g.*, LeDoux (2000), Maren and Quirk (2004)], and to study the therapeutic-like and neurobiological effects of medications such as antipsychotic drugs, as already described with the conditioned avoidance responding paradigm (Section 2.3.3.2, page 47). In rodents, Pavlovian conditioning takes place in a conditioning chamber (FIG. 1.12A). Animals learn to associate an initially neutral stimulus (*e.g.*, tone, light, lever) with an UCS (*e.g.*, food, electric shock). With this training, animals learn the contingency, and this is indicated by conditioned responses such as approaching a food port during CS presentation (*i.e.*, conditioned approach; FIG. 1.12A).

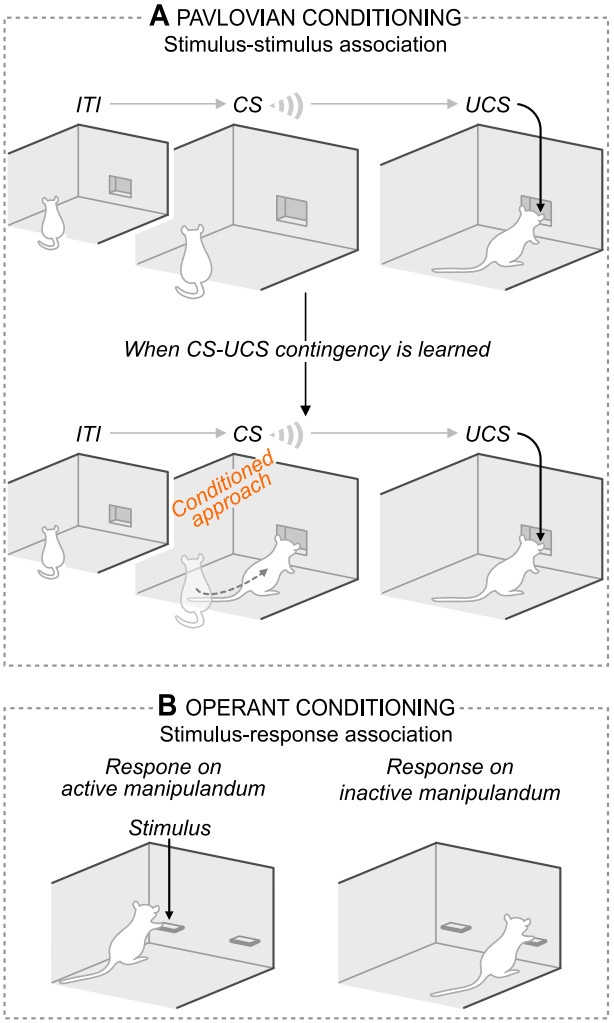

FIG. 1.12 — Pavlovian and operant conditioning procedures. (***A***) Using Pavlovian conditioning procedures, animals learn that an initially neutral stimulus predicts an impending unconditioned stimulus (UCS; reward or threat). Hence, through conditioning, the neutral stimulus becomes a conditioned stimulus (CS) and evokes conditioned responses (here, an animal approaches a receptacle where reward delivery is imminent, this is referred to as conditioned approach). (***B***) Using operant conditioning procedures, animals learn the association between an instrumental response (here, lever pressing) and stimulus delivery/presentation. The inactive manipulandum (here, an inactive lever) serves as a control of the instrumental procedure.

CS elicit behavioural responses not only through their ability to predict incoming UCS but also through their motivational effects. Thereby, CS act as powerful guides in everyday life toward unconditioned rewards and away from unconditioned threats. When they acquire too much power, CS can significantly impact maladaptive motivated behaviours in psychiatric disorders. Next is a description of behavioural paradigms that are used in laboratory animals to study different aspects of the motivational effects of appetitive CS. In addition to conditioned approach elicited by CS (FIG. 1.12A), these behavioural paradigms have been particularly useful to understand how the basolateral amygdala influences the response to appetitive CS. Then, a brief description on how to study the motivational effects of aversive CS is given, followed by a brief description on how CS with inappropriate level of motivational value can impact psychiatric disorders.

3.2. Behavioural Effects of Appetitive Conditioned Stimuli

As described in Section 1.3.2 (page 28), rewards are attractive, wanted and reinforce goal-directed behaviours because of their incentive motivational effects (Wise and Rompre, 1989; Robinson and Berridge, 1993; Wise, 2004). CS that predict reward availability can become rewarding themselves and thereby,

they can have the same motivational effects than unconditioned rewards (Bindra, 1974). The behavioural paradigms described here study these different aspects of incentive motivation.

In addition to Pavlovian conditioning, operant conditioning procedures can also be used to study the motivational effects of appetitive CS. In operant conditioning procedures, animals learn the contingency between an instrumental response (*e.g.*, pressing on a lever, making a nose poke in a port hole) and the presentation or delivery of a stimulus (CS or UCS; FIG. 1.12B) (Skinner, 1938). This procedure determines the extent to which a stimulus reinforces an instrumental response. Hence, Pavlovian conditioning establishes a stimulus-stimulus association, whereas operant conditioning establishes a stimulus-response association.

3.2.1. Conditioned Reinforcement Test: Conditioned Stimuli Can Be Pursued on their Own

The incentive motivational effects of CS make them attractive and therefore, they can be pursued on their own even in the absence of the associated UCS. The conditioned reinforcement test determines how much animals are willing to pursue appetitive CS alone. In this test, animals (that received prior CS-UCS conditioning) have typically access to two levers or two port-holes. Responses on one of the two instrument lead to CS presentation, and responses on the other do not (FIG. 1.13A) (Robbins, 1978; Taylor and Robbins, 1984). Because instrumental conditioning is new for the animals, learning of that task is solely reinforced by CS presentations. When CS gain incentive motivational value, their presentation is sufficient to reinforce instrumental responses. As a result, animals make more instrumental responses leading to CS presentation than instrumental responses without consequences (no CS).

3.2.2. Auto-shaping: Conditioned Stimuli Are Not Attractive for Everyone

While CS become strong predictors of incoming UCS, they do not necessarily gain incentive value. Indeed, there is a great variability among individuals in the attribution of incentive salience to CS. Auto-shaping is a behavioural measure that allows to identify animals that attribute strong incentive motivational value to CS and animals that do not. Auto-shaping is based on the conditioned response that animals develop throughout CS-UCS conditioning (Hearst and Jenkins, 1974; Lajoie and Bindra, 1976; Flagel *et al.*, 2009; Robinson *et al.*, 2014). In this type of test, animals are given

Pavlovian conditioning procedures, and auto-shaping is observable when the CS is localisable and spatially separated from where the reward is delivered. In studies using rodents, the CS is typically

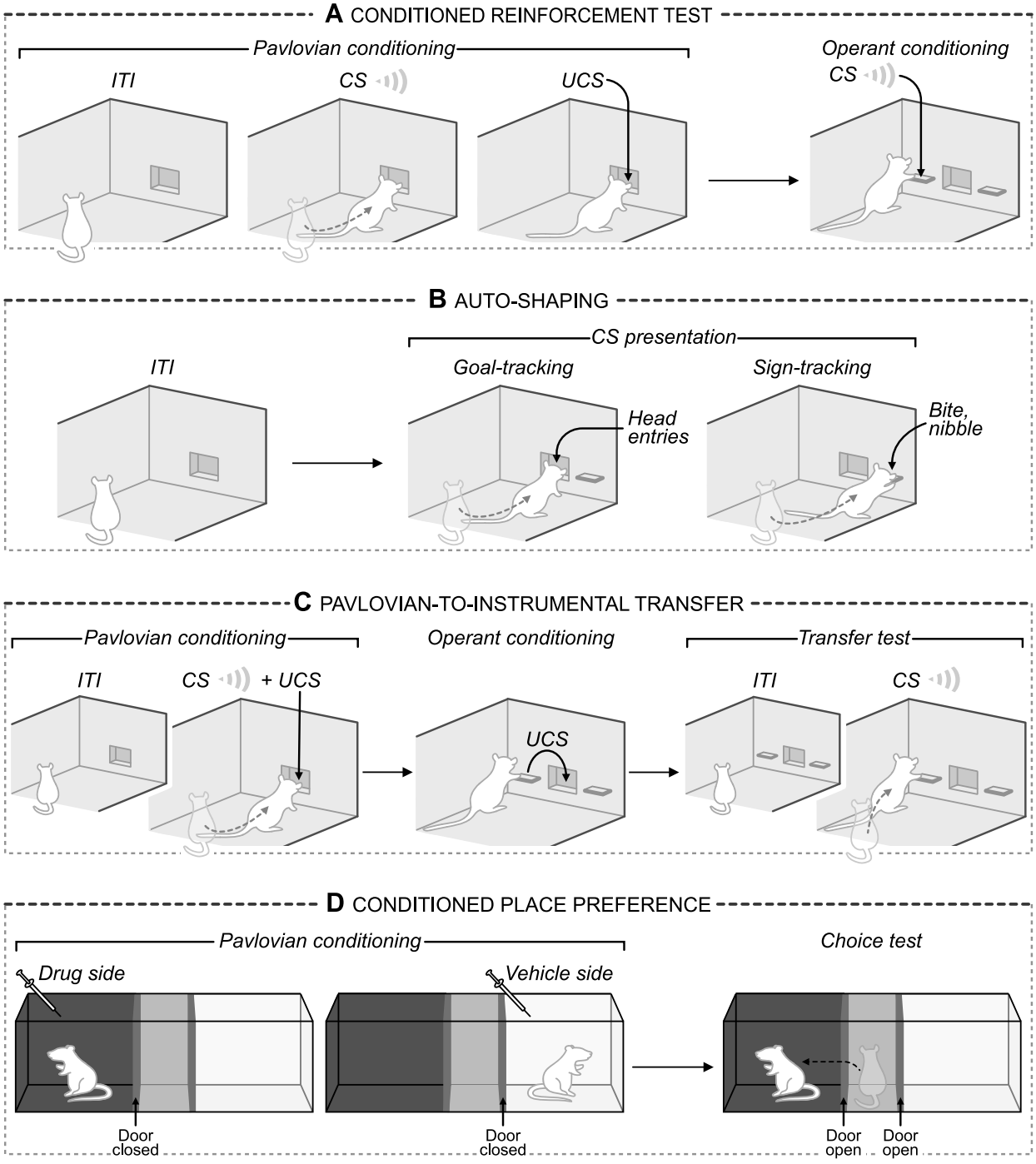

FIG. 1.13 — Behavioural measures of the motivational effects of appetitive conditioned stimuli (CS).
(see next page →)

(FIG. 1.13 →) (***A***) Animals that receive prior CS-unconditioned stimuli (UCS) conditioning are free to make instrumental responses for CS presentation alone. This serves as a measure of incentive motivation for CS. (***B***) Auto-shaping measures the type of conditioned responses animals acquire during appetitive conditioning. Upon CS presentation (here, a lever), goal-trackers approach the port where reward delivery is imminent, whereas sign-trackers interact with the CS. (***C***) During the transfer test in Pavlovian-to-instrumental procedures, CS allows to internally represent the action-UCS association and to motivate UCS seeking, even if instrumental responses and CS have never been associated before. (***D***) In conditioned place preference procedures, animals associate one context with a reward (here, a drug injection) and another context with a control procedure (here, a vehicle injection). After conditioning, animals are free to explore all compartments and time spent in the reward compartment relative to the no-reward compartment is compared. ITI, inter-trial interval.

a lever that is introduced for a few seconds before reward delivery (FIG. 1.13B). The lever is inactive and thereby, it does not influence reward delivery. Nonetheless, some animals interact more and more with the lever over time (*e.g.*, biting, nibble), and approach very little the location where the reward is soon to be delivered. These animals are referred to as *sign-trackers*, where 'sign' refers to the CS and 'tracker' refers to the orientation of behaviour (here, toward the appetitive CS). Some other animals show the opposite behaviour: they interact more and more with the reward receptacle during CS presentation (*e.g.*, head entries in a food receptacle) and show very little interest for the lever throughout CS-UCS conditioning. These animals are referred to as *goal-trackers*, where 'goal' refers to the reward. Some animals show a mix of both conditioned approach behaviours. Sign-trackers assign a greater incentive motivational value to CS than the other animals, as indicated by a gradual increase in the intensity of the interaction with the CS throughout appetitive conditioning. In line with this, sign-trackers also show strong incentive motivation for CS in the conditioned reinforcement test relative to goal-trackers and animals showing a mix of both type of conditioned responses (Robinson and Flagel, 2009).

3.2.3. Pavlovian-to-instrumental Transfer: Conditioned Stimuli Alone Guide Reward Seeking

Appetitive CS alone are sufficient to internally represent the associated UCS, but also actions necessary for acquiring that UCS. In addition to these effects, CS presentation alone is sufficient to invigorate UCS seeking. The Pavlovian-to-instrumental transfer paradigm allows to study these CS effects. In this behavioural paradigm, animals receive Pavlovian and operant conditioning in separate occasions, in any order (FIG. 1.13C) (Cardinal *et al.*, 2002; Balleine and Killcross, 2006;

Cartoni *et al.*, 2016). Then, animals receive a transfer test. During this test, instrumental responses are not reinforced by the UCS, and CS presentations are typically non-contingent. Instrumental responses are compared between periods of CS presentation and in-between periods of CS presentation (inter-trial interval, ITI). The instrumental response and the CS have never been associated before, but CS presentation is sufficient to internally represent the action-UCS association, and to motivate instrumental responses even in the absence of the UCS. This is indicated by an increase in instrumental responses made during CS presentation relative to ITI, and this is referred to as the transfer effect.

3.2.4. Conditioned Place Preference: Motivational Salience of Contextual Conditioned Stimuli

Conditioned place preference determines if contextual CS acquire incentive salience. This type of procedure is commonly used to study the motivational effects of drug of abuse, but conditioned place preference can be elicited by natural rewards like food as well (Bozarth, 1987; Stolerman, 1992; Bardo and Bevins, 2000). In conditioned place preference procedures, animals receive CS-UCS conditioning in a two- or three-compartment conditioning chamber (FIG. 1.13D illustrates a three-compartment chamber). Each compartment is accessible via a door and has distinct sensory features (*e.g.*, floor texture, pattern on the walls, olfactory cues). These features are chosen in order for animals to not have any preference for one compartment over another before conditioning. During the conditioning phase, the doors are close, and animals can explore only one compartment. Every other day, exploration of a compartment is associated with the subjective effects of an UCS (*e.g.*, drug injection) or an UCS itself (*e.g.*, food). On the remaining days, animals associate the other compartment with a control procedure (*e.g.*, vehicle injection). During the choice test, doors are open, and animals are free to explore all compartments. No UCS is presented/received on the test day. Hence, animal exploration is under the control of contextual CS only. When the compartment previously associated with the reward has acquired incentive salience, animals are attracted to this compartment and spend more time in it relative to the no-reward compartment.

3.3. Behavioural Effects of Aversive Conditioned Stimuli

Like appetitive CS, aversive CS have motivational properties. Indeed, aversive CS alone are sufficient to evoke an aversive motivational state that promotes defensive behaviours such as

avoidance (Masterson and Crawford, 1982). The motivational effects of aversive CS can be studied in similar behavioural paradigms than the ones used for the study of appetitive CS. For instance, animals avoid a contextual CS associated with an aversive UCS, as determined in a conditioned place avoidance paradigm (Bozarth, 1987). Similarly, discrete aversive CS elicit avoidance, and this can be measured in a conditioned avoidance test paradigm, as described in Section 2.3.3.2 (page 47). Furthermore, aversive CS alone can also be sufficient to reinforce the learning of a new instrumental response, as shown in the escape from fear paradigm (McAllister and McAllister, 1971). In this task, animals receive CS-UCS conditioning in one compartment of a two-compartment conditioning chamber. Animals do not have access to the other compartment in the Pavlovian conditioning phase. Subsequently, animals learn that making instrumental responses during CS presentation allows them to access the other compartment. Animals learn this even if the UCS is not delivered. Hence, the aversive motivational effects of CS are sufficient to reinforce the learning of a new instrumental task, like appetitive CS can do in the conditioned reinforcement test.

3.4. Conditioned Stimuli in Psychiatric Disorders

As mentioned earlier, CS can promote maladaptive motivated behaviours, and this is well characterized in the context of drug addiction. In addicted individuals, drug-predictive CS are sufficient to elicit craving for drugs, and this represents a major challenge to maintain abstinence from drug taking (Sinha and Li, 2007). Drug-predictive CS act as powerful motivators in laboratory animals as well. For instance, CS motivate drug seeking even if it was extinguished, as shown with Pavlovian-to-instrumental transfer procedures (Kruzich *et al.*, 2001). Similarly, discrete or contextual CS are sufficient to reinstate drug seeking in animals that chronically self-administered drug injections paired with CS presentations, as shown in the behavioural paradigm of cue (or CS)-induced reinstatement of extinguished drug seeking (FIG. 1.14) (Grimm and See, 2000; Grimm *et al.*, 2001; Crombag *et al.*, 2002). This behavioural paradigm has also been extensively used to study the influence of the basolateral amygdala in the behavioural effects of appetitive CS (see next section). Additionally, drugs of abuse themselves influence the motivational effects of CS. For instance, drugs such as d-amphetamine and nicotine enhance incentive motivation for CS, as shown with the conditioned reinforcement test paradigm (Robbins *et al.*, 1983; Olausson *et al.*, 2004). The aversive motivational effects of CS can gain excessive power in psychiatric disorders including

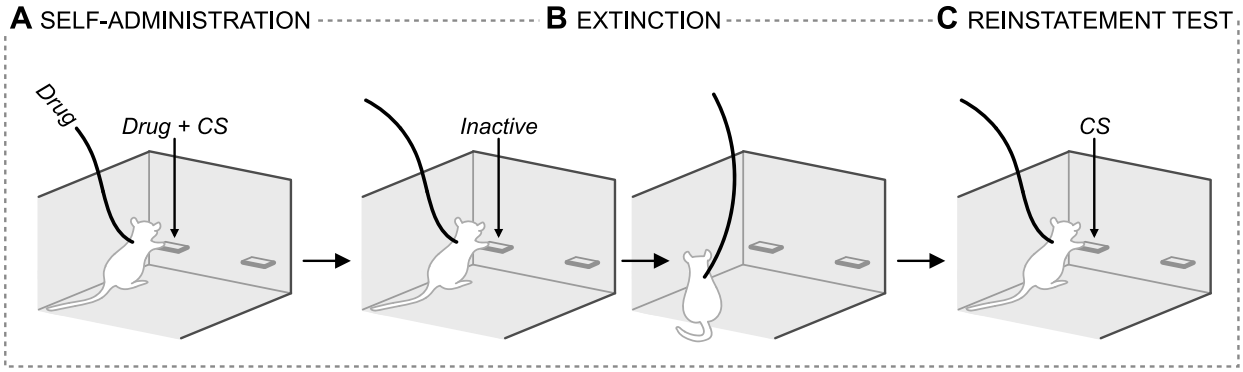

FIG. 1.14 — Paradigm of conditioned stimulus (CS)-induced reinstatement of extinguished drug seeking. This behavioural paradigm does not involve Pavlovian conditioning but solely operant conditioning. (***A***) Animals chronically self-administer a drug through instrumental responses (here, example of drug delivery via an intravenous catheter). Drug delivery is paired with a cue. (***B***) Then, animals receive extinction training, where instrumental responses are no longer reinforced by the drug and CS. During this phase, animals progressively reduce instrumental responses. (***C***) When drug seeking is extinguished, the ability of the CS to reinstate instrumental responses is evaluated. This behavioural paradigm can also be used for non-drug rewards such as food.

anxiety. For instance, contextual CS can help to discriminate what outcome is predicted by discrete CS, and in post-traumatic stress disorder, impaired processing of contextual CS would make discrete CS elicit inadequate responses (Maren *et al.*, 2013).

4. THE BASOLATERAL AMYGDALA IN APPETITIVE CONDITIONING

One of the goals of the present thesis was to better understand the neural substrates mediating the behavioural effects of appetitive CS, a work that could give insights on potential mechanisms underlying antipsychotic-evoked dopamine supersensitivity. Here a special focus was placed on the basolateral nucleus of the amygdala. How this nucleus influences the behavioural response to CS has been extensively studied in fear conditioning paradigms [*e.g.*, Fanselow and LeDoux (1999), Maren and Quirk (2004)]. Comparatively, the role of this nucleus has been studied to a lesser extent in appetitive conditioning. This characterisation has mainly focused on the necessity of the basolateral amygdala in the behavioural response to appetitive CS. Next is a description of what is the basolateral nucleus of the amygdala, and how this nucleus influences the ability of appetitive CS to guide behaviour.

4.1. What Is the Basolateral Amygdala?

The amygdala is found in the temporal lobe and represents a complex of multiple nuclei, including the basolateral nucleus and the central nucleus (Krettek and Price, 1978a). These nuclei are further subdivided. The basolateral amygdala is composed of the lateral nucleus, basal nucleus (also called the basolateral amygdala, but called basal nucleus here for clarity) and basomedial nucleus, whereas the central amygdala is composed of the centromedial and centrolateral nuclei (Krettek and Price, 1978a; McDonald, 1998; Sah *et al.*, 2003). The basolateral amygdala is a cortical-like structure. It is mainly composed of glutamatergic projection neurons with soma that are typically of a pyramidal shape, highly similar to pyramidal neurons of the cortex (McDonald, 1982; Washburn and Moises, 1992; McDonald, 1996). The basolateral amygdala contains a low density of GABAergic interneurons that make axo-somatic and axo-dendritic contacts with projection neurons (McDonald, 1982; Carlsen, 1988; McDonald and Augustine, 1993). Different subpopulations of interneurons are found in the basolateral amygdala. These subpopulations have specific neurochemical signatures based on peptides they express, such as somatostatin- or parvalbumin-expressing interneurons (McDonald and Pearson, 1989; McDonald and Mascagni, 2002). The central amygdala is a striatal-like structure mainly composed of GABAergic projection neurons (Carlsen, 1988; McDonald and Augustine, 1993). The amygdala complex is composed of other subdivisions, such as the intercalated nucleus, that is found in between the basolateral and central nuclei, as well as the medial nucleus, the periamygdaloid cortex, the amygdala-hippocampal area and the anterior amygdaloid area (Pitkanen *et al.*, 1997).

The basolateral amygdala receives projections from the prelimbic, infralimbic, orbitofrontal, anterior cingulate, olfactory, auditory, visual and somatic cortices, as well as from other brain areas such as the hippocampus, the insula, the thalamus and the hypothalamus (McDonald, 1998; Sah *et al.*, 2003). The basolateral amygdala also receives noradrenergic projections from the locus coeruleus, dopaminergic projections from the VTA and SNc, and serotoninergic projections from the dorsal and medial raphe nuclei (Fallon *et al.*, 1978; Vertes, 1991; Vertes *et al.*, 1999). Within the amygdala complex, the basal and basomedial subdivisions of the basolateral amygdala send projections to the lateral subdivision, as well as to the centromedial subdivision of the central amygdala (Pare *et al.*, 1995). The lateral nucleus of the basolateral amygdala is reciprocally connected with the basal and basomedial nuclei, and also sends projections to the centrolateral

subdivision of the central amygdala (Smith and Pare, 1994). The basolateral amygdala sends glutamatergic projections to several regions outside of the amygdala complex, including the nucleus accumbens, the olfactory tubercle, the ventral portion of the caudate-putamen and the hippocampus (Krettek and Price, 1978b; McDonald, 1996). Cortical regions sending projections to the basolateral amygdala also receive projections from that nucleus. Thereby, the basolateral amygdala is reciprocally connected with several cortical regions including the prefrontal cortex (McDonald, 1998; Sah *et al.*, 2003).

4.2. How Does the Basolateral Amygdala Influence Appetitive Conditioning?

4.2.1. Basolateral Amygdala Neurons Fire in Response to Conditioned Stimuli

Basolateral amygdala neurons fire in response to appetitive CS that are associated with a wide range of rewards, from natural rewards, drug rewards to rewarding intra-cranial stimulations (Fuster and Uyeda, 1971; Sanghera *et al.*, 1979; Muramoto *et al.*, 1993; Uwano *et al.*, 1995; Carelli *et al.*, 2003; Tye and Janak, 2007; Ambroggi *et al.*, 2008; Tye *et al.*, 2008). Throughout conditioning, basolateral amygdala neurons become more and more responsive to CS as they become predictors of reward availability (Tye *et al.*, 2008). CS predicting reward availability increase basolateral amygdala activity to a greater extent than CS predicting the unavailability of rewards (Ambroggi *et al.*, 2008). Basolateral amygdala neurons seem to encode different effects of appetitive CS. In response to CS, some basolateral amygdala neurons could be particularly active when a conditioned response is emitted, rather than during CS presentations themselves (Lee *et al.*, 2016; Kyriazi *et al.*, 2018). Basolateral amygdala neurons can also fire during anticipation of impending reward delivery (Kyriazi *et al.*, 2018). Even in the absence of rewards, CS have the ability to elicit reward seeking, and they can also be sufficient to be pursued themselves, as previously described in Section 3.2.1 (page 62). Some basolateral amygdala neurons could encode reward seeking elicited by CS presentations, whereas some other neurons could encode the pursuit of CS alone (Tye and Janak, 2007).

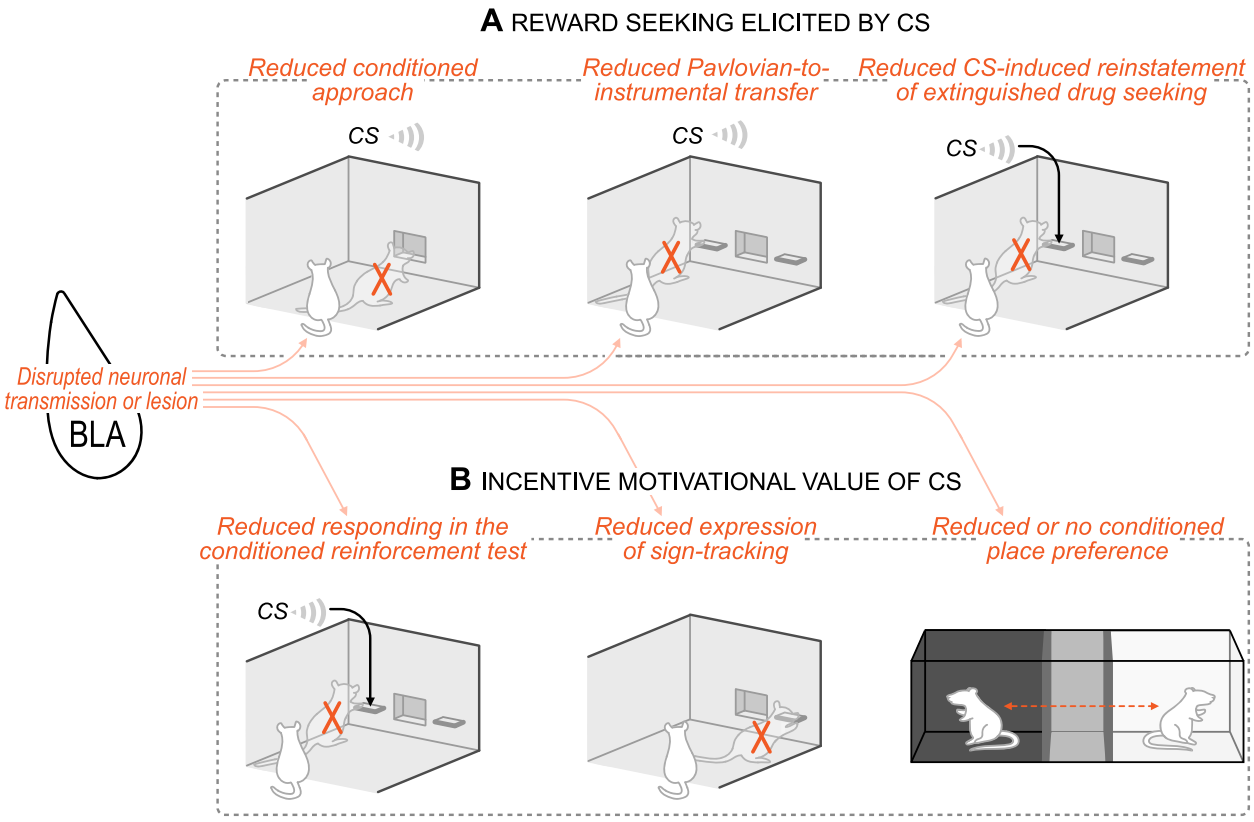

FIG. 1.15 — Intact neuronal activity within the basolateral amygdala is required for the motivational effects of appetitive conditioned stimuli (CS). Inhibition of basolateral amygdala activity impairs the ability of CS to (***A***) elicit seeking of the associated appetitive unconditioned stimulus (UCS) (as shown in conditioned approach behaviour, in the Pavlovian-to-instrumental transfer test, and in CS-induced reinstatement of extinguished drug seeking) and to (***B***) be salient and pursued (as assessed with the conditioned reinforcement test, auto-shaping procedures and the conditioned place preference test).

4.2.2. The Basolateral Amygdala Is Necessary for Conditioned Seeking

Basolateral amygdala neurons are responsive to appetitive CS, but how are these responses involved in the behavioural effects of appetitive CS? Intact neuronal activity within the basolateral amygdala is necessary for the ability of appetitive CS to guide behaviour toward unconditioned rewards (FIG. 1.15A). For instance, infusion of GABAergic agonists (Jones *et al.*, 2010b; Jones *et al.*, 2010a; Chaudhri *et al.*, 2013; Millan *et al.*, 2015), glutamate receptor antagonist (Burns *et al.*, 1994; Sciascia *et al.*, 2015) or D1-like receptor antagonist (Andrzejewski and Ryals, 2016) into the basolateral amygdala impairs conditioned approach. Also, glutamatergic mGluR5 receptor-mediated signalling within the basolateral amygdala is required for tempering the ability of contextual CS to potentiate conditioned approach elicited by discrete CS (Khoo *et al.*, 2019).

Instrumental conditioning guided by CS indicating whether or not reward is available requires intact neuronal activity within the basolateral amygdala as well, as this capacity is impaired by local infusion of GABAergic agonists (Ishikawa *et al.*, 2008; Jones *et al.*, 2010b; Jones *et al.*, 2010a). CS allows to internally represent action-reward association and to motivate reward seeking (as measured in the transfer test with Pavlovian-to-instrumental transfer procedures), and these CS effects require basolateral amygdala neurons. Indeed, lesion of the basolateral amygdala (Corbit and Balleine, 2005) or pharmacological inactivation of this nucleus by infusing locally GABAergic agonists (Gabriele and See, 2010) or tetrodotoxin (Kruzich and See, 2001) disrupts the transfer effect, indicating that CS no longer promote reward seeking. By infusing antagonist agents into the basolateral amygdala, it was shown that multiple neurotransmitter receptors are required for the acquisition and/or expression of this transfer effect, including D1-like and D2-like receptors (Berglind *et al.*, 2006), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartic acid (NMDA) receptors (Feltenstein and See, 2007; Malvaez *et al.*, 2015), muscarinic receptors (See *et al.*, 2003) and mu-opioid receptors (Lichtenberg and Wassum, 2017). Of particular importance for addiction is the ability of drug-associated CS to reinstate extinguished drug seeking, and intact neuronal activity within the basolateral amygdala is required for that CS effect. Indeed, pharmacological inhibition of the basolateral amygdala using tetrodotoxin (Fuchs and See, 2002; McLaughlin and See, 2003; Fuchs *et al.*, 2005) or lesion of the basolateral amygdala (Meil and See, 1997; Yun and Fields, 2003) impairs CS-induced reinstatement of extinguished drug seeking. This could involve dopamine- and glucocorticoid-mediated signalling within the basolateral amygdala, as local infusion of dopamine receptor antagonist (See *et al.*, 2001; Khaled *et al.*, 2014) or glucocorticoid receptor antagonist (Stringfield *et al.*, 2016) impairs this CS effect.

4.2.3. The Basolateral Amygdala Is Necessary for Conditioned Stimuli to Be Motivationally Salient

The ability of CS to elicit reward seeking requires basolateral amygdala neurons, but do these neurons influence the motivational salience of appetitive CS *per se*? The answer is yes, and FIG. 1.15B summarises important findings on this role of the basolateral amygdala. Antagonism of AMPA-mediated activity (Hitchcott and Phillips, 1997) or lesion of the basolateral amygdala (Cador *et al.*, 1989) disrupts incentive motivation for CS, as shown with the conditioned reinforcement test. Furthermore, intact neuronal activity within the basolateral amygdala is

required for contextual CS to be imbued with incentive salience, as shown with the conditioned place preference paradigm. For instance, lesion of the basolateral amygdala (Everitt *et al.*, 1991; Hiroi and White, 1991) or local infusion of dopamine receptor antagonist (Gremel and Cunningham, 2009; Lintas *et al.*, 2011), β2 or α1 receptor antagonist (Bernardi *et al.*, 2009) or muscarinic receptor antagonist (Schroeder and Packard, 2002) alters the acquisition and/or expression of a conditioned place preference. Similarly, selectively inducing apoptosis in basolateral amygdala neurons that are activated by nicotine abolishes the expression of an established conditioned place preference elicited by nicotine (Xue *et al.*, 2017). Furthermore, conditioned place preference elicited by d-amphetamine seems to engage neuronal plasticity within the basolateral amygdala, as indicated by an increase in the number of varicosities and the level of the protein Fos, the latter being positively correlated with the degree of conditioned place preference (Rademacher *et al.*, 2006). The role of the basolateral amygdala in the ability of CS to acquire motivational salience in auto-shaping procedures is less clear. Lesion of the basolateral amygdala does not prevent animals from acquiring sign-tracking behaviour (Chang *et al.*, 2012; Naeem and White, 2016), but this behaviour seems to be tempered over time in lesioned animals relative to animals with intact basolateral amygdala (Chang *et al.*, 2012). However, animals showing strong sign-tracking behaviour do not show increased activity within the basolateral amygdala, as suggested by similar levels of *c*-fos mRNA in the basolateral amygdala of sign-trackers, goal-trackers and controls (Flagel *et al.*, 2011a). Thereby, the available literature suggests that the basolateral amygdala seems to play a limited role in sign-tracking behaviour.

5. OPTOGENETIC METHODS FOR FINE NEURONAL MANIPULATIONS

The reports described in the previous section aimed at determining the necessity of intact neuronal transmission within the basolateral amygdala in the behavioural effects of appetitive CS. Comparatively, little is known on whether neuronal activity within the basolateral amygdala is *sufficient* to promote these motivational effects. This is an important aspect to explore given that, as described earlier, basolateral amygdala neurons fire in response to appetitive CS. In the present thesis, we took advantage of optogenetic methods to mimic the increased activity of basolateral amygdala neurons in response to appetitive CS in order to evaluate the impact on behavioural responses. Next is a description of what are optogenetic manipulations and the different methodological strategies that this technique offers.

5.1. What Is Optogenetics?

Optogenetics is a powerful technology that allows to reversibly manipulate cell activity with a very well-defined spatiotemporal resolution, whether in cell cultures or behaving mammals. Dr. Karl Deisseroth and colleagues were pioneers of optogenetic methods that are commonly used now, a success story that started in 2005 (Boyden *et al.*, 2005). Briefly, optogenetic methods combine optical technology with genetics to precisely modulate cell activity using opsins (*i.e.*, light-sensitive proteins) that are expressed in specific subset of cells through genetic manipulations. Attempts to control neuronal activity using optogenetic technologies were not as successful before, due to a lack of spatial resolution, the impossibility to use such techniques *in vivo*, and/or because these techniques required more methodological steps (Miller, 2006).

The first study on optogenetic methods as it is now commonly used was published by Deisseroth and colleagues (Boyden *et al.*, 2005). They established important foundations on the use of this new technology to manipulate neuronal activity. In this study, they transfected hippocampal neurons in culture with a lentivirus that delivers the gene of the opsin channelrhodopsin (ChR) 2, a light-sensitive channel that induce depolarisation (Nagel *et al.*, 2003). Fundamental findings made by Boyden *et al.* (2005) include: *i*) upon delivery of light, ChR2 induces action potentials within 1-3 milliseconds, *ii*) with adequate laser stimulation parameters, optically-driven action potentials have a *one-spike resolution* because ChR2 is rapidly and reliably activated and deactivated, *iii*) delivery of light pulses lasting a few milliseconds allows to control with high fidelity the pattern of neuronal firing, thereby the pattern of light pulses (and action potentials) can mimic physiological firing patterns, and *iv*) post-synaptic currents closely match the firing pattern controlled by ChR2, making optogenetics a useful tool to study neuronal pathways. Hence, Boyden *et al.* (2005) show that optogenetic methods allow precise neuronal manipulations, and this largely surpassed available tools at the time that have a lower spatiotemporal resolution, such as intra-cranial lesions and microinfusions of pharmacological agents that activate or inhibit neuronal activity.

These promising results led Deisseroth and colleagues to conclude that '*it is possible that ChR2 will be an effective tool for in vivo studies of circuit maps and behavior, even in mammals*' (Boyden *et al.*, 2005). This was first confirmed later that year in a study using *Caenorhabditis* elegans.

Indeed, Nagel *et al.* (2005) studied the effects of optogenetic stimulation of muscle cells and mechanosensory neurons using ChR2. They show that optogenetic stimulation of muscle cells causes muscle contraction, whereas optogenetic stimulation of mechanosensory neurons causes withdrawal (as if a mechanical stimulation is given). No more than two years later, *in vivo* optogenetic methods were successful in mammals as well, as reported in two studies. In a first study, Aravanis *et al.* (2007) showed that optogenetic stimulation of glutamatergic neurons of the vibrissal motor cortex increases the incidence of whiskers deflection, both in mice and rats. In a second study, Adamantidis *et al.* (2007) showed that optogenetic stimulation of orexin-expressing neurons in the lateral hypothalamus enhances the latency of asleep mice to awaken. Hence, these early investigations in rodents and *Caenorhabditis* elegans showed that *in vivo* optogenetic methods do allow to establish a causal link between increased activity of a given subpopulation of neurons and a behavioural effect. The introduction of optogenetic methods had and still has a large impact in the neuroscience field. A PubMed research with the word 'optogenetic' leads to 6,980 results (on September 15th 2020), even though this technique is only 14 years old (Deisseroth *et al.*, 2006). This illustrates well the revolution that optogenetics brought in research laboratories. Next is a description of how optogenetic methods have opened up many new avenues for neuroscience research, as scientists can shape the '*how, where, who and when*' of optogenetic manipulations.

5.2. Optogenetic Manipulation Strategies

5.2.1. How: Activation and Inhibition of Cell Activity, and More

Opsins determine how cell activity is altered. Opsins are activated by a specific wavelength of light. They are activated by light only when they are bound to the co-factor retinal (or vitamin-A aldehyde), forming a complex opsin-retinal termed rhodopsin (Spudich *et al.*, 2000; Zhang *et al.*, 2011). Opsins are composed of 7 transmembrane helices and are universally found across organisms, from microorganisms to vertebrates. Microbial opsins regulate phototaxis (*i.e.*, orientation of the behaviour toward or away from light) for photosynthesis. They bind to all-*trans* retinal. In presence of light of the appropriate wavelength, all-*trans* retinal absorbs a photon and isomerises, thereby all-*trans* retinal is converted to 13-*cis* retinal. This change of conformation activates the opsin. Microbial opsins used for optogenetic methods are ion pumps or channels.

Hence, when activated, these microbial opsins directly and quickly regulate ion conductance for a short duration. Retinal isomerisation is spontaneously and rapidly reversed and thereby, microbial opsins can be quickly re-activated in presence of light. Due to their interesting properties, several microbial opsins have been engineered for optogenetic method purposes to induce depolarisation or hyperpolarisation (Zhang *et al.*, 2011).

Ion channel opsins include ChR1 and ChR2. These opsins regulate phototaxis in the alga *Chlamydomonas*, a unicellular eukaryotic organism that is aquatic (Foster *et al.*, 1984). ChR1 is activated by green light (~500 nm) and ChR2 is activated by blue light (~470 nm) (Nagel *et al.*, 2002; Sineshchekov *et al.*, 2002; Nagel *et al.*, 2003). When activated, ChR1 allows a passive diffusion of protons, whereas ChR2 allows a passive diffusion of monovalent and divalent cations (Na^+, H^+, Ca^{2+}) (Nagel *et al.*, 2002; Nagel *et al.*, 2003). ChR2 is the prototypical ion channel opsin used for depolarisation (Boyden *et al.*, 2005). Chloride pump opsins are also commonly used for optogenetic manipulations. The first discovered was the halorhodopsin isolated from the bacteria *Halobacterium halobium* (Matsuno-Yagi and Mukohata, 1977). This chloride pump transports Cl^- in the cell and is activated by yellow light (~560-580 nm) (Matsuno-Yagi and Mukohata, 1977; Schobert and Lanyi, 1982). Halorhodopsins have been found in other microorganisms. The halorhodopsin express by the archea *Natronomonas pharaonic* (an opsin commonly termed NpHR) was the first used to block action potentials, as it shows a great affinity for Cl^- ions (Zhang *et al.*, 2007).

A great number of microbial opsin mutants have been engineered to meet scientist needs for optogenetic manipulations. They are fused to a fluorochrome to be easily detectable. Mutations of opsins influence their kinetic properties (detailed in Section 5.2.3, page 77). Mutations can also improve opsin expression, reliability and conductance. For instance, the H134R ChR2 mutant (used in the present thesis) has a greater ion conductance than wild type ChR2 (Nagel *et al.*, 2005), increasing its reliability to induce depolarisation. Other mutants have been developed using ChR1. For instance, ChR1-ChR2 chimeras, termed ChEF and ChIEF, have been developed to ameliorate ChR kinetic and reliability (Lin *et al.*, 2009). The NpHR mutant, eNpHR3.0, shows great expression at the membrane surface and thereby, is more reliable to inhibit action potentials than other NpHR mutants (Gradinaru *et al.*, 2010).

Vertebrate opsins are also used for optogenetic method purposes, but in a different way than microbial opsins as they have much different kinetic properties and effects on cell activity. Vertebrate opsins have slow kinetic properties, as they are G-protein coupled receptors that indirectly regulate ion conductance, and they are not readily re-activable like microbial opsins (Spudich *et al.*, 2000; Zhang *et al.*, 2011). Nonetheless, vertebrate opsins are still useful for optogenetic manipulations, because they allow to study the functional effects of specific intracellular signalling pathways (Zhang *et al.*, 2011). For instance, a chimera of bovine opsin expressing subunits of the adrenergic α_{1A} or β_2 receptor allows to optically control G_q or G_s protein-mediated intracellular signalling, respectively (Airan *et al.*, 2009). Such technology can be used for any type of cells, including non-excitable cells, making optogenetics a technology that can be virtually used for any cell types (Airan *et al.*, 2009).

5.2.2. Who and Where: From Cell Cultures to Intact Mammals, in Defined Cell Subtypes

As mentioned before, all types of organisms (from microorganisms to complex organisms) can be subjected to optogenetic manipulations. While optogenetic methods represent a powerful tool for research purposes, it is also considered to be used in humans as a therapeutic approach to treat pathologies, such as Parkinson's disease (Elkouzi *et al.*, 2019) and retinal diseases (Garita-Hernandez *et al.*, 2018). Such technology would allow to precisely target neurons with abnormal activity, while sparing unaffected neurons to avoid undesirable effects.

Genetic manipulations (viral transduction, transgenic animals) enable to express opsins in specific cell subtypes. Common engineered viral vectors used for opsin gene delivery are derived from adeno-associated viruses and lentiviruses (Zhang *et al.*, 2010). There are different types of adeno-associated virus (serotypes) that differ in the variant of capsid proteins they express at their shell, and this determines their respective ability to transduce specific type of host cells (Wu *et al.*, 2006). A common lentivirus used as a vector for gene delivery is derived from the human immunodeficiency virus type 1 (Trono, 2000). Lentiviruses and adeno-associated viruses allow long-term and stable transgene expression and can transduce neurons anterogradely or retrogradely (Trono, 2000; Zhang *et al.*, 2010). Additionally, rabies virus and herpes simplex virus 1 can also be used as viral vectors to retrogradely transduce neurons (Zhang *et al.*, 2010).

Viral vectors contain the gene of a promoter upstream to the opsin gene to limit their expression to cells where the promoter is active. Using transgenic animals has been proven important in optogenetic methods, because viral vectors have their own limitations. Indeed, viral vectors carry a limited amount of genetic information, and this is problematic when one wishes to use a large promoter. The Cre-*lox* system has helped to circumvent this problem. Cre is a deoxyribonucleic acid (DNA) recombinase enzyme that either excises DNA in between two *loxP* sites, or changes DNA orientation when the *loxP* sites are inverted (Sauer, 1998). Hence, viral vectors carrying inverted promotor and opsin genes in-between two inverted *loxP* sites allow to limit opsin expression to Cre-expressing cells. This approach is widely used in transgenic Cre animals. For instance, opsin expression can be limited to dopamine neurons in tyrosine hydroxylase-Cre (TH-Cre) animals (*i.e.*, Cre is active in cells where the tyrosine hydroxylase promotor is active, including dopamine neurons) (Tsai *et al.*, 2009; Witten *et al.*, 2011). Alternatively, the Cre-*lox* system can be used in wild type animals, and this is achieved by administering two viruses. One virus contains the Cre gene under the control of a promoter selectively active in the subset of neurons that one wishes to target (so that Cre is expressed in these neurons), and the other virus contains the inverted opsin gene in-between inverted *loxP* sites (so that opsin expression is limited to Cre-expressing neurons) (Gompf *et al.*, 2015).

In addition to genetic manipulations, where light is applied to the brain determines the specificity of the manipulation. When light is applied on the soma of opsin-expressing neurons, this allows to optically manipulate all the projections of these neurons (FIG. 1.16A; pathways 1→2, 1→3 and 1→4 are manipulated). When light is applied in a region where the opsin-expressing neurons project, this allows to optically manipulate only the projections to that region (FIG. 1.16B; only pathway 1→4 is manipulated).

5.2.3. When: Temporal Precision

Optogenetic manipulations have an excellent temporal resolution, whether used *in vitro*, *ex vivo* or *in vivo*, in anesthetised or awake animals. One benefit of this is that optogenetic stimulation can mimic physiological neuron activity. Engineering of new ChR2 mutants has allowed to extend the ability of ChR2 to mimic a great variety of physiological activities that neurons can have. For instance, Gunaydin *et al.* (2010) engineered new ChR2 mutants termed ChETA, that reliably induces action potentials with frequencies up to 200 Hz. Also, Berndt *et al.* (2009) developed ChR2

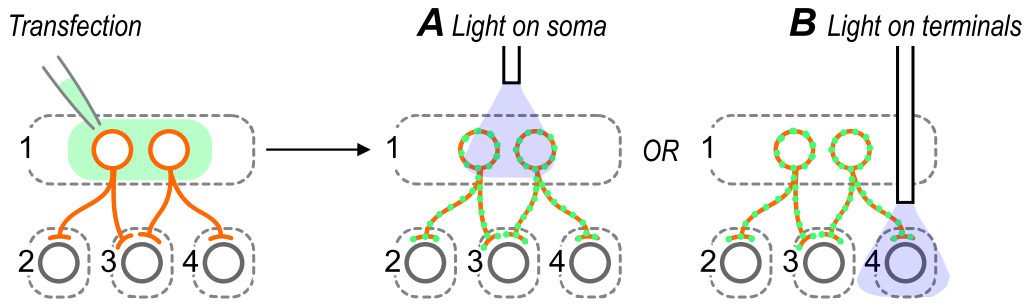

FIG. 1.16 — Optogenetic manipulation of neuronal pathways. In this example, the virus transfects the neurons of region 1. (***A***) Delivery of light in region 1 allows to optically manipulate pathways 1→2, 1→3 and 1→4, whereas (***B***) delivery of light on terminals in region 4 optically manipulates only pathway 1→4.

mutants, termed step-function opsin, that allow to sustainably depolarise neurons. Different wavelength of light respectively control the activation and inactivation of step-function opsins. Step-function opsins sustainably depolarise membranes for seconds after blue light application. These opsins can then be inactivated by green light application, so that the temporal effects of this opsin remain precise.

Another benefit of the precise temporal effects of optogenetic manipulations is that brain activity can be optically manipulated at specific times during behavioural tasks—which is of particular relevance for the present thesis. For instance, presentation of a CS or UCS (electric shock, food, drug) can be temporally coupled with optogenetic manipulations to determine the behavioural functions of the studied neuronal population [*e.g.*, Witten *et al.* (2010), Stuber *et al.* (2011), Tye *et al.* (2011), Burgos-Robles *et al.* (2017), Walsh *et al.* (2018)]. This type of application is especially interesting when neuronal activity is manipulated selectively during event when neuronal activity would be naturally enhanced or depressed [*e.g.*, Chang *et al.* (2016)]. Alternatively, one can evaluate the effects of an optogenetic manipulation in the absence of CS/UCS, to evaluate if the optogenetic manipulation is sufficient to trigger behavioural responses that would be normally elicited by CS and/or UCS [*e.g.*, such as with ICSS procedures (Kim *et al.*, 2012; Namburi *et al.*, 2015)]. Furthermore, optogenetic methods are used to study neuronal plasticity. Indeed, protocols of optogenetic stimulation that produce either long-term potentiation or depression allows to study the influence of neuronal plasticity in specific subsets of neurons on behaviour [*e.g.*, Lee *et al.* (2013), Nabavi *et al.* (2014)]. These few examples illustrate how valuable is the temporal precision of optogenetic manipulations in the study of behaviour.

6. PRESENT OBJECTIVES AND HYPOTHESES

As described in Section 2.4 (page 50), antipsychotic drugs can produce dopamine supersensitivity, and this has large consequences on the long-term management of schizophrenia symptoms and the quality of life of patients. Our current knowledge on how antipsychotic drugs produce these deleterious effects is limited. In Chapters II and III, the biological mechanisms underlying antipsychotic-evoked dopamine supersensitivity were investigated in rats using the typical antipsychotic drug haloperidol. In Chapter II, rats received continuous haloperidol treatment. In Chapter III, rats were either exposed transiently or continuously to haloperidol. We included a group treated transiently to increase our current knowledge on the long-term effects of extended antipsychotic dosing, as it represents a safe and potentially less harmful way to manage schizophrenia symptoms [see Annex, page 290; (Servonnet *et al.*, 2020a)]. In both Chapters II and III, the exaggerated psychomotor response to d-amphetamine served as an index to probe and study the behavioural and neurobiological effects of antipsychotic-evoked dopamine supersensitivity.

In Chapter II, we characterized *where* and *how* d-amphetamine acts to unveil the expression of antipsychotic-evoked dopamine supersensitivity. Injection of d-amphetamine through the systemic route reveals the expression of dopamine supersensitivity, but local infusion into the nucleus accumbens or caudate-putamen does not (El Hage *et al.*, 2015). Hence, actions of d-amphetamine outside of the striatum contribute to its ability to reveal the expression of dopamine supersensitivity. Furthermore, d-amphetamine stimulates dopamine but also noradrenaline and serotonin transmission (Rothman and Baumann, 2003). Whether stimulation of dopamine transmission alone is sufficient to reveal the expression of dopamine supersensitivity is largely unknown. Also, our current knowledge on how dopamine receptors are involved in the ability of d-amphetamine to reveal the expression of dopamine supersensitivity remains sparse. Within this context, the hypotheses of Chapter II are as follow:

> ***HYPOTHESES OF CHAPTER II*** *—i) The central effects of d-amphetamine are sufficient to reveal the expression of dopamine supersensitivity; ii) Enhancing dopamine transmission alone is sufficient to reveal the expression of antipsychotic-evoked dopamine supersensitivity; and iii) D1-like and D2-like-mediated signalling are both sufficient and necessary for revealing the expression of antipsychotic-evoked dopamine supersensitivity.*

As described in Section 2.4.4 (page 57), stress modulates dopaminergic functions, and this involves the stress hormones glucocorticoids. Hence, in Chapter III, we investigated whether antipsychotic-evoked dopamine supersensitivity enhances stress-like responses. This is important to explore because it could represent a mechanism by which dopamine supersensitivity worsens psychosis, given that stress promotes psychotic symptoms (Naeem *et al.*, 2006; McCutcheon *et al.*, 2019a). We first determined whether synthesis of the stress hormone corticosterone is required for the expression of antipsychotic-evoked dopamine supersensitivity. We then determined if dopamine-supersensitive rats show an increased behavioural response to stressors, as determined by *i*) greater avoidance in classical paradigms used to measure stress-related behaviours—*i.e.*, elevated plus-maze (Pellow *et al.*, 1985), open field (Katz *et al.*, 1981) and light-dark box (Crawley and Goodwin, 1980)—and by *ii*) a greater locomotor response to novelty (Piazza *et al.*, 1989). The hypothesis of Chapter III is as follows:

> ***Hypothesis of Chapter III*** *— The expression of antipsychotic evoked dopamine supersensitivity requires corticosterone synthesis and is accompanied with increased stress-related behaviour.*

In Chapter IV, we examined the role of the basolateral nucleus of the amygdala in the behavioural response to appetitive CS in animals with no prior antipsychotic treatment. This is important work because it could give novel insights on how antipsychotic-evoked dopamine supersensitivity enhances the motivational properties of appetitive CS. The evidence described in Section 4.2 (page 69) generally support that intact neuronal activity within the basolateral amygdala is necessary for the motivational effects of appetitive CS, including their ability to promote conditioned approach and to be attractive and rewarding on their own. Basolateral amygdala neurons show increased activity in response to appetitive CS presentation, but it remains largely unknown if this increased neuronal activity is sufficient to potentiate the behavioural response to appetitive CS. Hence, we determined the effects of *in vivo* optogenetic activation of basolateral amygdala neurons on *i*) conditioned approach behaviour, on *ii*) instrumental responses for CS presentation in the conditioned reinforcement test, as a measure of the incentive motivational value of CS, and on *iii*) ICSS, to determine whether optogenetic stimulation of basolateral amygdala neurons selectively

influence the motivational effects of CS, or does so because it is intrinsically rewarding. The hypothesis of Chapter IV is as follows:

> ***HYPOTHESIS OF CHAPTER IV*** *— Optogenetic activation of basolateral amygdala neurons is not rewarding on its own but potentiates conditioned approach and the incentive motivational value of CS.*

CHAPTER II. *Where and How Does d-Amphetamine Act to Reveal Antipsychotic-Induced Dopamine Supersensitivity in Rats?*

Preprint available on BioRxiv
https://doi.org/10.1101/2020.09.08.287664

Alice Servonnet[a], Florence Allain[b], Alice Gravel-Chouinard[a], Giovanni Hernandez[a,c], Casey Bourdeau Caporuscio[b], Mathilde Legrix[a], Daniel Lévesque[c], Pierre-Paul Rompré[a] and Anne-Noël Samaha[b,d]

[a]Department of Neurosciences, Faculty of Medicine; [b]Department of Pharmacology and Physiology, Faculty of Medicine; [c]Faculty of Pharmacy; [d]Groupe de recherche sur le système nerveux central, Faculty of Medicine, Université de Montréal, 2900 Edouard-Montpetit boulevard, Montreal (H3T 1J4), Quebec, Canada.

CONFLICT OF INTEREST

ANS was a scientific consultant for H. Lundbeck A/S as this research was being carried out. This has had no influence on the work. The remaining authors report no biomedical financial interests or potential conflicts of interest.

ABSTRACT

Antipsychotic treatment can produce a dopamine supersensitive state. In both schizophrenia patients and rodents, this is linked to antipsychotic treatment failure. In rodents, dopamine supersensitivity is often confirmed by an exaggerated behavioural response to the indirect monoamine agonist, d-amphetamine, after discontinuation of antipsychotic treatment. Here we investigated where and how d-amphetamine acts to trigger behavioural expression of dopamine supersensitivity, as this could uncover pathophysiological mechanisms underlying this supersensitivity. First, we examined the contributions of a central increase in dopamine/monoamine activity. Haloperidol-treated rats showed a potentiated psychomotor response to systemic d-amphetamine, confirming dopamine supersensitivity. However, they showed a normal psychomotor response to an increase in ventral midbrain dopamine impulse flow or to intracerebroventricular injection of d-amphetamine. This suggests that d-amphetamine's peripheral effects are required for a supersensitive response. Second, we determined the specific contributions of dopamine neurotransmission. The D2 agonist quinpirole, but not the D1 agonist SKF38393 or the dopamine reuptake blocker GBR12783 produced a supersensitive psychomotor response in haloperidol-treated rats. In these rats, the D1 antagonist SCH39166 decreased d-amphetamine-induced psychomotor activity, whereas the D2 antagonist sulpiride enhanced it. Thus, when d-amphetamine triggers a supersensitive response, this involves both D1- and D2-mediated transmission. Finally, we measured d-amphetamine-induced changes in D1- and D2-mediated intracellular signalling pathways in the striatum. In haloperidol-treated rats, a supersensitive response to d-amphetamine was linked to enhanced GSK3β activity and suppressed ERK1/2 activity in the nucleus accumbens, suggesting increased D2-mediated signalling. These findings provide new insights into the neurobiology of antipsychotic-evoked dopamine supersensitivity.

INTRODUCTION

Antipsychotic drugs attenuate schizophrenia symptoms by blunting dopamine D2 receptor activity. However, long-term antipsychotic treatment can produce neuroadaptations that lead to supersensitivity to dopamine receptor stimulation. Antipsychotic-induced dopamine supersensitivity is linked to antipsychotic treatment failure and to an exacerbation of psychosis symptoms (Asper *et al.*, 1973; MØller Nielsen *et al.*, 1974; Chouinard *et al.*, 1978; Margolese *et al.*, 2002; Chouinard and Chouinard, 2008; Fallon and Dursun, 2011; Chouinard *et al*., 2017). In animals, a widely-used index of antipsychotic-induced dopamine supersensitivity is an exaggerated locomotor response to d-amphetamine (Smith and Davis, 1975; Rebec *et al.*, 1982; Ericson *et al.*, 1996; Meng *et al.*, 1998; Samaha *et al.*, 2007; Samaha *et al.*, 2008; Carvalho *et al.*, 2009; Bedard *et al.*, 2011; El Hage *et al.*, 2015; Servonnet *et al.*, 2017; Amato *et al.*, 2018). In this context, d-amphetamine serves as a pharmacological tool to probe the functional consequences of an acute increase in striatal dopamine release, as seen during psychosis (Howes *et al.*, 2012). D-amphetamine is an indirect monoamine agonist (Rothman and Baumann, 2003) with multiple sites of action and neurochemical effects. The anatomical location and nature of the neurochemical effects through which d-amphetamine produces a supersensitive behavioural response in antipsychotic-treated rats are largely unknown. We investigated these effects here, as the answers could reveal underlying biological mechanisms and eventual therapeutic targets to suppress antipsychotic-evoked dopamine supersensitivity.

A first question concerns where d-amphetamine acts to trigger the expression of antipsychotic-evoked dopamine supersensitivity. In dopamine-supersensitive rats, infusing d-amphetamine into the striatum does not trigger expression of established supersensitivity, suggesting that d-amphetamine actions in extra-striatal sites are also required (El Hage *et al.*, 2015). Hence, we determined if increasing ventral tegmental area (VTA) dopamine impulse flow is sufficient to trigger a supersensitive psychomotor response. In other models where rats also show exaggerated d-amphetamine-induced psychomotor activity, this requires d-amphetamine actions in the periphery (Rivet *et al.*, 1989; Deroche *et al.*, 1992). Thus, we also determined whether limiting d-amphetamine's effects to the brain triggers a supersensitive psychomotor response in antipsychotic-treated rats.

A second question concerns the role of dopamine-mediated neurotransmission. D-amphetamine stimulates dopamine, but also noradrenaline and serotonin transmission (Millan *et al.*, 2002; Rothman and Baumann, 2003). Noradrenaline and serotonin also modulate the expression of antipsychotic-evoked dopamine supersensitivity (Obuchowicz, 1999; Charron *et al.*, 2015). Thus, we determined whether selective dopamine reuptake inhibition is sufficient to evoke a supersensitive response in antipsychotic-treated rats. Dopamine signals through dopamine D1-type and D2-type receptors. Selective D2 receptor stimulation evokes a supersensitive behavioural response in antipsychotic-treated rats (Obuchowicz, 1999; Hashimoto *et al.*, 2018), but whether D1 stimulation does the same is unknown. Thereby, we also examined whether stimulation of D1 receptors is sufficient to trigger a sensitised response. Furthermore, we determined whether D1 or D2 receptor antagonists suppress the exaggerated response to d-amphetamine in antipsychotic-treated rats. Lastly, we assessed the effects of d-amphetamine on D1- and D2-mediated signalling in the striatum by quantifying protein activity in the AKT/GSK3β- and cAMP/PKA-dependent pathways (Valjent *et al.*, 2000; Beaulieu *et al.*, 2004; Valjent *et al.*, 2005; Beaulieu *et al.*, 2007). This is because although injecting d-amphetamine into the striatum is not sufficient to produce an enhanced psychomotor response in dopamine-supersensitive rats (El Hage *et al.*, 2015), d-amphetamine-induced signalling in the striatum might still be necessary.

METHODS

See Supplement for further information on rats, drugs, intra-cranial manipulations, measurement of psychomotor activity and Western Blots. Experimental procedures were approved by the Université de Montréal's ethics committee and followed the guidelines of the Canadian Council on Animal Care.

Antipsychotic Treatment

Adult male Sprague-Dawley rats received haloperidol via osmotic minipumps (Alzet model 2ML2; Durect Corporation, Cupertino, CA) to achieve steady-state brain concentrations of the drug (Kapur *et al.*, 2003; Samaha *et al.*, 2007), as produced by standard antipsychotic treatment regimens in the clinic (Farde *et al.*, 1989; Remington *et al.*, 2006; Mamo *et al.*, 2008). We used 0.5 mg/kg/day haloperidol. This achieves 73% ± 14 SD striatal D2 receptor occupancy [unpublished observations,

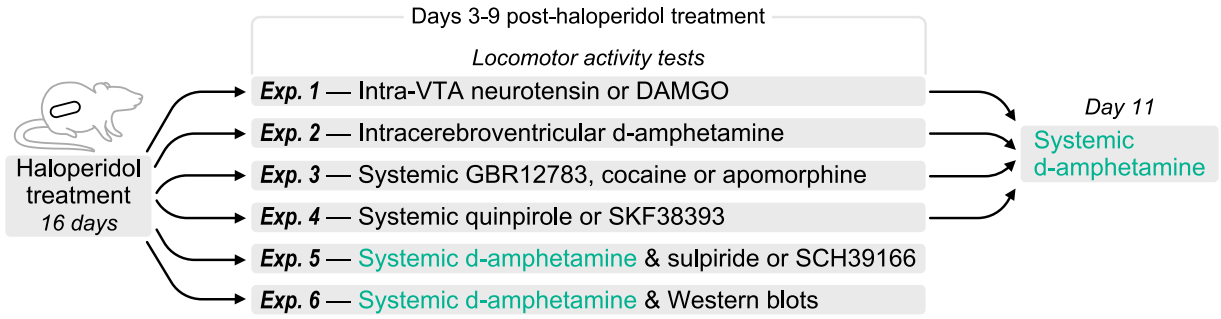

FIG. 2.1 — Experimental timelines.

see (Kapur *et al.*, 2003; Samaha *et al.*, 2007)], and this is within the occupancy range that is therapeutically-efficacious in patients (Farde *et al.*, 1992; Kapur *et al.*, 1999; Kapur *et al.*, 2000). Under isoflurane anaesthesia, minipumps were implanted subcutaneously (s.c.) for haloperidol-treated rats, and controls were sham-operated (Samaha *et al.*, 2007). Seventeen days later, minipumps were removed, and controls were sham-operated again.

Psychomotor Activity

Psychomotor activity was assessed using 2 measures: 1) photocell counts to measure horizontal locomotor activity and 2) observer ratings based on a 1-to-9 scale (Mattson *et al.*, 2007). Ratings between 1 and 4 indicate near-to-normal locomotor activity, 5 indicates hyperlocomotion without stereotypy, and ratings between 6 and 9 indicate stereotypy (Mattson *et al.*, 2007).

Experiments

FIG. 2.1A illustrates experimental timelines. Locomotion tests started at least 3 days after haloperidol discontinuation and were given every 48 hours, 1 test/day. All doses, routes of administration, number of rats per condition (per group) and the allocation of rats between the treatment conditions are detailed in Table I. In Exps. 1-5, injections were given in a counterbalanced order. In each experiment below, we confirmed antipsychotic-induced dopamine supersensitivity by measuring the psychomotor response to s.c. d-amphetamine (1.5 mg/kg).

Exp. 1: Increasing VTA dopamine impulse flow. We determined if enhancing VTA dopamine impulse flow produces a supersensitive psychomotor response in haloperidol-treated

rats. We evaluated the locomotor response to bilateral infusions of vehicle, neurotensin (1 nmol/hemisphere) or DAMGO (0.3 nmol/hemisphere) into the VTA, at concentrations that increase dopamine release in terminal regions (Kalivas and Duffy, 1990; Laitinen *et al.*, 1990). Neurotensin increases dopamine impulse flow by producing an inward current on dopamine neurons (Mercuri *et al.*, 1993), reducing D2 autoreceptor-mediated inhibition (Werkman *et al.*, 2000; Jomphe *et al.*, 2006; Thibault *et al.*, 2011), and enhancing glutamatergic inputs to dopamine neurons (Kempadoo *et al.*, 2013; Bose *et al.*, 2015). DAMGO, a μ-opioid receptor agonist (Chen *et al.*, 1993), inhibits GABA release thereby disinhibiting dopamine neuron activity (Kalivas and Duffy, 1990; Bergevin *et al.*, 2002).

Exp. 2: Intracerebroventricular d-amphetamine. We determined if limiting d-amphetamine's effects to the brain still triggers a supersensitive response in haloperidol-treated rats. We infused d-amphetamine bilaterally into the lateral ventricles [0, 50 or 150 μg/hemisphere (Lin *et al.*, 1983)], and measured psychomotor activity.

Exp. 3: Stimulation of dopamine transmission. We assessed whether selectively blocking dopamine reuptake with GBR12783 (Bonnet and Costentin, 1986) [0, 5 or 10 mg/kg (Le Pen *et al.*, 1996)] produces a supersensitive psychomotor response in haloperidol-treated rats. For comparison, we also assessed effects of the monoamine reuptake blocker cocaine (Rothman *et al.*, 2001) [0, 2.5 or 10 mg/kg (Kosten, 1997)] and the monoamine receptor agonist apomorphine (Millan *et al.*, 2002) [0, 0.25 or 0.5 mg/kg (Geyer *et al.*, 1987; Barros *et al.*, 1989)].

Exp. 4: Stimulation of D1 or D2 transmission. We determined whether selective D1 or D2 stimulation produces an enhanced psychomotor response in haloperidol-treated rats. Locomotion was recorded for 30 min before administration of a D1 agonist [SKF38393 (Seeman and Van Tol, 1994; Neumeyer *et al.*, 2003); 0, 1 or 10 mg/kg (Molloy and Waddington, 1987; Meller *et al.*, 1988)] or of a D2 agonist [quinpirole (Millan *et al.*, 2002); 0, 0.15 or 0.5 mg/kg (Benaliouad *et al.*, 2009; Hashimoto *et al.*, 2018)] and for 2 hours thereafter.

Exp. 5: Blockade of D1 or D2 transmission. We assessed whether D1 and/or D2 transmission is necessary for the expression of dopamine supersensitivity. Rats received the D2 antagonist sulpiride (Caley and Weber, 1995; Martelle and Nader, 2008) [0, 25 or 80 mg/kg (Fritts

Table I. Doses and route of administration of pharmacological agents, number of rats per condition (per group) and design of the allocation of rats between conditions

	Agent(s) or vehicle and route	Dose		n per group	Design
1	Intra-VTA vehicle	-	-	7	Within-subjects, all rats received neurotensin, DAMGO and vehicle injections
	Intra-VTA neurotensin	1	nmol/ hemisphere	7	
	Intra-VTA DAMGO	0.3		7	
2	I.c.v. vehicle	-	-	16-21	Between-subjects, one d-amphetamine dose per rat, all rats received vehicle
	I.c.v. d-amphetamine	50, 100	µg/ hemisphere	6-10	
3	S.c. or i.p. vehicle[a]	-	-	29-31	Between-subjects, each rat received no more than 3 agonist doses (*i.e.*, 3 out of 6 doses) and 2 vehicle injections
	S.c. GBR12783	5, 10	mg/kg	10-14	
	I.p. cocaine	2.5, 10		7-10	
	S.c. apomorphine	0.25, 0.5		9-10	
4	S.c. vehicle[a]	-	-	16	Between-subjects, each rat received one dose of each agonist (*i.e.*, 2 out of 4 doses) and one vehicle injection
	S.c. SKF38393	1, 10	mg/kg	8	
	S.c. quinpirole	0.15, 0.5		8	
5	S.c. vehicle & vehicle[a]	-	-	31-32	Between-subjects, each rat received 4 combinations: -1 vehicle/vehicle -1 antagonist/vehicle -1 vehicle/d-amphetamine -1 antagonist/d-amphetamine
	S.c. sulpiride & vehicle[a]	25, 80 (sul) & vehicle	mg/kg	7-8	
	S.c. SCH39166 & vehicle[a]	0.03, 0.1 (SCH) & vehicle		8	
	S.c. vehicle[a] & d-amphetamine	Vehicle & 1.5 (d-amph)		31-32	
	S.c sulpiride & d-amphetamine	25, 80 (sul) & 1.5 (d-amph)		8	
	S.c. SCH39166 & d-amphetamine	0.03, 0.1 (SCH) & 1.5 (d-amph)		7-8	
1 TO 6	S.c. saline	-	-	5-32	Within-subjects, each rat received saline and then d-amphetamine 30 or 60 min later
	S.c. d-amphetamine	1.5	mg/kg		

[a]*Vehicle of each agent were pooled together because there was no difference in their locomotor effects.*

et al., 1997; Wright *et al.*, 2013)] or the D1 antagonist SCH39166 (McQuade *et al.*, 1991) [0, 0.03 or 0.1 mg/kg (Batsche *et al.*, 1994; Scardochio and Clarke, 2013)], and 30 min later, they received d-amphetamine (0 or 1.5 mg/kg).

Exp. 6: D-amphetamine effects on D1- and D2-mediated signalling. We measured d-amphetamine-induced protein activity in the AKT/GSK3β- and cAMP/PKA-dependent pathways. Locomotion was recorded for 30 minutes, then control and haloperidol-treated rats received s.c. saline or d-amphetamine. One hour later, brains were extracted, samples were taken from the nucleus accumbens and the dorsal, ventrolateral and centromedial caudate-putamen. We quantified total and phosphorylated protein levels of DARPP-32, ERK1, ERK2, AKT and GSK3β using Western Blot procedures.

Statistical Analysis

Locomotor activity and protein levels were expressed as the percent change relative to vehicle-injected controls. Repeated measures or mixed-model ANOVA were used to analyse the influence of Injection (see description of levels in Result section) or Group (controls and haloperidol) on locomotion, psychomotor activity ratings or protein level (Group × Injection × Time, 'Injection' as a within-subjects variable in Exp. 1 and 'Injection' as between-subjects variables in Exps. 2-6). When interaction and/or main effects were significant ($p < 0.05$), effects were analysed further using Bonferroni's multiple comparisons' tests. Values in figures are mean ± SEM.

RESULTS

Across experiments, all haloperidol-treated groups developed dopamine supersensitivity, as indicated by enhanced d-amphetamine-induced locomotion relative to controls (FIG. 2.2; 'Injection': vehicle and d-amphetamine; Group × Injection interaction; 2.2B, $F_{1,11} = 25.95$; 2.2D, $F_{1,51} = 19.71$; 2.2E, $F_{1,30} = 8.1$; 2.2F, $F_{1,61} = 5.66$; 2.2G, $F_{1,20} = 18.76$; Group effect; 2.2B, $F_{1,11} = 5.31$; 2.2C, $F_{1,8} = 5.51$; 2.2D, $F_{1,51} = 9.05$; 2.2E, $F_{1,30} = 5.57$; 2.2F, $F_{1,61} = 7.88$; 2.2G, $F_{1,20} = 12.67$; Injection effect; 2.2B, $F_{1,11} = 92.63$; 2.2C, $F_{1,8} = 31.77$; 2.2D, $F_{1,51} = 497.5$; 2.2E, $F_{1,30} = 117.0$; 2.2F, $F_{1,61} = 260.4$; 2.2G, $F_{1,20} = 129$; 2.2-B-D-E-F-G; d-amph > veh in all groups; after d-amph, haloperidol > controls; all P's < 0.05). Haloperidol and control rats generally showed similar D-amphetamine-induced psychomotor activity ratings that indicate high levels of locomotor activity

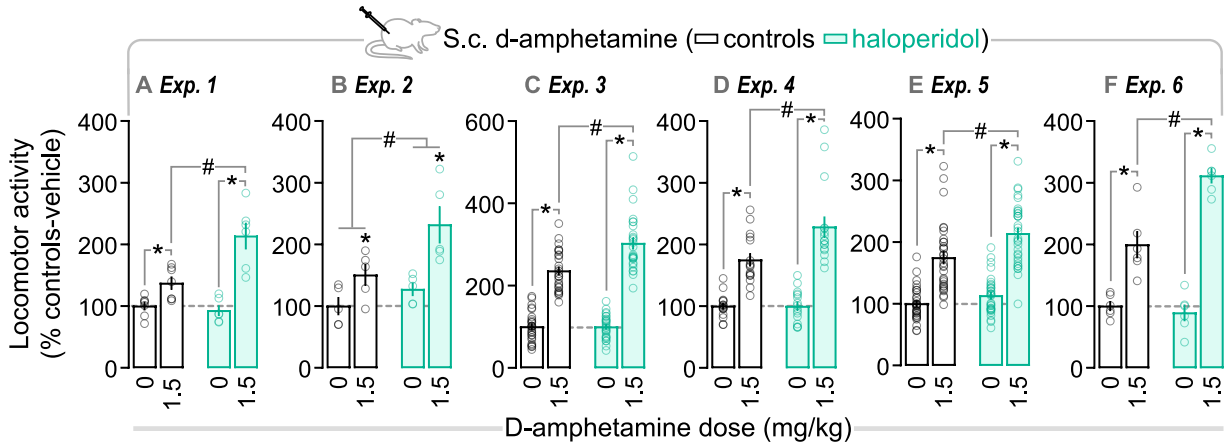

FIG. 2.2 — Chronic haloperidol treatment produced dopamine supersensitivity, as indicated by an exaggerated psychomotor response to d-amphetamine. (***A-F***) Locomotor response to subcutaneous (s.c.) d-amphetamine (0 or 1.5 mg/kg). Dotted lines indicate locomotion of vehicle-injected controls. *n*'s = 5-32/condition. **p* < 0.05; in (***B***), Injection effect. # *p* < 0.05; in (***B***), Group effect.

without notable stereotypy (FIG. 2.S1). Note that the scale used here to analyse psychomotor activity have only one score for hyperlocomotion without stereotypy, making unlikely to note group differences in d-amphetamine-induced psychomotor ratings here given that stereotypy was negligeable.

Exp. 1: Increasing VTA Dopamine Impulse Flow

Across groups, intra-VTA neurotensin enhanced locomotion and psychomotor activity ratings compared to vehicle, without significant group differences (FIG. 2.3B-C; 'Injection': vehicle and neurotensin; Injection × Time interaction on minutes 10-120, $F_{11,132} = 4.32$; Injection effect, $F_{1,12} = 14.41$; FIG. 2.3E; Injection effect; vehicle versus neurotensin, $F_{1,12} = 6.92$; all *P*'s < 0.05). Intra-VTA DAMGO also increased locomotor activity and ratings beyond vehicle similarly across groups (FIG. 2.3B-D; 'Injection': vehicle and DAMGO; Injection × Time interaction on minutes 10-120, $F_{11,132} = 7.75$; Injection effect, $F_{1,12} = 17.04$; FIG. 2.3E; Injection effect; vehicle versus DAMGO, $F_{1,12} = 13.91$; all *P*'s < 0.05).

Hence, in rats with established antipsychotic-evoked dopamine supersensitivity, increasing VTA dopamine impulse flow does not evoke an exaggerated psychomotor response.

Exp. 2: Intracerebroventricular D-amphetamine

Across groups, intracerebroventricular d-amphetamine dose-dependently increased locomotion and psychomotor activity ratings compared to vehicle, without group differences (FIGS. 2.3F-H; 'Injection': vehicle, 50 and 100 µg/hemisphere; minutes 10-120; Injection × Time interaction, $F_{22,726}$ = 6.38; Injection effect, $F_{2,66}$ = 29.49; FIG. 2.3I; Injection effect, $F_{2,66}$ = 50.43; all P's < 0.0001).

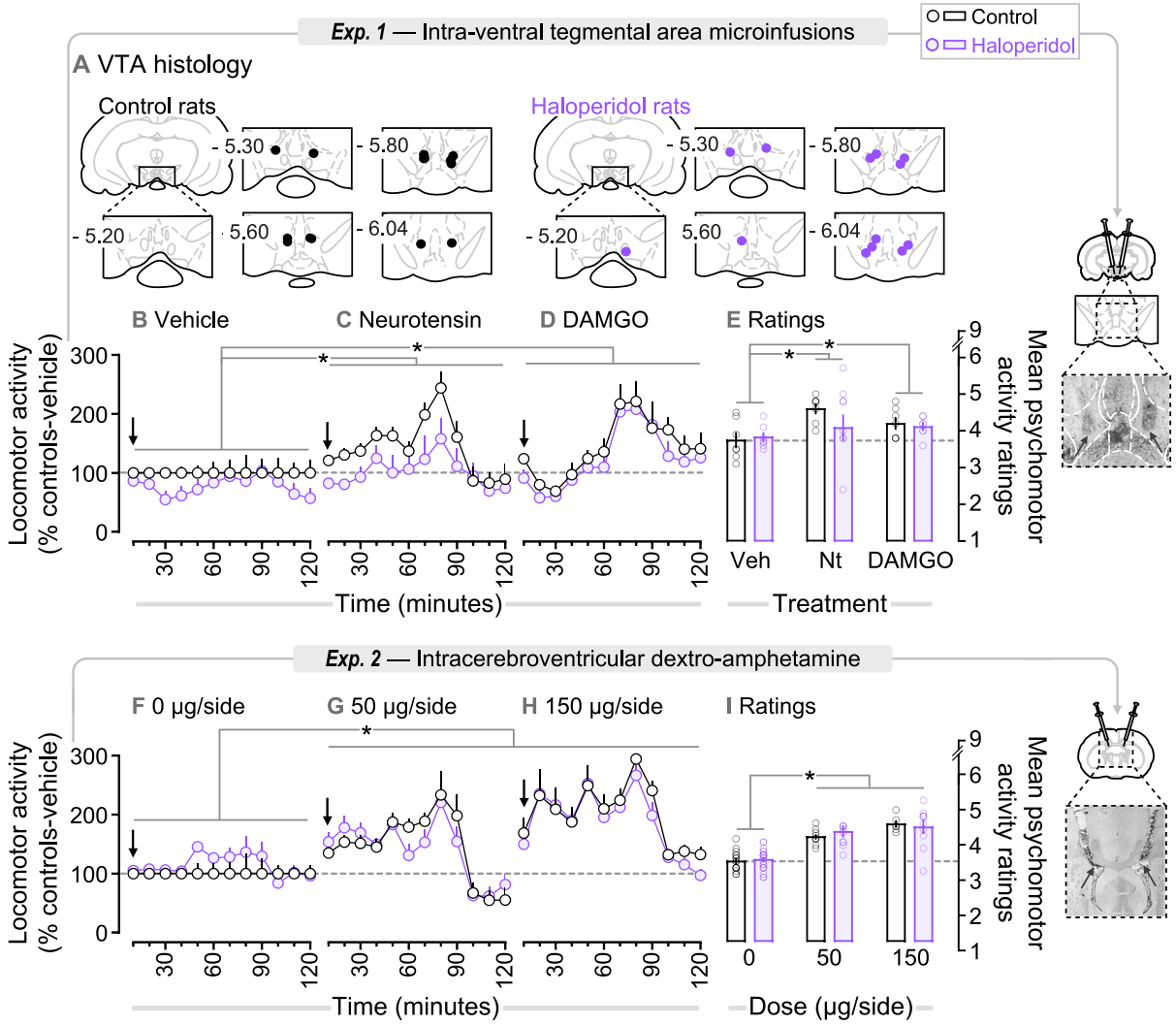

FIG. 2.3 — Neither increasing ventral tegmental area (VTA) dopamine impulse flow nor injecting d-amphetamine into the brain triggers the expression of dopamine supersensitivity. (***A***) VTA histology. (***B-E***) Psychomotor response to intra-VTA vehicle, neurotensin or DAMGO. (***F-I***) Psychomotor response to intracerebroventricular d-amphetamine. On the right, representative injector placements (arrows indicate injectors). Dotted lines indicate response of vehicle-injected controls. n's = 7-21/condition. *$p < 0.05$.

Hence, circumscribing d-amphetamine's effects to the brain does not evoke the expression of established dopamine supersensitivity.

Exp. 3: Stimulation of Dopamine Transmission

GBR12783 increased locomotion and psychomotor activity ratings above vehicle, without group differences (vehicle not shown; FIG. 2.4A-B versus vehicle; minutes 70-180; 'Injection': vehicle, 5 or 10 mg/kg; Injection × Time interaction, $F_{22,1133} = 5.87$; Injection effect, $F_{2,103} = 29.72$; 3C versus vehicle; Injection effect, $F_{2,91} = 41.4$; all P's < 0.0001).

Cocaine increased locomotion and ratings above vehicle, with a mildly enhanced response in haloperidol rats (FIG. 2.4D-E versus vehicle; minutes 70-180; 'Injection': vehicle, 2.5 and 10 mg/kg; Injection × Time interaction, $F_{22,957} = 5.98$; Injection effect, $F_{2,87} = 47.45$; Group effect, $F_{1,87} = 4.35$; 3F versus vehicle; Injection effect, $F_{2,75} = 53.45$; all P's < 0.05).

Similarly, apomorphine increased locomotor activity and ratings beyond vehicle, and these effects were enhanced in haloperidol rats (FIG. 2.4G-H versus vehicle; minutes 70-180; 'Injection': vehicle, 0.25 and 0.5 mg/kg; Injection × Time interaction, $F_{22,1023} = 6.4$; Injection effect, $F_{2,93} = 10.3$; Group × Time interaction, $F_{11,1023} = 3.05$; Group effect, $F_{1,93} = 8.05$; FIG. 2.4I versus vehicle; Group × Injection interaction, $F_{2,81} = 5.43$; Injection effect, $F_{2,81} = 189.8$; Group effect, $F_{1,81} = 28.48$; FIG. 2.4I; haloperidol > controls at both doses; all P's < 0.05).

Thus, in dopamine-supersensitive rats, monoamine agonists (cocaine and apomorphine) but not a selective dopamine reuptake inhibitor (GBR12783) produce a mildly enhanced psychomotor response.

Exp. 4: Stimulation of D1 or D2 Transmission

The D1 agonist SKF38393 evokes stereotypy but little hyperlocomotion (Meller *et al.*, 1988; Meyer and Shults, 1993; Hooks *et al.*, 1994). Accordingly, SKF38393 increased psychomotor activity ratings, and did so similarly across groups (vehicle not shown; FIG. 2.5A versus vehicle; 'Injection': vehicle, 1 and 10 mg/kg; Injection effect, $F_{2,58} = 15.89$, $p < 0.0001$) without increasing locomotion (FIG. 2.5B-C versus vehicle).

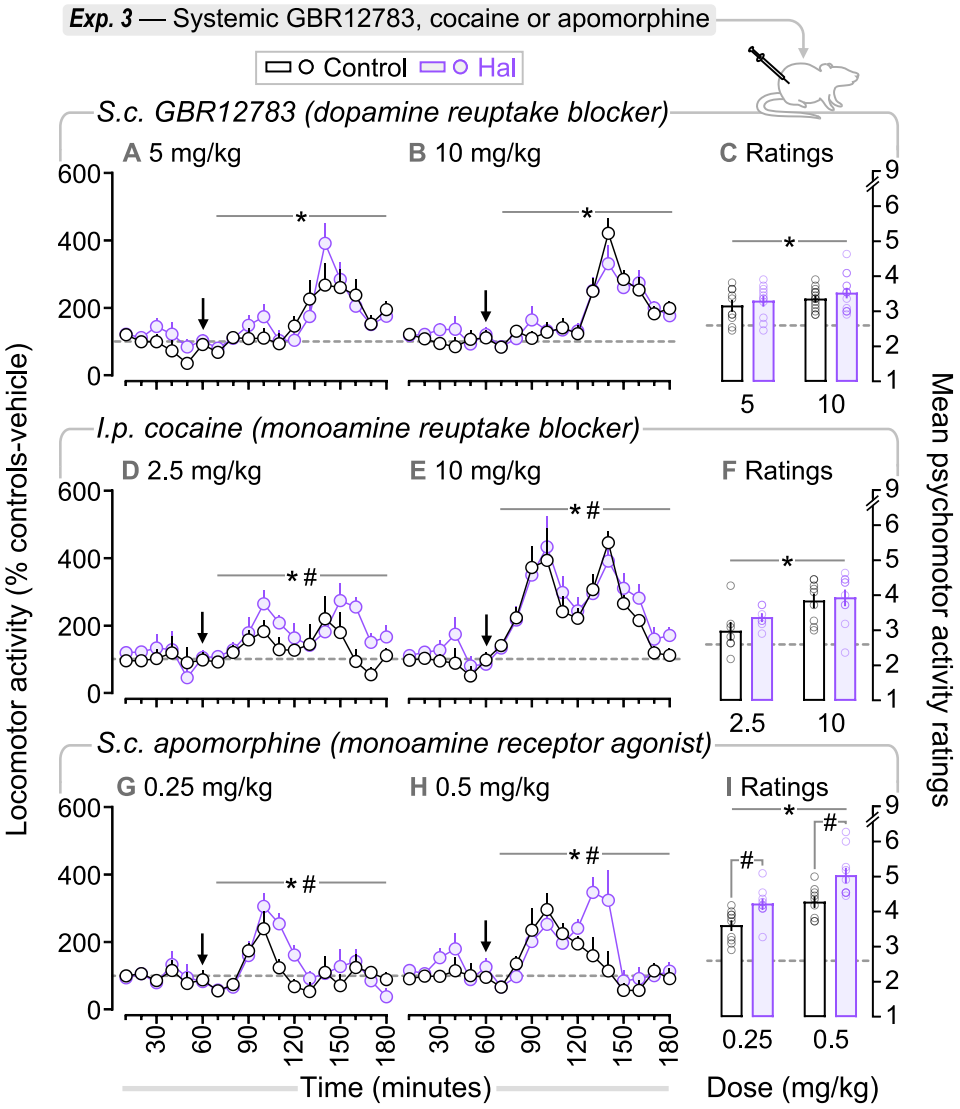

FIG. 2.4 — Psychomotor effects of GBR12783, cocaine and apomorphine in haloperidol-treated rats versus controls. Psychomotor response to (***A-C***) subcutaneous (s.c.) GBR12783, (***D-F***) intraperitoneal (i.p.) cocaine or (***G-I***) s.c. apomorphine. Dotted lines indicate response of vehicle-injected controls. *n*'s = 7-31/condition. #, *$p < 0.05$. In (***A-B***); *Injection × Time interaction and Injection effect. In (***C***); *Injection effect. In (***D-E***); *Injection × Time interaction and Injection effect, # Group effect. In (***F***); *Injection effect. In (***G-H***); *Injection × Time interaction and Injection effect, # Group × Time interaction and Group effect. In (***I***); *Injection effect.

The D2 agonist quinpirole dose-dependently increased locomotion and ratings relative to vehicle, and this effect was greatest in haloperidol rats (FIG. 2.5D versus vehicle; 'Injection': vehicle, 0.15 and 0.5 mg/kg; Injection effect, $F_{2,58} = 17.85$; Group effect, $F_{1,58} = 3.89$; FIG. 2.5E-F versus vehicle;

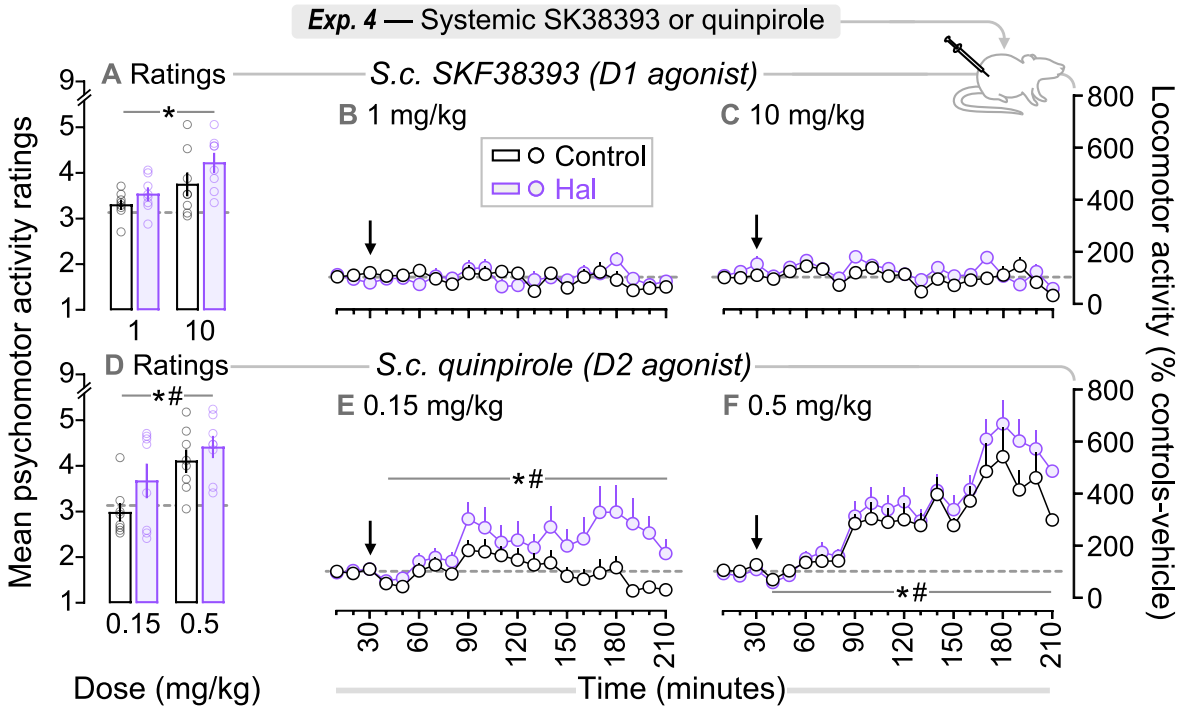

FIG. 2.5 — Stimulation of D2 but not D1 transmission is sufficient to reveal the expression of antipsychotic-evoked dopamine supersensitivity. (***A-F***) Psychomotor response subcutaneous (s.c.) quinpirole or SKF38393. Dotted lines indicate response of vehicle-injected controls. *n*'s = 7-32/condition. #, *$p < 0.05$. In (***A***); *Injection effect. In (***D***); *Injection effect, # Group effect. In (***E-F***), *Injection × Time interaction and Injection effect, # Group × Time interaction and Group effect.

minutes 40-210; Injection × Time interaction, $F_{34, 986} = 16.55$; Group × Time interaction, $F_{17,986} = 4.57$; Injection effect, $F_{2,58} = 32.11$; Group effect, $F_{1,58} = 6.8$; all *P*'s ≤ 0.05).

Hence, rats with antipsychotic-induced supersensitivity show an augmented behavioural response to D2, but not D1 receptor stimulation.

Exp. 5: Blockade of D1 or D2 Transmission

Haloperidol-treated rats showed greater d-amphetamine-induced ratings and locomotion than controls did (FIG. 2.6A; Group effect, $F_{1,88} = 14.48$; FIGS. 2.6B-C; minutes 40-150; Group × Time interaction, $F_{11,968} = 3.48$; Group effect, $F_{1,88} = 7.3$; all *P*'s < 0.01), confirming dopamine supersensitivity. Across groups, the D1 antagonist SCH39166 decreased both vehicle- (FIGS. 2.S2A-C) and d-amphetamine-induced locomotion and ratings (FIG. 2.6A; 'Injection': vehicle, 0.03 and 0.1 mg/kg; Injection effect, $F_{2,88} = 39.11$; FIGS. 2.6B-C; minutes 40-150; Injection × Time

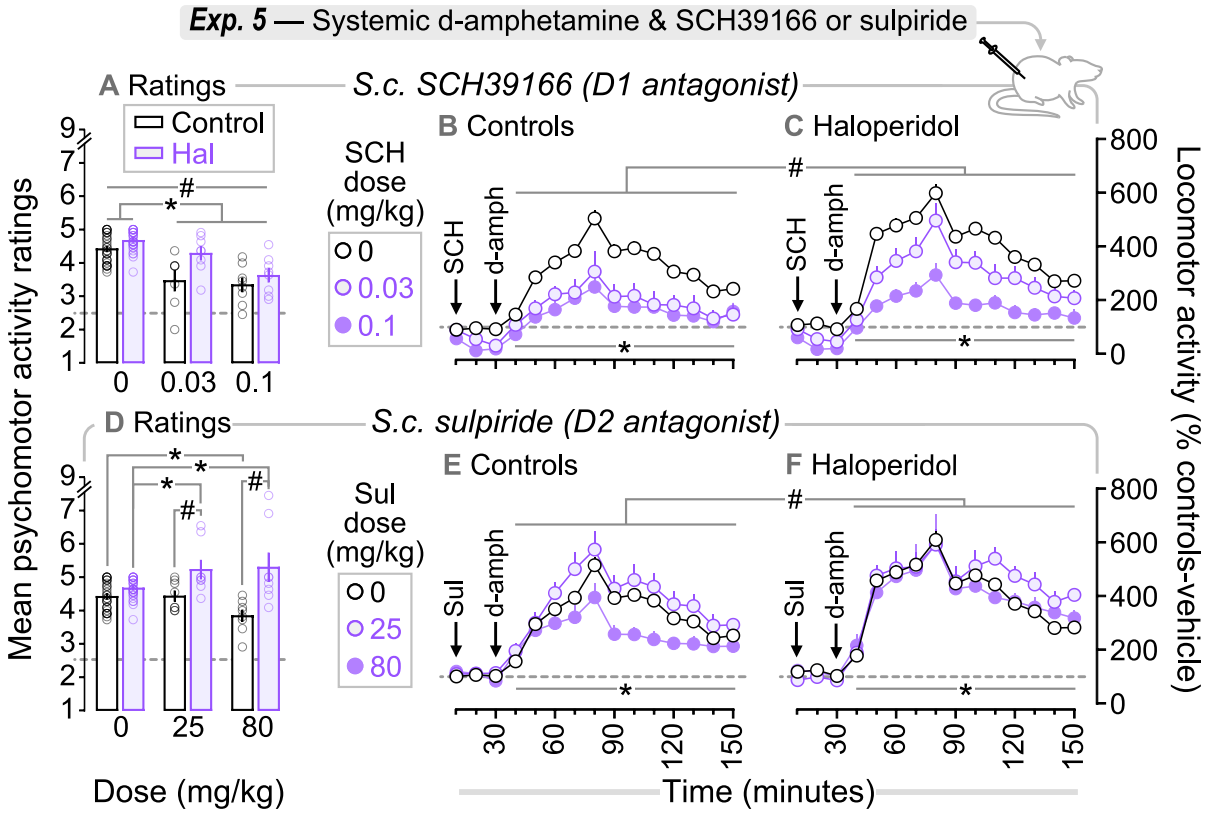

FIG. 2.6 — D1- but not D2-mediating signalling is necessary for the expression of antipsychotic-evoked dopamine supersensitivity. (***A-F***) Psychomotor response to s.c. SCH39166 or sulpiride and s.c. d-amphetamine. Dotted lines indicate response of vehicle-injected controls. *n*'s = 7-32/condition. #, **p* < 0.05. In (***A***); *Injection effect, # Group effect. In (***B-C***, ***E-F***); *Injection × Time interaction and Injection effect, # Group × Time interaction and Group effect.

interaction, $F_{22,968} = 6.33$; Injection effect, $F_{2,88} = 28.69$; all *P*'s < 0.0001). Notably, in haloperidol-treated rats, 0.03 mg/kg SCH39166 restored d-amphetamine-induced locomotion to control levels (compare light purple curve in FIG. 2.6C to white curve in FIG. 2.6B).

Across groups, the D2 antagonist sulpiride did not influence vehicle-induced ratings or locomotion (FIG. 2.S2D-F). Sulpiride *suppressed* d-amphetamine-induced psychomotor activity ratings in controls but, surprisingly, it *enhanced* this response in haloperidol-treated rats (FIG. 2.6D; 'Injection': vehicle, 25 and 80 mg/kg; Group × Injection interaction, $F_{2,89} = 8.47$; Group effect, $F_{1,89} = 38.25$; haloperidol > controls at both sulpiride doses; haloperidol rats, 0 < 25 and 80 mg/kg; control rats, 0 > 80 mg/kg; all *P*'s < 0.05). Sulpiride also influenced d-amphetamine-induced locomotion (FIGS. 2.6E-F; minutes 40-150; Injection × Time interaction, $F_{22,979} = 2.51$; Injection effect, $F_{2,89} = 3.09$; all *P*'s ≤ 0.05), with group differences in this effect. Specifically, sulpiride

decreased d-amphetamine-induced hyperlocomotion in controls but not in haloperidol rats (FIGS. 2.6E-F; Group × Time interaction, $F_{11,979} = 3.14$, Group effect, $F_{1,89} = 14.38$; all P's ≤ 0.05).

Thus, in dopamine-supersensitive rats, D1- but not D2-mediated activity is required for the expression of an enhanced psychomotor response to d-amphetamine. In parallel, D2 receptor blockade *potentiated* d-amphetamine-induced psychomotor activity in dopamine-supersensitive rats, suggesting that D2 receptor activity normally tempers the expression of dopamine supersensitivity.

Exp. 6: D-amphetamine Effects on D1- and D2-mediated Signalling

Caudate-putamen. D-amphetamine produced similar effects in haloperidol-treated and control rats. In the dorsal, ventrolateral and centromedial caudate-putamen, d-amphetamine had mixed effects on total proteins levels, but it either decreased or had no effect on phosphorylated/total protein ratios (FIGS. 2.S3-5). Thus, d-amphetamine did not increase protein phosphorylation in AKT/GSK3β- or cAMP/PKA-dependent pathways in the caudate-putamen. Some of our results differ from work showing that in otherwise naïve rats, d-amphetamine increases DARPP-32, ERK1/ERK2 and GSK3β activity, and decreases AKT activity in the striatum (Svenningsson *et al.*, 2003; Beaulieu *et al.*, 2004; Valjent *et al.*, 2005; Beaulieu *et al.*, 2006). However, these previous studies analysed the caudate-putamen as a whole or with nucleus accumbens included (Svenningsson *et al.*, 2003; Beaulieu *et al.*, 2004; Valjent *et al.*, 2005; Beaulieu *et al.*, 2006).

Nucleus accumbens. Relative to saline, d-amphetamine increased total GSK3β levels only in haloperidol-treated rats (FIG. 2.7C; 'Injection': vehicle and d-amphetamine; Group × Injection interaction, $F_{1,20} = 4.23$; Injection effect, $F_{1,20} = 14.61$; haloperidol rats, d-amph > saline; all P's ≤ 0.05). This reflects higher levels of non-phosphorylated (active) versus phosphorylated (inactive) GSK3β (Sutherland *et al.*, 1993), because d-amphetamine decreased pGSK3β/total GSK3β ratios across groups (FIG. 2.7D; Injection effect, $F_{1,20} = 7.57$, $p = 0.012$). D-amphetamine decreased total AKT levels and increased pAKT/total AKT ratios, with no group differences (Injection effect; FIG. 2.7E; $F_{1,15} = 13.01$; FIG. 2.7F; $F_{1,15} = 6.611$; all P's < 0.05). Hence, in the nucleus accumbens, d-amphetamine influences AKT similarly in dopamine-supersensitive and control rats, but d-amphetamine enhances GSK3β activity to a greater extent in dopamine-supersensitive rats.

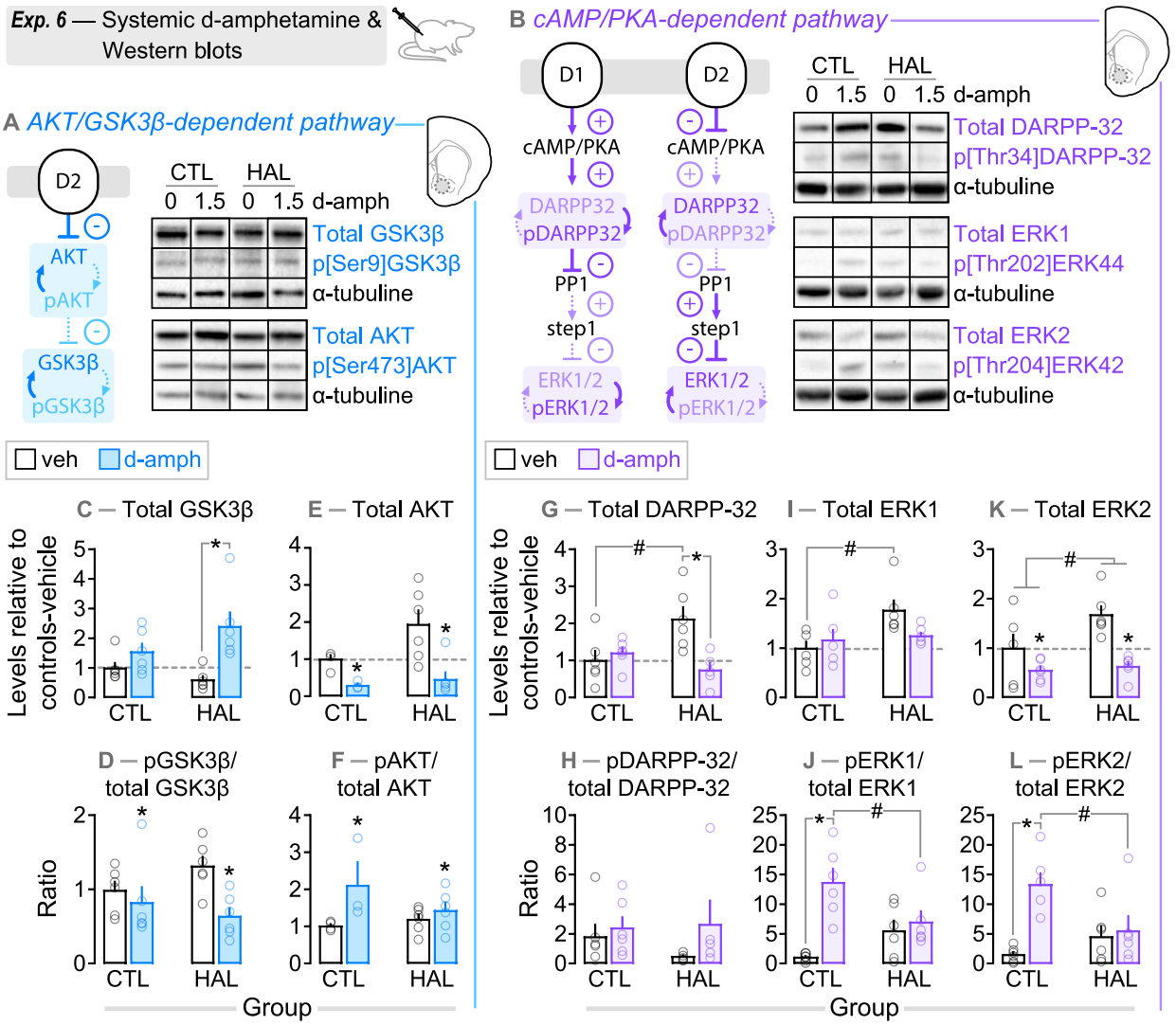

FIG. 2.7 — Dopamine-supersensitive rats have enhanced d-amphetamine-induced GSK3β activity and suppressed d-amphetamine-induced ERK1/2 activity in the nucleus accumbens. (***A-B***) AKT/GSK3β- and cAMP/PKA-dependent pathways and Western blots in accumbens tissue. Total protein levels and phosphorylated/total protein ratios within the (***C-F***) AKT/GSK3β- and (***G-L***) cAMP/PKA-dependent pathways. Dotted lines indicate mean protein level of vehicle-injected controls. *n*'s = 3-6/condition. # $p < 0.05$; in (***K***), Group effect. *$p < 0.05$; in (***D-F***, ***K***), Injection effect.

In haloperidol-treated rats, total DARPP-32 levels were increased at baseline and decreased after d-amphetamine (FIG. 2.7G; Group × Injection interaction, $F_{1,19} = 9.97$; Injection effect, $F_{1,19} = 5.47$; after saline, haloperidol rats > controls; Haloperidol rats, saline > d-amph; all *P*'s < 0.05). At baseline, total levels of both ERK1 and ERK2 were highest in haloperidol-treated rats (FIG. 2.7I; Group × Injection interaction, $F_{1,20} = 4.13$; Group effect, $F_{1,20} = 6.41$; haloperidol rats > controls after saline injection; FIG. 2.7K; Group effect, $F_{1,20} = 4.56$; all *P*'s ≤ 0.05). D-amphetamine

decreased total ERK2 levels similarly across groups (FIG. 2.7K; Injection effect, $F_{1,20} = 17.79$; all P's < 0.05). Hence, d-amphetamine-induced expression of dopamine supersensitivity potentially involves decreased total DARPP-32 levels in the accumbens, without distinct effects on total ERK1/ERK2 levels.

D-amphetamine enhanced the proportion of phosphorylated (active) versus total ERK1 and ERK2 levels in controls [see also (Svenningsson *et al.*, 2003; Valjent *et al.*, 2005; Beaulieu *et al.*, 2006)], but not in haloperidol-treated rats (FIG. 2.7J; Group × Injection interaction, $F_{1,20} = 9.73$; Injection effect, $F_{1,20} = 15.62$; FIG. 2.7L; Group × Injection interaction, $F_{1,20} = 8.41$; Injection effect, $F_{1,20} = 11.82$; FIGS. 2.7J-L; controls, d-amph > saline; after d-amph, controls > haloperidol rats; all P's ≤ 0.05). D-amphetamine did not change the proportion of phosphorylated DARPP-32 in either group (FIG. 2.7H; $p > 0.05$). Thus, in the accumbens, the expression of dopamine supersensitivity is potentially linked to suppressed phosphorylation of ERK1 and ERK2.

In summary, in dopamine-supersensitive rats, enhanced d-amphetamine-induced psychomotor activity is accompanied by enhanced GSK3β activity and decreased ERK activity in the nucleus accumbens.

DISCUSSION

In rats given a clinically-relevant antipsychotic treatment regimen, we examined where and how d-amphetamine acts to reveal the expression of dopamine supersensitivity. We report four main findings. First, systemic d-amphetamine reliably triggered the expression of established dopamine supersensitivity, whereas intracerebroventricular d-amphetamine infusion or an increase in VTA dopamine impulse flow did not. Second, dopamine-supersensitive rats showed an enhanced psychomotor response to selective D2, but not to D1 receptor stimulation or selective dopamine reuptake inhibition. Third, in dopamine-supersensitive rats, blocking D2 receptors *enhanced* the psychomotor response to d-amphetamine, whereas blocking D1 receptors *suppressed* d-amphetamine-induced responding. Fourth, in dopamine-supersensitive rats, d-amphetamine increased GSK3β levels in the nucleus accumbens, but d-amphetamine failed to increase ERK1/2 phosphorylation as it did in controls. These results give new insights into the mechanisms underlying the behavioural expression of antipsychotic-evoked dopamine supersensitivity.

Central Processes

Across experiments, rats withdrawn from haloperidol treatment showed an enhanced psychomotor response to systemic d-amphetamine, indicating dopamine supersensitivity (Smith and Davis, 1975; Ericson *et al.*, 1996; Samaha *et al.*, 2007). In these rats, increasing VTA dopamine impulse flow (by infusing neurotensin or DAMGO locally) produced control levels of hyperlocomotion and therefore, it did not trigger a sensitised response indicative of dopamine supersensitivity. It will be important to confirm these observations with more selective technics such as chemogenetics. Nonetheless, we extended the finding above by showing that restricting d-amphetamine's effects to the brain was also insufficient to reveal the expression of dopamine supersensitivity, as it produced a similar locomotor effect between antipsychotic-treated rats and controls. These observations converge with findings that intra-striatal d-amphetamine infusions also fail to trigger a sensitised response in antipsychotic-treated rats (El Hage *et al.*, 2015). Still, the above findings contrast with studies showing that antipsychotic-treated rats show a sensitized locomotor response to intra-striatal dopamine infusions (Halperin *et al.*, 1983, 1989). However, these previous studies used high and clinically unrepresentative antipsychotic doses (Kapur *et al.*, 2003). Using a clinically representative antipsychotic treatment regimen (Farde *et al.*, 1989; Kapur *et al.*, 2000; Kapur *et al.*, 2003; Mamo *et al.*, 2008), the present results and previous ones (El Hage *et al.*, 2015) suggest that once antipsychotic-evoked dopamine supersensitivity has developed, its behavioural expression is not revealed by increasing central dopamine or monoamine transmission. Thus, this suggests that in antipsychotic-treated rats, the psychomotor response to d-amphetamine is still centrally mediated, but the *exaggerated* response requires peripheral activity. This contrasts with previous findings showing that the expression of dopamine supersensitivity evoked by repeated d-amphetamine injections is centrally mediated, because a local infusion of this agonist into the striatum (Kolta *et al*., 1989; Paulson and Robinson, 1991) or into the lateral ventricles (Rebec and Segal, 1979) is sufficient to produce a sensitised psychomotor response. The peripheral effects mediating antipsychotic-evoked dopamine supersensitivity could involve adrenal glucocorticoids, as these are required for the expression of behavioural supersensitivity to d-amphetamine in other contexts [*e.g*., supersensitivity produced by stress (Deroche *et al.*, 1992)].

Dopamine Reuptake

Dopamine-supersensitive rats showed a normal psychomotor response to the dopamine reuptake blocker GBR12783, and only a marginally enhanced response to the monoamine reuptake blocker, cocaine. In contrast, these same rats showed markedly augmented d-amphetamine-induced psychomotor activity. GBR12783, cocaine and d-amphetamine all act at the dopamine transporter (DAT). D-amphetamine could trigger a more robust supersensitive response through more potent effects at the DAT [*e.g.*, by both blocking dopamine uptake and enhancing dopamine release (Rothman *et al.*, 2001)]. However, in another model of dopamine supersensitivity, repeated d-amphetamine injections potentiate the psychomotor response to cocaine and to the selective dopamine reuptake blocker GBR12909 (Bonate *et al.*, 1997; Vanderschuren *et al.*, 1999). Thereby, dopamine reuptake blockers can effectively evoke an exaggerated response in animals sensitive to the stimulating effects of d-amphetamine. Alternatively, d-amphetamine may be more potent to reveal the expression of antipsychotic-evoked dopamine supersensitivity relative to dopamine reuptake blocker through DAT-independent effects. For instance, in the caudate-putamen, d-amphetamine—but not cocaine—depletes dopamine-containing vesicles and enhances tonic dopamine release (Covey *et al.*, 2013). The effects of antipsychotic treatment on the processes above are not yet known, but antipsychotic-treated rats have potentially enhanced striatal DAT function (Amato *et al.*, 2018).

D2 and D1 Receptors

Our results suggest that dopamine-supersensitive rats have enhanced D2-mediated activity, extending prior observations of increased striatal D2 receptor density and function (Burt *et al.*, 1977; Clow *et al.*, 1980; Fleminger *et al.*, 1983; Samaha *et al.*, 2007; Samaha *et al.*, 2008). First, our dopamine-supersensitive rats showed an enhanced psychomotor response to a D2 receptor agonist. Second, acute D2 receptor blockade suppressed the psychomotor response to d-amphetamine in controls, but it *potentiated* d-amphetamine responding in dopamine-supersensitive rats. This potentiation could involve blockade of D2 autoreceptors, which would disinhibit dopamine synthesis/release and thus promote psychomotor activity. Indeed, chronic antipsychotic treatment can enhance presynaptic D2 receptor activity in the caudate-putamen (Calabresi *et al.*, 1992) [but not in the nucleus accumbens (Chesi *et al.*, 1995)]. This idea requires further investigations, because it could represent an important neurobiological mechanism by which antipsychotic-evoked dopamine supersensitivity produce a tolerance to antidopaminergic effects

over time in both rodents (Samaha *et al.*, 2007; Samaha *et al.*, 2008; Gill *et al.*, 2014) and schizophrenia patients (Chouinard and Jones, 1980; Chouinard *et al.*, 2017). Third, dopamine-supersensitive rats showed changes in nucleus accumbens cAMP/PKA- and GSK3β/AKT-dependent activity consistent with enhanced D2-mediated signalling. D2 receptor stimulation disinhibits GSK3β activity and inhibits both cAMP/PKA-dependent activity and ERK1/2 phosphorylation (Cross *et al.*, 1995; Nishi *et al.*, 1997; Beaulieu *et al.*, 2007). Our dopamine-supersensitive rats showed enhanced d-amphetamine-induced increases in GSK3β activity, but diminished d-amphetamine-induced ERK1/2 phosphorylation. Our biochemical and behavioural findings remain correlational. Moreover, we have shown previously that injecting d-amphetamine into the accumbens does not trigger the expression of established dopamine supersensitivity (El Hage *et al.*, 2015). However, future work can determine whether dopamine-mediated signalling in the accumbens is *necessary* for the expression of dopamine supersensitivity.

Our findings also suggest that D1 receptor activity could be required for the full expression of antipsychotic-induced supersensitivity. Dopamine-supersensitive rats showed a normal psychomotor response to a D1 agonist, and d-amphetamine failed to increase ERK1/2 activity in the accumbens in these rats, suggesting that D1 transmission is not potentiated. However, blocking D1 receptors normalized d-amphetamine-induced locomotion in dopamine-supersensitive rats. This extends findings that chronic stimulation of D1 (but not D2) receptors reverses the expression of antipsychotic-evoked dopamine supersensitivity (Marin and Chase, 1993; Braun *et al*., 1997). Similarly, chronic injections of a D1 agonist (Shuto *et al*., 2006) or blockade of D1 transmission (Ramos *et al*., 2004) reverse the expression of dopamine supersensitivity produced by repeated dopamine agonist injections. However, a caveat here is that D1 blockade also supressed basal locomotion in our rats, raising the possibility of non-specific motor effects. This requires further investigation. Nonetheless, our results show that dopamine-supersensitive rats remain responsive to the anti-dopaminergic effects of D1, but not D2 receptor blockade. Hence, D1 but not D2 receptors represent potential targets to temper the behavioural manifestations of antipsychotic-evoked dopamine supersensitivity.

CONCLUSIONS

Effective treatments to prevent the expression of antipsychotic-evoked dopamine supersensitivity depend on a better understanding of the biological mechanisms through which this supersensitivity is expressed. In this context, our findings both extend existing knowledge on the role of D2 receptors in antipsychotic-evoked dopamine supersensitivity and suggest two new underlying mechanisms. First, the expression of antipsychotic-evoked dopamine supersensitivity requires D1-mediated transmission. Second, beyond central processes, the expression of this supersensitivity likely involves peripheral mechanisms.

FUNDING AND DISCLOSURE

This work was supported by the Natural Sciences and Engineering Research Council of Canada (grant 355923) and the Canada Foundation for Innovation to ANS (grant 24326). ANS holds a salary award from the Fonds de la Recherche du Québec-Santé (grant 28988). ANS was a scientific consultant for H. Lundbeck A/S as this research was being carried out. This has had no influence on the work. The remaining authors report no biomedical financial interests or potential conflicts of interest.

REFERENCES OF CHAPTER II

Amato D, Canneva F, Cumming P, Maschauer S, Groos D, Dahlmanns JK, Gromer TW, Chiofalo L, Dahlmanns M, Zheng F, Kornhuber J, Prante O, Alzheimer C, von Horsten S, Muller CP (2018) A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failure reversal: the role of the dopamine transporter. Mol Psychiatry.

Asper H, Baggiolini M, Burki HR, Lauener H, Ruch W, Stille G (1973) Tolerance phenomena with neuroleptics catalepsy, apomorphine stereotypies and striatal dopamine metabolism in the rat after single and repeated administration of loxapine and haloperidol. Eur J Pharmacol 22:287-294.

Barros HM, Braz S, Carlini EA (1989) Behavioural manifestations elicited by apomorphine, influence of the route of administration. Pharmacology 38:335-340.

Batsche K, Ashby CR, Jr., Lee C, Schwartz J, Wang RY (1994) The behavioral effects of the stereoisomers of 4-methylaminorex, a psychostimulant, in the rat. The Journal of pharmacology and experimental therapeutics 269:1029-1039.

Beaulieu JM, Gainetdinov RR, Caron MG (2007) The Akt-GSK-3 signaling cascade in the actions of dopamine. Trends Pharmacol Sci 28:166-172.

Beaulieu JM, Sotnikova TD, Gainetdinov RR, Caron MG (2006) Paradoxical striatal cellular signaling responses to psychostimulants in hyperactive mice. The Journal of biological chemistry 281:32072-32080.

Beaulieu JM, Sotnikova TD, Yao WD, Kockeritz L, Woodgett JR, Gainetdinov RR, Caron MG (2004) Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. Proc Natl Acad Sci U S A 101:5099-5104.

Bedard AM, Maheux J, Levesque D, Samaha AN (2011) Continuous, but not intermittent, antipsychotic drug delivery intensifies the pursuit of reward cues. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 36:1248-1259.

Benaliouad F, Kapur S, Natesan S, Rompre PP (2009) Effects of the dopamine stabilizer, OSU-6162, on brain stimulation reward and on quinpirole-induced changes in reward and locomotion. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 19:416-430.

Bergevin A, Girardot D, Bourque MJ, Trudeau LE (2002) Presynaptic mu-opioid receptors regulate a late step of the secretory process in rat ventral tegmental area GABAergic neurons. Neuropharmacology 42:1065-1078.

Bonnet JJ, Costentin J (1986) GBR 12783, a potent and selective inhibitor of dopamine uptake: biochemical studies in vivo and ex vivo. Eur J Pharmacol 121:199-209.

Bose P, Rompre PP, Warren RA (2015) Neurotensin enhances glutamatergic EPSCs in VTA neurons by acting on different neurotensin receptors. Peptides 73:43-50.

Burt DR, Creese I, Snyder SH (1977) Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 196:326-328.

Calabresi P, De Murtas M, Mercuri NB, Bernardi G (1992) Chronic neuroleptic treatment: D2 dopamine receptor supersensitivity and striatal glutamatergic transmission. Ann Neurol 31:366-373.

Caley CF, Weber SS (1995) Sulpiride: an antipsychotic with selective dopaminergic antagonist properties. Ann Pharmacother 29:152-160.

Carvalho RC, Fukushiro DF, Helfer DC, Callegaro-Filho D, Trombin TF, Zanlorenci LH, Sanday L, Silva RH, Frussa-Filho R (2009) Long-term haloperidol treatment (but not risperidone) enhances addiction-related behaviors in mice: role of dopamine D2 receptors. Addiction biology 14:283-293.

Charron A, Hage CE, Servonnet A, Samaha AN (2015) 5-HT2 receptors modulate the expression of antipsychotic-induced dopamine supersensitivity. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 25:2381-2393.

Chen Y, Mestek A, Liu J, Hurley JA, Yu L (1993) Molecular cloning and functional expression of a mu-opioid receptor from rat brain. Mol Pharmacol 44:8-12.

Chesi AJ, Feasey-Truger KJ, Alzheimer C, ten Bruggencate G (1995) Dopamine autoreceptor sensitivity is unchanged in rat nucleus accumbens after chronic haloperidol treatment: an in vivo and in vitro voltammetric study. The European journal of neuroscience 7:2450-2457.

Chouinard G, Jones BD (1980) Neuroleptic-induced supersensitivity psychosis: clinical and pharmacologic characteristics. The American journal of psychiatry 137:16-21.

Chouinard G, Chouinard VA (2008) Atypical antipsychotics: CATIE study, drug-induced movement disorder and resulting iatrogenic psychiatric-like symptoms, supersensitivity rebound psychosis and withdrawal discontinuation syndromes. Psychother Psychosom 77:69-77.

Chouinard G, Jones BD, Annable L (1978) Neuroleptic-induced supersensitivity psychosis. The American journal of psychiatry 135:1409-1410.

Chouinard G, Samaha AN, Chouinard VA, Peretti CS, Kanahara N, Takase M, Iyo M (2017) Antipsychotic-Induced Dopamine Supersensitivity Psychosis: Pharmacology, Criteria, and Therapy. Psychother Psychosom 86:189-219.

Clow A, Theodorou A, Jenner P, Marsden CD (1980) Cerebral dopamine function in rats following withdrawal from one year of continuous neuroleptic administration. Eur J Pharmacol 63:145-157.

Covey DP, Juliano SA, Garris PA (2013) Amphetamine elicits opposing actions on readily releasable and reserve pools for dopamine. PLoS One 8:e60763.

Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378:785-789.

Deroche V, Piazza PV, Casolini P, Maccari S, Le Moal M, Simon H (1992) Stress-induced sensitization to amphetamine and morphine psychomotor effects depend on stress-induced corticosterone secretion. Brain research 598:343-348.

El Hage C, Bedard AM, Samaha AN (2015) Antipsychotic treatment leading to dopamine supersensitivity persistently alters nucleus accumbens function. Neuropharmacology.

Ericson H, Radesater AC, Servin E, Magnusson O, Mohringe B (1996) Effects of intermittent and continuous subchronic administration of raclopride on motor activity, dopamine turnover and receptor occupancy in the rat. Pharmacology & toxicology 79:277-286.

Fallon P, Dursun SM (2011) A naturalistic controlled study of relapsing schizophrenic patients with tardive dyskinesia and supersensitivity psychosis. J Psychopharmacol 25:755-762.

Farde L, Wiesel FA, Nordstrom AL, Sedvall G (1989) D1- and D2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. Psychopharmacology (Berl) 99 Suppl:S28-31.

Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G (1992) Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Archives of general psychiatry 49:538-544.

Fleminger S, Rupniak NM, Hall MD, Jenner P, Marsden CD (1983) Changes in apomorphine-induced stereotypy as a result of subacute neuroleptic treatment correlates with increased D-2 receptors, but not with increases in D-1 receptors. Biochem Pharmacol 32:2921-2927.

Fritts ME, Mueller K, Morris L (1997) Amphetamine-induced locomotor stereotypy in rats is reduced by a D1 but not a D2 antagonist. Pharmacol Biochem Behav 58:1015-1019.

Geyer MA, Russo PV, Segal DS, Kuczenski R (1987) Effects of apomorphine and amphetamine on patterns of locomotor and investigatory behavior in rats. Pharmacol Biochem Behav 28:393-399.

Gill KM, Cook JM, Poe MM, Grace AA (2014) Prior antipsychotic drug treatment prevents response to novel antipsychotic agent in the methylazoxymethanol acetate model of schizophrenia. Schizophr Bull 40:341-350.

Halperin R, Guerin JJ, Jr., Davis KL (1983) Chronic administration of three neuroleptics: effects of behavioral supersensitivity mediated by two different brain regions in the rat. Life Sci 33:585-592.

Halperin R, Guerin JJ, Jr., Davis KL (1989) Regional differences in the induction of behavioral supersensitivity by prolonged treatment with atypical neuroleptics. Psychopharmacology (Berl) 98:386-391.

Hashimoto T, Baba S, Ikeda H, Oda Y, Hashimoto K, Shimizu I (2018) Lack of dopamine supersensitivity in rats after chronic administration of blonanserin: Comparison with haloperidol. Eur J Pharmacol 830:26-32.

Hooks MS, Jones DN, Holtzman SG, Juncos JL, Kalivas PW, Justice JB, Jr. (1994) Individual differences in behavior following amphetamine, GBR-12909, or apomorphine but not SKF-38393 or quinpirole. Psychopharmacology (Berl) 116:217-225.

Howes OD, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A, Kapur S (2012) The nature of dopamine dysfunction in schizophrenia and what this means for treatment. Archives of general psychiatry 69:776-786.

Jomphe C, Lemelin PL, Okano H, Kobayashi K, Trudeau LE (2006) Bidirectional regulation of dopamine D2 and neurotensin NTS1 receptors in dopamine neurons. The European journal of neuroscience 24:2789-2800.

Kalivas PW, Duffy P (1990) Effect of acute and daily neurotensin and enkephalin treatments on extracellular dopamine in the nucleus accumbens. The Journal of neuroscience : the official journal of the Society for Neuroscience 10:2940-2949.

Kapur S, Zipursky RB, Remington G (1999) Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. The American journal of psychiatry 156:286-293.

Kapur S, VanderSpek SC, Brownlee BA, Nobrega JN (2003) Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. The Journal of pharmacology and experimental therapeutics 305:625-631.

Kapur S, Zipursky R, Jones C, Remington G, Houle S (2000) Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. The American journal of psychiatry 157:514-520.

Kempadoo KA, Tourino C, Cho SL, Magnani F, Leinninger GM, Stuber GD, Zhang F, Myers MG, Deisseroth K, de Lecea L, Bonci A (2013) Hypothalamic neurotensin projections promote reward by enhancing glutamate transmission in the VTA. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:7618-7626.

Kolta MG, Shreve P, Uretsky NJ (1989) Effect of pretreatment with amphetamine on the interaction between amphetamine and dopamine neurons in the nucleus accumbens. Neuropharmacology 28:9-14.

Kosten TA (1997) Enhanced neurobehavioral effects of cocaine with chronic neuroleptic exposure in rats. Schizophr Bull 23:203-213.

Laitinen K, Crawley JN, Mefford IN, De Witte P (1990) Neurotensin and cholecystokinin microinjected into the ventral tegmental area modulate microdialysate concentrations of dopamine and metabolites in the posterior nucleus accumbens. Brain research 523:342-346.

Le Pen G, Duterte-Boucher D, Costentin J (1996) Place conditioning with cocaine and the dopamine uptake inhibitor GBR12783. Neuroreport 7:2839-2842.

Lin MT, Chan HK, Chen CF, Teh GW (1983) Involvement of both opiate and catecholaminergic receptors in the behavioural excitation provoked by thyrotropin-releasing hormone: comparisons with amphetamine. Neuropharmacology 22:463-469.

Mamo D, Kapur S, Keshavan M, Laruelle M, Taylor CC, Kothare PA, Barsoum P, McDonnell D (2008) D2 receptor occupancy of olanzapine pamoate depot using positron emission tomography: an open-label study in patients with schizophrenia. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33:298-304.

Margolese HC, Chouinard G, Beauclair L, Belanger MC (2002) Therapeutic tolerance and rebound psychosis during quetiapine maintenance monotherapy in patients with schizophrenia and schizoaffective disorder. J Clin Psychopharmacol 22:347-352.

Marin C, Chase TN (1993) Dopamine D1 receptor stimulation but not dopamine D2 receptor stimulation attenuates haloperidol-induced behavioral supersensitivity and receptor up-regulation. Eur J Pharmacol 231:191-196.

Martelle JL, Nader MA (2008) A review of the discovery, pharmacological characterization, and behavioral effects of the dopamine D2-like receptor antagonist eticlopride. CNS Neurosci Ther 14:248-262.

Mattson BJ, Crombag HS, Mitchell T, Simmons DE, Kreuter JD, Morales M, Hope BT (2007) Repeated amphetamine administration outside the home cage enhances drug-induced Fos expression in rat nucleus accumbens. Behavioural brain research 185:88-98.

McQuade RD, Duffy RA, Anderson CC, Crosby G, Coffin VL, Chipkin RE, Barnett A (1991) [3H]SCH 39166, a new D1-selective radioligand: in vitro and in vivo binding analyses. J Neurochem 57:2001-2010.

Meller E, Bordi F, Bohmaker K (1988) Enhancement by the D1 dopamine agonist SKF 38393 of specific components of stereotypy elicited by the D2 agonists LY 171555 and RU 24213. Life Sci 42:2561-2567.

Meng ZH, Feldpaush DL, Merchant KM (1998) Clozapine and haloperidol block the induction of behavioral sensitization to amphetamine and associated genomic responses in rats. Brain Res Mol Brain Res 61:39-50.

Mercuri NB, Stratta F, Calabresi P, Bernardi G (1993) Neurotensin induces an inward current in rat mesencephalic dopaminergic neurons. Neurosci Lett 153:192-196.

Meyer ME, Shults JM (1993) Dopamine D1 receptor family agonists, SK&F38393, SK&F77434, and SK&F82958, differentially affect locomotor activities in rats. Pharmacol Biochem Behav 46:269-274.

Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. The Journal of pharmacology and experimental therapeutics 303:791-804.

MØller Nielsen I, Fjalland B, Pedersen V, Nymark M (1974) Pharmacology of neuroleptics upon repeated administration. Psychopharmacology (Berl) 34:95-104.

Molloy AG, Waddington JL (1987) Assessment of grooming and other behavioural responses to the D-1 dopamine receptor agonist SK & F 38393 and its R- and S-enantiomers in the intact adult rat. Psychopharmacology (Berl) 92:164-168.

Neumeyer JL, Kula NS, Bergman J, Baldessarini RJ (2003) Receptor affinities of dopamine D1 receptor-selective novel phenylbenzazepines. Eur J Pharmacol 474:137-140.

Nishi A, Snyder GL, Greengard P (1997) Bidirectional regulation of DARPP-32 phosphorylation by dopamine. The Journal of neuroscience : the official journal of the Society for Neuroscience 17:8147-8155.

Obuchowicz E (1999) Differential behavioural effect of quinpirole in neuroleptic-pretreated rats - role of alpha(1)-adrenoceptor. Eur J Pharmacol 384:101-109.

Paulson PE, Robinson TE (1991) Sensitization to systemic amphetamine produces an enhanced locomotor response to a subsequent intra-accumbens amphetamine challenge in rats. Psychopharmacology (Berl) 104:140-141.

Ramos M, Goni-Allo B, Aguirre N (2004) Studies on the role of dopamine D1 receptors in the development and expression of MDMA-induced behavioral sensitization in rats. Psychopharmacology (Berl) 177:100-110.

Rebec GV, Segal DS (1979) Enhanced responsiveness to intraventricular infusion of amphetamine following its repeated systemic administration. Psychopharmacology (Berl) 62:101-102.

Rebec GV, Peirson EE, McPherson FA, Brugge K (1982) Differential sensitivity to amphetamine following long-term treatment with clozapine or haloperidol. Psychopharmacology (Berl) 77:360-366.

Remington G, Mamo D, Labelle A, Reiss J, Shammi C, Mannaert E, Mann S, Kapur S (2006) A PET study evaluating dopamine D2 receptor occupancy for long-acting injectable risperidone. The American journal of psychiatry 163:396-401.

Rivet JM, Stinus L, LeMoal M, Mormede P (1989) Behavioral sensitization to amphetamine is dependent on corticosteroid receptor activation. Brain research 498:149-153.

Rothman RB, Baumann MH (2003) Monoamine transporters and psychostimulant drugs. Eur J Pharmacol 479:23-40.

Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI, Partilla JS (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. Synapse 39:32-41.

Samaha AN, Seeman P, Stewart J, Rajabi H, Kapur S (2007) "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:2979-2986.

Samaha AN, Reckless GE, Seeman P, Diwan M, Nobrega JN, Kapur S (2008) Less is more: antipsychotic drug effects are greater with transient rather than continuous delivery. Biological psychiatry 64:145-152.

Scardochio T, Clarke PB (2013) Inhibition of 50-kHz ultrasonic vocalizations by dopamine receptor subtype-selective agonists and antagonists in adult rats. Psychopharmacology (Berl) 226:589-600.

Seeman P, Van Tol HH (1994) Dopamine receptor pharmacology. Trends Pharmacol Sci 15:264-270.

Servonnet A, Minogianis EA, Bouchard C, Bedard AM, Levesque D, Rompre PP, Samaha AN (2017) Neurotensin in the nucleus accumbens reverses dopamine supersensitivity evoked by antipsychotic treatment. Neuropharmacology 123:10-21.

Shuto T, Kuroiwa M, Hamamura M, Yabuuchi K, Shimazoe T, Watanabe S, Nishi A, Yamamoto T (2006) Reversal of methamphetamine-induced behavioral sensitization by repeated administration of a dopamine D1 receptor agonist. Neuropharmacology 50:991-997.

Smith RC, Davis JM (1975) Behavioral supersensitivity to apomorphine and amphetamine after chronic high dose haloperidol treatment. Psychopharmacology communications 1:285-293.

Sutherland C, Leighton IA, Cohen P (1993) Inactivation of glycogen synthase kinase-3 beta by phosphorylation: new kinase connections in insulin and growth-factor signalling. Biochem J 296 (Pt 1):15-19.

Svenningsson P, Tzavara ET, Carruthers R, Rachleff I, Wattler S, Nehls M, McKinzie DL, Fienberg AA, Nomikos GG, Greengard P (2003) Diverse psychotomimetics act through a common signaling pathway. Science 302:1412-1415.

Thibault D, Albert PR, Pineyro G, Trudeau LE (2011) Neurotensin triggers dopamine D2 receptor desensitization through a protein kinase C and beta-arrestin1-dependent mechanism. The Journal of biological chemistry 286:9174-9184.

Valjent E, Corvol JC, Pages C, Besson MJ, Maldonado R, Caboche J (2000) Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:8701-8709.

Valjent E, Pascoli V, Svenningsson P, Paul S, Enslen H, Corvol JC, Stipanovich A, Caboche J, Lombroso PJ, Nairn AC, Greengard P, Herve D, Girault JA (2005) Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum. Proc Natl Acad Sci U S A 102:491-496.

Werkman TR, Kruse CG, Nievelstein H, Long SK, Wadman WJ (2000) Neurotensin attenuates the quinpirole-induced inhibition of the firing rate of dopamine neurons in the rat substantia nigra pars compacta and the ventral tegmental area. Neuroscience 95:417-423.

Wright JM, Dobosiewicz MR, Clarke PB (2013) The role of dopaminergic transmission through D1-like and D2-like receptors in amphetamine-induced rat ultrasonic vocalizations. Psychopharmacology (Berl) 225:853-868.

SUPPLEMENT

METHODS

Animals

Male Sprague-Dawley rats (200-275 g; Charles River Laboratories, Montreal, QC) were used. In Exps. 1-2, rats were housed 1/cage to avoid damage to intracerebral cannulae by conspecific. In Exps. 3-6, rats were housed 2/cage. All rats were housed on a reverse dark-light cycle (lights off at 8:30 am). All testing took place during the dark phase. Water/food were available *ad libitum*.

Drugs

D-amphetamine sulfate (Sigma-Aldrich, Dorset, UK), cocaine hydrochloride (Medisca Pharmaceutique, St-Laurent, QC), neurotensin acetate salt, DAMGO acetate salt (Bachem, Torrance, CA), (-)-quinpirole hydrochloride, SKF38393 hydrobromide and SCH39166 hydrobromide (R&D Systems, Minneapolis, MN) were dissolved in 0.9 % saline. Haloperidol (Sandoz, Boucherville, QC) was diluted in sterile water containing 0.5 % glacial acetic acid and pH was increased to ~5 using NaOH. (-)-Sulpiride (Sigma-Aldrich, Milwaukee, WI) was dissolved in 0.9 % saline containing ~1.4 % glacial acid acetic and pH was increased to ~6.5 using NaOH. GBR12783 dihydrochloride (R&D Systems) was dissolved in DMSO, diluted in 0.9 % saline (final concentration of DMSO is 10 %) and pH was increased to ~4 using NaOH. GBR12783 solubilised at higher pH values. Rats showed no visual/auditory signs of discomfort when receiving GBR12783 or its pH-matched vehicle. Apomorphine hydrochloride (Sigma, Oakville, ON) was dissolved in 0.9 % saline containing 0.1 % sodium L-ascorbate (Sigma, Oakville, ON). DAMGO, neurotensin and sulpiride solutions were frozen in aliquots and then thawed on testing days. GBR12783, apomorphine and its vehicle were prepared fresh on testing days. Haloperidol was administered via an osmotic minipump (Alzet model 2ML2; Durect Corporation, Cupertino, CA). Systemic injections were given s.c., except cocaine and its vehicle (i.p.). Systemic injections were given in a volume of 1 mL/kg, except for GBR12783 and its vehicle (4 mL/kg) and SKF38393 and its vehicle (3 mL/kg). Microinfusions in the lateral ventricles or in the ventral tegmental area (VTA) were given in a volume of 2 or 0.5 µL/hemisphere, respectively.

Intra-cerebral Procedures

Cannulae implantation. In Exps. 1-2, intra-cerebral cannulation was performed at the same time as minipump implantation or sham surgery. Rats weighing 325-350 g were anesthetized with isoflurane (5 % for induction, 2-3 % to maintain anaesthesia) and placed on a stereotaxic apparatus. Rats received penicillin (3000 IU, i.m.) and carprofen (1.5 mg, s.c.) at the beginning of surgery. A guide cannula (Exp. 1: 26 GA, model C315G; Exp. 2: 22 GA, model C313G; HRS Scientific, Montreal, Qc) was implanted in each cerebral hemisphere 2 mm above the VTA (A/P -5.9, M/L ±1.7, D/V -6.7, all mm relative to Bregma, M/L angle of 8°) or the lateral ventricles (A/P -1.1, M/L ±2.5, D/V -3.3, all mm relative to Bregma, M/L angle of 10°). Four stainless steel screws were anchored to the skull and dental cement secured the cannulae. Guide cannulae were sealed with obturators (Exp. 1: model C315CD; Exp. 2: model C313CD; HRS Scientific).

Intra-cerebral infusion. Microinfusions (0.5 µL/minute) were given via injectors protruding 2 mm beyond guide cannulae (Exp. 1: 33 GA, model C315I; Exp. 2: 28 GA, model C313I; HRS Scientific). The injectors were connected via tubing to 5-µL syringes placed on a microsyringe pump (HARVARD PHD, 2000: HARVARD Apparatus, Saint-Laurent, Canada). Following infusion, injectors were kept in place for an additional minute. On day 2 following minipump removal (before any behavioural testing), rats were brought to the testing room and were given an intra-cerebral infusion of 0.9 % saline for habituation. No behaviour was recorded.

Histology. In Exp. 2, rats received an intracerebroventricular infusion of ink prior to brain extraction to facilitate histological verification. In Exps. 1-2, brains were frozen in isopentane and stored at -20 °C until processing. Placement of injector tips was determined on 40-µm coronal slices using the atlas of Paxinos and Watson (1986). Data from rats with infusion sites outside of the targeted area were excluded from analysis.

Measurement of Psychomotor Activity

Psychomotor activity was measured using photocell counts and psychomotor activity ratings. Photocell counts—a measure of horizontal activity—were recorded in Plexiglas boxes (27 × 48 × 20 cm) equipped with 6 rows of photocells (3 cm above the box floor). An experimenter blind to condition rated behaviour on minutes 5, 10, 20, 40 and 60 in Exp. 1 or every 10 minutes in Exps.

2-5 (unless an injection was given on minutes 30 or 60) using the following scale (Mattson *et al.*, 2007) [modified from Ellinwood and Balster (1974)]: 1: asleep, 2: inactive, 3: normal in place activity, 4: alert, rearing, normal level of locomotion, 5: rearing, high level of locomotion, 6: slow patterned behaviours, no rearing, high level of locomotion, 7: faster patterned behaviours, no rearing, high level of locomotion, 8: highly repetitive patterned behaviours in a restricted area and 9: backing up, abnormally maintained posture. A psychomotor activity rating ≥ 6 indicates stereotypy.

Western Blot

Rats were briefly exposed to 5% isoflurane and brains were extracted. Two-mm coronal slices were cut, and bilateral tissue punches were taken from the slice at ~ +1.7 mm relative to Bregma in the nucleus accumbens, dorsal caudate-putamen, ventrolateral caudate-putamen and centromedial caudate-putamen using a 15-gauge sample corer. Striatal tissues were stored at -80°C until processing.

Striatal samples were mechanically homogenized in a lysis buffer (150 mM sodium chloride, 1 % triton X-100, 0.5 % sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM tris, pH = 7.4) containing protease and phosphatase inhibitors (Sigma-Aldrich, Oakville, ON). Homogenates were solubilized for 15 minutes on ice and then centrifuged at 16,000 g for 30 minutes at 4°C. The protein content of supernatants was measured using a BiCinchoninic acid Assay (BCA) protein assay kit (Thremo Fisher Scientific, Mississauga, ON, Canada). Equal amounts of protein in lysis buffer (10 µg) were dissolved in 25 µL of double distilled water (boiled at 95 °C for 5 minutes) containing loading buffer (4X; Bio-Rad Laboratories, Mississauga, ON, Canada) and reducing agent (20X; Bio-Rad Laboratories). Protein samples were loaded into a Bis-Tris 10 % pre-casted gels (Bio-Rad Laboratories). Proteins migrated for 60 minutes at 200 V and were then transferred to a polyvinylidene fluoride membrane (Bio-Rad Laboratories) for 2 hours (70 V, 4°C). Membranes were blocked in a solution of 5 % bovine serum albumin diluted in 0.1 % Tween 20/tris-buffered saline for 1 hour. Membranes were incubated overnight at 4 °C with the appropriate antibody (see Table SI for a detailed list of antibodies). Membranes were then rinsed 4 times for 5 minutes with 0.1 % Tween 20/tris-buffered saline at room temperature. Membranes were incubated with the appropriate secondary antibody conjugated with horseradish peroxidase (see Table 1) for 1 hour at room temperature. Immunoreactive bands were revealed using the

enhanced chemiluminescence reaction (Bio-Rad Laboratories) and the bands were placed against sensitive film (MidSci Scientific, Valley Parl, MI, USA) for a few seconds.

Densitometric levels were determined using Image Lab Software (Bio-Rad Laboratories). Background was subtracted for each band. The densitometric level of each band was normalized relative to the sum of densitometric levels across all tissue samples. Protein levels were then normalized relative to the corresponding level of the housekeeping protein α-tubuline. Protein levels were then normalized relative to the mean protein level of the control group that received saline injection prior to brain extraction. Using these values, we computed the ratio of phosphorylated protein levels over total protein levels.

RESULTS

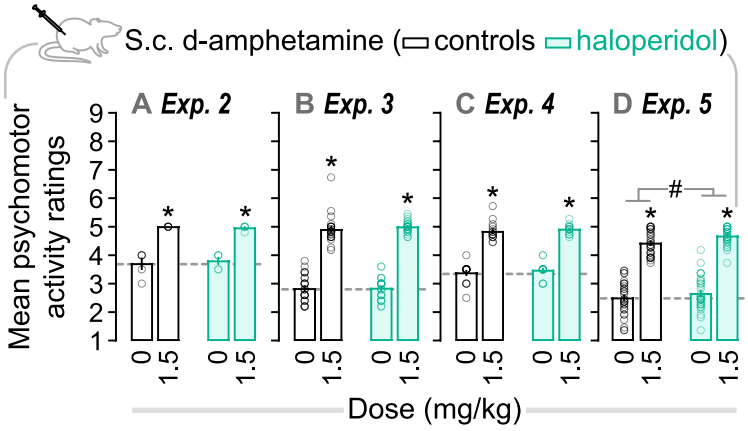

FIG. 2.S1 — D-amphetamine effects on psychomotor activity ratings. Across studies, d-amphetamine increased psychomotor activity ratings relative to vehicle ('Injection': vehicle and d-amphetamine; Injection effect; **A**, $F_{1,8} = 99.86$; **B**, $F_{1,51} = 744.4$; **C**, $F_{1,30} = 502.4$; **D**, $F_{1,61} = 556.3$; all P's < 0.0001). There were no group differences except in Exp. 5, where haloperidol rats had greater psychomotor activity ratings relative to controls (**D**; 'Group': controls and haloperidol; Group effect, $F_{1,61} = 5.03$, $p = 0.029$). Dotted lines indicate mean ratings of control rats receiving saline. n's = 5-32/condition. *$p < 0.05$, relative to vehicle ('0 mg/kg') in the same group; # $p < 0.05$, Group effect.

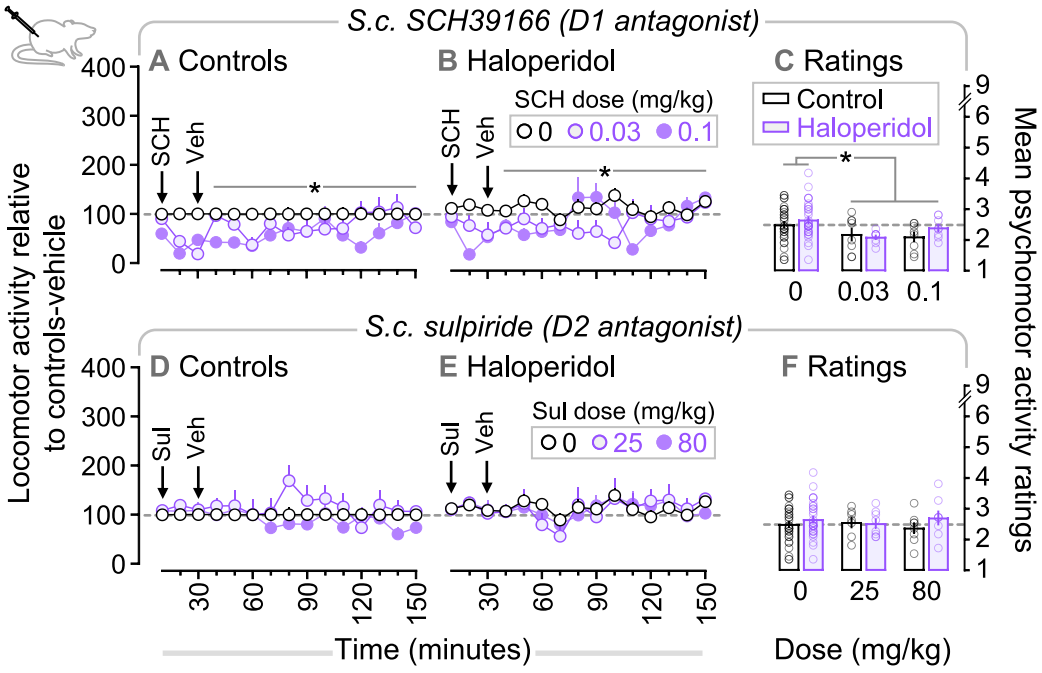

FIG. 2.S2 — Effects of the D1 antagonist SCH39166 and the D2 antagonist sulpiride on vehicle-induced locomotion and psychomotor activity ratings. SCH39166 reduced vehicle-induced locomotion and ratings similarly in haloperidol rats and controls (minutes 30-150; **A-B**; 'Injection', vehicle, 0.03 and 0.1 mg/kg; Injection × Time interaction, $F_{22,979} = 1.87$; Injection effect, $F_{2,89} = 7.96$; **C**; Injection effect, $F_{2,89} = 5.49$; all P's < 0.01). (**D-F**) In both groups, sulpiride had no influence on vehicle-induced locomotion or on ratings (all P's > 0.05). Dotted lines indicate response of control rats receiving vehicle. n's = 7-32/condition. *$p < 0.05$. In (A-B), Injection × Time interaction and Injection effect. In (C), Injection effect.

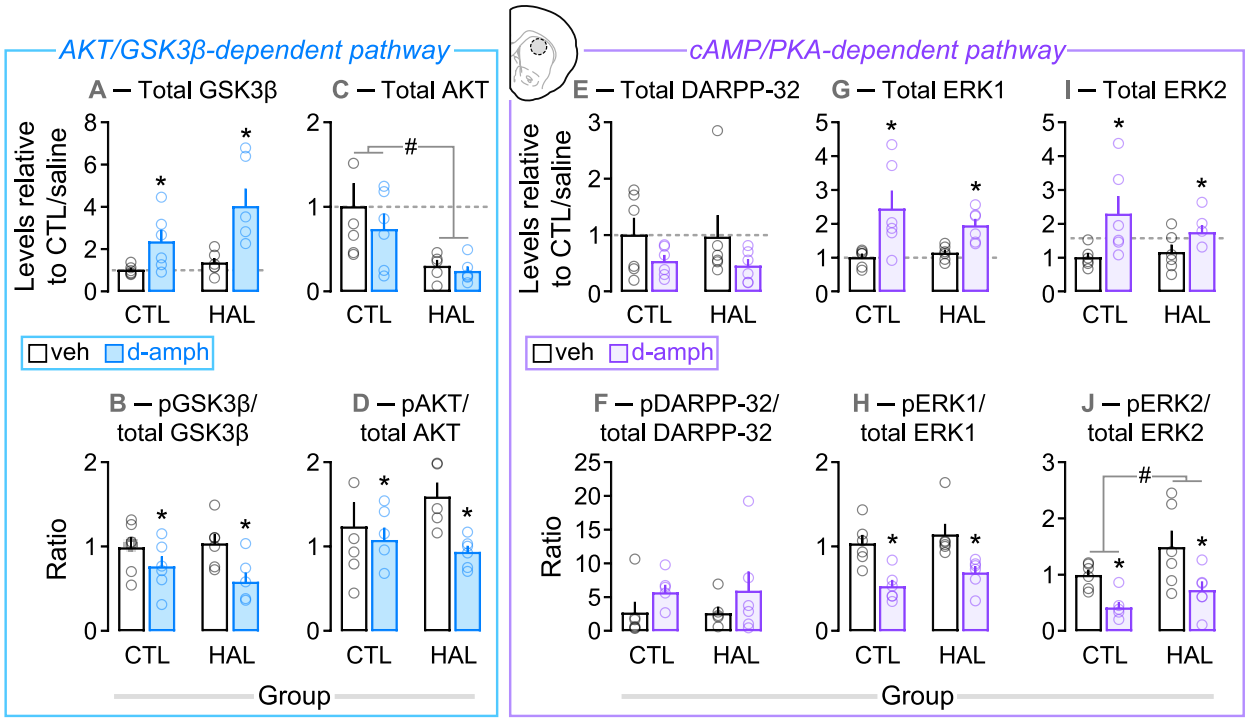

FIG. 2.S3 — cAMP/PKA- and AKT/GSK3β-dependent signalling in the dorsal caudate-putamen of haloperidol-treated (HAL) rats and control (CTL) rats. (**A**) Across groups, d-amphetamine increased GSK3β levels (Injection effect, $F_{1,20} = 15.19$, $p = 0.0009$), with no group differences. (**B**) D-amphetamine decreased pGSK3β/total GSK3β ratios similarly across groups (Injection effect, $F_{1,20} = 8.32$, $p = 0.009$). (**C**) Chronic haloperidol treatment decreased AKT level regardless of d-amphetamine injection (Group effect, $F_{1,19} = 11.11$, $p = 0.004$). (**D**) D-amphetamine decreased pAKT/total AKT ratios similarly across groups (Injection effect, $F_{1,19} = 4.65$, $p = 0.04$). There was no significant effect of haloperidol treatment or of d-amphetamine injection on (**E**) DARPP-32 or (**F**) pDARPP-32/total DARPP-32 ratios (all P's > 0.05). Across groups, d-amphetamine enhanced (**G**) ERK1 and (**I**) ERK2 levels, and decreased (**H**) pERK1/total ERK1 and (**J**) pERK2/total ERK2 ratios (Injection effect; ERK1, $F_{1,20} = 14.65$; ERK2, $F_{1,20} = 9.05$; pERK1/total ERK1 ratio, $F_{1,20} = 24.84$; pERK2/total ERK2 ratio, $F_{1,20} = 13.87$; all P's < 0.01). There were no group differences in these effects. (**J**) Additionally, chronic haloperidol treatment increased pERK2/total ERK2 ratios, regardless of d-amphetamine injection (Group effect, $F_{1,20} = 4.94$, $p = 0.038$). n's = 5-6/condition. In (**A-C-E-G-I**), dotted lines indicate the protein level of the control group injected with saline. *$p < 0.05$, relative to vehicle in the same group; # $p < 0.05$, Group effect.

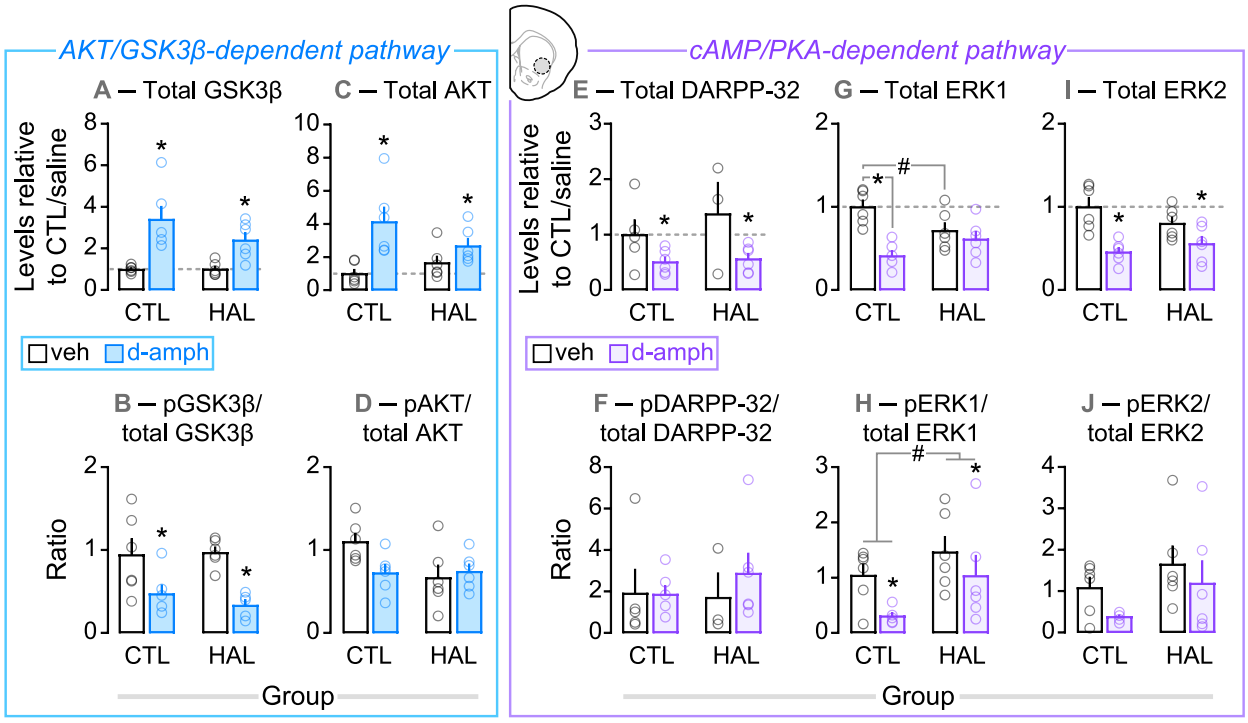

FIG. 2.S4 — cAMP/PKA- and AKT/GSK3β-dependent signalling in the ventrolateral caudate-putamen of haloperidol-treated (HAL) rats and control (CTL) rats. Across groups, d-amphetamine increased (**A**) GSK3β and (**C**) AKT levels (Injection effect; GSK3β, $F_{1,20} = 27.07$; AKT, $F_{1,20} = 14.35$; all P's ≤ 0.001), with no group differences. (**B**) D-amphetamine decreased pGSK3β/total GSK3β ratios similarly across groups (Injection effect, $F_{1,19} = 19.3$, $p = 0.0003$). (**D**) Neither haloperidol treatment nor d-amphetamine injection influenced pAKT/total AKT ratios ($p > 0.05$). Across groups, d-amphetamine decreased (**E**) DARPP-32 levels, (**I**) ERK2 levels and (**H**) pERK1/total ERK1 ratios (Injection effect; DARPP-32, $F_{1,16} = 8.11$; ERK2, $F_{1,20} = 21.63$; pERK1/total ERK1 ratio, $F_{1,20} = 5.23$; all P's < 0.05), with no group differences. There was no effect of haloperidol treatment or of d-amphetamine injection on (**F**) pDARPP-32/total DARPP-32 ratios or (**J**) pERK2/total ERK2 ratios (all P's > 0.05). (**G**) D-amphetamine decreased ERK1 levels in controls relative to both vehicle-injected control rats and haloperidol rats (Group × Injection interaction, $F_{1,20} = 8.53$; Injection effect, $F_{1,20} = 17.04$; controls, saline > d-amph; saline, controls > haloperidol rats; all P's < 0.05). (**H**) Chronic haloperidol increased pERK1/total ERK1 ratios regardless of d-amphetamine injection ($F_{1,20} = 5.07$, $p = 0.039$). n's = 3-6/condition. In (**A-C-E-G-I**), dotted lines indicate the protein level of the control group injected with saline. *$p <$ 0.05, relative to vehicle in the same group, unless indicated otherwise; # $p < 0.05$, Group effect.

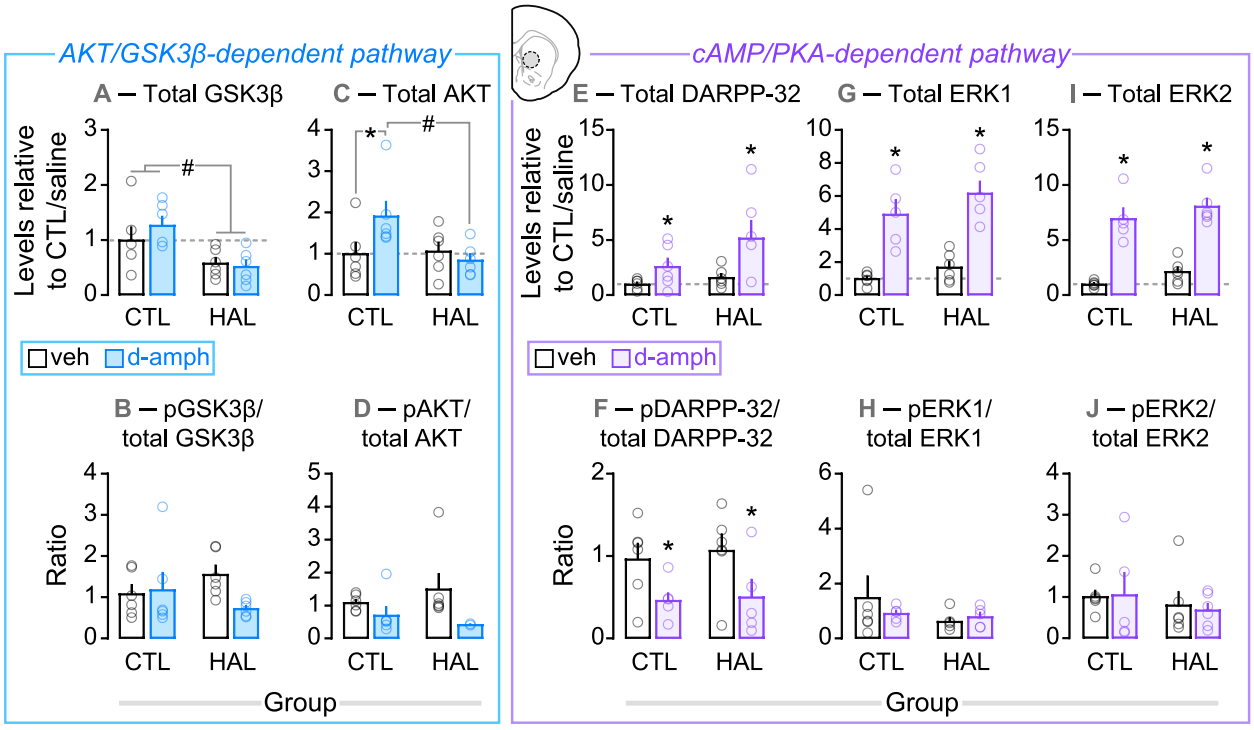

FIG. 2.S5 — cAMP/PKA- and AKT/GSK3β-dependent signalling in the centromedial caudate-putamen of haloperidol-treated (HAL) rats and control (CTL) rats. (**A**) Chronic haloperidol decreased GSK3β levels, and this was not influenced by d-amphetamine injection (Group effect, $F_{1,20}$ = 12.49, p = 0.002). There was no effect of haloperidol treatment or of d-amphetamine injection on (**B**) pGSK3β/total GSK3β and (**D**) pAKT/total AKT ratios (all P's > 0.05). (**C**) D-amphetamine increased AKT levels in control rats only (Group × Injection interaction, $F_{1,20}$ = 4.75; controls, d-amph > saline; d-amph, controls > haloperidol rats; all P's < 0.05). Across groups, d-amphetamine increased (**E**) DARPP-32, (**G**) ERK1 and (**I**) ERK2 levels (Injection effect; DARPP-32, $F_{1,20}$ = 8.03; ERK1, $F_{1,19}$ = 53.32; ERK2, $F_{1,19}$ = 95.27; all P's ≤ 0.01), with no group differences. (**F**) D-amphetamine decreased pDARPP-32/total DARPP-32 ratios similarly across groups (Injection effect, $F_{1,19}$ = 8.86, p = 0.008). D-amphetamine and haloperidol did not influence (**H**) pERK1/total ERK1 ratios or (**J**) pERK2/total ERK2 ratios (all P's > 0.05). n's = 2-6/condition. In (**A-C-E-G-I**), dotted lines indicate the protein level of the control group injected with saline. *p < 0.05, relative to vehicle in the same group, unless indicated otherwise; # p < 0.05, Group effect.

TABLE SI. List of antibodies used for Western Blots, and their respective dilution.

Antibody	Dilution	Provider*	Product no.
Rabbit monoclonal anti-GSK3β	1:1,000	CST	9315
Rabbit polyclonal anti-p[Ser9]GSK3β	1:500	CST	9336
Rabbit polyclonal anti-ERK1/2	1:50,000	CST	9102
Rabbit polyclonal anti-p[Thr202]ERK44/p[Thr204]ERK42	1:10,000	CST	9101
Mouse monoclonal anti-α-tubulin	1:50,000	Sigma-Aldrich	T5168
Goat anti-rabbit conjugated to horseradish peroxidase	1: 5,000 for phosphorylated kinase and 1:10,000 for non-phosphorylated kinase	CST	7074
Horse anti-mouse conjugated to horseradish peroxidase	1:150,000	CST	7076

*****Antibodies were purchased from CST (Cell Signalling Technology, New England BioLabs, Whitby, ON) or Sigma-Aldrich (Oakville, ON).

REFERENCES OF SUPPLEMENT SECTION – CHAPTER II

Ellinwood EH, Jr., Balster RL (1974) Rating the behavioral effects of amphetamine. Eur J Pharmacol 28:35-41.

Mattson BJ, Crombag HS, Mitchell T, Simmons DE, Kreuter JD, Morales M, Hope BT (2007) Repeated amphetamine administration outside the home cage enhances drug-induced Fos expression in rat nucleus accumbens. Behavioural brain research 185:88-98.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd Edition. Sydney ; Orlando: Academic Press.

CHAPTER III. *Potential Links Between Antipsychotic-Evoked Dopamine Supersensitivity and Increased Stress-Like Responses in Rats*

Unpublished results

Alice Servonnet[a], Laurence Théroux[a], Pierre-Paul Rompré[a] and Anne-Noël Samaha[b,c]

[a]Department of Neurosciences, Faculty of Medicine; [b]Department of Pharmacology and Physiology, Faculty of Medicine; [c]Groupe de recherche sur le système nerveux central, Faculty of Medicine, Université de Montréal; 2900 Edouard-Montpetit boulevard, Montreal (H3T 1J4), Quebec, Canada.

OBJECTIVES

As described in Section 2.4.4 (page 57), antipsychotic-evoked dopamine supersensitivity could increase stress-like responses. Here we investigated the contribution of the stress hormone corticosterone in the expression of antipsychotic-evoked dopamine supersensitivity, and we determined if dopamine-supersensitive rats show signs of increased stress-related behaviours. Hence, in Exp. 1, we determined whether corticosterone synthesis is necessary for the expression of already established dopamine supersensitivity, after haloperidol treatment cessation. We used the corticosterone inhibitor metyrapone to investigate this question. Metyrapone is an inhibitor of the enzyme 11β-hydroxylase, that catalyzes the conversion of deoxycorticosterone into corticosterone (Igaz *et al*., 2008). We then determined if the expression of dopamine supersensitivity during ongoing chronic haloperidol treatment (Exp. 2) or after chronic haloperidol treatment (Exp. 3) is accompanied with change in the behavioural response to stressors, as assessed by measuring avoidance in the elevated-plus maze (Pellow *et al*., 1985), the open field (Katz *et al*., 1981) and the light-dark box (Crawley and Goodwin, 1980). Lastly, in Exp. 4, we determined whether the expression of already established dopamine supersensitivity (after haloperidol treatment cessation) is linked to an increased locomotor response to novelty. This increased behavioural response seems linked to cross-sensitisation between the effects of stress and of dopamine agonists. Chronically-stressed animals are more likely to show a greater locomotor response to novelty relative to non-stressed controls (Marin *et al*., 2007). Interestingly, animals that have a greater locomotor response to novelty also show potentiated dopamine release in the nucleus accumbens in response to stress (Rouge-Pont *et al*., 1993) and an enhanced response to the psychomotor and rewarding effects of dopamine agonists (Piazza *et al*., 1989; Hooks *et al*., 1991; Hooks *et al*., 1994).

Exps. 1-3 included rats that were either exposed continuously (CONT-HAL) or transiently (TRANS-HAL) to haloperidol. CONT-HAL treatment was administered via s.c. osmotic minipump, whereas TRANS-HAL treatment was administered via daily s.c. injections (see FIG. 1.8B-C, page 46). As described in Chapter I (Section 2.4.3, page 56), transient antipsychotic exposure could reduce dose-dependent deleterious effects of antipsychotic drugs without compromising therapeutic efficacy. Hence, here we wished to better characterise the long-term

effects of transient treatment, as the current knowledge on this promising dosing strategy remains sparse.

METHODS

Animals

Male Sprague-Dawley rats (Charles river Laboratories; Montreal, Canada, for Exps. 1, 2 and 4; Kingston, New York, United-States, for Exp. 3) were housed 2/cage on a reversed 12-h light/dark cycle (lights off at 8:30 a.m.). Testing took place during the dark phase. Animals had *ad libitum* access to water and food. All experimental procedures were approved by the ethics committee of the Université de Montréal and followed the guidelines of the Canadian Council on Animal Care.

Drugs

Haloperidol (5 mg/mL; Sandoz, Boucherville, Canada) was diluted either in water containing 0.5 % glacial acetic acid (pH increased to ~5 using NaOH) for delivery via subcutaneous (s.c.) osmotic minipump (16 or 18 days of continuous delivery depending on minipump lot; Alzet, model 2ML2; Durect Corporation, Cupertino, California) or in 20 mM phosphate buffer saline (PBS) for delivery via daily s.c. injections (1 mL/kg). PBS was used to avoid irritation produced by repeated injections. Using PBS or water containing glacial acetic acid does not alter the behavioural effects of haloperidol (Samaha *et al*., 2008). Dextro-amphetamine sulfate (1.5 mg/kg; Sigma-Aldrich, Dorset, United Kingdom) was dissolved in 0.9 % saline (s.c., 1 mL/kg). Metyrapone (Abcam, Cambridge, Massachusetts) was dissolved in saline containing 50 % ethanol, and it was then diluted in saline for a final concentration of 10 % ethanol (s.c., 3 mL/kg).

Haloperidol Treatments

In patients, haloperidol produces therapeutic effects and has a low incidence to induce extrapyramidal effects at doses achieving ~65-80 % occupancy of striatal dopamine D2 receptors (Farde *et al*., 1992; Kapur *et al*., 2000). Here, CONT-HAL and TRANS-HAL rats received clinically representative haloperidol doses achieving similar peak levels of striatal D2 occupancy: 0.5 mg/kg/day for CONT-HAL treatment [73 % D2 occupancy ± 14 SD; unpublished observations;

see also (Kapur *et al*., 2003; Samaha *et al*., 2007)] and 0.05 mg/kg/injection for TRANS-HAL treatment [74 % D2 occupancy ± 7 SD, 2 hours post-injection; (Kapur *et al*., 2003)]. Continuous administration of haloperidol was achieved via s.c. osmotic minipump. CONT-HAL treatment mimics continuous exposure in schizophrenia patients produced by daily oral intake or intra-muscular depot of long-acting injectable antipsychotic (Farde *et al*., 1989; Remington *et al*., 2006; Mamo *et al*., 2008). TRANS-HAL rats received daily haloperidol injections to achieve transient plasma/brain levels of the antipsychotic, as D2 occupancy is above 65 % 2 hours following injection and then declines below 65 % 24 hours post-injection [19 % ± 31 SD; (Kapur *et al*., 2003)]. Hence, TRANS-HAL and CONT-HAL rats received haloperidol using the same route of administration (s.c.), treatment duration and peak levels of antipsychotic-evoked D2 occupancy, and the groups differed only in the within-day kinetics of haloperidol levels/D2 occupancy.

In the CONT-HAL group, s.c. osmotic minipumps were implanted under isoflurane anesthesia (Samaha *et al*., 2007). TRANS-HAL and control rats received a sham surgery (incision closed with wound clips). Starting the next day, TRANS-HAL rats received daily haloperidol injections, whereas CONT-HAL and control rats received daily PBS injections. Injections were given every day for 16-18 days. Seventeen or 19 days after minipump implantation/sham surgery, minipumps were removed from CONT-HAL rats, and controls and TRANS-HAL rats received a second sham surgery.

Measures of Psychomotor Activity

Psychomotor activity was measured in Plexiglas cages (27 × 48 × 20 cm) equipped with 6 rows of photocells placed 3 cm above the cage floor. We computed two indices of psychomotor activity; *i*) horizontal locomotion, measured as number of individual photocell beam breaks, and *ii*) psychomotor activity ratings given by an experimenter blind to condition. These ratings were given on minutes 10 and 20 (before d-amphetamine injection) and on minutes 40, 50, 60, 70 and 80 (after d-amphetamine injection), based on a 1-to-9 scale (Ellinwood and Balster, 1974; Mattson *et al*., 2007). On this scale, a rating ≥ 6 indicates stereotypy: 1) asleep, 2) inactive, 3) normal in place activity, 4) normal, alert, rearing, normal level of locomotor activity, 5) rearing, high level of locomotor activity, 6) slow patterned behaviours, no rearing, high level of locomotor activity, 7) faster patterned behaviours, no rearing, high level of locomotor activity, 8) highly repetitive patterned behaviours in a restricted area and 9) backing up, abnormally maintained posture.

Measures of Stress-like Behavioural Responses

As indices of stress-like behaviour, we measured avoidance of open arms in the elevated-plus maze, of the center of the open field and of the light compartment of the light-dark box. Rats were tested in each apparatus consecutively (5 min each, counterbalanced). The elevated-plus maze consisted of 4 arms: 2 open arms (45 × 10 cm, with a 0.5 cm Plexiglas border to keep rats from falling over the open arms) and 2 closed arms (45 × 10 cm, wall height of 30 cm). Arms were 50 cm above the floor. At the beginning of the test, rats were placed in the center, facing an open arm. The open field was 80 × 80 cm, with 4 × 4 squares drawn on the floor (20 × 20 cm each). The 4 squares in the middle represented the center. Wall height was 40 cm. Rats were placed in the periphery at the beginning of the test. For the light-dark box, the dark compartment was black and enclosed (20 × 30 cm floor) and the light compartment was wider, white and not enclosed (40 × 30 cm floor). Wall height was 30 cm. Rats were placed in the dark compartment at the beginning of the test, and had access to the light compartment through an opening in the wall separating the dark and light compartments. Rats were tested during the dark phase of the light-dark cycle, but tests were given in a room with lights on. This was to help keep rats from falling from the elevated-plus maze, and to also help them distinguish the dark and light compartments in the light-dark box. Thirty minutes before the tests, rats were moved to a room (adjacent to the testing room) with lights on for habituation. In Exp. 3, rats received their daily PBS or haloperidol injection before being moved to the habituation room. Tests were videotaped and an experimenter blind to conditions manually quantified time spent and entries made in each section. Entry in a section was counted each time rats placed the two front paws in a new section.

Exp. 1: Is Corticosterone Synthesis Necessary for the Expression of Established Antipsychotic-evoked Dopamine Supersensitivity?

If corticosterone synthesis is required for the expression of dopamine supersensitivity, then metyrapone should decrease to a greater extent d-amphetamine-induced psychomotor activity in CONT-HAL rats relative to controls and TRANS-HAL rats, because d-amphetamine-induced locomotion is highest in CONT-HAL rats.

On days 3-9 following minipump removal, rats received an s.c. injection of vehicle (saline alone or saline with 10 % ethanol) or metyrapone (50 or 100 mg/kg) in their home cage (FIG. 3.1A).

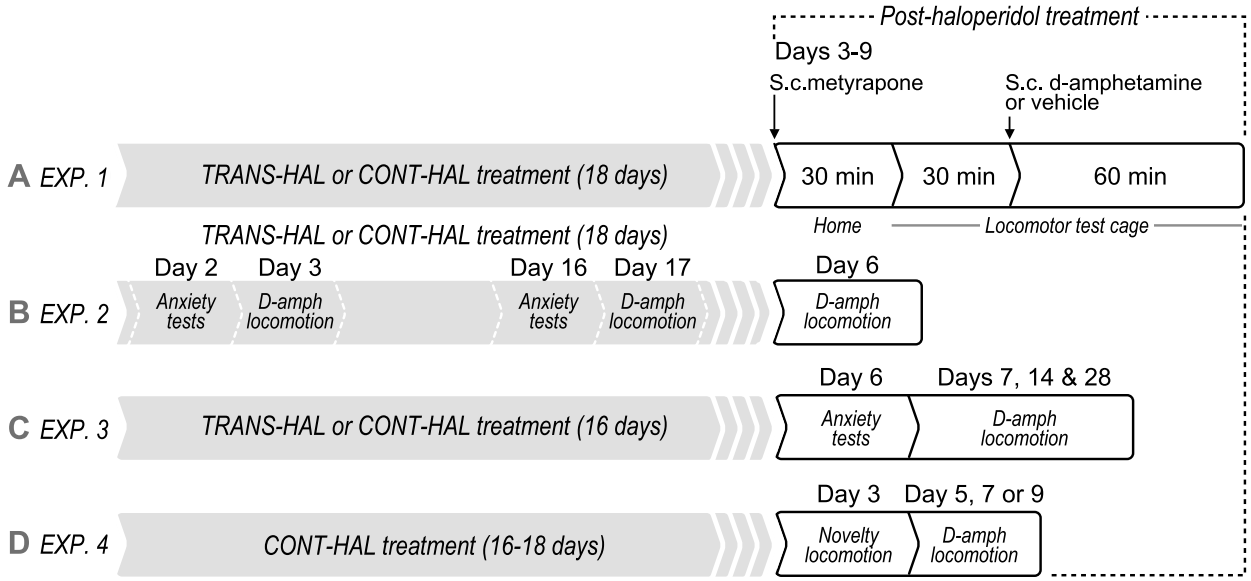

FIG. 3.1 — Experimental timelines. (***A***) Exp. 1. After cessation of transient (TRANS-HAL) or continuous (CONT-HAL) haloperidol treatment, the effects of the corticosterone synthesis inhibitor metyrapone (50 or 100 mg/kg) on d-amphetamine-induced psychomotor activity was evaluated. (***B***) In Exp. 2, stress-like behaviours were assessed on days 2 and 16 of ongoing, TRANS-HAL or CONT-HAL treatment. Changes in the anti-dopaminergic efficacy of ongoing antipsychotic treatment were assessed by measuring the ability of haloperidol treatment to suppress the psychomotor response to d-amphetamine on days 3 and 17 of haloperidol treatment. On day 6 after treatment cessation, we again measured the psychomotor response to d-amphetamine to assess expression of haloperidol-induced dopamine supersensitivity. (***C***) In Exp. 3, we measured stress-like behaviours 6 days after cessation of TRANS-HAL or CONT-HAL treatment. The expression of dopamine supersensitivity was assessed by measuring the psychomotor response to d-amphetamine on days 7, 14 and 28 after treatment cessation. (***D***) In Exp. 4, we sought to determine whether the dopamine supersensitivity evoked by CONT-HAL treatment also enhances the locomotor response to novelty. To this end, we analysed novelty- and d-amphetamine-induced locomotion in past experimental cohorts tested in our laboratory. Locomotor response to novelty was measured on day 3 after treatment cessation, and then d-amphetamine-induced locomotion was measured either on day 5, 7 or 9.

Metyrapone is commonly administered in vehicle containing ethanol (Reid *et al*., 1998; Bratt *et al*., 2001; Johnson and Yamamoto, 2009). In a pilot study, we found that a pre-treatment with metyrapone vehicle (saline/10 % ethanol) does not alter d-amphetamine-induced locomotion in antipsychotic-naïve animals. Here we included two vehicle conditions (saline alone or saline containing 10 % ethanol) to confirm that finding in rats with a history of chronic antipsychotic exposure. Because we found no difference between these two vehicles, we pooled the data. Thirty minutes after metyrapone administration, rats were moved to the testing room and placed in locomotor test cages. Locomotor activity was recorded, and on minute 30, rats received either s.c.

vehicle or d-amphetamine. Locomotor activity was recorded for an additional hour. Each rat received 4 combinations out of 8 possible combinations (1 combination/test): 1) vehicle + vehicle, 2) metyrapone (50 or 100 mg/kg) + vehicle, 3) d-amphetamine + vehicle and 4) metyrapone (50 or 100 mg/kg) + d-amphetamine (n = 6-15/condition, per group).

Exp. 2: Is the Development of Antipsychotic-evoked Dopamine Supersensitivity Paralleled with Increased Stress-like Behaviour?

If dopamine supersensitivity evoked by CONT-HAL treatment is paralleled by increased stress-like behaviour, then CONT-HAL rats should show control levels of stress-like behaviour early into treatment, but increased stress-like behaviour late into treatment when dopamine supersensitivity breakthrough. Furthermore, we expect that TRANS-HAL treatment does not increase stress-like behaviour at any time point, as there are no signs of breakthrough dopamine supersensitivity during transient exposure (Samaha *et al*., 2008).

Hence, here we evaluated stress-like behaviour at two time points during haloperidol treatment (FIG. 3.1B). First, we tested early into treatment (day 2), when dopamine supersensitivity has not yet developed (Samaha *et al*., 2007; Amato *et al*., 2018), and both TRANS-HAL and CONT-HAL treatments have significant anti-dopaminergic efficacy, as indicated by suppression of d-amphetamine-induced locomotor activity (Samaha *et al*., 2008). Second, we also tested late into treatment (day 16), when dopamine supersensitivity breaks through ongoing CONT-HAL but not TRANS-HAL treatment, as indicated by a loss of anti-dopaminergic efficacy in CONT-HAL rats only (Samaha *et al*., 2008). This treatment failure occurs even though a large proportion of striatal D2 receptors are still blocked by antipsychotics (Samaha *et al.*, 2007; Amato *et al.*, 2018). On the day following each set of stress tests (*i.e.*, days 3 and 17 of haloperidol treatment), we also assessed anti-dopaminergic efficacy. To this end, we evaluated whether TRANS-HAL and CONT-HAL treatments suppress the psychomotor response to d-amphetamine. Finally, on day 6 after discontinuation of haloperidol, we assessed the expression of antipsychotic-evoked dopamine supersensitivity, as indicated by an exaggerated psychomotor response to d-amphetamine (Smith and Davis, 1975; Vonvoigtlander *et al*., 1975; Ericson *et al*., 1996).

On days 2 and 16 of haloperidol treatment, TRANS-HAL rats (n = 10) received their daily haloperidol injection and CONT-HAL (n = 11) and control (n = 11) rats received their daily PBS

injection. Thirty minutes later, rats were tested in the stress tests described above. On days 3 and 17 of haloperidol treatment, TRANS-HAL rats received their daily haloperidol injection and CONT-HAL and control rats received their daily PBS injection. Locomotor activity was recorded for 30 minutes and then all rats received d-amphetamine. Locomotor activity was then recorded for an additional hour. On day 6 after haloperidol treatment cessation, we again assessed d-amphetamine-induced psychomotor activity, but all rats received a saline injection at the beginning of the test instead of PBS or haloperidol.

Exp. 3: Is the Expression of Antipsychotic-evoked Dopamine Supersensitivity Linked to Increased Stress-like Behaviour?

If dopamine supersensitivity is paralleled by increased stress-like behaviour, then CONT-HAL but not TRANS-HAL rats should show increased stress-like behaviour after treatment cessation relative to control rats. Also, because CONT-HAL treatment produces stronger signs of dopamine supersensitivity than TRANS-HAL treatment does (Ericson *et al*., 1996; Samaha *et al*., 2008), we predicted that the enhanced psychomotor response to d-amphetamine persists for a longer time after cessation of CONT-HAL treatment than TRANS-HAL treatment.

FIG. 3.1C illustrates experimental timeline. Here we evaluated stress-like behaviour of control, TRANS-HAL and CONT-HAL rats on day 6 after treatment cessation, when the expression of antipsychotic-evoked dopamine supersensitivity produced by CONT-HAL treatment is higher (Samaha *et al*., 2008; Bedard *et al*., 2011; Servonnet *et al*., 2017). On the day following the stress tests (day 7), we measured the psychomotor response to d-amphetamine to confirm the expression of antipsychotic-evoked dopamine supersensitivity. Furthermore, we also compared the persistence of any potential dopamine supersensitivity after cessation of TRANS-HAL or CONT-HAL treatment. Hence, d-amphetamine-induced psychomotor activity was measured again either on day 28 (rats received 2 d-amphetamine injections in total), or on days 14 and 28 (3 d-amphetamine injections in total). Rats that had received either 2 or 3 d-amphetamine injections in total showed a similar psychomotor response to d-amphetamine on day 28, indicating that receiving 2 or 3 injections did not significantly influence behaviour. Thus, we pooled these rats for analysis of psychomotor activity on day 28.

On day 6 following minipump removal, TRANS-HAL (n = 10), CONT-HAL (n = 11) and control (n = 11) rats were tested in the stress tests. On days 7, 14 and 28 following minipump removal, rats received a saline injection and locomotor activity was recorded for 30 minutes. The rats then received d-amphetamine, and locomotor activity was recorded for an additional hour.

Exp. 4: Do Dopamine-supersensitive Rats Show an Increased Locomotor Response to Novelty?

We determined whether CONT-HAL rats (n = 76) show an enhanced locomotor response to novelty relative to antipsychotic-naïve rats (n = 75) (FIG. 3.1D). Hence, we re-analysed data from previous cohorts (rats from Exps. 3-5 in Chapter II and rats from Exp. 1 here). We analysed the locomotor response to novelty on day 3 following CONT-HAL treatment cessation, which corresponds to rats' first exposure to the locomotion box. Depending on the cohort, some rats received a vehicle injection and others did not prior to the test. There was no difference in the locomotor response on the first day of test across cohorts, indicating that the different methodology (*i.e.*, injection prior to the test, vehicle type, experimenter, etc) did not significantly influence locomotor behaviour. Thereby, the cohorts were pooled together. In a subsequent test, we also analysed the locomotor response to saline and to d-amphetamine, when the locomotor test cage did not represent a novel environment anymore for the rats (n = 54/group). This test took place during the 3rd, 4th or 5th test that rats received, that correspond to 5, 7 or 9 days after treatment cessation, respectively.

Statistical Analysis

In Exp. 1, 4-way, mixed-model ANOVA was used to analyse the influence of Metyrapone (0, 50 and 50 mg/kg), D-amphetamine (0 and 1.5 mg/kg) or Group (control, TRANS-HAL and CONT-HAL) on locomotor activity (Group × Dose × Time; 'Time' as a within-subjects variable). Three-way ANOVA was used to analyse effects of Metyrapone, D-amphetamine or Group on psychomotor activity ratings or on the area under the curve (AUC) for metyrapone's effect on locomotion (Dose × Group; both as between-subjects variables).

In Exps. 2-3, mixed-model ANOVA was used to analyse the influence of Group (controls, TRANS-HAL and CONT-HAL) or Section (open field: center and periphery) or Compartment (light-dark

box: light and dark) or Arm (elevated-plus maze: open or closed) on time (Group × Section or Compartment or Arm; 'Compartment', 'Arm' and 'Section' as within-subjects variables). Mixed-model ANOVA was used to analyse the influence of Group or Arm on entries in the arms of the elevated-plus maze (Group × Arm; 'Arm' as a within-subjects variable). One-way ANOVA was used to analyse the influence of Group on entries in the center of the open field or in the light compartment of the light-dark box ('Group' as a between-subject variable).

In Exps. 2-4, mixed-model ANOVA was used to analyse the influence of Group (Exps. 2-3: controls, TRANS-HAL and CONT-HAL; Exp. 4: controls and CONT-HAL) on locomotor activity (Group × Time; 'Time' as a within-subjects variable). One-way ANOVA was used to measure the influence of Group on psychomotor activity ratings ('Group' as a between-subject variable).

Effects were further analysed using Bonferroni's multiple comparisons' tests when interaction and main effects were significant ($p < 0.05$). Values in figures are mean ± SEM.

RESULTS

Exp. 1: Is Corticosterone Synthesis Necessary for the Expression of Established Antipsychotic-evoked Dopamine Supersensitivity?

The influence of metyrapone pre-treatment on locomotor activity and ratings depended on whether animals received vehicle or d-amphetamine (FIGS. 3.2A-F; minutes 40-90; Metyrapone × D-amphetamine × Time interaction, $F_{10,730} = 6.91$; Metyrapone × D-amphetamine interaction, $F_{2,146} = 24.53$; insets in FIGS. 3.2A-F; Metyrapone × D-amphetamine interaction, $F_{2,146} = 15.98$; all P's < 0.0001). Thus, we analysed the influence of metyrapone on vehicle- and d-amphetamine-induced locomotion/ratings separately.

Across groups, metyrapone pre-treatment decreased both vehicle-induced locomotion (FIGS. 3.2A-C; minutes 40-90; Metyrapone × Time interaction, $F_{10,365} = 2.88$; Metyrapone effect, $F_{2,73} = 5.69$; all P's ≤ 0.005) and psychomotor activity ratings (insets in FIGS. 3.2A-C; Metyrapone effect, $F_{2,73} = 4.69$, $p = 0.012$). Additionally, CONT-HAL rats had generally greater vehicle-induced ratings (insets in FIGS. 3.2A-C; Group effect, $F_{2,73} = 8.08$, $p = 0.0007$) independently of metyrapone pre-treatment (no Metyrapone × Group interaction effect, $p > 0.05$).

Haloperidol increased d-amphetamine-induced locomotion (white curves in FIGS. 3.2D-F; minutes 40-90; Group × Time interaction, $F_{10,190} = 2.95$; Group effect, $F_{2,38} = 4.76$, all P's < 0.05). Specifically, CONT-HAL rats showed a greater locomotor response to d-amphetamine relative to control rats (white curves in FIGS. 3.2D versus 3.2F; Group × Time interaction, $F_{5,120} = 3.82$; Group effect, $F_{1,24} = 10.92$; all P's < 0.05). TRANS-HAL rats also showed a greater locomotor response

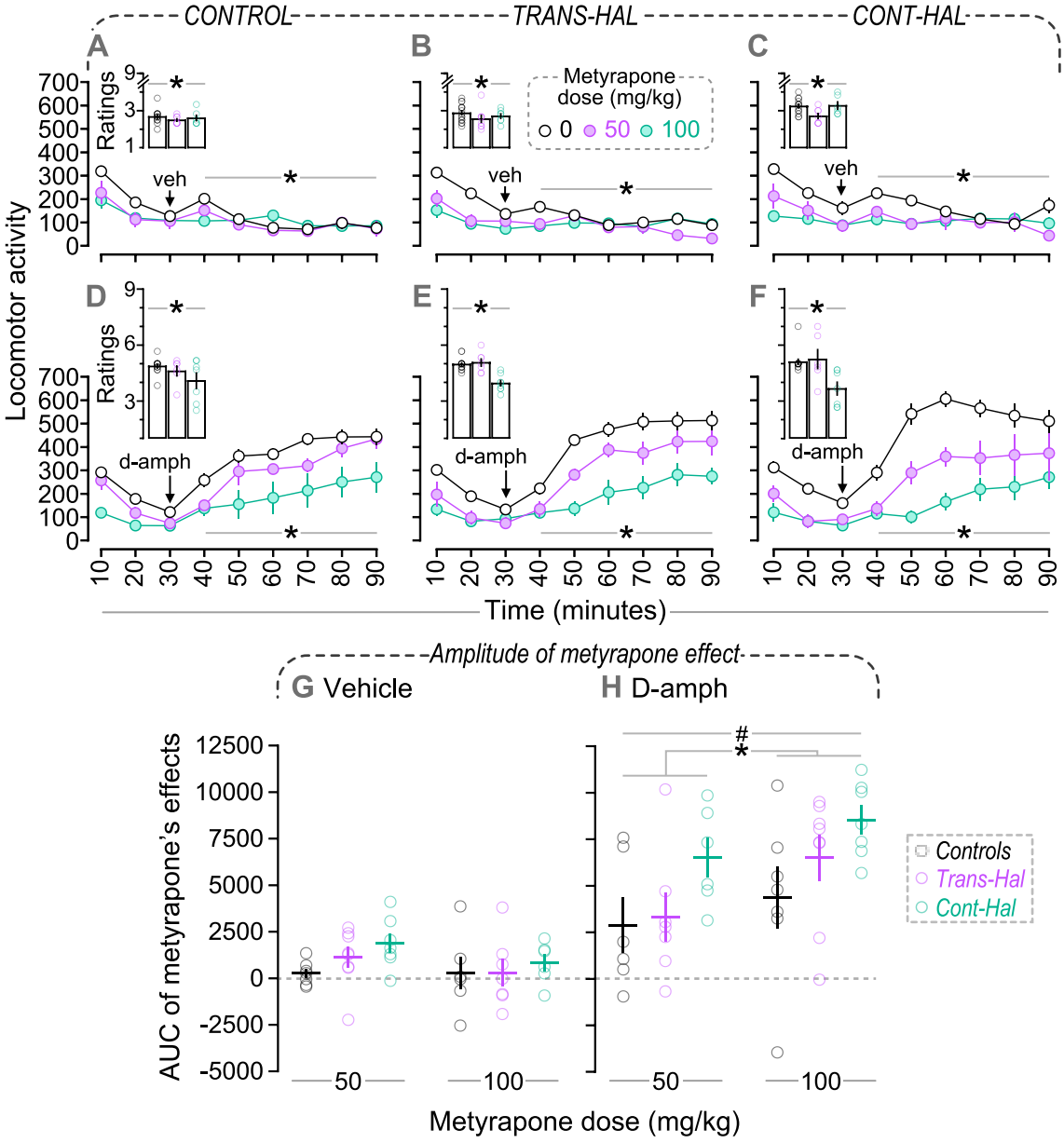

FIG. 3.2 — The corticosterone synthesis inhibitor metyrapone reduces the expression of established haloperidol-evoked dopamine supersensitivity. (***A-C***) Effects of subcutaneous (s.c.) (see next page →)

(FIG. 3.2 →) metyrapone on the response to s.c. vehicle. (***D***-***F***) Effects of s.c. metyrapone on the response to s.c. d-amphetamine. (***G***-***H***) Amplitude of metyrapone's effects on the locomotor response to vehicle and d-amphetamine, calculated as the area under the curve (AUC) of the locomotor response with 0 mg/kg metyrapone minus AUC of the locomotor response with 50 or 100 mg/kg metyrapone (metyrapone was co-administered with vehicle in ***G***, or with d-amphetamine in ***H***). *n*'s/condition = 6-15. #, *$p < 0.05$. In (***A***-***F***), overall effect of metyrapone across groups on locomotion induced by vehicle (***A***-***C*** analysed together) or d-amphetamine (***D***-***F*** analysed together): *Metyrapone × Time interaction and Metyrapone effect. Insets in (***A***-***F***), overall effect of metyrapone across groups on ratings induced by vehicle (insets in ***A***-***C*** analysed together) or d-amphetamine (insets ***D***-***F*** analysed together): *Metyrapone effect. In (***H***), overall *Metyrapone effect and # Group effect.

to d-amphetamine relative to control rats (white curves in FIGS. 3.2D versus 3.2E; Group × Time interaction, $F_{10,190} = 2.95$, $p = 0.002$). There was no group difference in psychomotor activity ratings (left histograms in insets of FIGS. 3.2D-F; $p > 0.05$). Hence, both modes of haloperidol treatment produced dopamine supersensitivity. Across groups, metyrapone pre-treatment decreased both d-amphetamine-induced locomotion (FIGS. 3.2D-F; minutes 40-90; Metyrapone × Time interaction, $F_{10,365} = 5.56$; Metyrapone effect, $F_{2,73} = 38.08$; all *P*'s < 0.0001) and psychomotor activity ratings (insets in FIGS. 3.2D-F; Metyrapone effect, $F_{2,73} = 19.81$, $p < 0.0001$), with no group differences.

Based on visual inspection of the locomotor curves in FIGS. 3.2A-C and FIGS. 3.2D-F, we also analysed the effects of metyrapone on AUC for both vehicle- and d-amphetamine-induced locomotion. This was determined by subtracting AUC at 50 or 100 mg/kg metyrapone from AUC at 0 mg/kg metyrapone. This calculation provides an index of how much metyrapone decreased locomotor activity, and this allowed to compare the amplitude of these suppressive effects between the groups. FIG. 3.2G shows the amplitude of metyrapone's effects on vehicle-induced locomotion. FIG. 3.2H shows the amplitude of metyrapone's effects on d-amphetamine-induced locomotion. The amplitude of metyrapone effect was dependent on whether rats received vehicle or d-amphetamine (FIGS. 3.2G-H; Metyrapone × D-amphetamine interaction, $F_{1,70} = 5.98$, $p = 0.017$) and thereby, we analysed the data presented on FIG. 3.2G (vehicle) and FIG. 3.2H (d-amphetamine) separately. Metyrapone suppressed vehicle-induced locomotion, and the amplitude of this effect was similar across metyrapone doses and groups (FIG. 3.2G; no Metyrapone effect nor Group effect, all *P*'s > 0.05). Metyrapone also suppressed d-amphetamine-induced locomotion, and the amplitude of this effect was dose-dependent (FIG. 3.2H; Metyrapone effect, $F_{1,35} = 4.46$, $p = 0.042$). There were also group differences in this effect (FIG. 3.2H; Group effect, $F_{2,35} = 4.52$, $p = 0.018$), with an amplitude that is greater in antipsychotic-treated rats. However, there was no Group ×

Metyrapone interaction effect ($p > 0.05$). This suggests that the effect of Group is similar across metyrapone doses and is not specific to a haloperidol treatment regimen. Hence, the results above suggest that antipsychotic-treated rats are more responsive to metyrapone's suppressive effects on d-amphetamine-induced locomotion.

SUMMARY OF EXP. 1

Both CONT-HAL and TRANS-HAL treatments produced dopamine supersensitivity. Metyrapone suppressed d-amphetamine-induced locomotor activity across groups, but that effect seemed greater in antipsychotic-treated rats. Hence, corticosterone synthesis could be necessary for revealing the expression of established antipsychotic-evoked dopamine supersensitivity.

Exp. 2: Is the Development of Antipsychotic-evoked Dopamine Supersensitivity Paralleled with Increased Stress-like Behaviour?

D-amphetamine-induced psychomotor effects. On day 3 of ongoing haloperidol treatment, TRANS-HAL and CONT-HAL rats showed similar levels of baseline locomotion (FIG. 3.3A) and psychomotor activity ratings (data not shown). Both groups also had reduced baseline locomotor counts relative to control rats (FIG. 3.3A; minutes 10-30; Group × Time interaction, $F_{16,174} = 2.34$; Group effect, $F_{8,87} = 10.81$; control rats > TRANS-HAL rats; Group effect, $F_{1,19} = 11.55$; control rats > CONT-HAL rats; Group × Time interaction, $F_{2,40} = 3.51$; Group effect, $F_{1,20} = 28.87$; all P's < 0.05). TRANS-HAL and CONT-HAL rats also showed supressed d-amphetamine-induced locomotion compared to control rats (FIG. 3.3A; minutes 40-90; Group × Time interaction, $F_{40,435} = 4.05$; Group effect, $F_{8,87} = 26.35$; control rats > TRANS-HAL rats; Group × Time interaction, $F_{5,95} = 13.81$; Group effect, $F_{1,19} = 90.72$; control rats > CONT-HAL rats; Group × Time interaction, $F_{5,100} = 6.23$; Group effect, $F_{1,20} = 38.82$; all P's < 0.0001. No other comparisons were significant). TRANS-HAL and CONT-HAL rats also had lower d-amphetamine-induced ratings compared to control rats (data not shown; Group effect, $F_{2,29} = 24.08$; controls > TRANS-HAL and CONT-HAL rats; all P's < 0.0005).

On day 17 of haloperidol treatment, TRANS-HAL but not CONT-HAL rats had reduced baseline locomotion compared to control rats (FIG. 3.3B; minutes 10-30; Group effect, $F_{2,29} = 7.58$; control

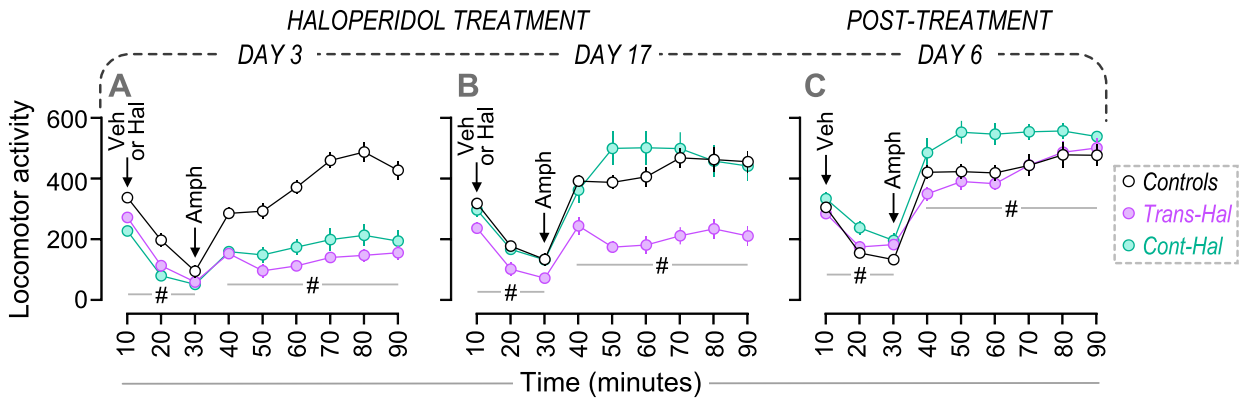

FIG. 3.3 — Breakthrough dopamine supersensitivity during ongoing continuous (CONT-HAL), but not transient (TRANS-HAL) haloperidol exposure. In Exp. 2, we measured haloperidol-induced suppression of the psychomotor response to d-amphetamine as an index of anti-dopaminergic efficacy. (***A***) Early into treatment (day 3), both CONT-HAL and TRANS-HAL exposure showed significant anti-dopaminergic efficacy, as indicated by suppression of d-amphetamine-induced psychomotor activity. (***B***) Late into treatment (day 17), TRANS-HAL treatment maintained this anti-dopaminergic efficacy, while CONT-HAL treatment lost efficacy. This indicates that CONT-HAL, but not TRANS-HAL treatment promotes breakthrough dopamine supersensitivity that undermines ongoing treatment efficacy. (***C***) After haloperidol treatment cessation, CONT-HAL but not TRANS-HAL rats showed an exaggerated psychomotor response to d-amphetamine relative to controls. This indicates persistent dopamine supersensitivity after CONT-HAL but not TRANS-HAL treatment. *n*'s/condition = 10-11. # $p < 0.05$, Group effect. In (***A***), controls > TRANS-HAL and CONT-HAL rats. In (***B***), controls and CONT-HAL rats > TRANS-HAL rats. In (***C***), CONT-HAL rats > controls and TRANS-HAL rats.

rats > TRANS-HAL rats; Group effect, $F_{1,19} = 15.09$; all P's < 0.005). TRANS-HAL and CONT-HAL rats had unchanged baseline psychomotor activity ratings compared to control rats (data not shown). Compared to control and CONT-HAL rats, TRANS-HAL rats also showed suppressed d-amphetamine-induced locomotion (FIG. 3.3B; minutes 40-90; Group × Time interaction, $F_{10,145} = 5.72$; Group effect, $F_{2,29} = 14.66$; control rats > TRANS-HAL rats; Group × Time interaction, $F_{5,95} = 2.8$; Group effect, $F_{1,19} = 35.24$; CONT-HAL rats > TRANS-HAL rats; Group × Time interaction, $F_{5,95} = 9.01$; Group effect, $F_{1,19} = 20.69$; all P's < 0.05), as well as suppressed d-amphetamine-induced ratings (data not shown; Group effect, $F_{2,29} = 8.82$; TRANS-HAL rats < control and CONT-HAL rats; all P's < 0.01). In contrast, CONT-HAL were no different from control rats on their response to d-amphetamine.

Hence, TRANS-HAL treatment maintained its anti-dopaminergic efficacy over time, while CONT-HAL treatment completely lost efficacy. These findings are consistent with the notion that CONT-

HAL, but not TRANS-HAL exposure promotes breakthrough dopamine supersensitivity during ongoing treatment, and that this compromises treatment efficacy (Samaha *et al*., 2008).

On day 6 after haloperidol treatment cessation, CONT-HAL but not TRANS-HAL rats showed greater vehicle-induced locomotion relative to control rats (FIG. 3.3C; minutes 10-30; Group effect, $F_{2,29}$ = 4.88; CONT-HAL rats > control rats; $F_{1,20}$ = 8.16; all *P*'s < 0.05). Vehicle-induced psychomotor activity ratings were similar across groups (data not shown; $p > 0.05$). Importantly, relative to control or TRANS-HAL rats, CONT-HAL rats also showed significantly more d-amphetamine-induced locomotion (FIG. 3.3C; minutes 40-90; Group × Time interaction, $F_{10,145}$ = 2; Group effect, $F_{2,29}$ = 6.06; CONT-HAL rats > control rats; Group effect, $F_{1,20}$ = 6.51; CONT-HAL rats > TRANS-HAL rats; Group effect, $F_{1,19}$ = 11.84; all *P*'s < 0.05). In contrast, TRANS-HAL rats were similar to control rats (FIG. 3.3C; minutes 40-90; purple versus white; $p > 0.05$). Psychomotor activity ratings followed a similar pattern of effects (data not shown; Group effect, $F_{2,29}$ = 3.48, p = 0.04). Thus, CONT-HAL, but not TRANS-HAL rats showed an exaggerated psychomotor response to d-amphetamine after discontinuation of antipsychotic treatment, indicating that CONT-HAL exposure promotes a dopamine supersensitive state that persists even after treatment cessation (Samaha *et al*., 2007; Bedard *et al*., 2011; Servonnet *et al*., 2017).

Open field. On day 2 of ongoing haloperidol treatment, all rats spent more time in the periphery relative to the center of the open field (FIG. 3.4A; Section effect, $F_{1,29}$ = 7657, $p < 0.0001$). There were no group differences in this effect. CONT-HAL rats entered less often in the center of the open field than control rats did (FIG. 3.4B; Group effect, $F_{2,29}$ = 6.05; controls > CONT-HAL rats; all *P*'s < 0.01). On day 16 of haloperidol treatment, all rats spent more time in the periphery than they did in the center of the open field (FIG. 3.4C; Section effect, $F_{1,29}$ = 6040, $p < 0.0001$), with no group differences. There were no group differences in the number of entries into the center section of the open field (FIG. 3.4D; $p > 0.05$).

Light-dark box. On day 2 of ongoing haloperidol treatment, all rats spent more time in the dark compartment of the light-dark box relative to the light compartment (FIG. 3.4E; Compartment effect, $F_{1,29}$ = 386.7, $p < 0.0001$), with no group differences. Similarly, there were no group differences in the number of times rats entered into the light compartment (FIG. 3.4F; $p > 0.05$). On day 16 of haloperidol treatment, all rats spent more time in the dark relative to the light compartment (FIG. 3.4G; Compartment effect, $F_{1,29}$ = 77.01, $p < 0.0001$), and this effect was similar

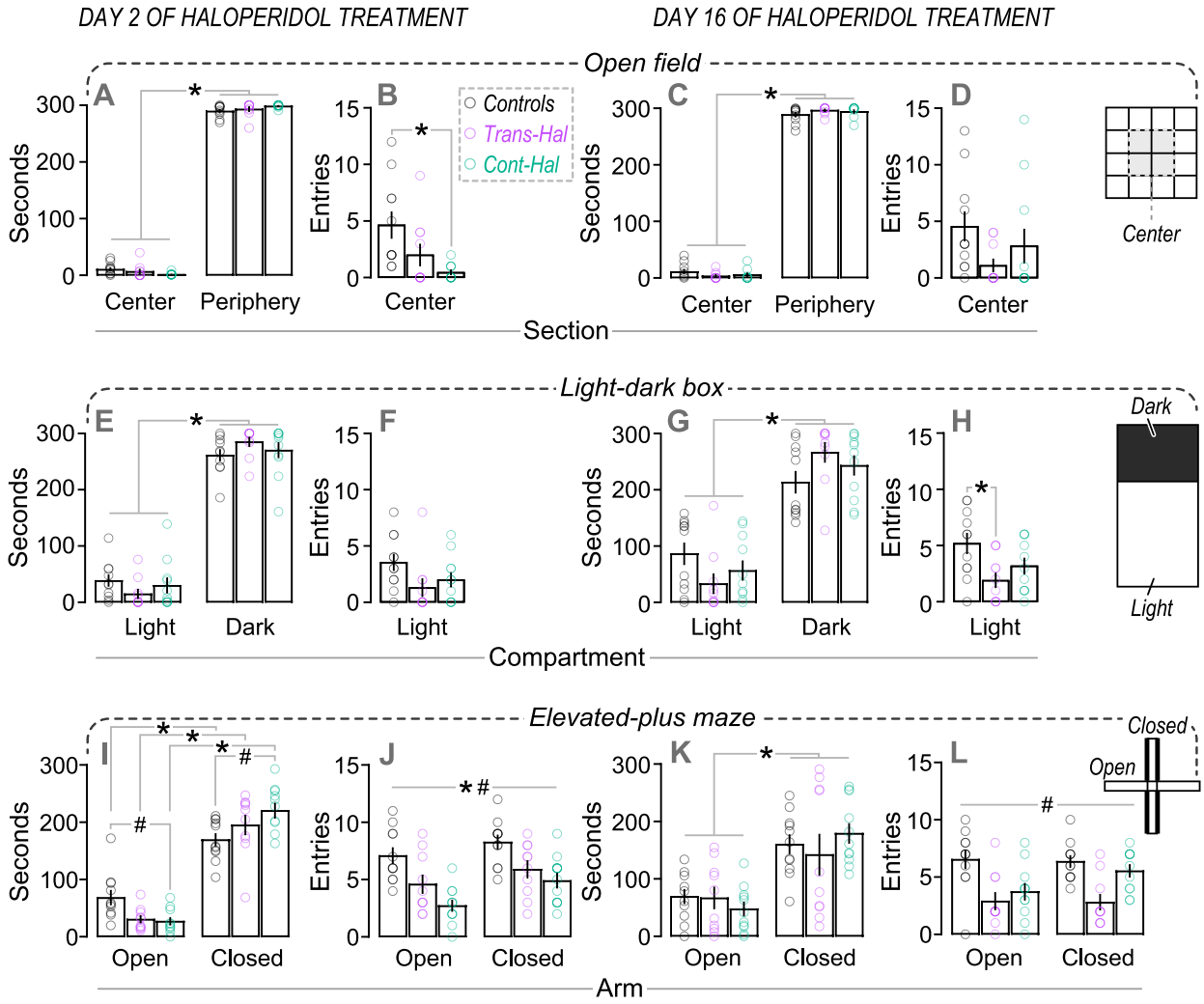

FIG. 3.4 — Stress-related behaviours measured in the open field, light-dark box and elevated-plus maze during transient (TRANS-HAL) or continuous (CONT-HAL) haloperidol treatment. In Exp. 2, stress-like behaviours were assessed in control, TRANS-HAL and CONT-HAL rats on days 2 (***A***, ***B***, ***E***, ***F***, ***I*** and ***J***) and 16 (***C***, ***D***, ***G***, ***H***, ***K*** and ***L***) of ongoing haloperidol treatment. Stress-like behaviours were measured using the (***A-D***) open field, (***E-H***) light-dark box and (***I-L***) elevated-plus maze. *n*'s/condition = 10-11. #,*$p < 0.05$. In (***J***), overall *Arm effect and # Group effect. In (***L***), overall # Group effect.

across groups. TRANS-HAL rats entered less often into the light compartment relative to control rats (FIG. 3.4H; Group effect, $F_{2,29} = 4.65$; controls > TRANS-HAL rats; all P's < 0.05). Hence, as seen in the open field test, 'breakthrough' dopamine supersensitivity late into CONT-HAL treatment (*i.e.*, day 16) is not paralleled by changes in stress-like behaviour measured in the light-dark box.

Elevated-plus maze. On day 2 of ongoing haloperidol treatment, rats spent more time in the closed relative to the open arms of an elevated-plus maze (FIG. 3.4I; Arm effect, $F_{1,29} = 179$, $p < 0.0001$), and there were group differences in this effect (Group × Arm interaction, $F_{2,29} = 5.88$, $p = 0.007$). Post-hoc analyses revealed that within each group, rats spent more time in the closed relative to the open arms (FIG. 3.4I; closed > open in all groups, all P's < 0.0001), and that CONT-HAL (but not TRANS-HAL) rats spent less time in the open arms and more time in the closed arms relative to control rats (FIG. 3.4I; white versus green; all P's < 0.05). Rats entered more often into the closed relative to the open arms (FIG. 3.4J; Arm effect, $F_{1,29} = 14.98$, $p = 0.0006$), and both CONT-HAL and TRANS-HAL rats entered less often into the maze arms (FIG. 3.4J; Group effect, $F_{2,29} = 12.35$, $p = 0.0001$), regardless of arm type (no Group × Arm interaction, $p > 0.05$).

On day 16 of haloperidol treatment, rats spent more time in the closed arms relative to the open arms (FIG. 3.4K; Arm effect, $F_{1,29} = 22.31$, $p < 0.0001$), and this was similar across groups (no Group effect, $p > 0.05$). Unlike on day 2, rats now entered just as often into the open versus closed arms (FIG. 3.4L; no Arm effect, $p > 0.05$). CONT-HAL and TRANS-HAL rats entered less often into the maze arms compared to control rats (FIG. 3.4L; Group effect, $F_{2,29} = 11$, $p = 0.0003$), regardless of arm type (no Group × Arm interaction, $p > 0.05$). Hence, CONT-HAL rats with confirmed dopamine supersensitivity (see FIG. 3.3B) show reduced exploratory behaviour in the elevated-plus maze, without changes in the time spent in open versus closed arms.

SUMMARY OF EXP. 2

Expression of dopamine supersensitivity: Both CONT-HAL and TRANS-HAL treatments produced antipsychotic-like effects early into treatment (day 3), as shown with suppression of d-amphetamine-induced locomotion (FIG. 3.3A). Later into treatment, CONT-HAL treatment produced dopamine supersensitivity. Thereby, only TRANS-HAL treatment still produced antidopaminergic effects on day 17 (FIG. 3.3B). After treatment cessation, only CONT-HAL rats had a greater psychomotor response to d-amphetamine relative to controls and TRANS-HAL rats (FIG. 3.3C), revealing the expression of dopamine supersensitivity.

Stress-related behaviours: On day 2, CONT-HAL rats showed reduced exploration in the open field (FIG. 3.4B) and in the elevated-plus maze (FIGS. 3.4I-J). These effects did not parallel dopamine supersensitivity and could rather reflect the suppressive effects of haloperidol on

locomotor activity (see FIG. 3.3A). Similarly, TRANS-HAL treatment likely reduced exploratory behaviour in the elevated-plus maze due to the suppressive effects of haloperidol on spontaneous locomotor activity (FIG. 3.4J). Late into CONT-HAL treatment, breakthrough dopamine supersensitivity was not associated with changes in stress-like behaviours measured in the open field and light-dark box (FIGS. 3.4C-D and 4G-H), but with reduced exploratory behaviour in the elevated-plus maze (FIG. 3.4L). This effect is not due to a reduction in spontaneous locomotor activity, because CONT-HAL treatment did no longer produced that effect (FIG. 3.3B). Rather, it could reflect greater avoidance. TRANS-HAL treatment also reduced exploratory behaviour in the elevated-plus maze (FIG. 3.4L), but it could be influenced by the suppressive effects of haloperidol on spontaneous locomotor activity (FIG. 3.3B). In the following experiment, stress-like behaviour was measured after treatment cessation, when the expression of dopamine supersensitivity is higher. However, this also allowed to avoid confounding effects produced by the decrease in spontaneous locomotion produced by haloperidol treatment.

Exp. 3: Is the Expression of Antipsychotic-evoked Dopamine Supersensitivity Linked to Increased Stress-like Behaviour?

Psychomotor response to d-amphetamine. We measured the psychomotor response to d-amphetamine on days 7, 14 and 28 after haloperidol discontinuation. There were no group differences in either vehicle- or d-amphetamine-induced psychomotor activity ratings (data not shown; Group effects, all P's > 0.05). There were also no group differences in vehicle-induced locomotion (FIGS. 3.5A-C; minutes 10-30; all P's > 0.05). On day 7 following haloperidol treatment discontinuation, a technical issue prevented recording of locomotor activity during the first 10 min following d-amphetamine injection for half of the rats in each group, so we analyzed minutes 50 to 90 instead of minutes 40 to 90 for that day. On day 7 following cessation of haloperidol treatment, both CONT-HAL and TRANS-HAL rats showed greater d-amphetamine-induced locomotion than control rats did (FIG. 3.5A; minutes 50-90; Group effect, $F_{2,29} = 11.33$; CONT-HAL rats > control rats, $F_{1,20} = 35.63$; TRANS-HAL rats > control rats, $F_{1,19} = 4.86$; all P's < 0.05). However, from day 14 onwards, TRANS-HAL rats returned to control levels (minutes 40-90; FIGS. 3.5B-C; purple versus white; all P's < 0.05), while CONT-HAL rat still showed an

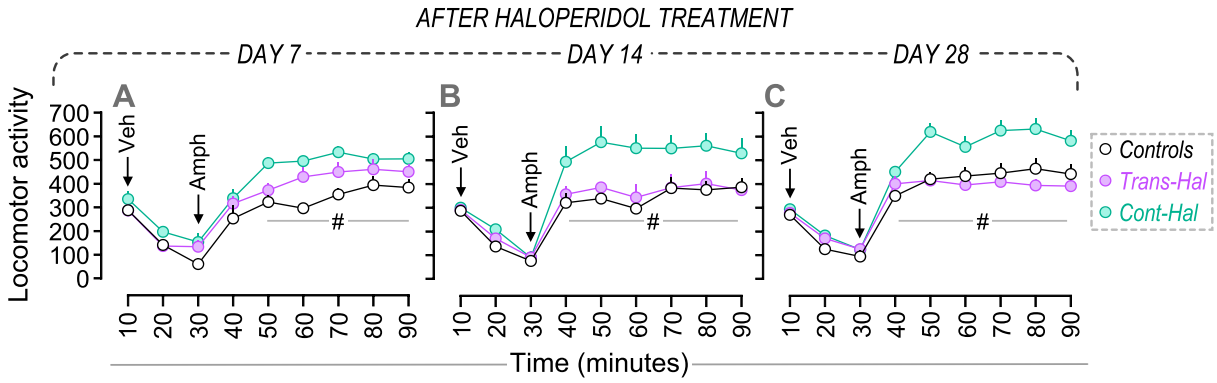

FIG. 3.5 — Both transient (TRANS-HAL) and continuous (CONT-HAL) haloperidol produced a dopamine supersensitive state, but supersensitivity was much more persistent after CONT-HAL exposure. In Exp. 3, the psychomotor response to d-amphetamine was measured in control, TRANS-HAL and CONT-HAL rats on days (***A***) 7, (***B***) 14 and (***C***) 28 after cessation of haloperidol treatment. CONT-HAL rats showed a potentiated psychomotor response to d-amphetamine relative to controls at all time points, whereas TRANS-HAL rats differed from control rats only on day 7, returning to control levels from day 14 on. *n*'s/condition = 5-11. # $p < 0.05$, Group effect.

enhanced AMPH-induced response compared to control rats and now also compared to TRANS-HAL rats (minutes 40-90; FIG. 3.5B; Group effect, $F_{2,14} = 8.79$; CONT-HAL rats > control rats, $F_{1,10} = 14.26$; CONT-HAL rats > TRANS-HAL rats, $F_{1,9} = 8.43$; FIG. 3.5C; Group × Time interaction, $F_{10,145} = 2.34$; Group effect, $F_{2,29} = 9.95$; CONT-HAL rats > control rats; Group effect, $F_{1,20} = 11.23$; CONT-HAL rats > TRANS-HAL rats, Group × Time interaction, $F_{5,95} = 4.28$; Group effect, $F_{1,19} = 16.66$; all P's < 0.05). Hence, both haloperidol treatments produced dopamine supersensitivity, but this effect abated with time after treatment cessation in TRANS-HAL rats, while it persisted in CONT-HAL rats.

Open field. Rats spent more time in the periphery than in the center of the open field (FIG. 3.6A; Section effect, $F_{1,29} = 15690$, $p < 0.0001$), with no group differences. There were also no group differences in the number of times rats entered into the center of the open field (FIG. 3.6B; Group effect, $p > 0.05$).

Light-dark box. Rats spent more time in the dark compartment than in the light compartment (FIG. 3.6C; Compartment effect, $F_{1,29} = 124.8$, $p < 0.0001$), and this was similar across groups. Rats also entered a similar number of times into the light compartment across groups (FIG. 3.6D; no Group effect; $p > 0.05$).

Figure 6

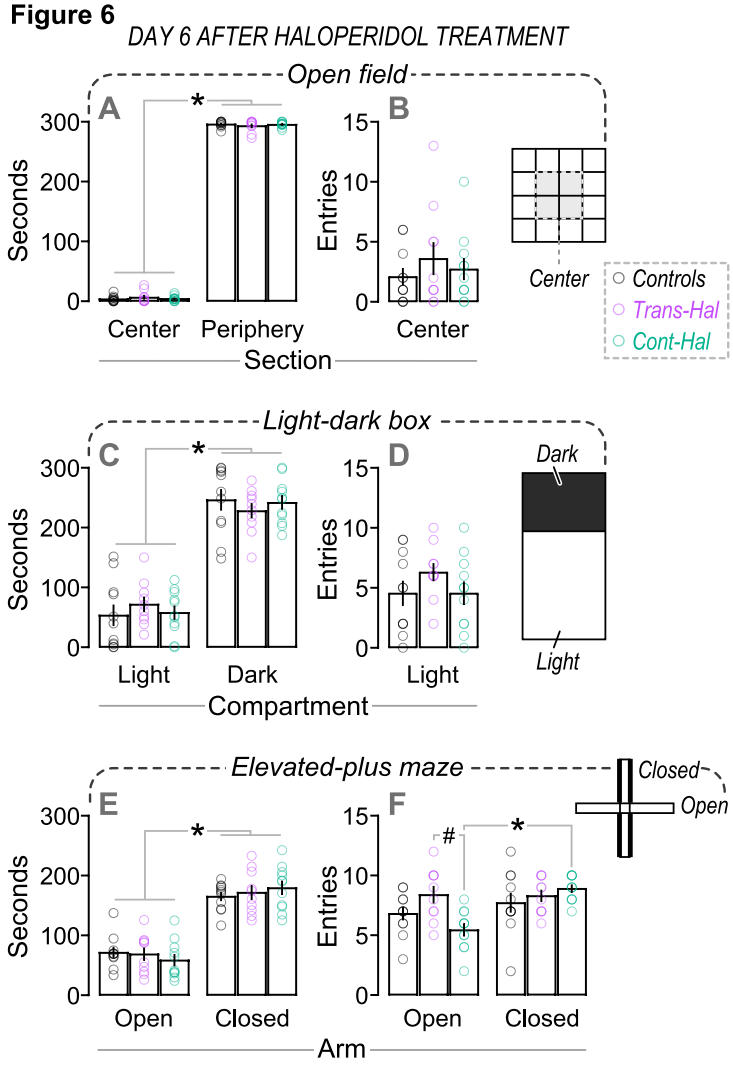

FIG. 3.6 — Stress-related behaviours measured in the open field, light-dark box and elevated-plus maze after cessation of transient (TRANS-HAL) or continuous (CONT-HAL) haloperidol treatment. In Exp. 3, stress-like behaviours were assessed in control, TRANS-HAL and CONT-HAL rats on day 6 after cessation of haloperidol treatment. Stress-like behaviours measured in (***A-B***) the open field, (***C-D***) the light-dark box and (***E-F***) the elevated-plus maze. *n*'s/condition = 10-11. #,*$p < 0.05$.

Elevated-plus maze. Rats spent more time in the open arms than in the closed arms (FIG. 3.6E; Arm effect, $F_{1,29} = 96.63$, $p < 0.001$), and this was similar across groups. There were group differences in the number of entries into the closed arms. First, only CONT-HAL rats entered more often into the closed arms relative to the open arms (FIG. 3.6F; Group × Arm interaction, $F_{2,29} = 7.17$; Arm effect, $F_{1,29} = 12.94$; CONT-HAL rats, closed arms > open arms; all P's ≤ 0.01). Second, CONT-HAL rats also entered less often into the open arms relative to TRANS-HAL rats but not relative to control rats (FIG. 3.6F; open arms, TRANS-HAL rats > CONT-HAL rats, $p = 0.0019$).

SUMMARY OF EXP. 3

Expression of dopamine supersensitivity: CONT-HAL treatment produced more persistent dopamine supersensitivity than TRANS-HAL treatment did. Indeed, CONT-HAL rats had a greater locomotor response to d-amphetamine relative to controls at all time points tested (days 7, 14 and 28 after treatment cessation; FIGS. 3.5A-C). In contrast, TRANS-HAL rats showed an exaggerated locomotor response to d-amphetamine on day 7 after treatment cessation (FIG. 3.5A), but their response returned to control levels after this point (FIGS. 3.5B-C).

Stress-related behaviours: CONT-HAL and TRANS-HAL groups did not produce different effects on stress-like behaviours measured in the open field and light-dark box (FIGS. 3.6A-D). However, in the elevated-plus maze, CONT-HAL rats entered significantly less often into the open versus closed arms (FIG. 3.6F; similar to Exp. 2; FIG. 3.4L), while TRANS-HAL and control rats entered just as often into each set of arms (FIG. 3.6F).

Exp. 4: Do Dopamine-supersensitive Rats Show an Increased Locomotor Response to Novelty?

On day 3 after treatment cessation, CONT-HAL rats showed a greater locomotor activity relative to control rats (FIG. 3.7A; minutes 10-30; Group × Time interaction, $F_{2,298} = 3.66$; Group effect, $F_{1,149} = 8.37$; all P's < 0.05). This indicates that CONT-HAL rats have a potentiated locomotor response to novelty. On a subsequent test (either on day 5, 7 or 9 after CONT-HAL treatment cessation), the locomotor response to vehicle and d-amphetamine was evaluated when the locomotor test cage was not novel anymore. In response to vehicle, there is a significant Group × Time interaction (FIG. 3.7B; minutes 10-30; $F_{2,212} = 3.4$, $p = 0.035$), but further analysis revealed no difference across groups (no Group effect, $p > 0.05$). This suggests that on the first test, CONT-HAL rats showed a greater locomotor response to novelty specifically because they do not show a potentiated locomotor response when given repeated tests. However, CONT-HAL rats showed a greater locomotor response to d-amphetamine relative to control rats (FIG. 3.7C; minutes 10-60; Group × Time interaction, $F_{5,530} = 15.04$; Group effect, $F_{1,106} = 36.94$; all P's < 0.0001), indicating haloperidol treatment produced a dopamine supersensitive state.

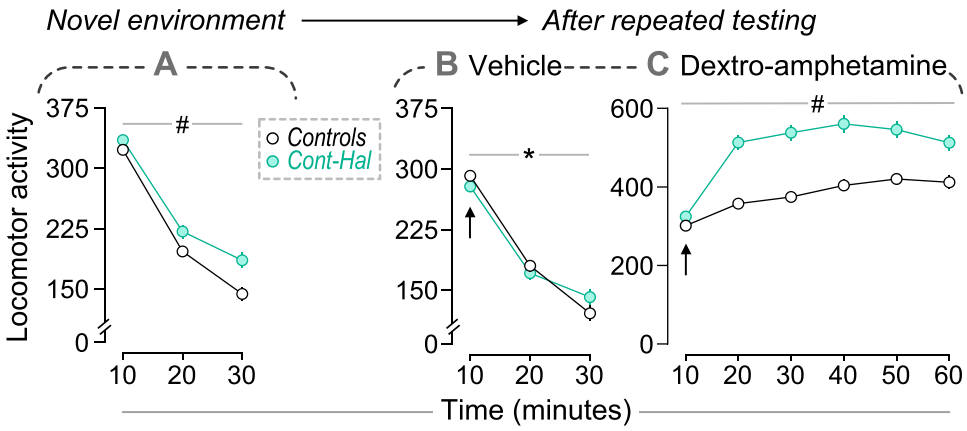

FIG. 3.7 — Continuous haloperidol treatment (CONT-HAL) enhances the locomotor response to both novelty and d-amphetamine. (***A***) Compared to antipsychotic-naïve rats, CONT-HAL rats showed a greater locomotor response to novelty on day 3 after treatment cessation. (***B***) In a subsequent test that was given either on day 5, 7 or 9 after treatment cessation, CONT-HAL and control rats have a comparable response to vehicle, indicating that CONT-HAL rats have specifically an enhanced locomotor response to novelty. (***C***) In the same test, CONT-HAL rats showed a greater locomotor response to d-amphetamine, indicating that haloperidol treatment produced dopamine supersensitivity. *n*'s/condition = 54-76. #,*$p < 0.05$. In (***A***, ***C***), # Group effect and Group × Time interaction. In (***B***), * Group × Time interaction.

SUMMARY OF EXP. 4

Rats with a history of CONT-HAL treatment had an exaggerated locomotor response to both environmental novelty and d-amphetamine, suggesting that antipsychotic-evoked dopamine supersensitivity also produces an enhanced response to the mild stress associated with entry into a new environment.

MAIN FINDINGS

The expression of antipsychotic-evoked dopamine supersensitivity is linked with some enhanced stress-like responses. First, metyrapone seemed to supress to a greater extent the psychomotor response to d-amphetamine of antipsychotic-treated rats. A possible interpretation for this finding is that the expression of antipsychotic-evoked dopamine supersensitivity requires the synthesis of the stress hormone corticosterone. Second, CONT-HAL rats show reduced exploratory behaviour in the elevated-plus maze when dopamine supersensitivity breaks through during ongoing antipsychotic exposure, and also after treatment cessation. Furthermore, CONT-HAL rats have a

greater locomotor response to novelty than antipsychotic-naïve rats. Dopamine supersensitivity is also linked to normal stress-like behaviour, as shown in the open field and the light-dark box.

REFERENCES OF CHAPTER III

Amato D, Canneva F, Cumming P, Maschauer S, Groos D, Dahlmanns JK, Gromer TW, Chiofalo L, Dahlmanns M, Zheng F, Kornhuber J, Prante O, Alzheimer C, von Horsten S, Muller CP (2018) A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failure reversal: the role of the dopamine transporter. Mol Psychiatry.

Bedard AM, Maheux J, Levesque D, Samaha AN (2011) Continuous, but not intermittent, antipsychotic drug delivery intensifies the pursuit of reward cues. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 36:1248-1259.

Bratt AM, Kelley SP, Knowles JP, Barrett J, Davis K, Davis M, Mittleman G (2001) Long term modulation of the HPA axis by the hippocampus. Behavioral, biochemical and immunological endpoints in rats exposed to chronic mild stress. Psychoneuroendocrinology 26:121-145.

Crawley J, Goodwin FK (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 13:167-170.

Ellinwood EH, Jr., Balster RL (1974) Rating the behavioral effects of amphetamine. Eur J Pharmacol 28:35-41.

Ericson H, Radesater AC, Servin E, Magnusson O, Mohringe B (1996) Effects of intermittent and continuous subchronic administration of raclopride on motor activity, dopamine turnover and receptor occupancy in the rat. Pharmacology & toxicology 79:277-286.

Farde L, Wiesel FA, Nordstrom AL, Sedvall G (1989) D1- and D2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. Psychopharmacology (Berl) 99 Suppl:S28-31.

Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G (1992) Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Archives of general psychiatry 49:538-544.

Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB, Jr. (1991) Individual differences in locomotor activity and sensitization. Pharmacol Biochem Behav 38:467-470.

Hooks MS, Jones DN, Holtzman SG, Juncos JL, Kalivas PW, Justice JB, Jr. (1994) Individual differences in behavior following amphetamine, GBR-12909, or apomorphine but not SKF-38393 or quinpirole. Psychopharmacology (Berl) 116:217-225.

Igaz P, Tombol Z, Szabo PM, Liko I, Racz K (2008) Steroid biosynthesis inhibitors in the therapy of hypercortisolism: theory and practice. Curr Med Chem 15:2734-2747.

Johnson BN, Yamamoto BK (2009) Chronic unpredictable stress augments +3,4-methylenedioxymethamphetamine-induced monoamine depletions: the role of corticosterone. Neuroscience 159:1233-1243.

Kapur S, VanderSpek SC, Brownlee BA, Nobrega JN (2003) Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. The Journal of pharmacology and experimental therapeutics 305:625-631.

Kapur S, Zipursky R, Jones C, Remington G, Houle S (2000) Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. The American journal of psychiatry 157:514-520.

Katz RJ, Roth KA, Carroll BJ (1981) Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. Neurosci Biobehav Rev 5:247-251.

Mamo D, Kapur S, Keshavan M, Laruelle M, Taylor CC, Kothare PA, Barsoum P, McDonnell D (2008) D2 receptor occupancy of olanzapine pamoate depot using positron emission tomography: an open-label study in patients with schizophrenia. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33:298-304.

Marin MT, Cruz FC, Planeta CS (2007) Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. Physiol Behav 90:29-35.

Mattson BJ, Crombag HS, Mitchell T, Simmons DE, Kreuter JD, Morales M, Hope BT (2007) Repeated amphetamine administration outside the home cage enhances drug-induced Fos expression in rat nucleus accumbens. Behavioural brain research 185:88-98.

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149-167.

Piazza PV, Deminiere JM, Le Moal M, Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. Science 245:1511-1513.

Reid MS, Ho LB, Tolliver BK, Wolkowitz OM, Berger SP (1998) Partial reversal of stress-induced behavioral sensitization to amphetamine following metyrapone treatment. Brain research 783:133-142.

Remington G, Mamo D, Labelle A, Reiss J, Shammi C, Mannaert E, Mann S, Kapur S (2006) A PET study evaluating dopamine D2 receptor occupancy for long-acting injectable risperidone. The American journal of psychiatry 163:396-401.

Rouge-Pont F, Piazza PV, Kharouby M, Le Moal M, Simon H (1993) Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals

predisposed to amphetamine self-administration. A microdialysis study. Brain research 602:169-174.

Samaha AN, Seeman P, Stewart J, Rajabi H, Kapur S (2007) "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:2979-2986.

Samaha AN, Reckless GE, Seeman P, Diwan M, Nobrega JN, Kapur S (2008) Less is more: antipsychotic drug effects are greater with transient rather than continuous delivery. Biological psychiatry 64:145-152.

Servonnet A, Minogianis EA, Bouchard C, Bedard AM, Levesque D, Rompre PP, Samaha AN (2017) Neurotensin in the nucleus accumbens reverses dopamine supersensitivity evoked by antipsychotic treatment. Neuropharmacology 123:10-21.

Smith RC, Davis JM (1975) Behavioral supersensitivity to apomorphine and amphetamine after chronic high dose haloperidol treatment. Psychopharmacology communications 1:285-293.

Uchida H, Suzuki T (2014) Dose and dosing frequency of long-acting injectable antipsychotics: a systematic review of PET and SPECT data and clinical implications. J Clin Psychopharmacol 34:728-735.

Vonvoigtlander PF, Losey EG, Triezenberg HJ (1975) Increased sensitivity to dopaminergic agents after chronic neuroleptic treatment. The Journal of pharmacology and experimental therapeutics 193:88-94.

CHAPTER IV. *Optogenetic Activation of the Basolateral Amygdala Promotes Both Appetitive Conditioning and the Instrumental Pursuit of Reward Cues*

Article published in the Journal of Neuroscience
DOI: https://doi.org/10.1523/JNEUROSCI.2196-19.2020

Running title: Basolateral amygdala and reward cue properties

Alice Servonnet[a], Giovanni Hernandez[a,b], Cynthia El Hage[c], Pierre-Paul Rompré[a] and Anne-Noël Samaha[c,d]

[a]Department of Neurosciences, Faculty of Medicine, Université de Montréal; 2900 Edouard-Montpetit boulevard, Montreal (H3T 1J4), Quebec, Canada; [b]Department of Psychiatry, Douglas Mental Health University Institute, McGill University; 6875 Boulevard Lasalle, Montreal (H4H 1R3), Quebec, Canada; [c]Department of Pharmacology and Physiology, Faculty of Medicine, Université de Montréal; 2900 Edouard-Montpetit boulevard, Montreal (H3T 1J4), Quebec, Canada; [d]Groupe de recherche sur le système nerveux central, Faculty of Medicine, Université de Montréal; 2900 Edouard-Montpetit boulevard, Montreal (H3T 1J4), Quebec, Canada

CONFLICT OF INTEREST

ANS was a scientific consultant for H. Lundbeck A/S as this research was being carried out. This has had no influence on the work. The remaining authors report no biomedical financial interests or potential conflicts of interest.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Science and Engineering Research Council of Canada (grant number 355923) and the Canada Foundation for Innovation to ANS (grant number 24326). ANS holds a salary award from the Fonds de la Recherche du Québec-Santé (grant number 28988). We thank Nadia Chaudri, Franca Lacroix, Franz Villaruel, Ivan Trujillo-Pisanty, Jonathan Britt, Sean J. Reed, Mike J.F. Robinson and Kent C. Berridge for generous advice on implementation of *in vivo* optogenetics procedures in our laboratory. We also thank Ellie-Anna Minogianis for technical support.

ABSTRACT

Reward-associated stimuli can both evoke conditioned responses and acquire reinforcing properties in their own right, becoming avidly pursued. Such conditioned stimuli (CS) can guide reward-seeking behavior in adaptive (*e.g.*, locating food) and maladaptive (*e.g.*, binge eating) ways. The basolateral amygdala (BLA) regulates conditioned responses evoked by appetitive CS, but less is known about how the BLA contributes to the instrumental pursuit of CS. Here we studied the influence of BLA neuron activity on both behavioral effects. Water-restricted male rats learned to associate a light-tone cue (CS) with water delivery into a port. During these Pavlovian conditioning sessions, we paired CS presentations with photo-stimulation of channelrhodopsin-2 (ChR2)-expressing BLA neurons. BLA photo-stimulation potentiated CS-evoked port entries during conditioning, indicating enhanced conditioned approach and appetitive conditioning. Next, new rats received Pavlovian conditioning without photo-stimulation. These rats then received instrumental conditioning sessions where they could press an inactive lever or an active lever that produced CS presentation, without water delivery. Rats pressed more on the active versus inactive lever, and pairing CS presentation with BLA-ChR2 photo-stimulation intensified responding for the CS. This suggests that BLA-ChR2 photo-stimulation enhanced CS incentive value. In a separate experiment, rats did not reliably self-administer BLA-ChR2 stimulations, suggesting that BLA neurons do not carry a primary reward signal. Last, intra-BLA infusions of d-amphetamine also intensified lever-pressing for the CS. The findings suggest that BLA-mediated activity facilitates CS control over behavior by enhancing both appetitive Pavlovian conditioning and instrumental pursuit of CS.

SIGNIFICANCE STATEMENT

Cues paired with rewards can guide animals to valuable resources such as food. Cues can also promote dysfunctional reward-seeking behavior, as in overeating. Reward-paired cues influence reward seeking through two major mechanisms. First, reward-paired cues evoke conditioned anticipatory behaviors to prepare for impending rewards. Second, reward-paired cues are powerful motivators and they can evoke pursuit in their own right. Here we show that increasing neural activity in the basolateral amygdala enhances both conditioned anticipatory behaviors and pursuit of reward-paired cues. The basolateral amygdala therefore facilitates cue-induced control over

behavior by both increasing anticipation of impending rewards and making reward cues more attractive.

INTRODUCTION

Initially neutral cues (sights, sounds, or places) that predict rewards such as food and water exert powerful control over behavior. For instance, reward-paired cues [conditioned stimuli (CS)] can acquire incentive motivational value (Bolles, 1972; Bindra, 1978), thereby "*goading an individual into action*" (Flagel *et al*., 2009). In this regard, CS can (1) elicit approach and attention, allowing animals to prepare for impending rewards (Hearst and Jenkins, 1974), (2) energize ongoing reward-seeking behaviors (Rescorla and Solomon, 1967), (3) trigger reinstatement of extinguished reward-seeking behavior (de Wit and Stewart, 1981), and (4) reinforce learning of new instrumental behaviors (Mackintosh, 1974; Cardinal *et al*., 2002). Through these effects, CS guide behavior toward rewards necessary for survival. However, changes in the response to CS can contribute to excessive reward-seeking behaviors (as in addiction) or conversely, low levels of appetitive behavior (as in depression).

Prior studies have examined the role of the basolateral amygdala (BLA) in the capacity of CS to both evoke conditioned approach and influence instrumental behavior. BLA lesions (Burns *et al*., 1993) and optogenetic stimulation of BLA→nucleus accumbens shell neurons (Millan *et al*., 2017) both attenuate CS-evoked conditioned responses. Similar effects are seen with optogenetic inhibition of either BLA neurons expressing the *Ppp1r1b* gene (Kim *et al*., 2016) or BLA→nucleus accumbens core neurons (Stuber *et al*., 2011). The BLA is also thought to be necessary for the expression of CS-controlled instrumental behavior. Decreasing BLA function with lesions (Cador *et al*., 1989; Everitt *et al*., 1991; Brown and Fibiger, 1993; Burns *et al*., 1993; White and McDonald, 1993; McDonald and Hong, 2004; McDonald *et al*., 2010), pharmacological agents (Grimm and See, 2000; Kantak *et al*., 2002; McLaughlin and See, 2003; Rogers *et al*., 2008; Gabriele and See, 2010) or optogenetic methods (Stefanik and Kalivas, 2013) suppresses CS-controlled instrumental behavior. However, these studies used tasks that potentially confound the motivational effects of the CS and those of the unconditioned stimulus (UCS), and/or neuronal manipulation methods that do not allow control of neural activity coincident with CS occurrence (*e.g.*, lesions/pharmacological agents).

In this context, key questions remain. First, how does increased BLA-mediated neuronal activity during CS presentation influence respectively, CS-evoked conditioned behaviors and the instrumental pursuit of CS? BLA neurons fire in response to CS presentations during appetitive conditioning (Tye and Janak, 2007; Ambroggi *et al*., 2008; Tye *et al*., 2008). The functional significance of this is not fully understood. Second, CS can motivate behavior through many dissociable psychological processes (Cardinal *et al*., 2002), what processes might BLA-dependent activity regulate? Increased BLA activity might mediate the specific incentive value attributed to the CS. If so, then increased BLA activity should alter CS motivational properties preferentially when it is explicitly paired with CS presentations. The BLA might also arouse a general motivational state, thereby "setting the occasion" to perform a CS-controlled goal-directed behavior (Lajoie and Bindra, 1976; Rescorla, 1988). If so, then increased BLA activity should alter CS incentive value, even when increased BLA activity is explicitly unpaired with CS presentations.

We addressed these questions using *in vivo* optogenetics combined with Pavlovian and instrumental conditioning procedures. First, we determined whether photo-stimulation of BLA neurons is intrinsically rewarding, as assessed by self-stimulation behavior. We compared self-stimulation of BLA neurons with self-stimulation of adjacent central amygdala (CeA) neurons, as rodents will self-stimulate into the CeA (Seo *et al*., 2016; Baumgartner *et al*., 2017; Kim *et al*., 2017). Second, we determined how photo-stimulation of BLA neurons influences appetitive conditioned responses, as assessed by CS-evoked approach behavior that indicates expectation of the primary reward (Tolman, 1932; Hearst and Jenkins, 1974). Finally, we assessed how photo-stimulation of BLA neurons influences CS-controlled instrumental behavior, by measuring the capacity of a CS to support the spontaneous learning of a new instrumental behavior (Mackintosh, 1974; Robbins, 1978; Cardinal *et al*., 2002).

MATERIAL AND METHODS

Animals

Male Sprague-Dawley rats (Charles River Laboratories; 200–275 g on arrival) were housed individually on a 12 h light/dark cycle (lights off at 8:30 A.M.). They were tested during the dark phase of the circadian cycle. Food and water were available *ad libitum*, except in Experiments 3–

4, where water access was restricted to 2 h/d. This was to facilitate Pavlovian conditioning using water as the UCS (see 'Pavlovian conditioning' section). The Université de Montréal approved all procedures involving animals and procedures followed the guidelines of the Canadian Council on Animal Care.

Intracerebral Surgery

Rats weighing 325–375 g were anesthetized with isoflurane and placed on a stereotaxic apparatus. For photo-stimulation of amygdala neurons in Experiments 1–3, rats received bilateral infusions of AAV5-hSyn1-hChR2(H134R)-eYFP (provided by Dr. Karl Deisseroth; UNC Vector Core, NC) into either the BLA (mm relative to Bregma, AP: −2.8, ML: ±5.0; mm relative to skull surface, DV: −8.4) or CeA (mm relative to Bregma, AP: −2.6, ML: ±4.3; mm relative to skull surface, DV: −7.9). Control rats received an optically inactive AAV-eYFP virus (AAV5-hSyn1-eYFP, UNC Vector Core). The hSyn promoter is neuron-specific and allows gene expression in both excitatory and inhibitory neurons (Dittgen *et al*., 2004). Using a glass pipette (tip diameter, ∼50 µm) coupled to a Nanoject II (Drummond Scientific), we administered 27 microinjections of 36.8 nl each (23 nl/s, at 10 s intervals; total volume of ∼1 µl/hemisphere) into each brain region. After the infusions, the glass pipette was left in place for 10 more min. In Experiment 4, guide cannulae (26 GA, model C315G, HRS Scientific) were implanted 2 mm dorsal to the BLA (mm relative to Bregma, AP: −2.4, ML: ±5.5; mm relative to skull surface: DV −6.6) or dorsal to the amygdala, without targeting the BLA specifically, as a neuroanatomical control (referred to as “Amygdala”; mm relative to Bregma, AP: −2.3, ML: ±5.1; mm relative to skull surface, DV: −6.2). In Experiment 1, the craniectomy was sealed with bone wax (Ethicon). In Experiments 2–3, an optic fiber implant (∼300 µm core diameter, numerical aperture = 0.39; Thorlabs; glued with epoxy to a ferrule, model F10061F340, Fiber Instrument Sales) was implanted in each hemisphere, 0.2 mm dorsal to the virus injection site. Four to 6 stainless steel screws were then anchored to the skull, and optic fiber implants or cannulae were fixed with dental cement. Optic fiber implants were protected with a sleeve and a dummy. Guide cannulae were sealed with obturators (model C315CD, HRS Scientific). Optogenetic manipulations started at least 4 weeks following virus injection, to allow sufficient viral expression (Zhang *et al*., 2010).

In Vivo *Electrophysiology*

We used *in vivo* electrophysiology to confirm laser-induced action potentials in channelrhodopsin-2 (ChR2)-expressing neurons in the BLA and CeA. Anesthetized rats (urethane, 1.2 g/kg, i.p.) were placed inside a Faraday cage on a stereotaxic frame equipped with a body temperature controller. Optrodes were implanted above the BLA and CeA. Optrodes were constructed using an extracellular Parylene-coated tungsten electrode (1 MΩ, ~125 μm outer diameter; FHC) glued with epoxy to an optical fiber (~300 μm core diameter, numerical aperture = 0.39) with a ~0.5 mm offset to ensure illumination of recorded neurons. A reference electrode (insulated silver wire, 0.25 mm diameter) was lowered into the back of the brain close to the cerebellum. The optrode and reference electrodes were fixed with stainless steel screws anchored to the skull and bee wax. The optrode was lowered by hydraulic microdrive into the BLA or the CeA to record single action potentials elicited by laser stimulation (465 nm blue diode laser). Optrodes were linked to the laser via patch-cords built as described by Trujillo-Pisanty *et al*. (2015).

The signal recorded from each optrode was fed into a high impedance headstage connected to a microelectrode amplifier (Model 1800, A-M Systems). During photo-stimulation, the low- and high-pass filters were set at 300 Hz and 5 kHz, respectively. To reduce the possibility of photoelectric artifacts, we grounded the laser head and patch-cord. Action potentials were displayed on an oscilloscope (Tektronix, Model TDS 1002). The signal was digitalized and stored using DataWave recording (USB 16 channels) and DataWave SciWorks Experimenter Package (DataWave Technologies).

Pavlovian Conditioning

Training and testing took place in standard operant chambers (Med Associates) where a fan and a house-light were on. Rats had restricted water access for at least 3 d (2 h/d). Starting on the next day, they were trained to associate a light-tone cue (FIG. 4.1A; CS) with water delivery (UCS; 100 μl) into a recessed receptacle, using Pavlovian conditioning procedures. The light-tone cue consisted of illumination of two discrete lights for 5 s, combined with the extinction of the house-light. This was immediately followed by an 1800 Hz, 85-dB tone. The tone lasted 0.18 s and was coincident with water delivery. The CS-UCSs were presented on a variable interval of 60 s, 20 or 30 times/session. To determine the extent to which rats learned the CS-UCS contingency, we measured CS-evoked conditioned approach behavior. To this end, we quantified the number of

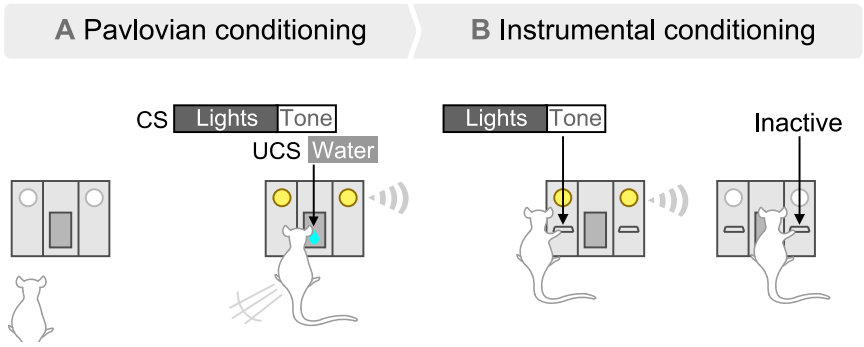

FIG. 4.1 — Pavlovian and Instrumental conditioning procedures. ***A***, During Pavlovian conditioning, rats with limited access to water (2 h/d) learned that a cue (lights + tone, CS) predicts water (100 µl) delivery into a recessed dish. We assessed the acquisition of CS-evoked conditioned approach behaviour by analyzing the ratio of the number of nose-pokes into the dish made during each 5 s cue presentation (CSR) over that made during the 5 s before each CS presentation (PCSR). ***B***, After Pavlovian conditioning, rats were given instrumental conditioning sessions during which they were presented with two levers for the first time. Pressing the active lever produced the CS, whereas pressing the inactive lever had no programmed outcome. No water was delivered during instrumental conditioning sessions.

nose-pokes into the recessed water receptacle during each 5 s light cue presentation [conditioned stimulus response (CSR)] versus during the 5 s period preceding each CS presentation [preconditioned stimulus response (PCSR)]. We computed a CSR/PCSR ratio for each animal, on each conditioning session.

Instrumental Conditioning

To assess the capacity of the CS to control instrumental behavior, we determined whether after Pavlovian CS-UCS conditioning, rats would spontaneously learn a new instrumental response (lever-pressing) to earn CS presentations, without the UCS. This procedure dissociates incentive motivation for the CS versus that for the UCS, because the instrumental response is new and not previously reinforced by the UCS (Mackintosh, 1974; Robbins, 1978; Cardinal *et al*., 2002). First, rats were placed in the operant chambers for a lever habituation session, during which they could sample two test levers for the first time. As shown in Figure 4.1B, pressing the active lever produced the CS, without water delivery, according to a random-ratio 2 (RR2) schedule. Pressing on the active lever during CS presentation or on the inactive lever had no programmed consequences but was recorded. The lever habituation session ended after 10 active lever presses or 40 min. To measure the incentive motivational value of the CS, rats received additional

instrumental test sessions. During these sessions, conditions were the same as during the lever habituation session, except that lever presses were not limited. Sessions ended after 20 or 40 min. We refer to these sessions as "operant responding for the CS".

Experiment 1: Effects of Photo-stimulation on Action Potentials in ChR2-expressing BLA and CeA Neurons in Vivo

As shown in Figure 4.2A, rats received either the ChR2-eYFP (n = 4) or eYFP (n = 1) virus into the BLA of one hemisphere and into the CeA of the contralateral hemisphere. At least 4 weeks later, rats were anesthetized and *in vivo* neuronal firing was measured following photo-stimulation [squared light pulses of 5 ms delivered at 1, 10, 20, or 40 Hz at 10 mW; based on studies by Huff *et al*. (2013) and Robinson *et al*. (2014)]. These are the photo-stimulation parameters used in the behavioral studies below, with frequencies ≤20 Hz, at which we observed excellent ChR2 fidelity. Importantly, BLA neurons also fire *in vivo* at frequencies ≤20 Hz in behavioral tasks involving reward cues (Tye and Janak, 2007; Ambroggi *et al*., 2008; Tye *et al*., 2008).

Experiment 2: Effects of Photo-stimulating ChR2-expressing BLA or CeA Neurons on Lever-pressing Behavior

If photo-stimulation of BLA neurons is intrinsically rewarding, it could reinforce lever pressing behavior and this would confound interpretation of subsequent results. Thus, here we determined whether otherwise naive rats would reliably lever press for photo-stimulation of BLA. We also evaluated self-stimulation of CeA neurons, because photo-stimulation of CeA ChR2 has been reported to sustain self-stimulation (Seo *et al*., 2016; Baumgartner *et al*., 2017; Kim *et al*., 2017). As shown in Figure 4.3A, rats received bilateral injections of the ChR2-eYFP or eYFP virus into the BLA or CeA. Experimental rats were ChR2-eYFP rats (n = 5/subregion) allowed to lever press for photo-stimulation. Control rats included (1) rats expressing ChR2-eYFP in the BLA (n = 3) or CeA (n = 2) that could lever press but this did not produce photo-stimulation, and (2) rats expressing eYFP in the BLA (n = 3) or CeA (n = 2) and allowed to lever press for photo-stimulations. Throughout the study, lever-pressing behavior was similar across control groups. Thus, they were pooled together for final analysis (n = 10). Photo-stimulation was bilateral except where noted otherwise.

As shown in Figure 4.3A, the rats were allowed to press a lever to obtain a 5.18 s laser stimulation (20 Hz frequency, unless stated otherwise) paired with a 5.18 s presentation of the light-tone stimulus described above. Importantly, rats were previously naive to the light-tone stimulus, such that this stimulus had not previously been paired with water or any other outcome in these rats. During all sessions, active lever presses during photo-stimulation and inactive lever presses had no programmed consequences, but both were recorded. Daily sessions ended after self-administration of 30 stimulations or 30 min, unless stated otherwise. First, for at least two sessions (1 session/d), pressing the active lever produced photo-stimulation under a fixed-ratio of 1 (FR1) schedule of reinforcement. The rats were then tested under RR2 and RR4 schedules, with two sessions/schedule. Then, rats were given two sessions where photo-stimulation was available under a progressive ratio 5 schedule of reinforcement (PR5). During these sessions, the number of active lever presses required to earn each successive photo-stimulation increased by a factor of 5, and sessions ended after 30 stimulations or 30 min (Rossi *et al*., 2013). Extinction responding was then evaluated during two 40 min sessions, based on the study by Ilango *et al*. (2014). During minutes 0–5 and 20–25 of the extinction sessions, lever pressing was reinforced with photo-stimulation under RR2. For the remaining min of each session, lever pressing produced the light-tone stimulus, without photo-stimulation. At the 20 min mark, a single, noncontingent photo-stimulation combined with the tone-light cue indicated that photo-stimulation was available once again. Next, we assessed the influence of laser stimulation frequency on lever pressing behavior during three sessions (5, 10, and 20 Hz, 1 frequency/session/d, counterbalanced). We then assessed reversal learning for two sessions during which the active and inactive levers were switched. If photo-stimulation of BLA or CeA neurons is reinforcing, then ChR2-BLA rats and ChR2-CeA rats should stop responding on the newly non-reinforced lever, and increase responding on the newly reinforced lever. Last, the rats were given a final test session to determine whether unilateral photo-stimulations are sufficient to reinforce lever-pressing behavior. The stimulated hemisphere was counterbalanced within each group. After the extinction sessions, one rat in the BLA-ChR2 group was excluded from subsequent testing because of increasing aggressive behavior.

In this and subsequent experiments, the experimenter observed each rat during testing. Some rats experienced seizures with repeated photo-stimulation of ChR2-containing BLA neurons (rats in the other groups did not show seizure activity). This is consistent with the amygdala kindling model of epilepsy and neuronal plasticity (Goddard *et al*., 1969; McNamara *et al*., 1980; Fisher, 1989).

Rats that experienced seizures were eliminated from final data analyses (Experiment 2, $n = 0$; Experiment 3a, $n = 3$; Experiment 3b, $n = 1$), except for one rat in Experiment 3a (see next section).

Experiment 3a: Effects of Photo-stimulating ChR2-expressing BLA Neurons During Pavlovian CS-UCS Conditioning on CS-evoked Conditioned Approach

Experiment 2 showed that rats reliably lever pressed for photo-stimulation of CeA but not BLA neurons. Thus, we pursued the following experiments with BLA manipulations only, as the reinforcing effects of CeA photo-stimulation could confound data interpretation. We first determined whether photo-stimulation of BLA neurons during Pavlovian conditioning changes CS-evoked conditioned approach behavior, as measured by the CSR/PCSR ratio described above. As shown in Figure 4.4A, a new cohort of rats was prepared for optogenetic manipulations in the BLA as described. The rats then received Pavlovian conditioning under one of the following three conditions: (1) "No Laser", where the CS was presented alone (ChR2, $n = 11$; eYFP, $n = 5$), (2) "Paired laser", where photo-stimulation was paired with each CS presentation (ChR2, $n = 3$; eYFP, $n = 3$), and (3) "Unpaired laser", where photo-stimulation and CS presentation were explicitly unpaired, by administering laser stimulation half-way between each CS-UCS presentation (ChR2, $n = 3$). The Unpaired laser group served to determine whether increased BLA neuronal activity had to coincide with CS presentation to influence CS-evoked conditioned approach. If so, then CSR/PCSR ratios in the Unpaired laser group should be similar to those in the ChR2-No Laser or eYFP rats. One Unpaired-ChR2 rat had a seizure on Session 9. Therefore, the effects of BLA photo-stimulation on CSR/PCSR ratios were analyzed on Sessions 1–8, with this rat included. There were no behavioral differences between ChR2-No laser, eYFP-Paired laser and eYFP-No laser rats under any test condition, and they were pooled into one group (controls, $n = 19$).

Experiment 3b: Effects of Photo-stimulating ChR2-expressing BLA Neurons During Operant Responding for a CS

Rats naive to laser stimulation (control rats from Experiment 3a, including 7 eYFP rats, and 8 ChR2 rats) received sessions where they could lever press for presentations of the CS, with or without CS-paired BLA photo-stimulation (0, 5, 10, or 20 Hz, one frequency/session, counterbalanced), as shown in Figure 4.5A. We then determined whether photo-stimulation of BLA neurons must be explicitly paired with CS presentations to alter operant responding for the CS. If so, then explicitly

unpairing photo-stimulation and CS presentation during operant responding for the CS should have no or reduced effects on lever-pressing for that CS, compared with effects seen when photo-stimulation and CS presentation are paired. To address this, all rats were given an operant responding session during which photo-stimulation was explicitly unpaired with CS presentation (photo-stimulation applied 3 s after each CS presentation).

Experiment 4: Effects of Intra-amygdala D-amphetamine Infusions on the Incentive Motivational Effects of a CS

Experiment 3b showed that photo-stimulation of BLA neurons potentiates operant responding for a CS, suggesting that changes in BLA neuron activity influences the incentive motivational effects of the CS. Here we sought to extend these findings by using a pharmacological approach to influence BLA neuron activity. Thus, we determined whether injecting d-amphetamine into the BLA (n = 20) also changes operant responding for a CS. We also determined whether, within the amygdala, effects of d-amphetamine on CS incentive properties are specific to the BLA. To this end, we assessed the effects of infusing d-amphetamine into the amygdala, but without targeting the BLA specifically (n = 15). We predicted that d-amphetamine infused specifically into the BLA would enhance operant responding for a CS, based on work showing that intra-BLA infusions of d-amphetamine increase cue-induced reinstatement of extinguished cocaine seeking (Ledford *et al*., 2003). As shown in Figure 4.7A, following Pavlovian CS-UCS conditioning, intra-cerebral cannulae were implanted bilaterally. The rats were then given at least 2 weeks to recover. Rats then received a reminder Pavlovian conditioning session. Right after this session, rats received intracerebral saline infusions to habituate them to the infusion procedure. No behavior was recorded. On the next day, rats received a lever habituation session. Starting on the next day, rats received intracerebral saline or d-amphetamine (10 or 30 μg/hemisphere; Sigma-Aldrich; 1 injection/d, given every other day) and they were then allowed to lever press for the CS during a 40 min test session. This session length was chosen based on our previous work with intra-nucleus accumbens d-amphetamine injections (El Hage *et al*., 2015). Each rat received a maximum of three intracerebral injections to minimize tissue damage. This included (1) a saline microinjection for habituation, (2) a saline microinjection before testing, and (3) a d-amphetamine microinjection (10 or 30 μg/hemisphere) before testing (10 μg/hemisphere: n = 11 in BLA group, n = 7 in Amygdala group; 30 μg/hemisphere: n = 9 in BLA group, n = 8 in Amygdala group). Therefore, each rat

received only one d-amphetamine microinjection. For intracerebral injections, injectors (33 GA, model C315I, HRS Scientific) were inserted to extend 2 mm beyond the cannulae. Microinjections were given in a volume of 0.5 µl/hemisphere and were infused over 1 min using a microsyringe pump (HARVARD PHD 200, HARVARD Apparatus). Injectors were left in place for an additional min after the infusion.

Histology

In Experiments 2–3, rats were anesthetized with urethane (1.2 g/kg, i.p.) and were transcardially perfused with PBS and 4% paraformaldehyde. Brains were then extracted and kept at room temperature for 1 week in a 30% sucrose/4% paraformaldehyde solution, and then stored at −80°C. In Experiment 4, rats were anesthetized with isoflurane (5%), brains were extracted and stored at −20°C. Forty µm-thick coronal slices were cut in a cryostat and optic fiber or injector placement was estimated using the Paxinos and Watson atlas (Paxinos and Watson, 1986).

Statistics

In Experiment 2, mixed-model ANOVA was used to analyze group differences in self-administered photo-stimulations and lever pressing behavior (Group × Session: "Session" as a within-subjects variable; Group × Time: "Time" as a within-subjects variable; Group × Laser Frequency: "Frequency" as a within-subjects variable). One-way ANOVA was used to analyze group differences in both active lever presses during the PR5 session and the number of self-administered unilateral stimulations. In Experiment 3a, mixed-model ANOVA was used to analyze group differences in average CSR/PCSR ratios (Group × Session: Session as a within-subjects variable). In Experiment 3b, mixed-model ANOVA was used to analyze group differences in lever pressing for the CS (Group × Session or Lever Type: Session and "Lever Type" as within-subjects variables). In Experiment 4, one-way ANOVA was used to analyze CSR/PCSR ratios across sessions. The effects of d-amphetamine on lever pressing were analyzed using mixed-model ANOVA (Dose × Lever Type: Lever Type as a within-subjects variable). When an interaction and/or main effects were significant ($p < 0.05$), effects were analyzed further using Bonferroni's multiple-comparisons tests. Values in figures are mean ± SEM.

RESULTS

Experiment 1: Effects of Photo-stimulation on Action Potentials in ChR2-expressing BLA and CeA Neurons in Vivo

Figure 4.2, B and C, shows ChR2-eYFP expression in the BLA and CeA. As seen in Figure 4.2, D and E, photo-stimulation of BLA or CeA neurons induced action potentials on average 100% of the time at 1, 10, and 20 Hz stimulation frequencies. However, at 40 Hz, spike fidelity decreased, and photo-stimulation produced action potentials only ∼45% of the time. In line with these observations, Figure 4.2, F and G, shows that the frequencies of neuron firing and photo-stimulation were closely matched at laser frequencies ≤20 Hz. However, at a stimulation frequency of 40 Hz, BLA and CeA neurons fired only at ∼18 Hz. This loss of fidelity is in accordance with the kinetic properties of ChR2(H134R), the ChR2 mutant used here. Indeed, when 5 ms pulses are given at a 40 Hz stimulation frequency, pulses are spaced by 20 ms, and this is shorter than the combined opening (∼3 ms) and closing (∼18 ms) rates of ChR2(H134R) (Lin *et al*., 2009). Importantly, laser application produced action potentials in ChR2-expressing BLA or CeA neurons (FIG. 4.2H), but not in eYFP-expressing BLA or CeA neurons (FIG. 4.2I). Thus, photo-stimulation reliably induced action potentials only in ChR2-expressing BLA or CeA neurons, and spike fidelity was excellent at laser frequencies ≤20 Hz. Thus, we used frequencies ≤20 Hz in the following studies.

Experiment 2: Effects of Photo-stimulating ChR2-expressing BLA or CeA Neurons on Lever-pressing Behavior

Here, we determined whether rats would reliably press on a lever for photo-stimulation of ChR2-expressing BLA or CeA neurons (FIG. 4.3A). Pressing on the active lever produced photo-stimulation paired with a light-tone cue, under FR1, RR2, and RR4 schedules of reinforcement (1 schedule/session). Pressing on the inactive lever had no programmed consequences.

Laser self-stimulation. Figure 4.3B shows estimated optic fiber placements in the CeA and BLA. Figure 4.3C shows that across different reinforcement schedules, CeA-ChR2 rats self-administered more laser stimulations than control rats (main effect of Group: $F_{(2,17)} = 5.5$, $p = 0.014$; CeA-ChR2 versus control rats: $F_{(1,13)} = 7.5$, $p = 0.017$). Accordingly, as seen in Figure 4.3D, CeA-ChR2 rats also pressed more on the active lever than control rats (main effect of Group: $F_{(2,17)} = 5.53$, $p = 0.014$; CeA-ChR2 versus control rats: $F_{(1,13)} = 7.42$, $p = 0.017$). In contrast, BLA-ChR2

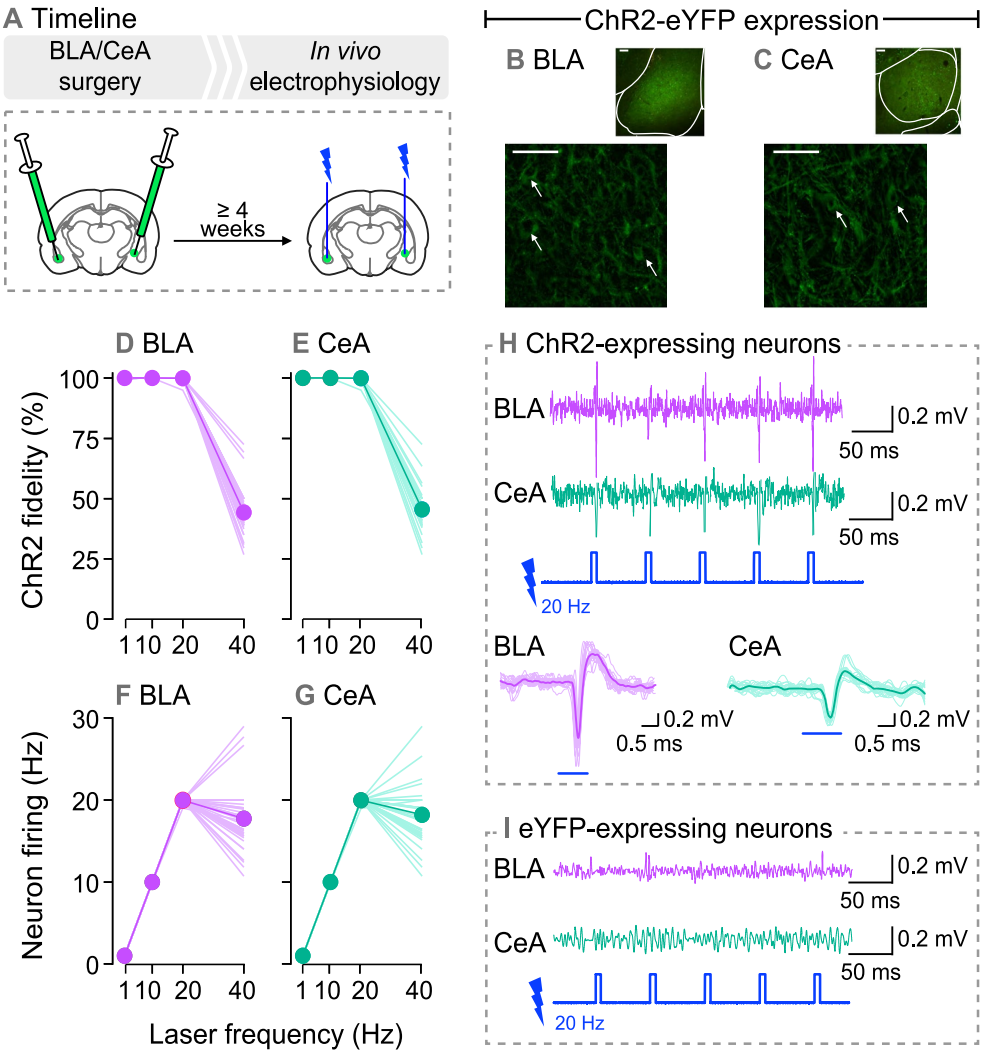

FIG. 4.2 — Photo-stimulation reliably induces action potentials only in BLA and CeA neurons expressing ChR2. ***A***, In Experiment 1, rats received AAV5-hSyn1-hChR2(H134R)-eYFP (ChR2-eYFP) for transduction and activation of BLA or CeA neurons. Control rats received an optically inactive virus lacking ChR2 (AAV5-hSyn1-eYFP) in the BLA or CeA. At least 4 weeks later, we measured action potentials evoked by photo-stimulation using *in vivo* electrophysiology. ***B***, ***C***, ChR2-eYFP expression is shown in the BLA and CeA, respectively. Scale bars, 50 µm. Arrows indicate cell bodies. When laser-light is delivered, ChR2 reliably induced action potentials in (***D***) BLA and (***E***) CeA neurons, with stimulation frequencies ranging between 1 and 20 Hz. ChR2 fidelity was reduced at 40 Hz. Accordingly, firing frequency of (***F***) BLA and (***G***) CeA neurons matched laser stimulation frequency only between 1 and 20 Hz. Recordings in 4 rats/region; 10 observations/rat. Data are means, with each line representing individual observations. Examples of *in vivo* recordings show that laser-light induced action potentials in (***H***) ChR2-expressing BLA and CeA neurons but not in (***I***) eYFP-expressing BLA and CeA neurons.

and control rats earned a similar number of photo-stimulations and pressed a similar number of times on the active lever (FIG. 4.3C,D; all p values > 0.05). Presses on the inactive lever did not

differ between groups (FIG. 4.3E; $p > 0.05$), suggesting that photo-stimulation of either BLA or CeA neurons did not produce nonspecific motor effects. Figure 4.3F shows the number of active lever presses for photo-stimulation under a PR5 schedule of reinforcement. Under this schedule, CeA-ChR2 rats pressed more on the active lever relative to BLA-ChR2 or control rats (main effect of Group: $F_{(2,17)} = 3.77$, $p = 0.007$; CeA-ChR2 > controls, $p = 0.014$; CeA-ChR2 > BLA-ChR2, $p = 0.013$). BLA-ChR2 rats and controls were not different ($p > 0.05$). Thus, across a range of schedules of reinforcement, rats self-administered cued photo-stimulations of CeA neurons, but not BLA neurons. The findings suggest that photo-stimulation of CeA, but not BLA neurons is reinforcing.

Extinction responding. We assessed lever-pressing behavior under extinction conditions during a 40 min session where photo-stimulation was only available from minutes 0–5 and 20–25, under a RR2 schedule. As shown in Figure 4.3G (top), BLA-ChR2 rats did not differ from controls during this session (all p values > 0.05). Presses on the inactive lever also did not differ between groups (FIG. 4.3G, bottom; $p > 0.05$). However, Figure 4.3G (top) also shows that when photo-stimulation was available in the first 5 min of the session, CeA-ChR2 rats pressed more on the active lever relative to controls and BLA-ChR2 rats (Group × Time interaction: $F_{(14,119)} = 4.14$, $p < 0.0001$; main effect of Group: $F_{(2,17)} = 7.69$, $p = 0.004$; CeA-ChR2 vs control rats: $F_{(1,13)} = 10.3$, $p = 0.007$; minutes 0–5, CeA-ChR2 > controls, $p < 0.0001$; CeA-ChR2 vs BLA-ChR2, $F_{(1,8)} = 5.11$, $p = 0.054$; *post hoc* comparisons on minutes 0–5, CeA-ChR2 > BLA-ChR2, $p = 0.0001$. No other comparisons were significant). CeA-ChR2 rats also extinguished their lever-pressing behavior during the extinction session (FIG. 4.3G, top; main effect of Time: $F_{(7,119)} = 6.12$, $p < 0.0001$; minutes 0–5 vs each subsequent 5 min block, all p values < 0.0001). Thus, only CeA-ChR2 rats lever-pressed for photo-stimulation when it was available, and decreased responding when it was not. In contrast, BLA-ChR2 rats and control rats lever-pressed very little, regardless of photo-stimulation availability.

Self-stimulation as a function of laser stimulation frequency. Figure 4.3H shows the influence of stimulation frequency (5, 10, and 20 Hz) on self-administration of photo-stimulations. Sessions stopped after 30 stimulations or 30 min. As a measure of the rate of responding, we analyzed the number of photo-stimulations earned per min. Relative to control rats, CeA-ChR2 rats earned more photo-stimulations/min at 10 and 20 Hz (FIG. 4.3H; Frequency × Group interaction:

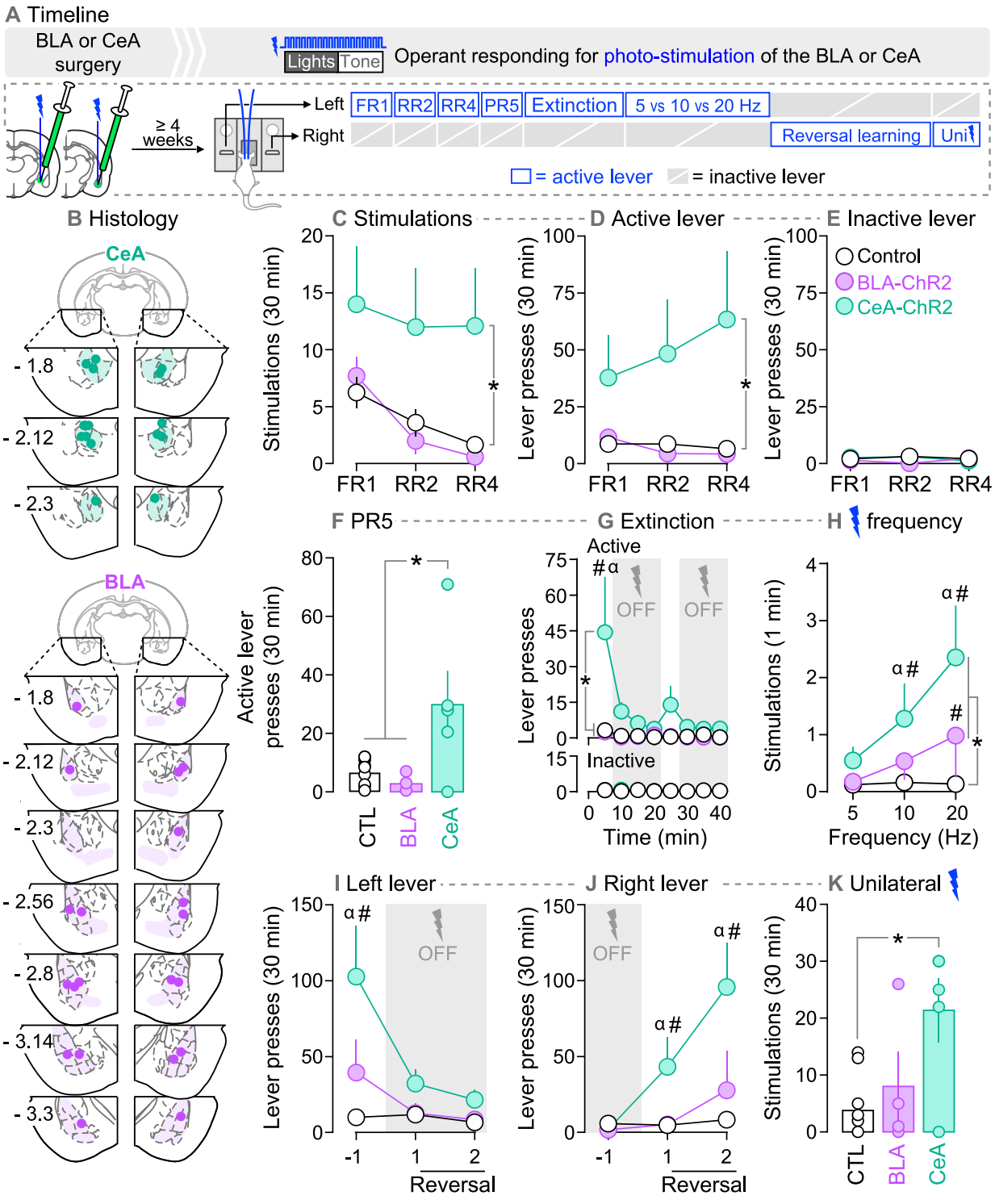

FIG. 4.3 — Photo-stimulation of neurons in the CeA, but not BLA, is reinforcing. (*see next page*) →

$F_{(4,32)} = 4.08$; $p = 0.009$; main effect of Group: $F_{(2,16)} = 5.76$, $p = 0.013$; CeA-ChR2 rats vs controls: $F_{(1,13)} = 10.67$, $p = 0.006$; CeA-ChR2 > controls at 10 Hz, $p = 0.046$, at 20 Hz, $p < 0.0001$). CeA-

(*FIG. 4.3*) → ***A***, In Experiment 2, rats received AAV5-hSyn1-hChR2(H134R)-eYFP or an optically inactive control virus lacking ChR2 (AAV5-hSyn1-eYFP) in the BLA or CeA of both hemispheres. Optic fibers were also implanted bilaterally, above virus injection sites. ***B***, Estimated optic fiber placements in the CeA and BLA (anteroposterior position is shown in mm relative to Bregma). At least 4 weeks after surgery, rats were allowed to press on two levers. Pressing the active lever produced photo-stimulation of BLA or CeA neurons, paired with presentation of a light-tone cue. Pressing the inactive lever had no programmed consequence. ***C***–***E***, Lever pressing was measured under FR1, RR2, and RR4 schedules of laser reinforcement. Responding was also assessed under (***F***) a PR5 schedule of laser reinforcement, and during a (***G***) within-session extinction test. ***H***, Effects of laser stimulation frequency on stimulations earned/min. ***I***, ***J***, Lever pressing under reversal learning conditions. ***K***, Effects of unilateral stimulation, under a RR2 schedule of laser reinforcement. *$p < 0.05$. ***G***, $^{\#}p < 0.05$ versus control rats and BLA-ChR2 rats; α $p < 0.05$, first 5 min block versus all other 5 min blocks in CeA-ChR2 rats. ***H***, $^{\#}p < 0.05$ versus control rats at the same frequency; α $p < 0.05$ versus 5 Hz in CeA-ChR2 rats. ***I***, $^{\#}p < 0.05$ versus control rats in Session −1; α $p < 0.05$ versus Sessions 1 and 2 in CeA-ChR2 rats. ***J***, $^{\#}p < 0.05$ versus control rats in the same test session; α $p < 0.05$ versus Session −1 in CeA-ChR2 rats. n = 4–10/group. Values are mean ± SEM. Individual data are shown on histograms.

ChR2 rats also earned more photo-stimulations/min as stimulation frequency was increased (FIG. 4.3H; main effect of Frequency: $F_{(2,32)} = 9.31$, $p = 0.0006$; CeA-ChR2 rats, 10 > 5 Hz, $p = 0.019$, 20 > 5 Hz, $p < 0.0001$). BLA-ChR2 rats earned more photo-stimulations/min relative to controls only at the highest frequency tested (main effect of Group: $F_{(1,12)} = 6.18$, $p = 0.029$; BLA-ChR2 > controls, at 20 Hz, $p = 0.013$). No other comparisons were statistically significant. Thus, compared with control rats, BLA-ChR2 rats earned more photo-stimulations/min at 20 Hz, whereas CeA-ChR2 rats earned more photo-stimulations/min at both 10 and 20 Hz. Furthermore, only CeA-ChR2 rats increased their self-stimulation behavior with increasing laser frequency.

Reversal learning. Here we determined whether photo-stimulation of CeA or BLA neurons supports reversal learning. Figure 4.3I shows pressing on a lever that produced laser stimulation on Session −1, but not on subsequent sessions. Figure 4.3J shows pressing on a lever that did not produce laser stimulation on Session −1, but did so on subsequent sessions. As seen in Figure 4.3I, CeA-ChR2 but not BLA-ChR2 rats pressed more on the reinforced lever relative to control rats (Group × Session interaction: $F_{(4,32)} = 5.46$, $p = 0.002$; main effect of Group: $F_{(2,16)} = 10.05$, $p = 0.002$; CeA-ChR2 vs controls: $F_{(1,13)} = 19.47$, $p = 0.0007$; CeA-ChR2 > controls on Session −1, $p < 0.0001$). CeA-ChR2 rats also pressed significantly less on this lever after reversal versus before (main effect of Session: $F_{(2,32)} = 11.98$, $p = 0.0001$; CeA-ChR2 rats, Session −1 > Session 1: $p = 0.0002$, Session −1 > Session 2: $p < 0.0001$). As seen in Figure 4.3J, after lever reversal, CeA-

ChR2 but not BLA-ChR2 rats pressed more on the newly reinforced lever relative to controls (Group × Session interaction: $F_{(4,32)} = 5.94$, $p = 0.001$; main effect of Group: $F_{(2,16)} = 8.59$, $p = 0.003$; CeA-ChR2 rats > controls, Session 1: $p = 0.033$, Session 2: $p < 0.0001$). CeA-ChR2 rats also pressed more on this lever after reversal versus before (main effect of Session: $F_{(2,32)} = 11.84$, $p = 0.0001$; CeA-ChR2 rats, Session −1 > Session 1: $p = 0.035$, Session −1 > Session 2: $p < 0.0001$). In summary, photo-stimulation of CeA neurons both reliably reinforced lever-pressing behavior and supported reversal learning, whereas photo-stimulation of BLA neurons supported neither response.

Unilateral laser stimulation. Last, we determined whether unilateral photo-stimulation of CeA or BLA neurons was reinforcing. Figure 4.3K shows that CeA-ChR2 but not BLA-ChR2 rats earned more unilateral laser stimulations relative to controls (main effect of Group: $F_{(2,16)} = 6.24$, $p = 0.01$; CeA-ChR2 > controls, $p = 0.009$). Therefore, unilateral stimulation of CeA, but not BLA neurons sustains self-stimulation.

In summary, across different schedules of reinforcement, operant testing conditions and photo-stimulation parameters, rats did not reliably self-administer photo-stimulation of BLA neurons. In contrast, rats reliably self-administered photo-stimulation of CeA neurons, indicating that it is reinforcing. These findings show that photo-stimulation of BLA versus CeA neurons has dissociable effects, and that CeA but not BLA neurons carry a primary reward signal.

Experiment 3a: Effects of Photo-stimulating ChR2-expressing BLA Neurons During Pavlovian CS-UCS Conditioning on CS-evoked Conditioned Approach

Figure 4.4B shows estimated optic fiber placements in the BLA. We first determined the effects of BLA photo-stimulation on CS-evoked conditioned approach behavior (FIG. 4.4A). This was assessed by analyzing the ratio of nose-pokes into the water receptacle during each 5 s light cue presentation (CSR), versus during the 5 s period preceding each CS presentation (PCSR). Figure 4.4C shows the effects of photo-stimulation of ChR2-expressing BLA neurons on the CSR/PCSR ratio over Pavlovian conditioning sessions. Average CSR/PCSR ratios progressively increased over sessions in all groups, indicating that rats learned the CS-UCS contingency (FIG. 4.4C; main effect of Session: $F_{(3,66)} = 20.12$, $p < 0.0001$). Pairing photo-stimulation of BLA neurons with CS presentations ("ChR2-Paired laser" group) potentiated conditioned approach behavior relative to

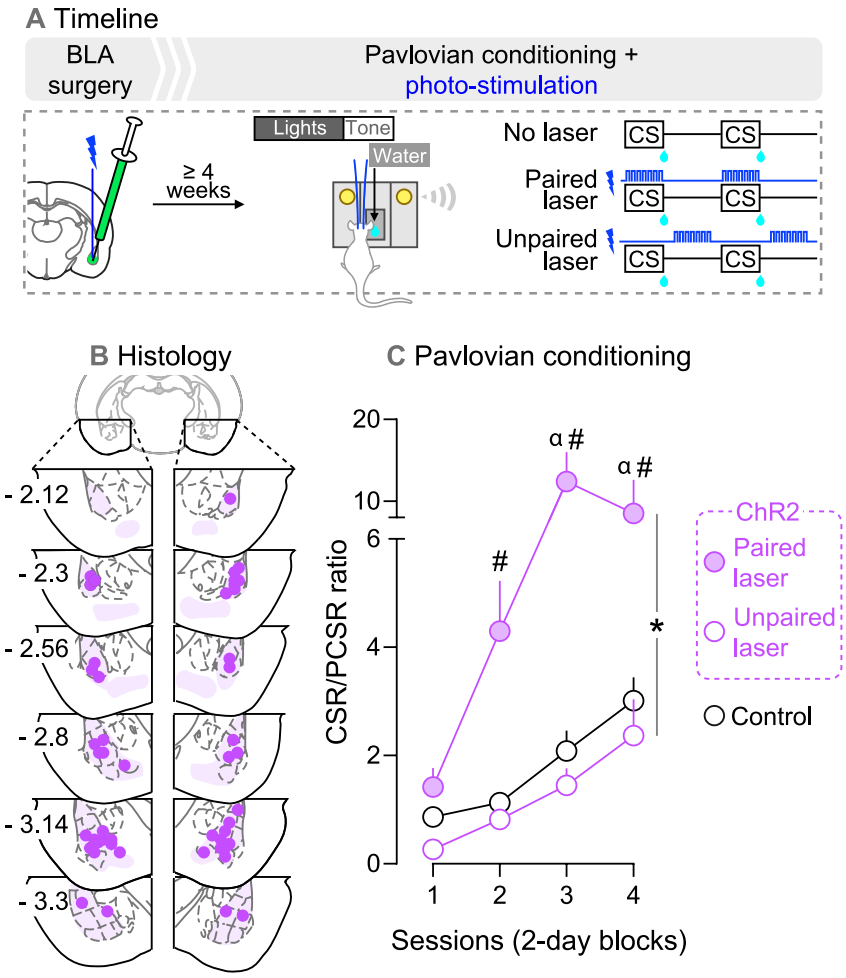

FIG. 4.4 — Photo-stimulation of BLA neurons during CS presentation potentiates CS-evoked conditioned approach. ***A***, In Experiment 3a, rats received AAV5-hSyn1-hChR2(H134R)-eYFP or an optically inactive control virus lacking ChR2 (AAV5-hSyn1-eYFP) in the BLA of both hemispheres. Optic fibers were also implanted bilaterally, above virus injection sites. ***B***, Estimated optic fiber placements in the BLA (anteroposterior position is shown in mm relative to Bregma). At least 4 weeks after surgery, rats were water-restricted (2 h/d) and received Pavlovian conditioning sessions where a light-tone CS predicted water (100 µl) delivery (UCS) into a recessed dish. During conditioning sessions, photo-stimulation of BLA neurons was either explicitly paired or unpaired with CS presentation, in independent groups of rats. Control rats included ChR2 and eYFP rats that did not receive photo-stimulations and eYFP rats that received photo-stimulations. ***C***, CS-paired but not CS-unpaired BLA photo-stimulation enhanced CSR/PCSR ratios (ratio of nose-pokes into the water dish during each 5 s CS presentation versus nose-pokes made during the 5 s period preceding each CS presentation). This indicates enhanced Pavlovian learning. n = 3–19/group. *p < 0.05 versus ChR2-Unpaired laser group and control group; $^{\#}p < 0.05$ versus control group on the same session; α $p < 0.05$ versus ChR2-Unpaired laser group on the same session. Values are mean ± SEM.

all other conditions (FIG. 4.4C; Group × Session interaction: $F_{(6,66)} = 8.31$, $p < 0.0001$; main effect of Group: $F_{(2,22)} = 21.81$, $p < 0.0001$; ChR2 Paired laser > Controls, Session 2: $p = 0.025$, Session

3: $p < 0.0001$, Session 4: $p < 0.0001$; ChR2 Paired laser > ChR2 Unpaired laser, Session 3: $p < 0.0001$, Session 4: $p = 0.0004$). No other comparisons were significant. Thus, photo-stimulation of BLA neurons potentiated CS-evoked conditioned approach behavior over time, but only if photo-stimulation was explicitly paired with CS presentation.

Experiment 3b: Effects of Photo-stimulating ChR2-expressing BLA Neurons During Operant Responding for a CS

Here, we sought to determine whether BLA photo-stimulation would potentiate instrumental pursuit of the CS. To this end, we used rats from Experiment 3a that had undergone Pavlovian CS-UCS conditioning without laser stimulation. These are rats with ChR2-expressing BLA neurons that had not received laser photo-stimulation, and rats with eYFP-expressing BLA neurons. We determined in these rats whether BLA photo-stimulation during operant responding for the CS enhances responding for that CS (FIG. 4.5A). Figure 4.5B shows presses on an active lever that produced CS presentation and on an inactive lever, during a session where rats did not receive laser stimulation. Across groups, rats pressed more on the active versus inactive lever (main effect of Lever Type: $F_{(1,13)} = 13.86$, $p = 0.003$). This indicates that the CS acquired incentive properties. There was neither a main effect of Group nor a Group × Lever Type interaction effect (all p values > 0.05). Thus, without laser stimulation, ChR2 and eYFP rats show similar incentive motivation for the CS.

Figure 4.5C shows presses on the active and inactive levers when CS presentations were paired with BLA photo-stimulation at different laser frequencies (5, 10, or 20 Hz). Figure 4.5C shows that both ChR2 and eYFP rats pressed more on the active versus inactive lever (main effect of Lever Type: $F_{(1,26)} = 18.3$, $p = 0.001$; eYFP rats: $F_{(1,12)} = 6.31$, $p = 0.027$; ChR2 rats: $F_{(1,14)} = 12.19$, $p = 0.004$). Thus, both groups showed incentive motivation for the CS under these conditions. In addition, ChR2 rats pressed more on the active lever than did eYFP rats (FIG. 4.5C; main effect of Group: $F_{(1,13)} = 5.39$, $p = 0.04$; no other comparisons were significant). This suggests that photo-stimulation of BLA neurons potentiates the expression of incentive motivation for the CS. Figure 4.5D shows lever-pressing behavior when pressing the active lever produced the CS and photo-stimulation 3 s later, such that the CS and photo-stimulation were unpaired. Only ChR2 rats pressed more on the active versus inactive lever (FIG. 4.5D; Group × Lever Type interaction: $F_{(1,13)} = 5.79$, $p = 0.032$; main effect of Lever Type: $F_{(1,13)} = 20.13$, $p = 0.0006$; ChR2 rats: active > inactive lever,

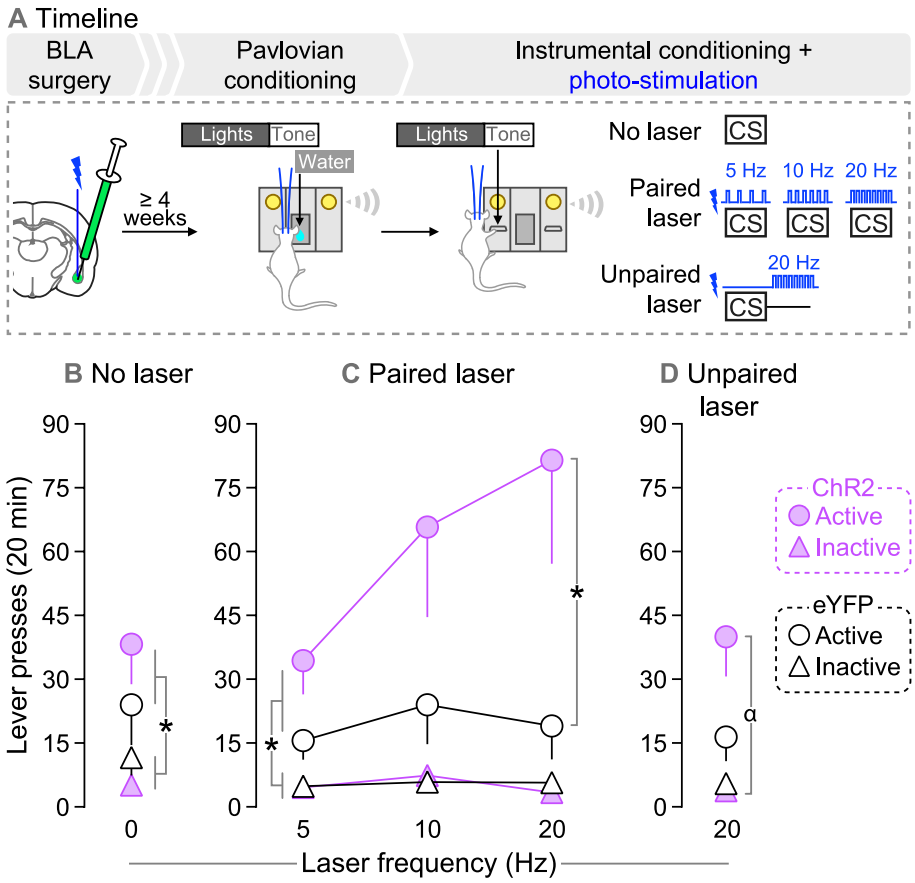

FIG. 4.5 — Photo-stimulation of BLA neurons potentiates incentive motivation for a CS. ***A***, In Experiment 3b, rats that had not received photo-stimulation of BLA neurons during previous Pavlovian CS-UCS conditioning (eYFP rats and ChR2-No laser control rats from Experiment 3a) were used to assess the effects of photo-stimulation of BLA neurons during instrumental responding for the CS. ***B***, During a session without laser stimulation, both groups pressed more on the active versus inactive lever, and there were no group differences in lever-pressing behavior. ***C***, During sessions where BLA photo-stimulation was paired with each earned CS presentation, ChR2 rats pressed more on the active lever than eYFP rats did. This indicates that photo-stimulation of BLA neurons during CS presentation enhances the incentive motivational value of the CS. ***D***, During sessions where BLA photo-stimulation was explicitly unpaired with each earned CS presentation, ChR2 rats still pressed more on the active versus inactive lever, but lever-pressing behavior did not differ between ChR2 and eYFP rats. n = 7–8/group. *$p < 0.05$; α $p < 0.05$ active lever presses versus inactive lever presses in ChR2 rats. Values are mean ± SEM.

$p = 0.0004$). However, ChR2 rats did not press more on the active lever than eYFP rats ($p > 0.05$). No other comparisons were significant. Last, BLA photo-stimulation, when it was either paired or unpaired with CS presentation, did not influence nose pokes into the water receptacle (data not shown, all p values > 0.05). This suggests that BLA photo-stimulation did not increase the urge to consume the associated water UCS. Together, these results show that photo-stimulation of BLA

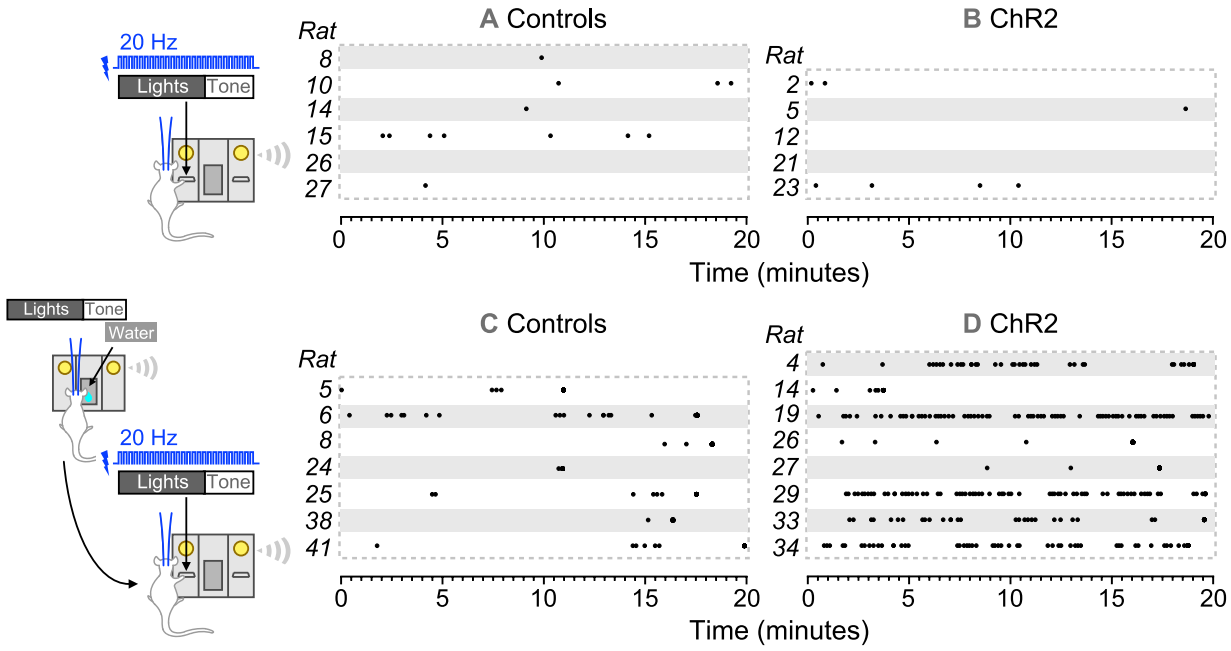

FIG. 4.6 — Photo-stimulation of BLA neurons potentiates motivation for a discrete environmental stimulus if and only if this stimulus was previously associated with a primary reward. Each dot indicates reinforcements earned by individual rats that were lever pressing for presentations of a light-tone stimulus. Data are shown for individual control rats (***A***, ***C***) and individual rats receiving photo-stimulation of ChR2-expressing BLA neurons paired with each stimulus presentation (***B***, ***D***). ***A***, ***B***, When the light-tone stimulus had not previously been associated with a primary reward, BLA photo-stimulation did not change the number of stimulus presentations earned. ***C***, ***D***, When the light-tone stimulus had previously been associated with a water reward, BLA photo-stimulation enhanced responding.

neurons during operant responding for the CS potentiated incentive motivation for that CS, and that this effect was strongest when photo-stimulation was explicitly paired with each CS presentation.

Together, the results of Experiments 2 and 3b indicate that BLA photo-stimulation increases instrumental pursuit of a discrete stimulus, if and only if that stimulus reliably predicts a primary reward (water). That is, BLA photo-stimulation selectively potentiates the pursuit of environmental stimuli that possess conditioned incentive properties. Figure 4.6 highlights this effect. It shows reinforcements earned by individual rats lever pressing for presentations of a light-tone stimulus not previously associated with a reward (FIG. 4.6A,B; rats from Experiment 2) or a light-tone stimulus previously associated with a water reward (FIG. 4.6C,D; rats from Experiment 3b). When the light-tone stimulus had no relationship with a primary reward, BLA photo-stimulation did not

significantly change the number of stimulus presentations earned (FIG. 4.6A,B). After the light-tone stimulus had been paired with water, control rats pursued this CS more avidly (FIG. 4.6, compare A, C), and BLA photo-stimulation potentiated this effect (FIG. 4.6, compare C, D).

Additionally, photo-stimulation of BLA neurons increased both CS-evoked conditioned approach (FIG. 4.4C; Experiment 3a) and operant responding for the CS (FIG. 4.5C; Experiment 3b). These two CS effects rely on common but also partially dissociable neurobiological and psychological processes (Flagel *et al*., 2011; Tabbara *et al*., 2016). In agreement, in the control rats represented in Figure 4.4C, there was no significant correlation between average CSR/PCSR ratios over the last 4 d of Pavlovian conditioning and active lever presses during a subsequent instrumental conditioning session (without laser; data not shown; $r^2 = 0.002$, $p = 0.85$).

Experiment 4: Effects of Intra-amygdala D-amphetamine Infusions on the Incentive Motivational Effects of a CS

After CS-UCS Pavlovian conditioning, rats were given instrumental responding tests where they could lever-press for the CS (FIG. 4.7A). Immediately before these tests, rats received bilateral infusions of d-amphetamine (0, 10, or 30 μg/hemisphere) into the BLA or into the amygdala without targeting the BLA specifically. Figure 4.7B shows estimated location of injector tips when both cannulae were specifically in the BLA (top) or simply in the amygdala, but without targeting the BLA exclusively (bottom). The rats learned the CS-UCS contingency, as indicated by a progressive increase in CSR/PCSR ratio (FIG. 4.7C; main effect ofu Session: $F_{(4,76)} = 11.12$, $p < 0.0001$; 7*F*; main effect of Session: $F_{(4,56)} = 5.04$, $p = 0.002$). Figure 4.7D,E–G,H show that rats in both experimental groups pressed more on the active versus inactive lever (FIG. 4.7D,E; Dose × Lever Type interaction: $F_{(2,37)} = 5.31$, $p = 0.009$; main effect of Lever Type: $F_{(1,37)} = 142.4$; $p < 0.0001$; 7*G*,*H*; main effect of Lever Type: $F_{(1,27)} = 25.61$, $p < 0.0001$). Thus, all rats spontaneously learned a new operant response to produce the CS, indicating that the CS acquired incentive value. d-Amphetamine influenced active lever pressing only when infused specifically into the BLA, such that active lever pressing was greatest at 30 μg/hemisphere d-amphetamine (FIG. 4.7D; main effect of Dose: $F_{(2,37)} = 4.5$, $p = 0.018$; 30 vs 0 μg, $p = 0.0002$; 30 vs 10 μg, $p = 0.027$). In contrast, d-amphetamine did not alter lever-pressing behavior in rats that received infusions into the amygdala, without specifically targeting the BLA (FIG. 4.7G,H; all p values > 0.05). No other comparisons were statistically significant. Last, neither intra-BLA nor intra-amygdala d-amphetamine altered

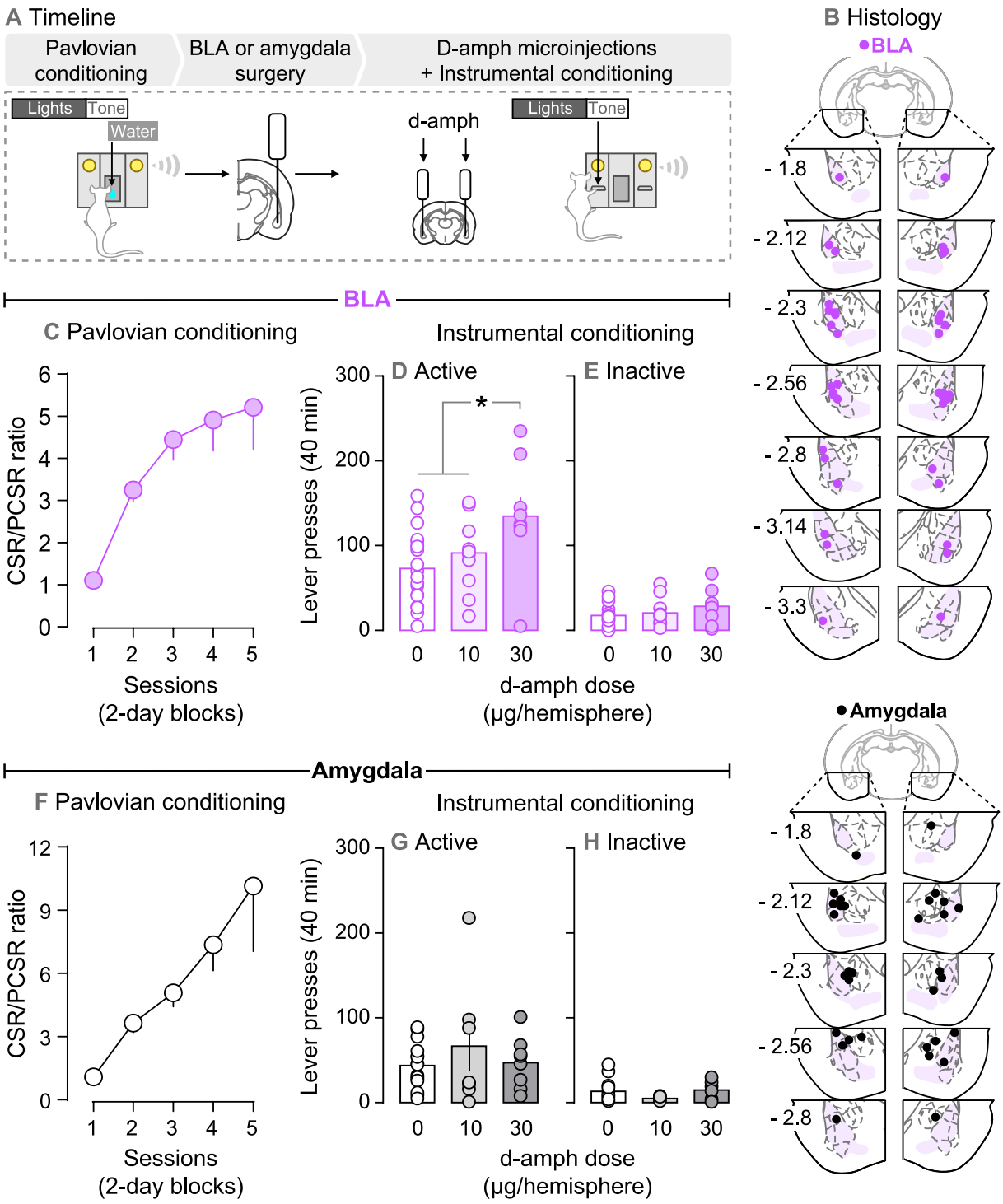

FIG. 4.7 — Bilateral infusions of d-amphetamine into the BLA intensify the incentive value of a CS. ***A***, In Experiment 4, rats received Pavlovian conditioning. Bilateral cannulae were then (*see next page*) →

the number of nose pokes into the water receptacle (data not shown, all p values > 0.05). This suggests that d-amphetamine infusions into the amygdala did not increase the urge to consume the

(*FIG. 4.7*) → implanted specifically into the BLA (BLA group) or into the amygdala without targeting the BLA specifically (Amygdala group). ***B***, Estimated injector tip placements in BLA rats and in Amygdala rats (anteroposterior position is shown in mm relative to Bregma). ***C***, ***F***, During Pavlovian conditioning, rats reliably learned the CS-unconditioned stimulus contingency, as indicated by increasing CSR/PCSR ratios over sessions (ratio of nose-pokes into the water receptacle made during each 5 s CS presentation versus during the 5 s period preceding each CS presentation). Next, we assessed the effects of intracerebral d-amphetamine infusions (0, 10, or 30 μg/hemisphere) on instrumental responding for the CS. Both (***D***, ***E***) BLA and (***G***, ***H***) Amygdala rats pressed more on the active versus inactive lever, indicating that the CS acquired incentive motivational value. ***D***, d-Amphetamine influenced responding for the CS only when the drug was infused into the BLA. n = 7–20/group. *$p < 0.05$. Values are mean ± SEM. Individual data are shown on histograms.

associated water UCS. Thus, the findings show that intra-BLA d-amphetamine intensified incentive motivation for the CS.

DISCUSSION

We evaluated the contributions of the BLA to appetitive Pavlovian conditioning and to the instrumental pursuit of a reward-predictive CS. First, photo-stimulation of BLA neurons was not intrinsically reinforcing, whereas photo-stimulation of neurons in the adjacent CeA was. Second, photo-stimulation of BLA neurons during Pavlovian CS-UCS conditioning enhanced CS-evoked conditioned approach, indicating potentiated anticipation of the primary reward. Third, photo-stimulation of BLA neurons potentiated operant responding for the CS, suggesting enhanced CS incentive value. Finally, intra-BLA infusions of d-amphetamine also augmented operant responding for the CS, suggesting that a local increase in monoamine neurotransmission is also involved in enhanced conditioned incentive motivation. Thus, increased neuronal activity within the BLA facilitates cue-controlled behavior by both increasing cue-induced anticipation of impending rewards and making reward cues more attractive.

Photo-stimulation of CeA, but not BLA Neurons, Is Reinforcing

Rats reliably lever pressed for photo-stimulation of CeA, but not BLA neurons, suggesting that CeA neurons carry a primary reward signal. Our findings agree with earlier work showing that electrical stimulation of CeA cells is reinforcing (Prado-Alcalá and Wise, 1984; Kane *et al*., 1991). CeA neurons are mostly GABAergic, but they express different neuropeptides and have different

anatomical connections. More recent studies show that stimulation of specific neuronal populations in the CeA can also be reinforcing. This includes CeA neurons expressing corticotropin-releasing hormone, somatostatin, neurotensin, and/or tachykinin 2 (Baumgartner *et al*., 2017; Kim *et al*., 2017), and CeA→medial prefrontal cortex neurons (Seo *et al*., 2016). In contrast, using photo-stimulation of CeA neurons without regards to cell subtype as done here, Berridge and colleagues report that CeA photo-stimulation is not reinforcing (Robinson *et al*., 2014; Warlow *et al*., 2017). This could involve the CeA subregion where photo-stimulation was applied. Robinson *et al*. (2014) and Warlow *et al*. (2017) implanted optic fibers in the posterior CeA, whereas we implanted in the anterior CeA. Our rats did not reliably self-administer photo-stimulation of BLA neurons. Rats will electrically self-stimulate some BLA subregions (Prado-Alcalá and Wise, 1984; Kane *et al*., 1991), and studies using optogenetic methods suggest that self-stimulation depends on the BLA circuit targeted. For instance, photo-stimulation of BLA→nucleus accumbens terminals is reinforcing (Stuber *et al*., 2011; Britt *et al*., 2012; Namburi *et al*., 2015), but photo-stimulation of BLA→medial CeA terminals produces avoidance (Namburi *et al*., 2015). The absence of BLA self-stimulation here could involve the hSyn promoter we used. It confers neuron-specific transgene expression, but it does not target neuron subtypes.

Via distinct cell types and connections, amygdala nuclei and subregions exert many functions, including both appetitive and defensive behaviors (Gallagher and Chiba, 1996). Future studies will be important to examine roles of specific CeA and BLA neuron subtypes and projections in appetitive behavior. As this research unfolds, our results support the idea that while the BLA and CeA are connected and can play similar roles in motivational processes (Wassum *et al*., 2011), they also have distinct appetitive functions (Corbit and Balleine, 2005; Robinson *et al*., 2014; Warlow *et al*., 2017).

Photo-stimulation of BLA Neurons during CS-UCS Conditioning Enhances CS-evoked Conditioned Approach

During Pavlovian conditioning, we paired photo-stimulation of BLA neurons with CS presentation. This potentiated CS-evoked conditioned approach, as shown by more CS-triggered visits to the water dish. This suggests enhanced anticipation of the CS-associated water reward. Explicitly unpairing BLA stimulation and CS presentation did not influence CS-evoked conditioned approach. Thus, increasing BLA neuron activity when a CS is presented amplifies associative CS-

UCS learning. Increased CS-triggered visits to the water dish could suggest that BLA photo-stimulation enhances the appetitive value of water. This is possible, but unlikely, because BLA lesions do not alter water consumption (Cador *et al*., 1989). Instead, enhanced CS-evoked conditioned approach likely involves changes in how BLA neurons represent the CS and/or how they encode the CS-UCS association. CS-triggered conditioned approach behaviors can reflect both the predictive and incentive effects of CS. BLA stimulation could increase CS-triggered visits to the water dish by enhancing either or both effects. For instance, rats might visit the water dish during CS presentation because the CS is evoking an incentive urge to drink the associated water (Weingarten, 1983). If so, then BLA photo-stimulation during the CS could increase visits to the water dish by enhancing this conditioned incentive urge (Holland *et al*., 2002). Similarly, the water dish is also a CS in our experiments, less predictive than the light-tone CS, but more proximal to the water UCS. As such, BLA photo-stimulation could have increased water dish visits by enhancing the incentive value of the dish. We do not believe this is the case, because BLA photo-stimulation increased the number of water dish visits only when this stimulation was explicitly paired with the light-tone CS.

Photo-stimulation of BLA Neurons or D-amphetamine Infusion into the BLA Enhances CS Incentive Value

Once the CS had been imbued with incentive value through prior association with an appetitive UCS, BLA photo-stimulation amplified the expression of this incentive motivation (as measured by lever-pressing reinforced by the CS alone). Infusing d-amphetamine into the BLA had the same effect, suggesting that increases in monoamine-mediated neurotransmission in the BLA are involved (Ledford *et al*., 2003; Bernardi *et al*., 2009; Gremel and Cunningham, 2009; Lintas *et al*., 2011). This extends lesion studies showing that the BLA is necessary for operant responding reinforced by a CS (Cador *et al*., 1989; Burns *et al*., 1993). BLA photostimulation or d-amphetamine infusions into the BLA could have enhanced instrumental responding for the CS by potentiating the appetitive value of the associated water reward. This is unlikely, because neither manipulation influenced the number of water dish visits during instrumental tests. In addition, the increased lever pressing during tests of instrumental responding for the CS likely does not involve any intrinsically reinforcing effects of BLA photo-stimulation. Indeed, our BLA photo-stimulation parameters did not reliably support self-stimulation behavior. Instead, the BLA stores information

about CS value, which is then used to guide behavior (Cardinal *et al*., 2002). As such, stimulation of BLA neurons could enhance operant responding for a CS by potentiating the incentive value of the CS itself or of the CS-associated reward representation (Mogenson, 1987; Everitt and Robbins, 1992).

Conclusions

Increased neuronal activity in BLA-dependent circuits amplifies control over behavior by an appetitive cue, and this involves two overlapping, but also dissociable psychological mechanisms. A first mechanism involves enhanced CS-UCS associative learning, such that the CS triggers increased conditioned approach, and increased anticipation of the primary reward. This prepares animals to engage with the forthcoming reward. The second mechanism involves amplified incentive motivation to pursue the CS, such that animals show enhanced instrumental responding for the CS. Thus, when reward cues are present in the environment, increased recruitment of BLA-dependent pathways could promote excessive pursuit of associated rewards both by augmenting anticipation for these rewards and making reward-paired cues more attractive in their own right.

REFERENCES OF CHAPTER IV

Ambroggi F, Ishikawa A, Fields HL, Nicola SM (2008) Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. Neuron 59:648-661.

Baumgartner HM, Olney JJ, Warlow SM, Schulkin J, Berridge KC (2017) Investigating corticotropin releasing factor in mediating appetitive behavior. Published abstract for the Society for Neuroscience Annual Meeting, Washington, DC.

Bernardi RE, Ryabinin AE, Berger SP, Lattal KM (2009) Post-retrieval disruption of a cocaine conditioned place preference by systemic and intrabasolateral amygdala beta2- and alpha1-adrenergic antagonists. Learn Mem 16:777-789.

Bindra D (1978) How adaptive behavior is produced: a perceptual-motivation alternative to response reinforcement. Behavioral and Brain Sciences 1:41-52.

Bolles RC (1972) Reinforcement, expectancy, and learning. Psychological Review 79:394-409.

Britt JP, Benaliouad F, McDevitt RA, Stuber GD, Wise RA, Bonci A (2012) Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens. Neuron 76:790-803.

Brown EE, Fibiger HC (1993) Differential effects of excitotoxic lesions of the amygdala on cocaine-induced conditioned locomotion and conditioned place preference. Psychopharmacology 113:123-130.

Burns LH, Robbins TW, Everitt BJ (1993) Differential effects of excitotoxic lesions of the basolateral amygdala, ventral subiculum and medial prefrontal cortex on responding with conditioned reinforcement and locomotor activity potentiated by intra-accumbens infusions of D-amphetamine. Behavioural brain research 55:167-183.

Cador M, Robbins TW, Everitt BJ (1989) Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. Neuroscience 30:77-86.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26:321-352.

Corbit LH, Balleine BW (2005) Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:962-970.

de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 75:134-143.

Dittgen T, Nimmerjahn A, Komai S, Licznerski P, Waters J, Margrie TW, Helmchen F, Denk W, Brecht M, Osten P (2004) Lentivirus-based genetic manipulations of cortical neurons and their optical and electrophysiological monitoring in vivo. Proceedings of the National Academy of Sciences of the United States of America 101:18206-18211.

El Hage C, Bedard AM, Samaha AN (2015) Antipsychotic treatment leading to dopamine supersensitivity persistently alters nucleus accumbens function. Neuropharmacology.

Everitt B, Robbins T (1992) Amygdala-Ventral Striatal Interactions and Reward-related Processes. The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction New York, NY, US: Wiley-Liss.:401-409.

Everitt BJ, Morris KA, O'Brien A, Robbins TW (1991) The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience 42:1-18.

Fisher RS (1989) Animal models of the epilepsies. Brain Res Brain Res Rev 14:245-278.

Flagel SB, Akil H, Robinson TE (2009) Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. Neuropharmacology 56 Suppl 1:139-148.

Flagel SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I, Akers CA, Clinton SM, Phillips PE, Akil H (2011) A selective role for dopamine in stimulus-reward learning. Nature 469:53-57.

Gabriele A, See RE (2010) Reversible inactivation of the basolateral amygdala, but not the dorsolateral caudate putamen, attenuates consolidation of cocaine-cue associative learning in a reinstatement model of drug-seeking. The European journal of neuroscience 32:1024-1029.

Gallagher M, Chiba AA (1996) The amygdala and emotion. Current opinion in neurobiology 6:221-227.

Goddard GV, McIntyre DC, Leech CK (1969) A permanent change in brain function resulting from daily electrical stimulation. Exp Neurol 25:295-330.

Gremel CM, Cunningham CL (2009) Involvement of amygdala dopamine and nucleus accumbens NMDA receptors in ethanol-seeking behavior in mice. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 34:1443-1453.

Grimm JW, See RE (2000) Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 22:473-479.

Hearst E, Jenkins HM (1974) Sign-tracking: The Stimulus-reinforcer Relation and Directed Action. Psychonomic Society, Austin.

Holland PC, Petrovich GD, Gallagher M (2002) The effects of amygdala lesions on conditioned stimulus-potentiated eating in rats. Physiol Behav 76:117-129.

Huff ML, Miller RL, Deisseroth K, Moorman DE, LaLumiere RT (2013) Posttraining optogenetic manipulations of basolateral amygdala activity modulate consolidation of inhibitory avoidance memory in rats. Proceedings of the National Academy of Sciences of the United States of America 110:3597-3602.

Ilango A, Kesner AJ, Broker CJ, Wang DV, Ikemoto S (2014) Phasic excitation of ventral tegmental dopamine neurons potentiates the initiation of conditioned approach behavior: parametric and reinforcement-schedule analyses. Front Behav Neurosci 8:155.

Kane F, Coulombe D, Miliaressis E (1991) Amygdaloid self-stimulation: a movable electrode mapping study. Behav Neurosci 105:926-932.

Kantak KM, Black Y, Valencia E, Green-Jordan K, Eichenbaum HB (2002) Dissociable effects of lidocaine inactivation of the rostral and caudal basolateral amygdala on the maintenance and reinstatement of cocaine-seeking behavior in rats. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:1126-1136.

Kim J, Pignatelli M, Xu S, Itohara S, Tonegawa S (2016) Antagonistic negative and positive neurons of the basolateral amygdala. Nat Neurosci 19:1636-1646.

Kim J, Zhang X, Muralidhar S, LeBlanc SA, Tonegawa S (2017) Basolateral to Central Amygdala Neural Circuits for Appetitive Behaviors. Neuron 93:1464-1479 e1465.

Lajoie J, Bindra D (1976) An interpretation of autoshaping and related phenomena in terms of stimulus-incentive contingencies alone. Can J Psychol 30:157-173.

Ledford CC, Fuchs RA, See RE (2003) Potentiated reinstatement of cocaine-seeking behavior following D-amphetamine infusion into the basolateral amygdala. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 28:1721-1729.

Lin JY, Lin MZ, Steinbach P, Tsien RY (2009) Characterization of engineered channelrhodopsin variants with improved properties and kinetics. Biophys J 96:1803-1814.

Lintas A, Chi N, Lauzon NM, Bishop SF, Gholizadeh S, Sun N, Tan H, Laviolette SR (2011) Identification of a dopamine receptor-mediated opiate reward memory switch in the basolateral amygdala-nucleus accumbens circuit. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:11172-11183.

Mackintosh NJ (1974) The psychology of animal learning. London ; New York: Academic Press.

McDonald RJ, Hong NS (2004) A dissociation of dorso-lateral striatum and amygdala function on the same stimulus-response habit task. Neuroscience 124:507-513.

McDonald RJ, Yim TT, Lehmann H, Sparks FT, Zelinski EL, Sutherland RJ, Hong NS (2010) Expression of a conditioned place preference or spatial navigation task following muscimol-induced inactivations of the amygdala or dorsal hippocampus: A double dissociation in the retrograde direction. Brain research bulletin 83:29-37.

McLaughlin J, See RE (2003) Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology 168:57-65.

McNamara JO, Byrne MC, Dasheiff RM, Fitz JG (1980) The kindling model of epilepsy: a review. Progress in neurobiology 15:139-159.

Millan EZ, Kim HA, Janak PH (2017) Optogenetic activation of amygdala projections to nucleus accumbens can arrest conditioned and unconditioned alcohol consummatory behavior. Neuroscience 360:106-117.

Mogenson GJ (1987) Limbic-motor integration. Prog Psychobiol Physiol Psvchol 12:117-170.

Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichmann R, Holden SS, Mertens KL, Anahtar M, Felix-Ortiz AC, Wickersham IR, Gray JM, Tye KM (2015) A circuit mechanism for differentiating positive and negative associations. Nature 520:675-678.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd Edition. Sydney ; Orlando: Academic Press.

Prado-Alcala R, Wise RA (1984) Brain stimulation reward and dopamine terminal fields. I. Caudate-putamen, nucleus accumbens and amygdala. Brain research 297:265-273.

Rescorla RA (1988) Pavlovian conditioning. It's not what you think it is. Am Psychol 43:151-160.

Rescorla RA, Solomon RL (1967) Two-process learning theory: Relationships between Pavlovian conditioning and instrumental learning. Psychol Rev 74:151-182.

Robbins TW (1978) The acquisition of responding with conditioned reinforcement: effects of pipradrol, methylphenidate, d-amphetamine, and nomifensine. Psychopharmacology 58:79-87.

Robinson MJ, Warlow SM, Berridge KC (2014) Optogenetic excitation of central amygdala amplifies and narrows incentive motivation to pursue one reward above another. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:16567-16580.

Rogers JL, Ghee S, See RE (2008) The neural circuitry underlying reinstatement of heroin-seeking behavior in an animal model of relapse. Neuroscience 151:579-588.

Rossi MA, Sukharnikova T, Hayrapetyan VY, Yang L, Yin HH (2013) Operant self-stimulation of dopamine neurons in the substantia nigra. PLoS One 8:e65799.

Seo DO, Funderburk SC, Bhatti DL, Motard LE, Newbold D, Girven KS, McCall JG, Krashes M, Sparta DR, Bruchas MR (2016) A GABAergic Projection from the Centromedial Nuclei of the Amygdala to Ventromedial Prefrontal Cortex Modulates Reward Behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience 36:10831-10842.

Stefanik MT, Kalivas PW (2013) Optogenetic dissection of basolateral amygdala projections during cue-induced reinstatement of cocaine seeking. Front Behav Neurosci 7:213.

Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, Tye KM, Kempadoo KA, Zhang F, Deisseroth K, Bonci A (2011) Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. Nature 475:377-380.

Tabbara RI, Maddux JM, Beharry PF, Iannuzzi J, Chaudhri N (2016) Effects of sucrose concentration and water deprivation on Pavlovian conditioning and responding for conditioned reinforcement. Behav Neurosci 130:231-242.

Tolman EC (1932) Purposive behavior in animals and men. London, England: Century/Random House UK.

Trujillo-Pisanty I, Sanio C, Chaudhri N, Shizgal P (2015) Robust optical fiber patch-cords for in vivo optogenetic experiments in rats. MethodsX 2:263-271.

Tye KM, Janak PH (2007) Amygdala neurons differentially encode motivation and reinforcement. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:3937-3945.

Tye KM, Stuber GD, de Ridder B, Bonci A, Janak PH (2008) Rapid strengthening of thalamo-amygdala synapses mediates cue-reward learning. Nature 453:1253-1257.

Warlow SM, Robinson MJF, Berridge KC (2017) Optogenetic Central Amygdala Stimulation Intensifies and Narrows Motivation for Cocaine. The Journal of neuroscience : the official journal of the Society for Neuroscience 37:8330-8348.

Wassum KM, Cely IC, Balleine BW, Maidment NT (2011) Micro-opioid receptor activation in the basolateral amygdala mediates the learning of increases but not decreases in the incentive value of a food reward. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:1591-1599.

Weingarten HP (1983) Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. Science 220:431-433.

White NM, McDonald RJ (1993) Acquisition of a spatial conditioned place preference is impaired by amygdala lesions and improved by fornix lesions. Behavioural brain research 55:269-281.

Zhang F, Gradinaru V, Adamantidis AR, Durand R, Airan RD, de Lecea L, Deisseroth K (2010) Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. Nature protocols 5:439-456.

CHAPTER V. General Discussion

1. ANTIPSYCHOTIC-EVOKED DOPAMINE SUPERSENSITIVITY: NEW INSIGHTS AND PERSPECTIVES

Antipsychotic-evoked dopamine supersensitivity could increase the risk of treatment failure and psychotic relapse in schizophrenia patients (Chouinard and Jones, 1980). In laboratory animals, antipsychotic-evoked dopamine supersensitivity also produces a tolerance to antipsychotic-like effects and exacerbates the behavioural response to dopamine stimulation. In Chapters II and III, we used the exaggerated psychomotor response to the dopamine/monoamine agonist d-amphetamine to probe the behavioural and neurochemical effects of dopamine supersensitivity produced by a continuous exposure to the antipsychotic drug haloperidol. In a first experiment, we tested the hypothesis that the central effects of d-amphetamine are sufficient to reveal the expression of antipsychotic-evoked dopamine supersensitivity. Second, given that d-amphetamine enhances noradrenaline and serotonin transmissions as well (Rothman and Baumann, 2003), we tested the hypothesis that enhancing dopamine transmission using a selective indirect dopamine agonist is sufficient to trigger a sensitised response. To extend the characterization on the specific contributions of dopamine transmission, we also tested the hypothesis that D1-like and D2-like-mediated signalling are both sufficient and necessary for unveiling the expression of dopamine supersensitivity. Last, we tested the hypothesis that antipsychotic-evoked dopamine supersensitivity enhances stress-related responses.

1.1. Present Findings

FIGS. 5.1 and 5.2 summarise the main findings of Chapters II and III, respectively. In a set of experiments, we examined the contribution of D1-like- and D2-like-mediated signalling (FIG. 5.1A). As described in Section 2.4 in the Introduction (page 50), antipsychotic-evoked dopamine supersensitivity is linked to increased levels of striatal D2-like and D2-like^{HIGH} receptors. In accordance with this and previous reports (Obuchowicz, 1999; Hashimoto *et al.*, 2018), we showed that stimulation of D2-like receptors is sufficient to evoke the expression of dopamine supersensitivity. Furthermore, we showed that D2-like-mediated signalling is not required, because D2-like antagonism *exacerbated* the exaggerated psychomotor response to d-amphetamine. Hence, altering D2-like transmission promotes a sensitised psychomotor response, whether D2-like transmission is stimulated or blocked. We examined intracellular signalling pathways mediated by

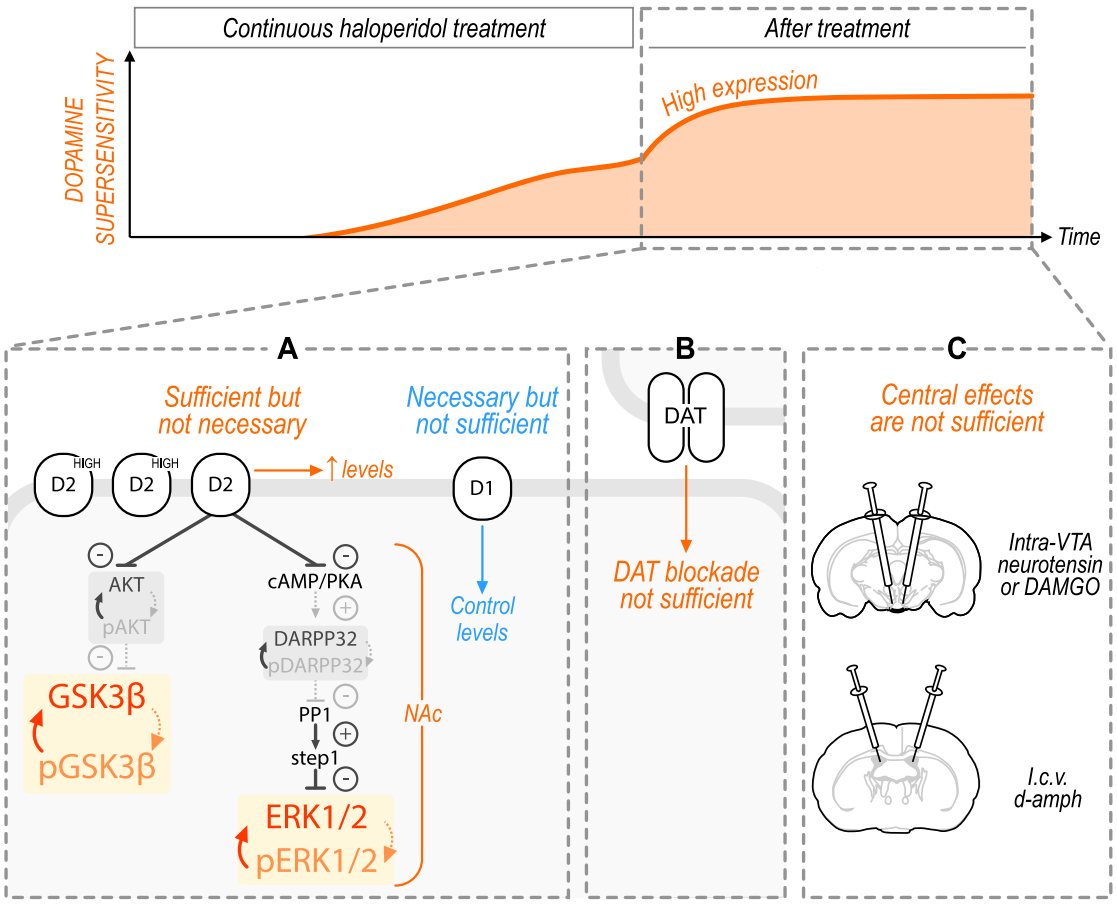

FIG. 5.1 — Main findings of Chapter II. (***A***) D1-like and D2-like receptors contribute distinctively to dopamine supersensitivity. D2-like transmission is sufficient but not necessary to reveal the expression of established antipsychotic-evoked dopamine supersensitivity, whereas D1-like transmission is not sufficient but necessary. Furthermore, dopamine supersensitivity is accompanied by an increased in D2 transmission in the nucleus accumbens (but not caudate-putamen), as suggested by an enhanced activity of GSK3β and reduced activity of ERK1/2. (***B***) Blockade of dopamine reuptake is not sufficient to trigger a sensitised psychomotor response in dopamine-supersensitive rats. (***C***) Neither increasing ventral tegmental area (VTA) dopamine impulse flow nor infusing d-amphetamine into the lateral ventricles is sufficient to unveil the expression of dopamine supersensitivity.

dopamine receptors and found converging evidence that D2-like transmission is increased in animals with a history of chronic antipsychotic exposure. In the nucleus accumbens (but not caudate-putamen), we found that d-amphetamine enhances GSK3β activity to a greater extent in antipsychotic-treated animals, while its ability to activate extracellular signal-regulated kinases (ERK) 1 and 2 is suppressed. Because activation of D2-like receptors activates GSK3β and inhibits ERK1/2 (Beaulieu *et al.*, 2007), these d-amphetamine effects are likely reflecting an increase in D2-like transmission. Regarding D1-like receptors, we showed that their activity is not sufficient

but necessary for unveiling the expression of antipsychotic-evoked dopamine supersensitivity. In the dorsal, ventrolateral and centromedial caudate-putamen or nucleus accumbens, we found no changes in protein activity of the cAMP/PKA-dependent pathway that would otherwise suggest enhanced D1-like transmission in dopamine-supersensitive rats. This is consistent with the observation that chronic antipsychotic exposure does not elevate the striatal level of D1-like receptors (Fleminger *et al.*, 1983; Jiang *et al.*, 1990; Kestler *et al.*, 2001). The results above are similar to previous findings that stimulation of D2-like but not D1-like receptors reveals the expression of psychomotor sensitisation produced by repeated d-amphetamine injections (Levy *et al.*, 1988; Vanderschuren *et al.*, 1999), and that this supersensitivity is accompanied by an increase in the density of D2-like/D2-like^{HIGH} in the striatum (Seeman *et al.*, 2007). We also found that selective blockade of dopamine reuptake is insufficient to reveal the expression of dopamine supersensitivity (FIG. 5.1B).

In another set of experiments, we analysed the contribution of the central effects of d-amphetamine (FIG. 5.1C). Previous studies showed that while a systemic administration of d-amphetamine effectively reveals the expression of antipsychotic-evoked dopamine supersensitivity, a local infusion into the nucleus accumbens or caudate-putamen does not, as dopamine-supersensitive rats show a similar psychomotor response than antipsychotic-naïve animals (El Hage *et al.*, 2015). One possibility is that dopamine transmission must be enhanced at multiple sites. Thus, in the present thesis, we determined whether mesocorticolimbic dopamine transmission is sufficient to reveal the expression of antipsychotic-evoked dopamine supersensitivity. We found that the psychomotor response to an increase in VTA dopamine impulse flow (achieved by a local infusion of neurotensin or DAMGO) is similar between dopamine-supersensitive and antipsychotic-naïve rats. This indicates that mesocorticolimbic dopamine transmission is not sufficient to reveal the expression of antipsychotic-evoked dopamine supersensitivity. Furthermore, we also found that an intracerebroventricular infusion of d-amphetamine (to enhance monoamine transmission only in the central nervous system) is not sufficient to trigger a sensitised response in dopamine-supersensitive rats. This indicates that peripheral processes play a necessary role in the expression of antipsychotic-evoked dopamine supersensitivity.

Last, we found that dopamine supersensitivity correlates with some signs of increased stress-like responses. Indeed, we found that inhibition of the synthesis of the stress hormone corticosterone

supresses the exaggerated psychomotor response to d-amphetamine produced by antipsychotic-evoked dopamine supersensitivity (FIG. 5.2A). Furthermore, dopamine supersensitivity correlates with some alterations in stress-related behaviours. Indeed, avoidance behaviour is unchanged in the open field and the light-dark box, but dopamine-supersensitive rats show decreased exploratory behaviour in the elevated-plus maze and an increased locomotor response to environmental novelty (FIG. 5.2B).

1.2. Implications and Future Directions

1.2.1. Dopamine Receptors

The present findings increment our understanding of the important role of D2-like transmission in antipsychotic-evoked dopamine supersensitivity. However, important mechanistic questions remain. For instance, why does D2-like antagonism exacerbate the expression of dopamine supersensitivity? This is likely an effect involving blockade of presynaptic D2 receptors. Indeed, if D2 autoreceptor number/function is greater in dopamine-supersensitive rats, then these receptors would normally temper the behavioural manifestations of dopamine supersensitivity, and their blockade would exacerbate this supersensitivity. Such adaptations on the presynaptic side could represent an important mechanism involved in treatment tolerance produced by dopamine supersensitivity. Given the important implications, future studies should analyse D2 autoreceptor in dopamine-supersensitive rats, as little is known so far. For instance, it is unknown whether the elevated number of striatal D2-like/D2-like^{HIGH} receptors associated with dopamine supersensitivity (Samaha *et al.*, 2007; Samaha *et al.*, 2008) concerns the presynaptic and/or postsynaptic side. Regarding D2 autoreceptor function, there are mixed observations. An *ex vivo* experiment that used fast-scan voltammetry showed no evidence that D2 autoreceptor function is altered in the nucleus accumbens of animals chronically exposed to haloperidol (Chesi *et al.*, 1995). Indeed, the enhancement of dopamine release produced by sulpiride application or the suppression of dopamine release produced by application of the D2 agonist quinpirole did not differ between antipsychotic-treated and antipsychotic-naïve animals (Chesi *et al.*, 1995). However, another *ex vivo* experiment showed alterations in D2 autoreceptor function in antipsychotic-treated animals. In control animals, quinpirole reduces the amplitude of excitatory post-synaptic currents in the caudate-putamen, without altering the electrophysiological properties of neurons on the post-

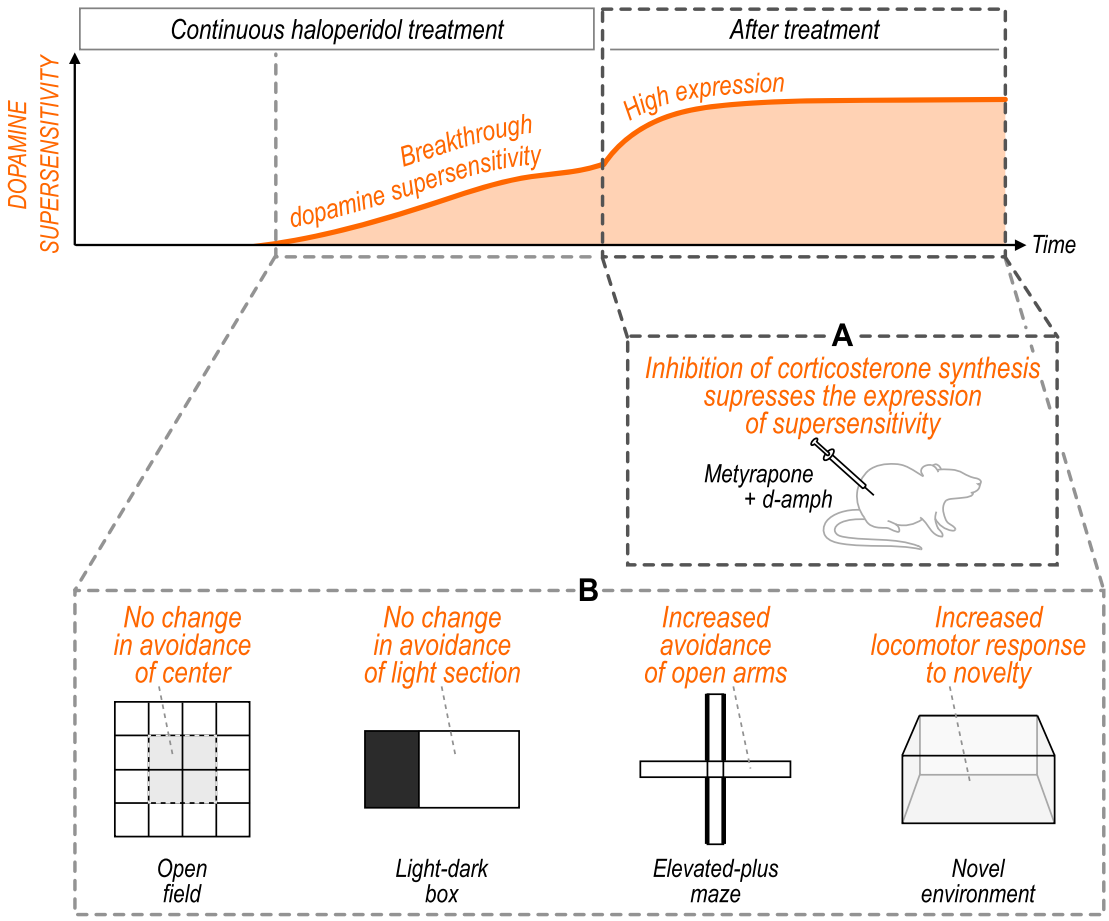

FIG. 5.2 — Main findings of Chapter III. (***A***) Corticosterone synthesis is necessary to reveal the expression of dopamine supersensitivity. (***B***) Dopamine-supersensitive rats show signs of heightened stress-like behaviour in the elevated-plus maze and in their locomotor response to novelty, but not in the open field or in the light-dark box.

synaptic side (Calabresi *et al.*, 1992). Thus, this inhibitory effect is likely mediated by presynaptic D2 receptors, and it can be used to probe their function. Interestingly, Calabresi *et al.* (1992) showed that chronic exposure to haloperidol largely potentiates the ability of quinpirole to inhibit excitatory post-synaptic currents, suggesting that the function of D2 autoreceptors is potentiated. The discrepancy in the results above could be due to the use of different methodological procedures, haloperidol treatment regimen and/or that D2 autoreceptor function was studied in distinct subregion of the striatum (Calabresi *et al.*, 1992; Chesi *et al.*, 1995). Future studies should be designed to compare D2 autoreceptor function in different striatal subregions of dopamine-supersensitive rats. Such studies should also include animals chronically exposed to an antipsychotic treatment regimen that is unlikely to produce dopamine supersensitivity. Indeed,

even without behavioural signs of dopamine supersensitivity, this type of treatment regimen can still elevate D2-like receptor function as well, even if it is to a lesser extent (FIG. 1.11, page 55) (Samaha *et al.*, 2008). Thus, comparing treatment regimen producing or not dopamine supersensitivity would allow to delineate changes related to D2-like receptors that are linked to supersensitivity versus other antipsychotic-evoked changes.

Also, antipsychotic-evoked dopamine supersensitivity is linked to changes in protein activity in the cAMP/PKA- and GSK3β/AKT-dependent intracellular signalling pathways suggestive of increased D2-like transmission. However, this remains a correlational observation. Future studies should assess the functional effects of increased GSK3β activity and decreased ERK1/2 activity in the nucleus accumbens of dopamine-supersensitive animals. There are some evidence suggesting that modulating GSK3β activity in the nucleus accumbens could temper the behavioural manifestations of antipsychotic-evoked dopamine supersensitivity. For instance, inhibition of GSK3α/β activity in the nucleus accumbens reduces the behavioural effects of d-amphetamine (Wickens *et al.*, 2017). The neurotensin type 1 receptor agonist PD149163 reduces both the psychomotor response to d-amphetamine and GSK3β activity in the nucleus accumbens (Vadnie *et al.*, 2016). Interestingly, neurotensin function in the nucleus accumbens is enhanced in dopamine-supersensitive animals. Indeed, neurotensin infused into the nucleus accumbens reduces the psychomotor effects of d-amphetamine (Ervin *et al.*, 1981), and this effect is greatest in animals that received an antipsychotic treatment producing dopamine supersensitivity relative to animals that received an antipsychotic treatment regimen unlikely to produce this supersensitivity (Servonnet *et al.*, 2017). Thereby, neurotensin function in the nucleus accumbens seems greater in dopamine-supersensitive rats, and this could rely on the ability of neurotensin to suppress GSK3β activity in the nucleus accumbens. This needs further exploration. How a suppression of ERK1/2 activity contributes to antipsychotic-evoked dopamine supersensitivity is more puzzling, because administration of an inhibitor of ERK1/2 activity into the nucleus accumbens reduces the behavioural effects of d-amphetamine (Gerdjikov *et al.*, 2004). This requires further investigations.

A striking observation in the present thesis is that continuous antipsychotic drug exposure alters the dopamine system in a way that render animals tolerant to the antidopaminergic effects of D2-like but not D1-like antagonism. Similarly, following chronic antipsychotic exposure, repeated stimulations of D1-like but not D2-like receptors reverse the behavioural expression of dopamine

supersensitivity and the increase in D2-like receptor number in the striatum (Marin and Chase, 1993; Braun *et al.*, 1997). Similarly, repeated D1-like agonist injections (Shuto *et al.*, 2006) or blockade of D1-like-mediated signalling (Ramos *et al.*, 2004) also reverse the expression of supersensitivity produced by repeated dopamine agonists injections. Hence, D1-like but not D2-like-mediated signalling represents a potential target to temper the effects of antipsychotic-evoked dopamine supersensitivity. An important aspect that has not been studied in the present thesis is the combined effects of mediating D1-like and D2-like receptor transmissions. Antipsychotic-treated animals were sensitive to the psychomotor effects of the D2-like receptor agonist quinpirole, the D1-like/D2-like receptor agonist apomorphine but not the D1-like receptor agonist SKF38393. Apomorphine is also an agonist of serotonin and noradrenergic receptors (Millan *et al.*, 2002), and does not allow to measure the interaction between stimulation of D1-like and D2-like receptors. Thus, future studies should measure the interaction between stimulation of D1-like and D2-like transmission, especially given that simultaneous stimulation of these receptors has a synergic effect on behaviour. For instance, administration of both D1-like and D2-like agonists produces strong psychomotor effects with doses of agonists that produce mild psychomotor effects when given alone (Rouillard and Bedard, 1988). Furthermore, because we found that D1-like antagonism blocked the expression of already established dopamine supersensitivity, future studies should also determine if co-administering antipsychotic drugs with a D1-like antagonist can prevent the development or reverse the expression of breakthrough dopamine supersensitivity during antipsychotic exposure. Braun *et al.* (1997) reported previously that concomitant, repeated administration of a D1-like antagonist and a D2-like antagonist exacerbate the expression of dopamine supersensitivity. However, using a clinically-relevant regimen of antipsychotic treatment and testing different type and dose of D1-like antagonist may yield different outcomes.

1.2.2. Dopamine Transporters

Stimulation of D2-like receptors is sufficient to evoke a sensitised response in dopamine-supersensitive animals. Thus, enhancing dopamine concentration in the synaptic cleft should be sufficient to elicit the expression of antipsychotic-induced dopamine supersensitivity. However, we found that selective blockade of dopamine reuptake is insufficient to reveal the expression of dopamine supersensitivity. We also found a weak sensitised response to cocaine in haloperidol-treated rats. This contrasts with previous findings showing that animals rendered supersensitive to

the behavioural effects of d-amphetamine following repeated administrations are also supersensitive to the psychomotor effects of cocaine and of the selective dopamine reuptake blocker GBR12909 (Bonate *et al.*, 1997; Vanderschuren *et al.*, 1999). As discussed in Chapter II, antipsychotic-evoked dopamine supersensitivity could produce alterations in dopamine reuptake functions that could consequently minimise the psychomotor effects of a dopamine reuptake blocker. This would explain why dopamine-supersensitive rats show control levels of hyperlocomotion in response to GBR12783. In line with this idea, dopamine supersensitivity seems linked to an enhancement in DAT function in the striatum. Indeed, tolerance to antipsychotic-like effects, that is provoked by breakthrough dopamine supersensitivity, is reversed by blockade of DAT (Amato *et al.*, 2018).

1.2.3. Central Versus Peripheral Processes

Increasing VTA dopamine impulse flow is not sufficient to reveal the expression of dopamine supersensitivity, because antipsychotic-treated animals show control level of hyperlocomotion in response to neurotensin or DAMGO infused into the VTA. There are at least a few explanations for this observation. First, chronic antipsychotic treatment could alter the functional effects of neurotensin and DAMGO in the VTA. However, it is noteworthy that these molecules increase dopamine impulse flow via distinct mechanisms. DAMGO inhibits local GABA release by activation of μ-opioid receptors located on GABA terminals, and this consequently disinhibits dopamine neuron activity (Kalivas and Duffy, 1990; Chen *et al.*, 1993; Bergevin *et al.*, 2002). Neurotensin acts on neurotensin type 1 receptor and activates dopamine neuron activity by inhibition of D2 autoreceptor activity and activation of local glutamate transmission (Werkman *et al.*, 2000; Jomphe *et al.*, 2006; Kempadoo *et al.*, 2013; Bose *et al.*, 2015). The probability that chronic antipsychotic exposure produces changes that would decrease the functional effects of both neurotensin and DAMGO is low. Nonetheless, future studies should confirm these findings using techniques such as chemogenetics or optogenetics to increase dopamine neuron activity in a selective manner. Also, these techniques are well suited to determine if mesocorticolimbic dopamine transmission is *necessary* for the ability of d-amphetamine to reveal antipsychotic-evoked dopamine supersensitivity. This is an important aspect given that, as described in the previous section, dopamine transmission is altered in the striatum of dopamine-supersensitive rats, and these alterations are unlikely sufficient but could be necessary for the expression of dopamine

supersensitivity. Another possible explanation as to why dopamine-supersensitive rats are not supersensitive to the psychomotor effects of intra-VTA neurotensin and DAMGO involves somatodendritic release of dopamine. Indeed, activation of VTA dopamine neurons enhances local dopamine release, and thus this activates D2 autoreceptors located on VTA dopamine neurons. If these receptors function/number is enhanced in dopamine-supersensitive rats, then this should minimise the activating effects of neurotensin and DAMGO, leading to control levels of psychomotor activity. I am not aware of studies that measured D2 autoreceptor number or function in the VTA, and future studies should address this given that, as in the striatum, there could be compensatory changes due to chronic antagonism of D2-like transmission. Chronic haloperidol does not alter dopamine availability, at least in the striatum (Compton and Johnson, 1988; Ichikawa and Meltzer, 1992; Samaha *et al.*, 2007). Hence, we do not expect that neurotensin- and DAMGO-induced dopamine release would differ between antipsychotic-treated animals and controls.

Another explanation regarding why antipsychotic-treated animals do not show an exaggerated locomotor response to intra-VTA neurotensin or DAMGO implicates that peripheral processes are necessary for unveiling a sensitised response, making intra-cerebral manipulations insufficient. We explored that possibility by evaluating the psychomotor response to d-amphetamine infused into the lateral ventricles, to restrict its effect to the central nervous system. We found that an intracerebroventricular administration of d-amphetamine produced a similar psychomotor response in antipsychotic-treated and antipsychotic-naïve animals, which contrasts with the sensitised response evoked by an administration through the systemic route. In line with this finding, it was also shown that infusing d-amphetamine into the nucleus accumbens or the caudate-putamen does not evoke a sensitised psychomotor response in antipsychotic-treated rats (El Hage *et al.*, 2015). Thus, the psychomotor response to d-amphetamine is still centrally mediated in dopamine-supersensitive rats, but peripheral effects are required for producing a sensitised response. In another model of dopamine supersensitivity, repeated injections of d-amphetamine evoke psychomotor sensitisation over time, and infusion of d-amphetamine into the lateral ventricles (Rebec and Segal, 1979), the nucleus accumbens or the caudate-putamen (Kolta *et al.*, 1989; Paulson and Robinson, 1991) is sufficient to evoke a sensitised response. Hence, the expression of supersensitivity is centrally mediated when psychomotor sensitisation is produced by repeated psychostimulant injections, and seems peripherally mediated when this sensitisation is produced by chronic antipsychotic exposure. Future studies should address how antipsychotic drugs

influence peripheral processes that promote dopamine supersensitivity over time. The peripheral effects of antipsychotic drugs are likely insufficient to promote dopamine supersensitivity. Indeed, chronic administration of the D2-like antagonist domperidone, that does not cross the blood-brain-barrier (Laduron and Leysen, 1979), does not potentiate the psychomotor response to apomorphine (Rupniak *et al.*, 1983). However, it remains to be determined whether the peripheral effects of d-amphetamine are sufficient to evoke a sensitised response in dopamine-supersensitive rats. So far, very little is known about the effects of stimulating peripheral dopamine/monoamine transmission on locomotor activity. An injection through the systemic route of dopamine [which does not cross the blood-brain-barrier (Bertler *et al.*, 1963)] *decreases* locomotor activity in otherwise naïve rats (Butcher and Engel, 1969). Also, the cocaine analog cocaine methiodide does not cross the blood-brain-barrier as well, and it produces no-to-very little locomotor effects when administered through the systemic route in control animals (Hemby *et al.*, 1994; Brown and Kiyatkin, 2006). Nonetheless, the manipulations above may lead to different effects in dopamine-supersensitive rats and future studies could address this.

1.2.4. Stress-like Responses

As presented in Chapter III, there are some evidence that antipsychotic-evoked dopamine supersensitivity increased the biological and behavioural effects of stress. This is important to consider and to further explore given that worsening of psychosis produced by dopamine supersensitivity (Chouinard *et al.*, 1978) could be promoted by an increased vulnerability to stress [an already known contributing factor to psychosis relapse (Naeem *et al.*, 2006; McCutcheon *et al.*, 2019a)]. First, we found that the corticosterone synthesis inhibitor metyrapone seems to supress to a greater extent d-amphetamine-induced psychomotor activity in antipsychotic-treated rats, suggesting that corticosterone is necessary for the full expression of dopamine supersensitivity. However, these findings should be interpreted cautiously. Because haloperidol-treated animals showed a greater psychomotor response to d-amphetamine, this may in itself make metyrapone's effects appear greater because the response is simply greater. This is especially important to consider given that the locomotor response to the co-administration of d-amphetamine and metyrapone is similar between haloperidol-treated rats and controls—*i.e.*, the response of haloperidol-treated animals is not below control level. Still, d-amphetamine-induced locomotion is higher in haloperidol-treated rats. Because metyrapone reduced this exaggerated locomotor

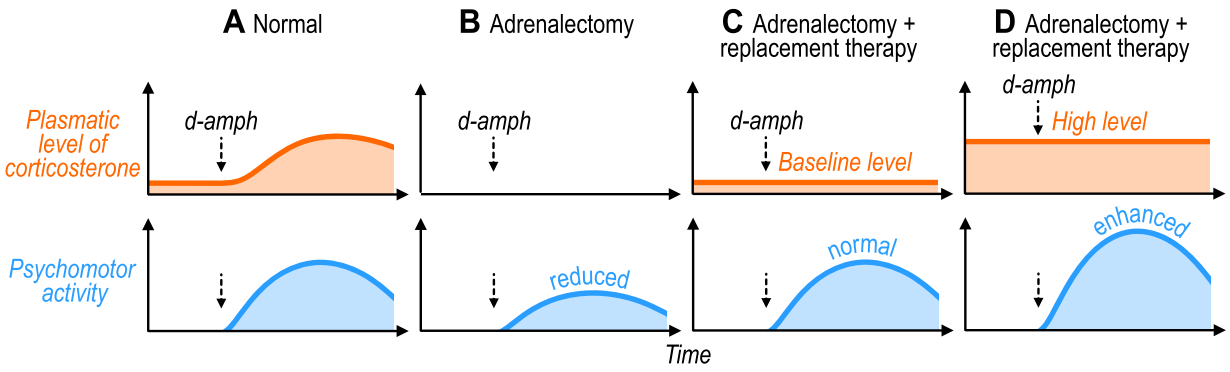

FIG. 5.3 — Relationship between the plasmatic levels of corticosterone and the psychomotor effects of d-amphetamine. (***A***) D-amphetamine enhances the plasmatic level of corticosterone. (***B***) Adrenalectomy supresses both the plasmatic level of corticosterone and the psychomotor response to d-amphetamine. (***C***) Adrenalectomy paired with corticosterone replacement therapy—that achieves sustained level of corticosterone mimicking baseline concentration—is sufficient to restore the psychomotor response to d-amphetamine. Hence, d-amphetamine-induced elevation in the plasmatic level of corticosterone is not necessary for its psychomotor effects. Furthermore, (***D***) high dosage of corticosterone replacement therapy in adrenalectomized animals exacerbates the psychomotor response to d-amphetamine.

response in a way that attained control levels, this in itself should require that metyrapone produces greater effects in antipsychotic-treated animals. While it remains unclear whether metyrapone's effects were greater in haloperidol-treated rats, the present results still encourage further investigations on corticosterone, because metyrapone effectively tempered the expression of antipsychotic-evoked dopamine supersensitivity.

Thus, what do the present findings suggest on the potential involvement of corticosterone in antipsychotic-evoked dopamine supersensitivity? D-amphetamine administration enhances the plasmatic level of corticosterone (FIG. 5.3A) (Swerdlow *et al.*, 1993). Thereby, one possibility is that this enhancement is greater in dopamine-supersensitive rats, and this participates in the exacerbated psychomotor response to d-amphetamine. However, the available literature suggests that this is unlikely. In fact, d-amphetamine-induced elevation in corticosterone blood levels is *not necessary* for its psychomotor effects. Adrenalectomy supresses the plasmatic level of corticosterone and reduces the psychomotor response to d-amphetamine (FIG. 5.3B) (Cador *et al.*, 1993). Combining adrenalectomy with corticosterone replacement therapy to achieve sustained plasmatic concentration of corticosterone (mimicking baseline level) is sufficient to restore the psychomotor response to d-amphetamine (FIG. 5.3C) (Cador *et al.*, 1993). Hence, the psychomotor

effects of d-amphetamine are restored even if d-amphetamine does not elevate the plasmatic level of corticosterone. Interestingly, when adrenalectomized animals receive a high dose of corticosterone replacement therapy, this exacerbates their psychomotor response to d-amphetamine (FIG. 5.3D) (Cador *et al.*, 1993). Hence, our antipsychotic-treated rats could have greater circulating levels of corticosterone at baseline and not in response to d-amphetamine. However, it was previously shown that late into continuous haloperidol treatment [when dopamine supersensitivity has already developed (Samaha *et al.*, 2007; Amato *et al.*, 2018)], antipsychotic-treated animals have comparable plasmatic level of corticosterone relative to controls (Lin *et al.*, 2006). Nonetheless, it is possible that *after* treatment cessation, rats with a history of continuous haloperidol treatment have elevated plasmatic levels of corticosterone. It is also possible that dopamine-supersensitive rats have normal circulating levels of corticosterone, but that the stress produced by the test procedures increases these circulating levels beyond control concentrations. This is a possibility to consider given that we administered metyrapone in the colony room prior to the test [in accordance with previous methods (Marrow *et al.*, 1999)]. Thereby, this approach prevented any increase in corticosterone provoked by the stress of the experimental procedure. How antipsychotic-evoked dopamine supersensitivity influences the plasmatic levels of corticosterone (whether when unstressed, stressed or in response to d-amphetamine) requires further investigations.

Additionally, metyrapone crosses the blood-brain-barrier (Stith *et al.*, 1976), and therefore our results do not allow to delineate the specific contribution of central versus adrenal corticosterone synthesis (Croft *et al.*, 2008). However, it is likely that adrenal corticosterone is necessary for two reasons: *i*) in Chapter II, we showed that the peripheral effects of d-amphetamine are necessary for the expression of antipsychotic-evoked dopamine supersensitivity, and *ii*) adrenalectomy reduces the psychomotor response to d-amphetamine, and this is restored by corticosterone replacement therapy (FIG. 5.3B) (Cador *et al.*, 1993). The necessity of adrenal versus neuronal corticosterone synthesis in the expression of antipsychotic-evoked dopamine supersensitivity could be determined in adrenalectomized rats. Also, metyrapone produces other effects than inhibiting corticosterone synthesis. For instance, metyrapone also inhibits aldosterone synthesis (Igaz *et al.*, 2008), and this may have influenced the expression of antipsychotic-evoked dopamine supersensitivity. However, the available literature suggests that aldosterone does not seem to promote at least some behavioural effects of psychostimulant drugs, such as the rewarding effects of cocaine (Mantsch *et*

al., 1998) and methamphetamine-induced psychomotor activity (Kobayashi and Arai, 1976). Metyrapone also inhibits the activity of the enzyme debrisoquine 4-hydroxylase (CYP2D6) (Wolff and Strecker, 1985), which is involved in the metabolization of amphetamines (de la Torre *et al.*, 2004). Here, metyrapone *reduced* d-amphetamine-induced locomotion and in opposition, a reduction in amphetamine metabolization would enhance its psychomotor effects. Hence, the inhibition of debrisoquine 4-hydroxylase may have mitigated the suppressive effects of metyrapone on d-amphetamine-induced locomotion. Another important aspect that should be explored is how antipsychotic-evoked dopamine supersensitivity influences corticosterone-mediated signalling in the brain, and how this hormone influences dopamine transmission in dopamine-supersensitive rats. Corticosterone signals via two receptors, glucocorticoid and mineralocorticoid receptors (de Kloet, 2000). In rats, glucocorticoid receptors are found throughout the brain [including on VTA dopaminergic neurons (Harfstrand *et al.*, 1986)], whereas mineralocorticoid receptors are found in a limited number of regions, including the dopamine-rich region septum (Reul and de Kloet, 1986). Activation of corticosterone receptors promote mesocorticolimbic dopamine transmission and the behavioural effects of dopamine agonists (Marinelli and Piazza, 2002). It remains undetermined if antipsychotic-evoked dopamine supersensitivity alters the number and/or function of corticosterone receptors. Interestingly, corticosterone administered alone is sufficient to stimulate locomotor activity (Piazza *et al.*, 1996; Sandi *et al.*, 1996), and exaggerated circulating levels of corticosterone is also sufficient to enhance the psychomotor response to d-amphetamine (Piazza *et al.*, 1991; Cador *et al.*, 1993). Hence, here we showed that corticosterone is *necessary* for revealing the expression of antipsychotic-evoked dopamine supersensitivity, and future studies should determine if enhancing corticosterone transmission is *sufficient*.

We also found some changes in stress-related behavioural responses in dopamine-supersensitive rats, during ongoing haloperidol treatment when dopamine supersensitivity breaks through and after treatment cessation when the expression of dopamine supersensitivity persists. Their avoidance of the center of the open field was similar to antipsychotic-naïve animals. However, the level of avoidance in the control group was very high (they spent a few seconds in the center over 5 minutes). Thereby, any further decrease could be impossible to observe. In the light-dark box, the degree of avoidance of the light compartment was more moderate in control rats, but dopamine-supersensitive rats still did not differ from that response. However, we found signs of increased stress-like behavioural responses in the elevated-plus maze. Indeed, continuously-treated rats show

decreased exploratory behaviour in the elevated-plus maze, both late into treatment when dopamine supersensitivity breaks through and after treatment cessation. Avoidance behaviour in the tests above may involve stress-like but also anxiety-like effects. Thereby, any change in avoidance that correlates with antipsychotic-evoked dopamine supersensitivity may be linked to increased stress- and/or anxiety-like behaviour. Even if stress and anxiety are often intertwined processes, they have distinct features. For instance, stress involves increased arousal, tension and secretion of glucocorticoids, whereas anxiety involves apprehension, fear and is GABA-dependant (Bystritsky and Kronemyer, 2014). Because we found changes in the elevated-plus maze, future studies could clarify the respective contributions of stress and anxiety by evaluating the effects of anxiolytic drugs [they are GABAergic agonists that enhance exploration of the open arms (Pellow and File, 1986)] or measuring stress-related physiological responses [*e.g.*, the open arms elevate corticosterone secretion (Pellow *et al.*, 1985)]. Also, an important issue with the stress tests conducted during ongoing antipsychotic treatment is that the treatment itself reduced locomotor behaviour. Because the tests rely on exploratory behaviour, the reduced locomotor activity produced by haloperidol are likely producing confounding effects. Regarding continuous haloperidol treatment, the results presented in Chapter III show that this regimen strongly reduces spontaneous locomotor activity early into treatment when dopamine supersensitivity has not developed yet, but not later into treatment when dopamine supersensitivity breaks through. Thereby, the correlation between breakthrough dopamine supersensitivity and the greater avoidance of open arms is unlikely influenced by the suppressive effects of haloperidol on exploratory behaviour. However, such suppressive effects make it difficult to determine if rats show enhanced stress-like responses early into treatment, when dopamine supersensitivity has not developed yet. One solution to resolve this issue would be to continuously expose rats to haloperidol for a few days (short enough to not produce dopamine supersensitivity), and then give the stress tests following cessation of that short treatment to avoid the direct suppressive effects of haloperidol on locomotor activity. Regarding the transient haloperidol treatment, this regimen reduces spontaneous locomotion throughout the entire treatment. Thereby, exploratory behaviour in the stress tests is likely impaired by this. This issue could be avoided by giving the stress tests before transiently-treated animals receive their next haloperidol injection (that is, at through), instead of after the injection as done in Chapter III.

It was previously shown that rats continuously exposed to haloperidol show an increased locomotor response to vehicle injection (Tadokoro *et al.*, 2012; Oda *et al.*, 2015), perhaps reflecting an increased locomotor response to novelty. Here we showed that continuously-treated rats show increased spontaneous locomotor activity in a novel environment, and that this returns to control levels with repeated testing. This increased locomotor activity could reflect that continuous haloperidol treatment enhances exploratory behaviour of a novel environment, and/or that habituation is slower in these animals. This behavioural response could reflect a state of cross-sensitisation between the effects of stress and of dopamine stimulation. Indeed, increased locomotor response to novelty is *i*) found in animals that have been chronically stressed (Marin *et al.*, 2007), *ii*) linked with enhanced dopamine release in the nucleus accumbens in response to stress (Rouge-Pont *et al.*, 1993), and *iii*) predictive of supersensitivity to the psychomotor and rewarding effects of dopamine agonists (Piazza *et al.*, 1989; Hooks *et al.*, 1991; Hooks *et al.*, 1994).

Because we found inconsistent changes in stress-related behavioural responses, future studies should investigate the influence of antipsychotic-evoked dopamine supersensitivity on other measures of stress-like behaviour such as conditioned fear (LeDoux, 2000). The effects of antipsychotic-evoked dopamine supersensitivity on conditioned stress responses have been studied in the conditioned avoidance paradigm. As described in the Introduction (Section 2.4.1, page 51), haloperidol decreases avoidance elicited by aversive CS, and this effect is lost over time with the emergence of dopamine supersensitivity (Samaha *et al.*, 2007; Samaha *et al.*, 2008). This lost of effect over time could be explained by the concomitant emergence of dopamine supersensitivity and increased stress-like responses. However, with these findings (Samaha *et al.*, 2007; Samaha *et al.*, 2008), it remains unclear if dopamine-supersensitive rats would show conditioned avoidance *beyond* control levels. Indeed, in this type of task, the effect of antipsychotic drug exposure is determined in well-trained animals, and this results in controls avoiding approximately 100% of CS presentations. It is therefore impossible to measure avoidance beyond this response in antipsychotic-treated rats, but other approaches would allow to avoid this issue. For instance, the effects of antipsychotic-evoked dopamine supersensitivity could be measured during extinction learning, where the CS is not followed by the aversive UCS anymore. Animals chronically stressed show decreased extinction behaviour in response to aversive CS (Miracle *et al.*, 2006). Therefore, antipsychotic-treated animals that developed dopamine supersensitivity may be more resistant to extinction learning as well.

2. How Do Appetitive Conditioned Stimuli Guide Behaviour? New Insights and Perspectives

Appetitive CS play a critical role in guiding everyday behaviour toward essential rewards, but they can also promote maladaptive motivated behaviours, such as following chronic antipsychotic drug exposure producing dopamine supersensitivity. Here we were interested in studying the role of the basolateral amygdala in the behavioural response to appetitive CS in antipsychotic-naïve rats, as this could give novel insights on the neurobiological mechanisms underlying the behavioural manifestations of antipsychotic-evoked dopamine supersensitivity. Furthermore, studying the behavioural effects of appetitive CS has also important implications for other psychiatric disorders such as addiction and depression, where motivational processes are abnormal. As described in the Introduction, basolateral amygdala neurons fire in response to appetitive CS, and disruption of neuronal transmission in that nucleus reduces the behavioural effects of appetitive CS (Section 4.2, page 69). However, it is largely undetermined whether increased activity of basolateral amygdala neurons in response to CS is sufficient to intensify their behavioural effects. Hence, here we tested the hypothesis that optogenetic stimulation of basolateral amygdala neurons is not reinforcing on its own, but it enhances both conditioned approach and the ability of appetitive CS to be salient and be pursued (Servonnet *et al.*, 2020b).

2.1. Present Findings and their Implications

Water-restricted rats were trained to associate a compound stimulus (lights and tone) with water delivery in a receptacle. Over the sessions, rats progressively increased the number of head entries into the water receptacle selectively during CS presentation. This indicates that the CS guided animals' behaviour toward the imminent delivery of water. We found that optogenetic stimulation of ChR2-expressing basolateral amygdala neurons during CS presentation potentiated conditioned approach toward the water receptacle (FIG. 5.4A). In contrast, we found that optogenetic stimulation of basolateral amygdala neurons halfway of ITI did not influence CS-UCS conditioning, as it led to control level of conditioned approach. Hence, increased activity in the basolateral amygdala is sufficient to enhance CS-elicited conditioned approach, and this effect is only observed when the stimulation coincides with CS presentation.

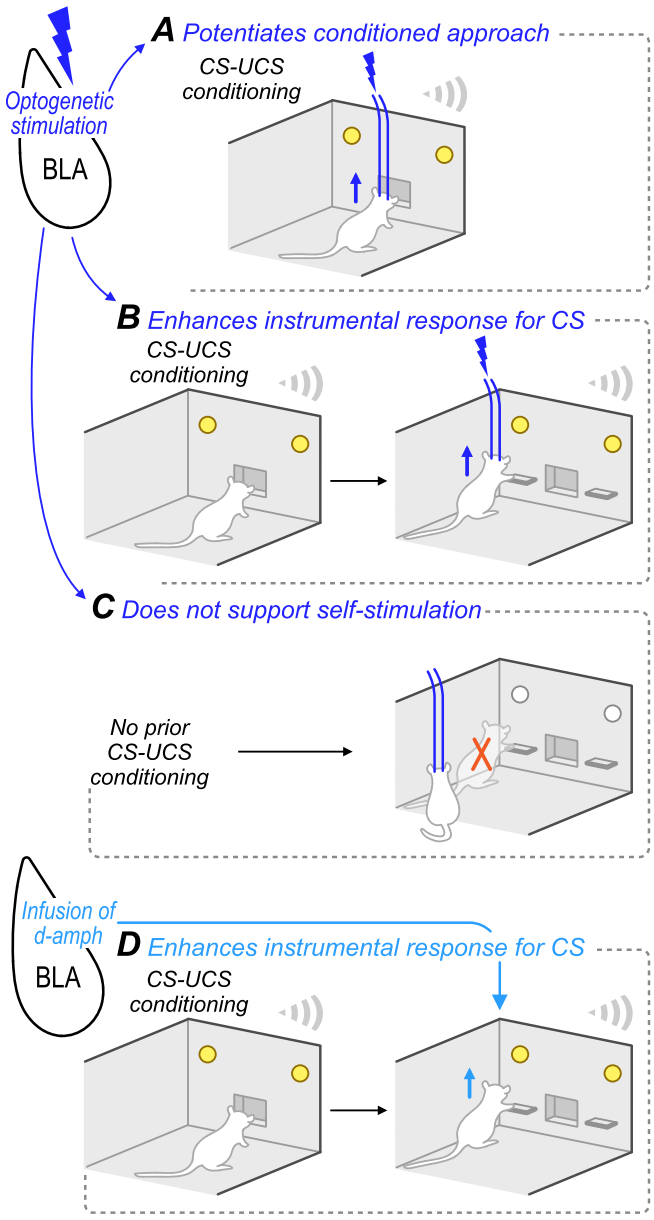

FIG. 5.4 — Main findings of Chapter IV. Optogenetic stimulation of basolateral amygdala neurons (***A***) enhances conditioned approach (***B***) and the incentive motivational value of conditioned stimuli (CS), (***C***) without being intrinsically rewarding. Similarly, (***D***) enhancing monoamine transmission in the basolateral amygdala is sufficient to promote incentive motivation for CS. BLA, basolateral amygdala; UCS, unconditioned stimulus.

Following CS-UCS conditioning, we determined whether CS presentation is sufficient to reinforce instrumental responses for CS presentation, as determined in the conditioned reinforcement test paradigm. We found that animals made more instrumental responses on a lever allowing CS presentation relative to an inactive lever. This discrimination indicates that the CS acquired incentive motivational value. Interestingly, we showed that optogenetic stimulation of basolateral amygdala neurons during CS presentation intensifies incentive motivation for CS presentation (FIG. 5.4B), as indicated by an increased number of active lever presses in animals expressing ChR2 relative to control animals. This effect is strongest when optogenetic stimulation is explicitly combined with CS presentation. Indeed, stimulations after CS presentations did not increase lever pressing behaviour beyond control level. This suggests that stimulating basolateral amygdala neurons does not elevate the motivational state of the animals *per se*, but instead selectively potentiates the incentive motivational effect of the CS. It is still noteworthy that during the test where photostimulations and CS presentations were explicitly unpaired, animals

expressing ChR2 but not controls pressed more on the active lever relative to the inactive lever. This may indicate that *i*) stimulating basolateral amygdala neurons outside of CS presentations still guides lever pressing behaviour (without intensifying that response), *ii*) the stimulation may be temporally too close to CS presentation and therefore it still influences the motivational effects of CS, or *iii*) there is a carry-over effect of the previous sessions during which photostimulations produced intense lever pressing. This could be further explored by analysing the effects of longer intervals between CS presentation and stimulation in animals prepared for this. In a separate study, we showed that photostimulation of basolateral amygdala neurons does not reinforce instrumental responses in animals that did not receive prior CS-UCS conditioning (FIG. 5.4C), while photostimulation of central amygdala neurons did [in line with previous findings (Seo *et al.*, 2016; Baumgartner *et al.*, 2017; Kim *et al.*, 2017)]. This suggests that stimulation of basolateral amygdala neurons is not intrinsically reinforcing, and that increasing their activity enhances the behavioural effects of appetitive CS selectively, and not because it is rewarding. Lastly, as converging evidence, we demonstrated that increasing monoamine transmission in the basolateral amygdala (achieved by a local infusion of d-amphetamine) is also sufficient to potentiate instrumental responses for CS presentation (FIG. 5.4D). These results show that increased activity of basolateral amygdala neurons is sufficient to promote the ability of CS to be attractive and pursued.

The implication of the findings above is that increased activity of the basolateral amygdala potentiates the ability of appetitive CS to be salient and to orient behaviour toward unconditioned rewards. Excessive activity of the basolateral amygdala could perhaps represent a mechanism by which CS have a greater propensity to promote maladaptive responses in psychiatric disorders, but also following antipsychotic drug exposure producing dopamine supersensitivity. That latter possibility is especially important given that there is already some evidence that schizophrenia patients show abnormal activity in the amygdala, and that this is linked to psychotic symptoms, a symptom worsened by dopamine supersensitivity (Chouinard *et al.*, 1978; Chouinard *et al.*, 2017). For instance, the amygdala of schizophrenia patients seems more easily activated by surrounding stimuli even if they are not emotionally salient (Anticevic *et al.*, 2012). Furthermore, the amygdala is more active when patients are at rest, especially patients with prominent psychotic symptoms (Pinkham *et al.*, 2015). Our results also show that infusing d-amphetamine into the basolateral amygdala is sufficient to enhance the incentive effect of CS. Thus, the reward-enhancing effects of d-amphetamine (Robbins *et al.*, 1983)—that are potentiated by antipsychotic-evoked dopamine

supersensitivity (Bedard *et al.*, 2011, 2013; El Hage *et al.*, 2015)—are critically regulated by the basolateral amygdala. Beside schizophrenia, the present findings are important for other psychiatric disorders as well, including drug addiction. For instance, future studies should determine the influence of enhanced activity in the basolateral amygdala on the behavioural effects of drug-predictive CS. Because of the important implications, I next discuss of potential mechanisms that could regulate the present observations, and what directions future studies should go to improve our understanding of these findings.

2.2. Future directions

2.2.1. How Does the Basolateral Amygdala Promote Conditioned Approach?

Our methods do not allow to determine which basolateral amygdala projections mediate conditioned approach. This is an important aspect that future studies should explore. Indeed, projection neurons are the most abundant neurons in the basolateral amygdala, and they represent a relatively homogeneous population (Namburi *et al.*, 2015), but there are some genetic signatures that may allow to distinguish neurons relative to their function. For instance, basolateral amygdala neurons expressing the *Ppp1r1b* gene seem particularly involved in incentive learning, whereas neurons expressing the *Rspo2* gene preferentially regulate aversive learning (Kim *et al.*, 2016). An important determinant in the functional role of basolateral amygdala neurons is their connectivity (Janak and Tye, 2015). Potential projections that could be involved in the ability of optogenetic stimulation of basolateral amygdala neurons to enhance conditioned approach include projections to the nucleus accumbens. For instance, appetitive CS preferentially increase the activity of basolateral amygdala neurons that send projections to the nucleus accumbens (Beyeler *et al.*, 2016). Additionally, appetitive CS promote neuronal plasticity in the basolateral amygdala-to-nucleus accumbens pathway, because throughout appetitive conditioning, synapses onto basolateral amygdala-to-nucleus accumbens neurons increase in strength (Namburi *et al.*, 2015). Of great interest for the present thesis is that the activity of that pathway is required for proper expression of conditioned approach, especially the projections to the core subdivision. Indeed, photoinhibition of basolateral amygdala terminals in the nucleus accumbens (mostly the core subdivision) during CS presentation (Stuber *et al.*, 2011)—but not after CS-UCS presentation (Namburi *et al.*, 2015)—reduces conditioned approach (FIG. 5.5A). Similarly, lesion of the basolateral amygdala reduces

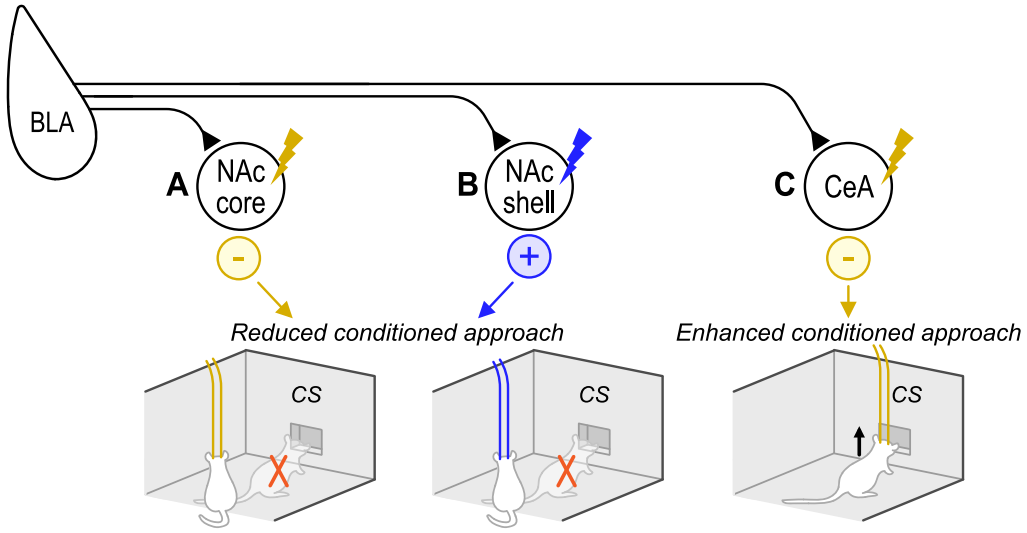

FIG. 5.5 — How do basolateral amygdala (BLA) pathways regulate conditioned approach elicited by appetitive conditioned stimuli (CS)? The current available literature shows that (***A***) photoinhibition of basolateral amygdala projections to the core subdivision of the nucleus accumbens (NAc) reduces conditioned approach, whereas (***B***) photoactivation of projections to the shell subdivision inhibits this CS effect. Furthermore, (***C***) photoinhibition of basolateral amygdala-to-central amygdala (CeA) neurons enhances conditioned approach.

CS-induced increased activity of nucleus accumbens neurons in the core but not shell subdivision (Jones *et al.*, 2010a), as well as CS-induced dopamine release in the nucleus accumbens core (Jones *et al.*, 2010b). Hence, basolateral amygdala-to-nucleus accumbens core neurons are necessary for conditioned approach, and future studies should determine if activation of these neurons is sufficient.

Basolateral amygdala projections to the cortex represent other interesting candidates, such as the projections to the orbitofrontal cortex. Appetitive conditioning enhances the correlated electrophysiological activity between the basolateral amygdala and the orbitofrontal cortex (Schoenbaum *et al.*, 2000). Furthermore, lesion of the basolateral amygdala impairs *i*) the ability of animals to discriminate distinct CS that predict whether an unconditioned reward is available, and *ii*) the encoding of CS-UCS association in the orbitofrontal cortex (Schoenbaum *et al.*, 2003). Projections to the prelimbic cortex could promote conditioned approach as well. Basolateral amygdala-to-prelimbic cortex neurons show enhanced protein levels of Fos late into appetitive conditioning but not early on (Keefer and Petrovich, 2017), suggesting that an increase in the activity of that pathway parallels learning of CS-UCS contingency. The impact on appetitive

conditioning of such increased activity remains to be determined because so far, the available literature highlights an important role of that pathway in fear conditioning rather than appetitive conditioning. Indeed, coherent electrophysiological activity between the basolateral amygdala and prelimbic cortex is greater during aversive conditioning relative to appetitive conditioning (Burgos-Robles *et al.*, 2017). Furthermore, photostimulation of basolateral amygdala-to-prelimbic cortex neurons is sufficient to elicit freezing, a typical conditioned response elicited by aversive CS (Burgos-Robles *et al.*, 2017). Besides projection neurons, basolateral amygdala interneurons could also influence appetitive conditioning. This has already been explored in fear conditioning procedures, where it was demonstrated that parvalbumin- and somatostatin-expressing interneurons of the basolateral amygdala regulate conditioned responses elicited by aversive CS (Wolff *et al.*, 2014). Their role in appetitive conditioning remains to be determined.

While the projections to the core subdivision of the nucleus accumbens could be sufficient to enhance conditioned approach, projections to the nucleus accumbens shell are not. In fact, optogenetic stimulation of basolateral amygdala-to-nucleus accumbens shell neurons *reduces* conditioned approach (FIG. 5.5B) (Millan *et al.*, 2017). Similarly, projections to the central amygdala are unlikely sufficient to promote conditioned approach. Indeed, work on the basolateral amygdala projections to the central amygdala showed that this pathway adjusts its level of activity during Pavlovian conditioning in accordance with UCS nature, and its activation promotes fear conditioning rather than appetitive conditioning. For instance, basolateral amygdala-to-central amygdala neurons are preferentially activated by aversive CS than appetitive CS (Beyeler *et al.*, 2016). Similarly, basolateral amygdala neurons projecting to the central amygdala receive weaker synaptic input during appetitive conditioning and stronger synaptic input during fear conditioning (Namburi *et al.*, 2015). Decreased plasticity in the basolateral-to-central amygdala pathway during appetitive conditioning is a necessary adaptation, because optogenetic inhibition of basolateral amygdala-to-central amygdala neurons potentiates conditioned approach (FIG. 5.5C) (Namburi *et al.*, 2015). Hence, the projections to the nucleus accumbens shell and the central amygdala are not (or unlikely) implicated in the ability of optogenetic stimulation of basolateral amygdala neurons to promote conditioned approach. Nonetheless, the studies above illustrate the importance to dissect basolateral amygdala pathways, because they can promote behavioural responses during CS-UCS conditioning through opposing mechanisms (*e.g.*, decreased versus increased neuronal plasticity).

2.2.2. How Do Basolateral Amygdala Neurons Intensify Incentive Motivation for Conditioned Stimuli?

As with conditioned approach, projections to the nucleus accumbens could play an important role. This is supported indirectly by the finding that optogenetic activation of basolateral amygdala-to-nucleus accumbens (core and/or shell) pathway is sufficient to reinforce ICSS (FIG. 5.6A) (Stuber *et al.*, 2011; Britt *et al.*, 2012; Namburi *et al.*, 2015). Hence, activation of this pathway is intrinsically rewarding, and could perhaps imbue CS with motivational salience. Furthermore, conditioned place preference elicited by cocaine correlates with greater protein levels of Fos in basolateral amygdala neurons projecting to the nucleus accumbens, suggesting that when a contextual CS is imbued with motivational salience, these neurons are more active (Miller and Marshall, 2005). Additionally, basolateral amygdala projections to the nucleus accumbens are required for the ability of CS to elicit instrumental responses in the CS-induced reinstatement of drug seeking paradigm. Indeed, decreasing the activity of basolateral amygdala projections in the nucleus accumbens via selective apoptosis (Keistler *et al.*, 2017), optogenetic inhibition (FIG. 5.6B) (Stefanik and Kalivas, 2013) or artificial induction of long-term depression using optogenetic methods (Lee *et al.*, 2013) reduces CS-induced reinstatement of drug seeking.

Activation of basolateral amygdala projections to the prefrontal cortex could also be sufficient to increase the motivational value of CS. For instance, basolateral amygdala-to-prelimbic cortex neurons show enhance levels of Fos following conditioned place preference elicited by cocaine (Miller and Marshall, 2005), suggesting increased activity of these neurons when appetitive CS have acquired incentive salience. Additionally, optogenetic inhibition of basolateral amygdala-to-prelimbic cortex neurons reduces CS-induced reinstatement of extinguished cocaine seeking (FIG. 5.6C) (Stefanik and Kalivas, 2013). In accordance with this finding, pharmacological inhibition (using lidocaine) of the basolateral amygdala of one hemisphere and of the contralateral prelimbic cortex reduces CS-induced reinstatement of extinguished cocaine seeking (Mashhoon *et al.*, 2010). Regarding the orbitofrontal cortex, photoinhibition of basolateral amygdala projections to that cortical area does not impair CS-induced reinstatement of extinguished drug seeking, suggesting that this projection is not required for that motivational effect of CS (FIG. 5.6D) (Arguello *et al.*, 2017). However, these neurons seems important in more complex settings when different CS and actions are presented/available. Indeed, chemogenetic inhibition of basolateral amygdala-to-

orbitofrontal cortex neurons disrupt instrumental responding elicited by CS when different rewards are each associated with specific CS and actions, as shown using outcome-specific Pavlovian-to-instrumental transfer (FIG. 5.6E) (Lichtenberg *et al.*, 2017). Interestingly, this intra-cranial manipulation does not influence reward seeking that is elicited by non-contingent delivery of the unconditioned reward, suggesting that basolateral amygdala-to-orbitofrontal cortex pathway is especially important to promote reward seeking elicited by CS but not by UCS themselves (Lichtenberg *et al.*, 2017).

The findings above are mostly correlational and/or suggestive that basolateral amygdala projection to the nucleus accumbens or prefrontal cortex could regulate incentive motivation for CS. However, it is important to note that when evaluating CS-induced reinstatement of extinguished reward seeking, animals lever press for CS presentation, but that instrumental response was previously reinforced by both drug injections/reward delivery and the CS (FIG. 1.14, page 67). Similarly, in Pavlovian-to-instrumental transfer procedures, CS elicit lever presses, but this

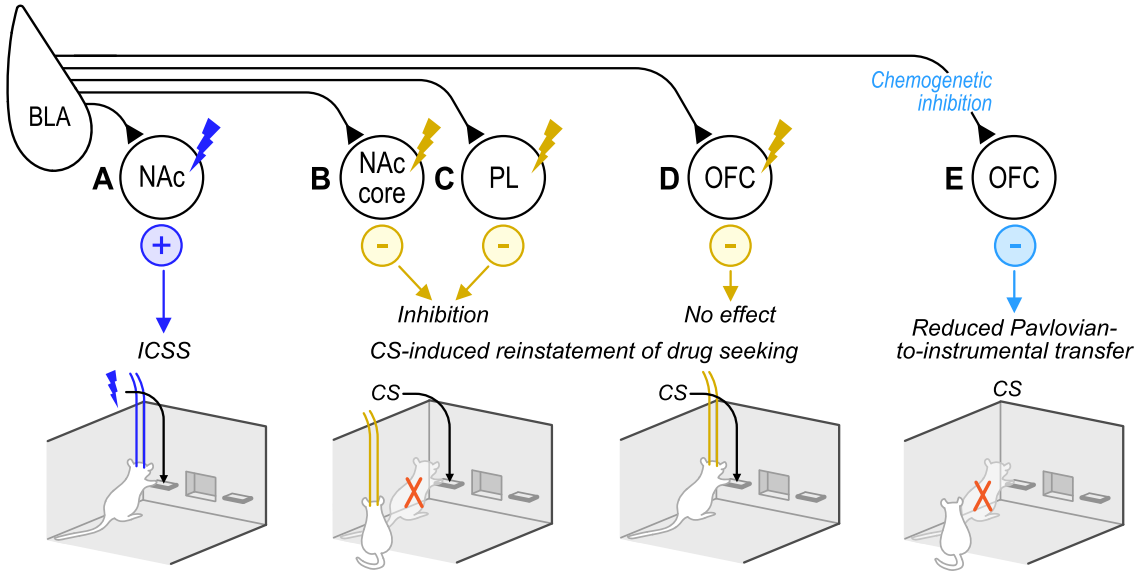

FIG. 5.6 — How do basolateral amygdala (BLA) pathways regulate motivated behaviours? (***A***) Optogenetic stimulation of basolateral amygdala projections to the nucleus accumbens is intrinsically reinforcing because it supports intra-cranial self-stimulation (ICSS). Photoinhibition of basolateral amygdala projections to the (***B***) nucleus accumbens (NAc) core or to the (***C***) prelimbic cortex (PL) but not to the (***D***) orbitofrontal cortex (OFC) inhibits conditioned stimulus (CS)-induced reinstatement of extinguished drug seeking. Additionally, (***E***) chemogenetic inhibition of basolateral amygdala-to-orbitofrontal cortex neurons abolishes outcome-specific Pavlovian-to-instrumental transfer.

instrumental response was previously reinforced by the unconditioned reward (FIG. 1.13C, page 63). These procedures adequately evaluate CS-elicited reward seeking. However, to adequately evaluate the incentive motivational value of CS themselves, future studies should determine how basolateral amygdala projections influence instrumental responses that are solely reinforced by CS and never by the associated UCS. Lastly, we found that d-amphetamine infusion into the basolateral amygdala is sufficient to enhance the incentive motivational effects of CS. To better understand this d-amphetamine effect, future studies should investigate the role of monoamine transmission in the basolateral amygdala in motivation for CS. The basolateral amygdala receives dopaminergic, noradrenergic and serotonergic projections (Fallon *et al.*, 1978; Vertes, 1991; Vertes *et al.*, 1999). Special attention should be given on dopamine projections coming from the VTA, because of their well-known role in motivation (Section 1.3.2 in the Introduction, page 28).

2.2.3. Why Does Increased Basolateral Amygdala Activity Enhance the Behavioural Effects of CS?

In addition to mechanistic questions, the present results bring questions regarding *why* optogenetic stimulation of basolateral amygdala neurons potentiate the behavioural effects of CS. For instance, there are various effects that can enhance conditioned approach. As already discussed in Chapter IV, increased neuronal activity in the basolateral amygdala could potentiate conditioned approach by increasing the motivational value of water [even if it is unlikely (Cador *et al.*, 1989)] or by enhancing the ability of CS to act as predictors of incoming reward availability. Another possibility is that stimulation of basolateral amygdala neurons is rewarding and thereby, this rewarding effect facilitate appetitive conditioning. However, we rule out that possibility by showing that optogenetic stimulation of basolateral amygdala neurons does not support ICSS, indicating that it is not intrinsically rewarding. Additionally, optogenetic stimulation of basolateral amygdala neurons could potentiate the motivational salience that CS acquire during Pavlovian conditioning, which would consequently facilitate their ability to elicit conditioned approach. Due to technical limitations (low number of rats, seizure), we could not determine whether optogenetic stimulation of basolateral amygdala neurons during appetitive conditioning subsequently potentiate incentive motivation for CS in the conditioned reinforcement test. Instead, we found that optogenetic stimulation of basolateral amygdala neurons potentiate the expression of incentive motivation for CS in animals that already show that incentive motivation, as assessed in the conditioned

reinforcement test that combined CS presentation with optogenetic stimulation of basolateral amygdala neurons. That observation does not allow to determine whether enhancing neuronal activity in the basolateral amygdala during appetitive conditioning promotes the ability of the CS to *acquire* incentive motivational value and consequently, to enhance conditioned approach. A behavioural paradigm that is well suited to examine this question is auto-shaping. If optogenetic stimulation of basolateral amygdala neurons promote the acquisition of incentive motivation for CS, then this would be indicated by an increase in sign-tracking behaviour during appetitive conditioning (Section 3.2.2 in the Introduction, page 62).

Finally, it was previously proposed that the basolateral amygdala directs the ability of animals to discriminate their response on levers in the conditioned reinforcement test, whereas this nucleus has little influence on the degree of motivation for CS (Cador *et al.*, 1989). Indeed, lesion of the basolateral amygdala reduces operant responding for CS, but it does not influence the ability of d-amphetamine infused into the nucleus accumbens core to intensify lever responding for CS (Cador *et al.*, 1989). Here, our rats already discriminated their response on the active and inactive levers. Therefore, if the intra-cranial manipulations we used here (optogenetics and local infusion of d-amphetamine) influence the ability of animals to discriminate their lever responding, then it should have a limited effect in rats already discriminating. However, the manipulations did further increase presses on the active lever, supporting that the basolateral amygdala regulates the *intensity* of the motivation for CS. During the test where photostimulation of basolateral amygdala neurons and CS presentations were explicitly unpaired, only ChR2-expressing rats discriminated their response on the levers but presses on the active lever were not intensified. This could reflect that photostimulation of basolateral amygdala neurons outside of CS presentations may guide lever pressing behaviour toward CS (even if it requires further confirmation, as suggested earlier). In any case, our findings are not necessarily at odds with Cador *et al.* (1989), because the basolateral amygdala may regulate both the ability of animals to discriminate their response and the intensity of this response.

3. GENERAL LIMITATIONS

So far, the present discussion has point out specific limitations of Chapters II, III and IV, but there are also more general limitations that require to be highlighted. First, the present work was

exclusively done in male rats but most evidently, the present problematics concern both sexes. In the context of antipsychotic-evoked dopamine supersensitivity, there are some findings suggesting that the consequences of this supersensitivity could be more important in females. In other models of dopamine supersensitivity, it has been shown that behavioural sensitisation produced by repeated psychostimulant injections is stronger in females relative to males (Becker, 1999). Also, tardive dyskinesia, that is thought to commonly co-occur with antipsychotic-evoked dopamine supersensitivity (Chouinard *et al.*, 1978; Chouinard and Jones, 1980; Fallon and Dursun, 2011; Fallon *et al.*, 2012), may be more common among women than men, as suggested by a meta-analysis including 39,187 patients (Yassa and Jeste, 1992). Another important aspect for the present thesis is that females and males can perform differently in Pavlovian and instrumental conditioning tasks (Dalla and Shors, 2009). Hence, it is urgent to extend the present work to females as well. In terms of methodological limitation, different control conditions were tested and were pooled together to simplify the analysis and presentation of data. When this was done, analysis were used to ensure that the different control conditions were statistically equivalent. However, in some experiments, this involved to compare control conditions with a low number of rats (such as in the ICSS experiment in Chapter IV), and this in itself may limit to find significant differences between controls.

There are additional limitations regarding the work on antipsychotic drugs (Chapters II and III). First, we only studied the antipsychotic haloperidol, and most of the work on antipsychotic-evoked dopamine supersensitivity has been done using haloperidol and also less frequently olanzapine (Smith and Davis, 1975; Vonvoigtlander *et al.*, 1975; Turrone *et al.*, 2003a; Samaha *et al.*, 2007; Bedard *et al.*, 2011; Gill *et al.*, 2014; Amato *et al.*, 2018). This work should be extended to other antipsychotic drugs, especially given that drug type is a determinant factor for the development of dopamine supersensitivity. Indeed, typical antipsychotic drugs (such as haloperidol) are more likely to promote dopamine supersensitivity (Smith and Davis, 1975; Vonvoigtlander *et al.*, 1975; Clow *et al.*, 1980; Montanaro *et al.*, 1982; Fleminger *et al.*, 1983; Calza *et al.*, 1990; Marin and Chase, 1993; Samaha *et al.*, 2007; Fukushiro *et al.*, 2008; Tadokoro *et al.*, 2012; Bedard *et al.*, 2013) than atypical antipsychotic drugs (Samaha *et al.*, 2007; Fukushiro *et al.*, 2008; Carvalho *et al.*, 2009; Tadokoro *et al.*, 2012; Bedard *et al.*, 2013). Second, we studied antipsychotic-evoked dopamine supersensitivity in otherwise neurologically-intact rats. We used d-amphetamine to acutely mimic the increase in dopamine transmission that accompanied psychotic symptoms

(Howes *et al.*, 2012), but this does not model the chronic condition that is schizophrenia. It was previously shown that in an animal model of schizophrenia-like symptoms (prenatal exposure to methylazoxymethanol acetate), chronic haloperidol exposure produces dopamine supersensitivity as suggested by drug tolerance (Gill *et al.*, 2014). However, important aspects remain to be characterized. For instance, future studies should determine the effects of antipsychotic-evoked dopamine supersensitivity on animals already supersensitive to the behavioural effects of dopamine agonists, as found in several models of schizophrenia-like symptoms (Archer *et al.*, 1988; Lipska *et al.*, 1993; Jentsch *et al.*, 1998; Tenn *et al.*, 2003).

Regarding the use of optogenetic methods, it allows to precisely modulate neuronal activity, but results should still be interpreted cautiously. For instance, optogenetic activation of a given neuronal population consequently alters the activity of other neuronal populations. This in itself could regulate the behavioural effects produced by the optogenetic stimulation, rather than being directly modulated by the neuronal population targeted by optogenetic manipulations (Bernard, 2020).

CONCLUSIONS

Preclinical and clinical studies suggest that antipsychotic-evoked dopamine supersensitivity produces antipsychotic failure, worsens psychotic symptoms and promotes maladaptive motivated behaviours. Thereby, one of the goals of the present thesis was to investigate the neurobiological substrates mediating the expression of antipsychotic-evoked dopamine supersensitivity in rats, a necessary work to develop treatment strategies with better long-term outcomes for schizophrenia patients. The present thesis reveals important new mechanisms: *i*) D2-like-mediated transmission promotes dopamine supersensitivity—whether transmission via these receptors is stimulated or blocked—and this could represent an important mechanism by which dopamine supersensitivity produces treatment tolerance and worsen psychosis; *ii*) D1-like receptors are potential target to temper the expression of dopamine supersensitivity, because dopamine-supersensitive animals are responsive to the antidopaminergic effects of D1-like blockade (unlike D2-like blockade), and stimulation of these receptors does not evoke a sensitised response; *iii*) we identified an unexpected and important role of peripheral processes in the expression of antipsychotic-evoked dopamine supersensitivity; and *iv*) the expression of antipsychotic-evoked dopamine supersensitivity is linked to enhanced stress-like responses, and this could have important implications for worsening of psychosis produced by dopamine supersensitivity given that stress exacerbates psychotic symptoms. Additionally, antipsychotic-evoked dopamine supersensitivity intensifies the motivational properties of appetitive CS. Thereby, the present thesis last investigated the neurobiological substrates mediating the behavioural effects of appetitive CS in a normal state (*i.e.*, no prior antipsychotic treatment), because such work could provide novel insights on the neurobiological effects of antipsychotic-evoked dopamine supersensitivity. We found that optogenetic stimulation of basolateral amygdala neurons potentiates the behavioural effects of appetitive CS, because it intensifies their incentive motivational value and their ability to increase the anticipation of impending unconditioned rewards. This suggests that excessive activity of basolateral amygdala neurons could attribute too much power to CS, and consequently this would confer them an increased ability to promote inadequate responses such as following antipsychotic drug exposure. Taken together, the present thesis identifies new neurobiological mechanisms underlying maladaptive processes evoked by antipsychotic drugs and conditioned rewards.

REFERENCES OF CHAPTERS I & V

Abi-Dargham A, Xu X, Thompson JL, Gil R, Kegeles LS, Urban N, Narendran R, Hwang DR, Laruelle M, Slifstein M (2012) Increased prefrontal cortical D(1) receptors in drug naive patients with schizophrenia: a PET study with [(1)(1)C]NNC112. J Psychopharmacol 26:794-805.

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M (2000) Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. Proc Natl Acad Sci U S A 97:8104-8109.

Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, Hwang DR, Keilp J, Kochan L, Van Heertum R, Gorman JM, Laruelle M (2002) Prefrontal dopamine D1 receptors and working memory in schizophrenia. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:3708-3719.

Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L (2007) Neural substrates of awakening probed with optogenetic control of hypocretin neurons. Nature 450:420-424.

Airan RD, Thompson KR, Fenno LE, Bernstein H, Deisseroth K (2009) Temporally precise in vivo control of intracellular signalling. Nature 458:1025-1029.

Akimoto K, Hamamura T, Otsuki S (1989) Subchronic cocaine treatment enhances cocaine-induced dopamine efflux, studied by in vivo intracerebral dialysis. Brain research 490:339-344.

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366-375.

Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266-271.

Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357-381.

Amato D, Canneva F, Cumming P, Maschauer S, Groos D, Dahlmanns JK, Gromer TW, Chiofalo L, Dahlmanns M, Zheng F, Kornhuber J, Prante O, Alzheimer C, von Horsten S, Muller CP (2018) A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failure reversal: the role of the dopamine transporter. Mol Psychiatry.

Ambroggi F, Ishikawa A, Fields HL, Nicola SM (2008) Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. Neuron 59:648-661.

Andrzejewski ME, Ryals C (2016) Dissociable hippocampal and amygdalar D1-like receptor contribution to discriminated Pavlovian conditioned approach learning. Behavioural brain research 299:111-121.

Antelman SM, Eichler AJ, Black CA, Kocan D (1980) Interchangeability of stress and amphetamine in sensitization. Science 207:329-331.

Anthony JC, Warner LA, Kessler RC (1996) Comparative Epidemiology of Dependence on Tobacco, Alcohol, Controlled Substances, and Inhalants: Basic Findings From the National Comorbidity Survey. Experimental and Clinical Psychopharmacology 2:244-268.

Anticevic A, Van Snellenberg JX, Cohen RE, Repovs G, Dowd EC, Barch DM (2012) Amygdala recruitment in schizophrenia in response to aversive emotional material: a meta-analysis of neuroimaging studies. Schizophr Bull 38:608-621.

Aravanis AM, Wang LP, Zhang F, Meltzer LA, Mogri MZ, Schneider MB, Deisseroth K (2007) An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology. J Neural Eng 4:S143-156.

Archer T, Fredriksson A, Sundstrom E, Luthman J, Lewander T, Soderberg U, Jonsson G (1988) Prenatal methylazoxymethanol treatment potentiates d-amphetamine- and methylphenidate-induced motor activity in male and female rats. Pharmacology & toxicology 63:233-239.

Arguello AA, Richardson BD, Hall JL, Wang R, Hodges MA, Mitchell MP, Stuber GD, Rossi DJ, Fuchs RA (2017) Role of a Lateral Orbital Frontal Cortex-Basolateral Amygdala Circuit in Cue-Induced Cocaine-Seeking Behavior. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 42:727-735.

Arnt J (1995) Differential effects of classical and newer antipsychotics on the hypermotility induced by two dose levels of D-amphetamine. Eur J Pharmacol 283:55-62.

Asper H, Baggiolini M, Burki HR, Lauener H, Ruch W, Stille G (1973) Tolerance phenomena with neuroleptics catalepsy, apomorphine stereotypies and striatal dopamine metabolism in the rat after single and repeated administration of loxapine and haloperidol. Eur J Pharmacol 22:287-294.

Axelrod J, Tomchick R (1958) Enzymatic O-methylation of epinephrine and other catechols. The Journal of biological chemistry 233:702-705.

Baker DA, Khroyan TV, O'Dell LE, Fuchs RA, Neisewander JL (1996) Differential effects of intra-accumbens sulpiride on cocaine-induced locomotion and conditioned place preference. The Journal of pharmacology and experimental therapeutics 279:392-401.

Balleine BW, Killcross S (2006) Parallel incentive processing: an integrated view of amygdala function. Trends Neurosci 29:272-279.

Ban TA (2007) Fifty years chlorpromazine: a historical perspective. Neuropsychiatr Dis Treat 3:495-500.

Barch DM, Carter CS (2005) Amphetamine improves cognitive function in medicated individuals with schizophrenia and in healthy volunteers. Schizophr Res 77:43-58.

Bardo MT, Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology (Berl) 153:31-43.

Baumgartner HM, Olney JJ, Warlow SM, Schulkin J, Berridge KC (2017) Investigating corticotropin releasing factor in mediating appetitive behavior. Published abstract for the Society for Neuroscience Annual Meeting, Washington, DC.

Beaulieu JM, Gainetdinov RR, Caron MG (2007) The Akt-GSK-3 signaling cascade in the actions of dopamine. Trends Pharmacol Sci 28:166-172.

Becker JB (1999) Gender differences in dopaminergic function in striatum and nucleus accumbens. Pharmacol Biochem Behav 64:803-812.

Bedard AM, Maheux J, Levesque D, Samaha AN (2011) Continuous, but not intermittent, antipsychotic drug delivery intensifies the pursuit of reward cues. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 36:1248-1259.

Bedard AM, Maheux J, Levesque D, Samaha AN (2013) Prior haloperidol, but not olanzapine, exposure augments the pursuit of reward cues: implications for substance abuse in schizophrenia. Schizophr Bull 39:692-702.

Beninger RJ (1989) Dissociating the effects of altered dopaminergic function on performance and learning. Brain research bulletin 23:365-371.

Bergevin A, Girardot D, Bourque MJ, Trudeau LE (2002) Presynaptic mu-opioid receptors regulate a late step of the secretory process in rat ventral tegmental area GABAergic neurons. Neuropharmacology 42:1065-1078.

Berglind WJ, Case JM, Parker MP, Fuchs RA, See RE (2006) Dopamine D1 or D2 receptor antagonism within the basolateral amygdala differentially alters the acquisition of cocaine-cue associations necessary for cue-induced reinstatement of cocaine-seeking. Neuroscience 137:699-706.

Bernard C (2020) Optogenetics: Keep Interpretations Light. eNeuro 7.

Bernardi RE, Ryabinin AE, Berger SP, Lattal KM (2009) Post-retrieval disruption of a cocaine conditioned place preference by systemic and intrabasolateral amygdala beta2- and alpha1-adrenergic antagonists. Learn Mem 16:777-789.

Berndt A, Yizhar O, Gunaydin LA, Hegemann P, Deisseroth K (2009) Bi-stable neural state switches. Nat Neurosci 12:229-234.

Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K, Seitelberger F (1973) Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J Neurol Sci 20:415-455.

Berridge KC (2018) Evolving Concepts of Emotion and Motivation. Front Psychol 9:1647.

Bertler A, Falck B, Rosengren E (1963) The Direct Demonstration of a Barrier Mechanism in the Brain Capillaries. Acta Pharmacol Toxicol (Copenh) 20:317-321.

Beyeler A, Namburi P, Glober GF, Simonnet C, Calhoon GG, Conyers GF, Luck R, Wildes CP, Tye KM (2016) Divergent Routing of Positive and Negative Information from the Amygdala during Memory Retrieval. Neuron 90:348-361.

Bindra D (1974) A motivational view of learning, performance, and behavior modification. Psychol Rev 81:199-213.

Bjorklund A, Dunnett SB (2007) Dopamine neuron systems in the brain: an update. Trends Neurosci 30:194-202.

Bobo WV, Woodward ND, Sim MY, Jayathilake K, Meltzer HY (2011) The effect of adjunctive armodafinil on cognitive performance and psychopathology in antipsychotic-treated patients with schizophrenia/schizoaffective disorder: a randomized, double-blind, placebo-controlled trial. Schizophr Res 130:106-113.

Bonate PL, Swann A, Silverman PB (1997) Context-dependent cross-sensitization between cocaine and amphetamine. Life Sci 60:PL1-7.

Bonci A, Hopf FW (2005) The dopamine D2 receptor: new surprises from an old friend. Neuron 47:335-338.

Bonnet JJ, Costentin J (1986) GBR 12783, a potent and selective inhibitor of dopamine uptake: biochemical studies in vivo and ex vivo. Eur J Pharmacol 121:199-209.

Bose P, Rompre PP, Warren RA (2015) Neurotensin enhances glutamatergic EPSCs in VTA neurons by acting on different neurotensin receptors. Peptides 73:43-50.

Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2005) Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci 8:1263-1268.

Bozarth MA (1987) Methods of assessing the reinforcing properties of abused drugs. New York: Springer-Verlag.

Braun AR, Laruelle M, Mouradian MM (1997) Interactions between D1 and D2 dopamine receptor family agonists and antagonists: the effects of chronic exposure on behavior and receptor binding in rats and their clinical implications. J Neural Transm (Vienna) 104:341-362.

Britt JP, Benaliouad F, McDevitt RA, Stuber GD, Wise RA, Bonci A (2012) Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens. Neuron 76:790-803.

Brock JW, Farooqui S, Ross K, Prasad C (1992) Localization of dopamine D2 receptor protein in rat brain using polyclonal antibody. Brain research 578:244-250.

Brown PL, Kiyatkin EA (2006) The role of peripheral Na(+) channels in triggering the central excitatory effects of intravenous cocaine. The European journal of neuroscience 24:1182-1192.

Brozoski TJ, Brown RM, Rosvold HE, Goldman PS (1979) Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. Science 205:929-932.

Bunzow JR, Van Tol HH, Grandy DK, Albert P, Salon J, Christie M, Machida CA, Neve KA, Civelli O (1988) Cloning and expression of a rat D2 dopamine receptor cDNA. Nature 336:783-787.

Burgos-Robles A, Kimchi EY, Izadmehr EM, Porzenheim MJ, Ramos-Guasp WA, Nieh EH, Felix-Ortiz AC, Namburi P, Leppla CA, Presbrey KN, Anandalingam KK, Pagan-Rivera PA, Anahtar M, Beyeler A, Tye KM (2017) Amygdala inputs to prefrontal cortex guide behavior amid conflicting cues of reward and punishment. Nat Neurosci 20:824-835.

Burns LH, Everitt BJ, Robbins TW (1994) Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 impairs acquisition but not performance of discriminated approach to an appetitive CS. Behav Neural Biol 61:242-250.

Burt DR, Creese I, Snyder SH (1977) Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 196:326-328.

Butcher LL, Engel J (1969) Peripheral factors in the mediation of the effects of L-dopa on locomotor activity. J Pharm Pharmacol 21:614-616.

Bystritsky A, Kronemyer D (2014) Stress and anxiety: counterpart elements of the stress/anxiety complex. Psychiatr Clin North Am 37:489-518.

Cador M, Robbins TW, Everitt BJ (1989) Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. Neuroscience 30:77-86.

Cador M, Dulluc J, Mormede P (1993) Modulation of the locomotor response to amphetamine by corticosterone. Neuroscience 56:981-988.

Caine SB, Geyer MA, Swerdlow NR (1995) Effects of D3/D2 dopamine receptor agonists and antagonists on prepulse inhibition of acoustic startle in the rat. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 12:139-145.

Calabresi P, De Murtas M, Mercuri NB, Bernardi G (1992) Chronic neuroleptic treatment: D2 dopamine receptor supersensitivity and striatal glutamatergic transmission. Ann Neurol 31:366-373.

Calza L, Giardino L, Piazza PV, Amato G (1990) Thioridazine chronic administration: a behavioural and autoradiographic study. Neurosci Lett 109:206-211.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26:321-352.

Cardno AG, Gottesman, II (2000) Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet 97:12-17.

Carelli RM, Williams JG, Hollander JA (2003) Basolateral amygdala neurons encode cocaine self-administration and cocaine-associated cues. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:8204-8211.

Carlezon WA, Jr., Devine DP, Wise RA (1995) Habit-forming actions of nomifensine in nucleus accumbens. Psychopharmacology (Berl) 122:194-197.

Carlsen J (1988) Immunocytochemical localization of glutamate decarboxylase in the rat basolateral amygdaloid nucleus, with special reference to GABAergic innervation of amygdalostriatal projection neurons. J Comp Neurol 273:513-526.

Carlsson A, Lindqvist M (1963) Effect of Chlorpromazine or Haloperidol on Formation of 3methoxytyramine and Normetanephrine in Mouse Brain. Acta Pharmacol Toxicol (Copenh) 20:140-144.

Carlsson A, Lindqvist M, Magnusson T (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180:1200.

Carpenter WT, Jr., Buchanan RW, Kirkpatrick B, Lann HD, Breier AF, Summerfelt AT (1999) Comparative effectiveness of fluphenazine decanoate injections every 2 weeks versus every 6 weeks. The American journal of psychiatry 156:412-418.

Cartoni E, Balleine B, Baldassarre G (2016) Appetitive Pavlovian-instrumental Transfer: A review. Neurosci Biobehav Rev 71:829-848.

Carvalho RC, Fukushiro DF, Helfer DC, Callegaro-Filho D, Trombin TF, Zanlorenci LH, Sanday L, Silva RH, Frussa-Filho R (2009) Long-term haloperidol treatment (but not risperidone) enhances addiction-related behaviors in mice: role of dopamine D2 receptors. Addiction biology 14:283-293.

Casey DE (1991) Neuroleptic drug-induced extrapyramidal syndromes and tardive dyskinesia. Schizophr Res 4:109-120.

Chang CY, Esber GR, Marrero-Garcia Y, Yau HJ, Bonci A, Schoenbaum G (2016) Brief optogenetic inhibition of dopamine neurons mimics endogenous negative reward prediction errors. Nat Neurosci 19:111-116.

Chang SE, Wheeler DS, Holland PC (2012) Roles of nucleus accumbens and basolateral amygdala in autoshaped lever pressing. Neurobiol Learn Mem 97:441-451.

Chaudhri N, Woods CA, Sahuque LL, Gill TM, Janak PH (2013) Unilateral inactivation of the basolateral amygdala attenuates context-induced renewal of Pavlovian-conditioned alcohol-seeking. The European journal of neuroscience 38:2751-2761.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807-821.

Chen Y, Mestek A, Liu J, Hurley JA, Yu L (1993) Molecular cloning and functional expression of a mu-opioid receptor from rat brain. Mol Pharmacol 44:8-12.

Chesi AJ, Feasey-Truger KJ, Alzheimer C, ten Bruggencate G (1995) Dopamine autoreceptor sensitivity is unchanged in rat nucleus accumbens after chronic haloperidol treatment: an in vivo and in vitro voltammetric study. The European journal of neuroscience 7:2450-2457.

Chouinard G, Jones BD (1980) Neuroleptic-induced supersensitivity psychosis: clinical and pharmacologic characteristics. The American journal of psychiatry 137:16-21.

Chouinard G, Jones BD, Annable L (1978) Neuroleptic-induced supersensitivity psychosis. The American journal of psychiatry 135:1409-1410.

Chouinard G, Samaha AN, Chouinard VA, Peretti CS, Kanahara N, Takase M, Iyo M (2017) Antipsychotic-Induced Dopamine Supersensitivity Psychosis: Pharmacology, Criteria, and Therapy. Psychother Psychosom 86:189-219.

Chu B, Kelley AE (1992) Potentiation of reward-related responding by psychostimulant infusion into nucleus accumbens: Role of dopamine receptor subtypes. Psychobiology 20:153-162.

Clelland CL, Drouet V, Rilett KC, Smeed JA, Nadrich RH, Rajparia A, Read LL, Clelland JD (2016) Evidence that COMT genotype and proline interact on negative-symptom outcomes in schizophrenia and bipolar disorder. Transl Psychiatry 6:e891.

Clow A, Jenner P, Marsden CD (1979) Changes in dopamine-mediated behaviour during one year's neuroleptic administration. Eur J Pharmacol 57:365-375.

Clow A, Theodorou A, Jenner P, Marsden CD (1980) Cerebral dopamine function in rats following withdrawal from one year of continuous neuroleptic administration. Eur J Pharmacol 63:145-157.

Cole SL, Robinson MJF, Berridge KC (2018) Optogenetic self-stimulation in the nucleus accumbens: D1 reward versus D2 ambivalence. PLoS One 13:e0207694.

Compton DR, Johnson KM (1988) Effects of acute and chronic clozapine and haloperidol on in vitro release of acetylcholine and dopamine from striatum and nucleus accumbens. The Journal of pharmacology and experimental therapeutics 248:521-530.

Cook L, Sepinwall J (1975) Reinforcement schedules and extrapolations to humans from animals in behavioral pharmacology. Fed Proc 34:1889-1897.

Cools R, D'Esposito M (2011) Inverted-U-shaped dopamine actions on human working memory and cognitive control. Biological psychiatry 69:e113-125.

Corbett D, Wise RA (1980) Intracranial self-stimulation in relation to the ascending dopaminergic systems of the midbrain: a moveable electrode mapping study. Brain research 185:1-15.

Corbit LH, Balleine BW (2005) Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:962-970.

Crawley J, Goodwin FK (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 13:167-170.

Creese I, Burt DR, Snyder SH (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192:481-483.

Croft AP, O'Callaghan MJ, Shaw SG, Connolly G, Jacquot C, Little HJ (2008) Effects of minor laboratory procedures, adrenalectomy, social defeat or acute alcohol on regional brain concentrations of corticosterone. Brain research 1238:12-22.

Crombag HS, Grimm JW, Shaham Y (2002) Effect of dopamine receptor antagonists on renewal of cocaine seeking by reexposure to drug-associated contextual cues. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 27:1006-1015.

Crow TJ (1972) A map of the rat mesencephalon for electrical self-stimulation. Brain research 36:265-273.

Dal Toso R, Sommer B, Ewert M, Herb A, Pritchett DB, Bach A, Shivers BD, Seeburg PH (1989) The dopamine D2 receptor: two molecular forms generated by alternative splicing. EMBO J 8:4025-4034.

Dalia A, Uretsky NJ, Wallace LJ (1998) Dopaminergic agonists administered into the nucleus accumbens: effects on extracellular glutamate and on locomotor activity. Brain research 788:111-117.

Dalla C, Shors TJ (2009) Sex differences in learning processes of classical and operant conditioning. Physiol Behav 97:229-238.

Daniel DG, Weinberger DR, Jones DW, Zigun JR, Coppola R, Handel S, Bigelow LB, Goldberg TE, Berman KF, Kleinman JE (1991) The effect of amphetamine on regional cerebral blood flow during cognitive activation in schizophrenia. The Journal of neuroscience : the official journal of the Society for Neuroscience 11:1907-1917.

Das S, Grunert M, Williams L, Vincent SR (1997) NMDA and D1 receptors regulate the phosphorylation of CREB and the induction of c-fos in striatal neurons in primary culture. Synapse 25:227-233.

Davidson LL, Heinrichs RW (2003) Quantification of frontal and temporal lobe brain-imaging findings in schizophrenia: a meta-analysis. Psychiatry research 122:69-87.

Davis KL, Kahn RS, Ko G, Davidson M (1991) Dopamine in schizophrenia: a review and reconceptualization. The American journal of psychiatry 148:1474-1486.

de Haan L, van Bruggen M, Lavalaye J, Booij J, Dingemans PM, Linszen D (2003) Subjective experience and D2 receptor occupancy in patients with recent-onset schizophrenia treated with low-dose olanzapine or haloperidol: a randomized, double-blind study. The American journal of psychiatry 160:303-309.

De Hert M, Detraux J, van Winkel R, Yu W, Correll CU (2011) Metabolic and cardiovascular adverse effects associated with antipsychotic drugs. Nat Rev Endocrinol 8:114-126.

de Kloet ER (2000) Stress in the brain. Eur J Pharmacol 405:187-198.

de la Torre R, Farre M, Navarro M, Pacifici R, Zuccaro P, Pichini S (2004) Clinical pharmacokinetics of amfetamine and related substances: monitoring in conventional and non-conventional matrices. Clin Pharmacokinet 43:157-185.

Dearry A, Gingrich JA, Falardeau P, Fremeau RT, Jr., Bates MD, Caron MG (1990) Molecular cloning and expression of the gene for a human D1 dopamine receptor. Nature 347:72-76.

Defagot MC, Malchiodi EL, Villar MJ, Antonelli MC (1997) Distribution of D4 dopamine receptor in rat brain with sequence-specific antibodies. Brain Res Mol Brain Res 45:1-12.

Deisseroth K, Feng G, Majewska AK, Miesenbock G, Ting A, Schnitzer MJ (2006) Next-generation optical technologies for illuminating genetically targeted brain circuits. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:10380-10386.

Demjaha A, Murray RM, McGuire PK, Kapur S, Howes OD (2012) Dopamine synthesis capacity in patients with treatment-resistant schizophrenia. The American journal of psychiatry 169:1203-1210.

Den Boer JA, van Megen HJ, Fleischhacker WW, Louwerens JW, Slaap BR, Westenberg HG, Burrows GD, Srivastava ON (1995) Differential effects of the D1-DA receptor antagonist SCH39166 on positive and negative symptoms of schizophrenia. Psychopharmacology (Berl) 121:317-322.

Desamericq G, Schurhoff F, Meary A, Szoke A, Macquin-Mavier I, Bachoud-Levi AC, Maison P (2014) Long-term neurocognitive effects of antipsychotics in schizophrenia: a network meta-analysis. Eur J Clin Pharmacol 70:127-134.

Duvarci S, Simpson EH, Schneider G, Kandel ER, Roeper J, Sigurdsson T (2018) Impaired recruitment of dopamine neurons during working memory in mice with striatal D2 receptor overexpression. Nat Commun 9:2822.

Duvauchelle CL, Levitin M, MacConell LA, Lee LK, Ettenberg A (1992) Opposite effects of prefrontal cortex and nucleus accumbens infusions of flupenthixol on stimulant-induced locomotion and brain stimulation reward. Brain research 576:104-110.

Eidne KA, Taylor PL, Zabavnik J, Saunders PT, Inglis JD (1989) D2 receptor, a missing exon. Nature 342:865.

El Hage C, Bedard AM, Samaha AN (2015) Antipsychotic treatment leading to dopamine supersensitivity persistently alters nucleus accumbens function. Neuropharmacology.

El Mestikawy S, Wallen-Mackenzie A, Fortin GM, Descarries L, Trudeau LE (2011) From glutamate co-release to vesicular synergy: vesicular glutamate transporters. Nat Rev Neurosci 12:204-216.

Elkouzi A, Vedam-Mai V, Eisinger RS, Okun MS (2019) Emerging therapies in Parkinson disease - repurposed drugs and new approaches. Nat Rev Neurol 15:204-223.

Erhart SM, Marder SR, Carpenter WT (2006) Treatment of schizophrenia negative symptoms: future prospects. Schizophr Bull 32:234-237.

Erickson JD, Eiden LE, Hoffman BJ (1992) Expression cloning of a reserpine-sensitive vesicular monoamine transporter. Proc Natl Acad Sci U S A 89:10993-10997.

Ericson H, Radesater AC, Servin E, Magnusson O, Mohringe B (1996) Effects of intermittent and continuous subchronic administration of raclopride on motor activity, dopamine turnover and receptor occupancy in the rat. Pharmacology & toxicology 79:277-286.

Ernest D, Vuksic O, Shepard-Smith A, Webb E (2017) Schizophrenia : an information guide. Centre for Addiction and Mental Health.

Ervin GN, Birkemo LS, Nemeroff CB, Prange AJ, Jr. (1981) Neurotensin blocks certain amphetamine-induced behaviours. Nature 291:73-76.

Everitt BJ, Morris KA, O'Brien A, Robbins TW (1991) The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience 42:1-18.

Fallon JH, Moore RY (1978) Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. J Comp Neurol 180:545-580.

Fallon JH, Koziell DA, Moore RY (1978) Catecholamine innervation of the basal forebrain. II. Amygdala, suprarhinal cortex and entorhinal cortex. J Comp Neurol 180:509-532.

Fallon P, Dursun SM (2011) A naturalistic controlled study of relapsing schizophrenic patients with tardive dyskinesia and supersensitivity psychosis. J Psychopharmacol 25:755-762.

Fallon P, Dursun S, Deakin B (2012) Drug-induced supersensitivity psychosis revisited: characteristics of relapse in treatment-compliant patients. Therapeutic advances in psychopharmacology 2:13-22.

Fanselow MS, LeDoux JE (1999) Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron 23:229-232.

Farde L, Wiesel FA, Nordstrom AL, Sedvall G (1989) D1- and D2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. Psychopharmacology (Berl) 99 Suppl:S28-31.

Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G (1992) Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Archives of general psychiatry 49:538-544.

Feldman RS, Meyer JS, Quenzer LF (1997) Principles of neuropsychopharmacology. Sunderland, Mass.: Sinauer Associates.

Feltenstein MW, See RE (2007) NMDA receptor blockade in the basolateral amygdala disrupts consolidation of stimulus-reward memory and extinction learning during reinstatement of cocaine-seeking in an animal model of relapse. Neurobiol Learn Mem 88:435-444.

Ferenczi EA, Zalocusky KA, Liston C, Grosenick L, Warden MR, Amatya D, Katovich K, Mehta H, Patenaude B, Ramakrishnan C, Kalanithi P, Etkin A, Knutson B, Glover GH, Deisseroth K (2016) Prefrontal cortical regulation of brainwide circuit dynamics and reward-related behavior. Science 351:aac9698.

Flagel SB, Akil H, Robinson TE (2009) Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. Neuropharmacology 56 Suppl 1:139-148.

Flagel SB, Cameron CM, Pickup KN, Watson SJ, Akil H, Robinson TE (2011a) A food predictive cue must be attributed with incentive salience for it to induce c-fos mRNA expression in cortico-striatal-thalamic brain regions. Neuroscience 196:80-96.

Flagel SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I, Akers CA, Clinton SM, Phillips PE, Akil H (2011b) A selective role for dopamine in stimulus-reward learning. Nature 469:53-57.

Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR (2007) New insights into the mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol 47:681-698.

Fleminger S, Rupniak NM, Hall MD, Jenner P, Marsden CD (1983) Changes in apomorphine-induced stereotypy as a result of subacute neuroleptic treatment correlates with increased D-2 receptors, but not with increases in D-1 receptors. Biochem Pharmacol 32:2921-2927.

Forbes NF, Carrick LA, McIntosh AM, Lawrie SM (2009) Working memory in schizophrenia: a meta-analysis. Psychol Med 39:889-905.

Ford CP (2014) The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. Neuroscience 282:13-22.

Foster KW, Saranak J, Patel N, Zarilli G, Okabe M, Kline T, Nakanishi K (1984) A rhodopsin is the functional photoreceptor for phototaxis in the unicellular eukaryote Chlamydomonas. Nature 311:756-759.

French ED, Vantini G (1984) Phencyclidine-induced locomotor activity in the rat is blocked by 6-hydroxydopamine lesion of the nucleus accumbens: comparisons to other psychomotor stimulants. Psychopharmacology (Berl) 82:83-88.

Fuchs RA, See RE (2002) Basolateral amygdala inactivation abolishes conditioned stimulus- and heroin-induced reinstatement of extinguished heroin-seeking behavior in rats. Psychopharmacology (Berl) 160:425-433.

Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, See RE (2005) The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 30:296-309.

Fukushiro DF, Carvalho Rde C, Ricardo VP, Alvarez Jdo N, Ribeiro LT, Frussa-Filho R (2008) Haloperidol (but not ziprasidone) withdrawal potentiates sensitization to the hyperlocomotor effect of cocaine in mice. Brain research bulletin 77:124-128.

Fusar-Poli P, Meyer-Lindenberg A (2013) Striatal presynaptic dopamine in schizophrenia, Part I: meta-analysis of dopamine active transporter (DAT) density. Schizophr Bull 39:22-32.

Fusar-Poli P, Papanastasiou E, Stahl D, Rocchetti M, Carpenter W, Shergill S, McGuire P (2015) Treatments of Negative Symptoms in Schizophrenia: Meta-Analysis of 168 Randomized Placebo-Controlled Trials. Schizophr Bull 41:892-899.

Fuster JM, Uyeda AA (1971) Reactivity of limbic neurons of the monkey to appetitive and aversive signals. Electroencephalogr Clin Neurophysiol 30:281-293.

Fuxe K, Agnati L, Kalia M, Goldstein M, Andersson K, Härfstrand A (1985) Dopaminergic Systems in the Brain and Pituitary. Basic and Clinical Aspects of Neuroscience.

Gabriele A, See RE (2010) Reversible inactivation of the basolateral amygdala, but not the dorsolateral caudate putamen, attenuates consolidation of cocaine-cue associative learning in a reinstatement model of drug-seeking. The European journal of neuroscience 32:1024-1029.

Garita-Hernandez M, Guibbal L, Toualbi L, Routet F, Chaffiol A, Winckler C, Harinquet M, Robert C, Fouquet S, Bellow S, Sahel JA, Goureau O, Duebel J, Dalkara D (2018) Optogenetic Light Sensors in Human Retinal Organoids. Front Neurosci 12:789.

Gerdjikov TV, Ross GM, Beninger RJ (2004) Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of ERK or p38 MAP kinases in rats. Behav Neurosci 118:740-750.

Gerfen CR, Herkenham M, Thibault J (1987) The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. The Journal of neuroscience : the official journal of the Society for Neuroscience 7:3915-3934.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Jr., Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429-1432.

Gianutsos G, Drawbaugh RB, Hynes MD, Lal H (1974) Behavioral evidence for dopaminergic supersensitivity after chronic haloperidol. Life Sci 14:887-898.

Gill KM, Cook JM, Poe MM, Grace AA (2014) Prior antipsychotic drug treatment prevents response to novel antipsychotic agent in the methylazoxymethanol acetate model of schizophrenia. Schizophr Bull 40:341-350.

Ginovart N, Kapur S (2012) Role of dopamine D(2) receptors for antipsychotic activity. Handbook of experimental pharmacology:27-52.

Giros B, el Mestikawy S, Bertrand L, Caron MG (1991) Cloning and functional characterization of a cocaine-sensitive dopamine transporter. FEBS Lett 295:149-154.

Giros B, el Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T, Caron MG (1992) Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. Mol Pharmacol 42:383-390.

Goldberg TE, Egan MF, Gscheidle T, Coppola R, Weickert T, Kolachana BS, Goldman D, Weinberger DR (2003) Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. Archives of general psychiatry 60:889-896.

Goldman-Rakic PS (1992) Dopamine-mediated mechanisms of the prefrontal cortex. Seminars in the Neurosciences 4:149-159.

Goldman-Rakic PS (1995) Cellular basis of working memory. Neuron 14:477-485.

Gompf HS, Budygin EA, Fuller PM, Bass CE (2015) Targeted genetic manipulations of neuronal subtypes using promoter-specific combinatorial AAVs in wild-type animals. Front Behav Neurosci 9:152.

Gong W, Neill DB, Lynn M, Justice JB, Jr. (1999) Dopamine D1/D2 agonists injected into nucleus accumbens and ventral pallidum differentially affect locomotor activity depending on site. Neuroscience 93:1349-1358.

Gradinaru V, Zhang F, Ramakrishnan C, Mattis J, Prakash R, Diester I, Goshen I, Thompson KR, Deisseroth K (2010) Molecular and cellular approaches for diversifying and extending optogenetics. Cell 141:154-165.

Greenfield JG, Bosanquet FD (1953) The brain-stem lesions in Parkinsonism. J Neurol Neurosurg Psychiatry 16:213-226.

Gremel CM, Cunningham CL (2009) Involvement of amygdala dopamine and nucleus accumbens NMDA receptors in ethanol-seeking behavior in mice. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 34:1443-1453.

Grimm JW, See RE (2000) Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 22:473-479.

Grimm JW, Hope BT, Wise RA, Shaham Y (2001) Neuroadaptation. Incubation of cocaine craving after withdrawal. Nature 412:141-142.

Gunaydin LA, Yizhar O, Berndt A, Sohal VS, Deisseroth K, Hegemann P (2010) Ultrafast optogenetic control. Nat Neurosci 13:387-392.

Hafner H, Maurer K, Loffler W, Riecher-Rossler A (1993) The influence of age and sex on the onset and early course of schizophrenia. Br J Psychiatry 162:80-86.

Harfstrand A, Fuxe K, Cintra A, Agnati LF, Zini I, Wikstrom AC, Okret S, Yu ZY, Goldstein M, Steinbusch H, et al. (1986) Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. Proc Natl Acad Sci U S A 83:9779-9783.

Harrison PJ, Owen MJ (2003) Genes for schizophrenia? Recent findings and their pathophysiological implications. Lancet 361:417-419.

Harvey RC, James AC, Shields GE (2016) A Systematic Review and Network Meta-Analysis to Assess the Relative Efficacy of Antipsychotics for the Treatment of Positive and Negative Symptoms in Early-Onset Schizophrenia. CNS drugs 30:27-39.

Hashimoto T, Baba S, Ikeda H, Oda Y, Hashimoto K, Shimizu I (2018) Lack of dopamine supersensitivity in rats after chronic administration of blonanserin: Comparison with haloperidol. Eur J Pharmacol 830:26-32.

Hearst E, Jenkins HM (1974) Sign-tracking: The Stimulus-reinforcer Relation and Directed Action. Psychonomic Society, Austin.

Hemby SE, Jones GH, Hubert GW, Neill DB, Justice JB, Jr. (1994) Assessment of the relative contribution of peripheral and central components in cocaine place conditioning. Pharmacol Biochem Behav 47:973-979.

Hippius H (1989) The history of clozapine. Psychopharmacology (Berl) 99 Suppl:S3-5.

Hiroi N, White NM (1991) The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. The Journal of neuroscience : the official journal of the Society for Neuroscience 11:2107-2116.

Hitchcott PK, Phillips GD (1997) Amygdala and hippocampus control dissociable aspects of drug-associated conditioned rewards. Psychopharmacology (Berl) 131:187-195.

Hollister LE, Krieger GE, Kringel A, Roberts RH (1955) Treatment of chronic schizophrenic reactions with reserpine. Ann N Y Acad Sci 61:92-100.

Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB, Jr. (1991) Individual differences in locomotor activity and sensitization. Pharmacol Biochem Behav 38:467-470.

Hooks MS, Jones DN, Holtzman SG, Juncos JL, Kalivas PW, Justice JB, Jr. (1994) Individual differences in behavior following amphetamine, GBR-12909, or apomorphine but not SKF-38393 or quinpirole. Psychopharmacology (Berl) 116:217-225.

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull 35:549-562.

Howes OD, Hird EJ, Adams RA, Corlett PR, McGuire P (2020) Aberrant salience, information processing and dopaminergic signalling in people at clinical high risk for psychosis. Biological psychiatry In press.

Howes OD, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A, Kapur S (2012) The nature of dopamine dysfunction in schizophrenia and what this means for treatment. Archives of general psychiatry 69:776-786.

Huang N, Ase AR, Hebert C, van Gelder NM, Reader TA (1997) Effects of chronic neuroleptic treatments on dopamine D1 and D2 receptors: homogenate binding and autoradiographic studies. Neurochemistry international 30:277-290.

Huang Q, Zhou D, Chase K, Gusella JF, Aronin N, DiFiglia M (1992) Immunohistochemical localization of the D1 dopamine receptor in rat brain reveals its axonal transport, pre- and postsynaptic localization, and prevalence in the basal ganglia, limbic system, and thalamic reticular nucleus. Proc Natl Acad Sci U S A 89:11988-11992.

Huhn M, Nikolakopoulou A, Schneider-Thoma J, Krause M, Samara M, Peter N, Arndt T, Backers L, Rothe P, Cipriani A, Davis J, Salanti G, Leucht S (2019) Comparative efficacy and tolerability of 32 oral antipsychotics for the acute treatment of adults with multi-episode schizophrenia: a systematic review and network meta-analysis. Lancet 394:939-951.

Hunt GE, Large MM, Cleary M, Lai HMX, Saunders JB (2018) Prevalence of comorbid substance use in schizophrenia spectrum disorders in community and clinical settings, 1990-2017: Systematic review and meta-analysis. Drug Alcohol Depend 191:234-258.

Ichikawa J, Meltzer HY (1992) The effect of chronic atypical antipsychotic drugs and haloperidol on amphetamine-induced dopamine release in vivo. Brain research 574:98-104.

Igaz P, Tombol Z, Szabo PM, Liko I, Racz K (2008) Steroid biosynthesis inhibitors in the therapy of hypercortisolism: theory and practice. Curr Med Chem 15:2734-2747.

Ikemoto S (2007) Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. Brain Res Rev 56:27-78.

Ikemoto S, Glazier BS, Murphy JM, McBride WJ (1997) Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. The Journal of neuroscience : the official journal of the Society for Neuroscience 17:8580-8587.

Ilango A, Kesner AJ, Keller KL, Stuber GD, Bonci A, Ikemoto S (2014) Similar roles of substantia nigra and ventral tegmental dopamine neurons in reward and aversion. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:817-822.

Ishikawa A, Ambroggi F, Nicola SM, Fields HL (2008) Contributions of the amygdala and medial prefrontal cortex to incentive cue responding. Neuroscience 155:573-584.

Janak PH, Tye KM (2015) From circuits to behaviour in the amygdala. Nature 517:284-292.

Javitch JA, Snyder SH (1984) Uptake of MPP(+) by dopamine neurons explains selectivity of parkinsonism-inducing neurotoxin, MPTP. Eur J Pharmacol 106:455-456.

Jentsch JD, Taylor JR, Roth RH (1998) Subchronic phencyclidine administration increases mesolimbic dopaminergic system responsivity and augments stress- and psychostimulant-induced hyperlocomotion. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 19:105-113.

Jiang LH, Kasser RJ, Altar CA, Wang RY (1990) One year of continuous treatment with haloperidol or clozapine fails to induce a hypersensitive response of caudate putamen neurons to dopamine D1 and D2 receptor agonists. The Journal of pharmacology and experimental therapeutics 253:1198-1205.

Johnson RG, Jr. (1987) Proton pumps and chemiosmotic coupling as a generalized mechanism for neurotransmitter and hormone transport. Ann N Y Acad Sci 493:162-177.

Jomphe C, Lemelin PL, Okano H, Kobayashi K, Trudeau LE (2006) Bidirectional regulation of dopamine D2 and neurotensin NTS1 receptors in dopamine neurons. The European journal of neuroscience 24:2789-2800.

Jones JL, Day JJ, Wheeler RA, Carelli RM (2010a) The basolateral amygdala differentially regulates conditioned neural responses within the nucleus accumbens core and shell. Neuroscience 169:1186-1198.

Jones JL, Day JJ, Aragona BJ, Wheeler RA, Wightman RM, Carelli RM (2010b) Basolateral amygdala modulates terminal dopamine release in the nucleus accumbens and conditioned responding. Biological psychiatry 67:737-744.

Kalivas PW, Duffy P (1990) Effect of acute and daily neurotensin and enkephalin treatments on extracellular dopamine in the nucleus accumbens. The Journal of neuroscience : the official journal of the Society for Neuroscience 10:2940-2949.

Kamin LJ (1967) Predictability, surprise, attention and conditioning. Symposium on Punishment Technical Report No. 13.

Kane JM, D'Souza DC, Patkar AA, Youakim JM, Tiller JM, Yang R, Keefe RS (2010) Armodafinil as adjunctive therapy in adults with cognitive deficits associated with schizophrenia: a 4-week, double-blind, placebo-controlled study. The Journal of clinical psychiatry 71:1475-1481.

Kapur S (2003) Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. The American journal of psychiatry 160:13-23.

Kapur S, Remington G (1996) Serotonin-dopamine interaction and its relevance to schizophrenia. The American journal of psychiatry 153:466-476.

Kapur S, Seeman P (2001) Does fast dissociation from the dopamine d(2) receptor explain the action of atypical antipsychotics?: A new hypothesis. The American journal of psychiatry 158:360-369.

Kapur S, Wadenberg ML, Remington G (2000a) Are animal studies of antipsychotics appropriately dosed? Lessons from the bedside to the bench. Canadian journal of psychiatry Revue canadienne de psychiatrie 45:241-246.

Kapur S, Mizrahi R, Li M (2005) From dopamine to salience to psychosis--linking biology, pharmacology and phenomenology of psychosis. Schizophr Res 79:59-68.

Kapur S, VanderSpek SC, Brownlee BA, Nobrega JN (2003) Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. The Journal of pharmacology and experimental therapeutics 305:625-631.

Kapur S, Zipursky R, Jones C, Remington G, Houle S (2000b) Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. The American journal of psychiatry 157:514-520.

Kapur S, Zipursky R, Jones C, Shammi CS, Remington G, Seeman P (2000c) A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D2 receptor occupancy. Archives of general psychiatry 57:553-559.

Karlsson P, Smith L, Farde L, Harnryd C, Sedvall G, Wiesel FA (1995) Lack of apparent antipsychotic effect of the D1-dopamine receptor antagonist SCH39166 in acutely ill schizophrenic patients. Psychopharmacology (Berl) 121:309-316.

Katz RJ, Roth KA, Carroll BJ (1981) Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. Neurosci Biobehav Rev 5:247-251.

Katzung B (1992) Basic & Clinical Pharmacology. Appleton & Lange.

Kavanagh DJ, McGrath J, Saunders JB, Dore G, Clark D (2002) Substance misuse in patients with schizophrenia: epidemiology and management. Drugs 62:743-755.

Keefer SE, Petrovich GD (2017) Distinct recruitment of basolateral amygdala-medial prefrontal cortex pathways across Pavlovian appetitive conditioning. Neurobiol Learn Mem 141:27-32.

Keistler CR, Hammarlund E, Barker JM, Bond CW, DiLeone RJ, Pittenger C, Taylor JR (2017) Regulation of Alcohol Extinction and Cue-Induced Reinstatement by Specific Projections among Medial Prefrontal Cortex, Nucleus Accumbens, and Basolateral Amygdala. The Journal of neuroscience : the official journal of the Society for Neuroscience 37:4462-4471.

Kelley AE, Delfs JM (1991) Dopamine and conditioned reinforcement. I. Differential effects of amphetamine microinjections into striatal subregions. Psychopharmacology (Berl) 103:187-196.

Kelley AE, Lang CG, Gauthier AM (1988) Induction of oral stereotypy following amphetamine microinjection into a discrete subregion of the striatum. Psychopharmacology (Berl) 95:556-559.

Kelly PH, Iversen SD (1976) Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. Eur J Pharmacol 40:45-56.

Kelly PH, Seviour PW, Iversen SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain research 94:507-522.

Kempadoo KA, Tourino C, Cho SL, Magnani F, Leinninger GM, Stuber GD, Zhang F, Myers MG, Deisseroth K, de Lecea L, Bonci A (2013) Hypothalamic neurotensin projections promote reward by enhancing glutamate transmission in the VTA. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:7618-7626.

Kestler LP, Walker E, Vega EM (2001) Dopamine receptors in the brains of schizophrenia patients: a meta-analysis of the findings. Behavioural pharmacology 12:355-371.

Khaled MA, Pushparaj A, Di Ciano P, Diaz J, Le Foll B (2014) Dopamine D3 receptors in the basolateral amygdala and the lateral habenula modulate cue-induced reinstatement of nicotine seeking. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 39:3049-3058.

Khan ZU, Gutierrez A, Martin R, Penafiel A, Rivera A, de la Calle A (2000) Dopamine D5 receptors of rat and human brain. Neuroscience 100:689-699.

Khoo SY, LeCocq MR, Deyab GE, Chaudhri N (2019) Context and topography determine the role of basolateral amygdala metabotropic glutamate receptor 5 in appetitive Pavlovian responding. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 44:1524-1533.

Kim J, Pignatelli M, Xu S, Itohara S, Tonegawa S (2016) Antagonistic negative and positive neurons of the basolateral amygdala. Nat Neurosci 19:1636-1646.

Kim J, Zhang X, Muralidhar S, LeBlanc SA, Tonegawa S (2017) Basolateral to Central Amygdala Neural Circuits for Appetitive Behaviors. Neuron 93:1464-1479 e1465.

Kim KM, Baratta MV, Yang A, Lee D, Boyden ES, Fiorillo CD (2012) Optogenetic mimicry of the transient activation of dopamine neurons by natural reward is sufficient for operant reinforcement. PLoS One 7:e33612.

Kirrane RM, Mitropoulou V, Nunn M, New AS, Harvey PD, Schopick F, Silverman J, Siever LJ (2000) Effects of amphetamine on visuospatial working memory performance in schizophrenia spectrum personality disorder. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 22:14-18.

Kirschner M, Aleman A, Kaiser S (2017) Secondary negative symptoms - A review of mechanisms, assessment and treatment. Schizophr Res 186:29-38.

Kobayashi M, Arai E (1976) Effect of cortisone, aldosterone and nialamide on "amphetamine stereotypies" and brain methamphetamine levels of adrenalectomized rats. Psychopharmacologia 46:317-324.

Kolta MG, Shreve P, Uretsky NJ (1989) Effect of pretreatment with amphetamine on the interaction between amphetamine and dopamine neurons in the nucleus accumbens. Neuropharmacology 28:9-14.

Kosten TA (1997) Enhanced neurobehavioral effects of cocaine with chronic neuroleptic exposure in rats. Schizophr Bull 23:203-213.

Krause M, Zhu Y, Huhn M, Schneider-Thoma J, Bighelli I, Nikolakopoulou A, Leucht S (2018) Antipsychotic drugs for patients with schizophrenia and predominant or prominent negative symptoms: a systematic review and meta-analysis. Eur Arch Psychiatry Clin Neurosci 268:625-639.

Kravitz AV, Tye LD, Kreitzer AC (2012) Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nat Neurosci 15:816-818.

Krettek JE, Price JL (1978a) A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connections. J Comp Neurol 178:255-280.

Krettek JE, Price JL (1978b) Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. J Comp Neurol 178:225-254.

Krueger BK (1990) Kinetics and block of dopamine uptake in synaptosomes from rat caudate nucleus. J Neurochem 55:260-267.

Kruzich PJ, See RE (2001) Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine-seeking behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:RC155.

Kruzich PJ, Congleton KM, See RE (2001) Conditioned reinstatement of drug-seeking behavior with a discrete compound stimulus classically conditioned with intravenous cocaine. Behav Neurosci 115:1086-1092.

Kupchik YM, Brown RM, Heinsbroek JA, Lobo MK, Schwartz DJ, Kalivas PW (2015) Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. Nat Neurosci 18:1230-1232.

Kyriazi P, Headley DB, Pare D (2018) Multi-dimensional Coding by Basolateral Amygdala Neurons. Neuron 99:1315-1328 e1315.

Laborit H, Huguenard P, Alluaume R (1952) Un nouveau stabilisateur végétatif (le 4560 RP). Presse Med 60:206-208.

Laduron PM, Leysen JE (1979) Domperidone, a specific in vitro dopamine antagonist, devoid of in vivo central dopaminergic activity. Biochem Pharmacol 28:2161-2165.

Lajoie J, Bindra D (1976) An interpretation of autoshaping and related phenomena in terms of stimulus-incentive contingencies alone. Can J Psychol 30:157-173.

Langston JW, Ballard P, Tetrud JW, Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219:979-980.

Laruelle M, Abi-Dargham A (1999) Dopamine as the wind of the psychotic fire: new evidence from brain imaging studies. J Psychopharmacol 13:358-371.

Laruelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R (1999) Increased dopamine transmission in schizophrenia: relationship to illness phases. Biological psychiatry 46:56-72.

Laruelle M, Frankle WG, Narendran R, Kegeles LS, Abi-Dargham A (2005) Mechanism of action of antipsychotic drugs: from dopamine D(2) receptor antagonism to glutamate NMDA facilitation. Clin Ther 27 Suppl A:S16-24.

Lasser RA, Dirks B, Nasrallah H, Kirsch C, Gao J, Pucci ML, Knesevich MA, Lindenmayer JP (2013) Adjunctive lisdexamfetamine dimesylate therapy in adult outpatients with predominant negative symptoms of schizophrenia: open-label and randomized-withdrawal phases. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 38:2140-2149.

LeDoux JE (2000) Emotion circuits in the brain. Annu Rev Neurosci 23:155-184.

Lee BR, Ma YY, Huang YH, Wang X, Otaka M, Ishikawa M, Neumann PA, Graziane NM, Brown TE, Suska A, Guo C, Lobo MK, Sesack SR, Wolf ME, Nestler EJ, Shaham Y, Schluter

OM, Dong Y (2013) Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. Nat Neurosci 16:1644-1651.

Lee SC, Amir A, Headley DB, Haufler D, Pare D (2016) Basolateral amygdala nucleus responses to appetitive conditioned stimuli correlate with variations in conditioned behaviour. Nat Commun 7:12275.

Levesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, Schott D, Morgat JL, Schwartz JC, Sokoloff P (1992) Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[3H]hydroxy-N,N-di-n-propyl-2-aminotetralin. Proc Natl Acad Sci U S A 89:8155-8159.

Levey AI, Hersch SM, Rye DB, Sunahara RK, Niznik HB, Kitt CA, Price DL, Maggio R, Brann MR, Ciliax BJ (1993) Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. Proc Natl Acad Sci U S A 90:8861-8865.

Levy AD, Kim JJ, Ellison GD (1988) Chronic amphetamine alters D-2 but not D-1 agonist-induced behavioral responses in rats. Life Sci 43:1207-1213.

Lewis DA, Levitt P (2002) Schizophrenia as a disorder of neurodevelopment. Annu Rev Neurosci 25:409-432.

Leyton M, Stewart J (1990) Preexposure to foot-shock sensitizes the locomotor response to subsequent systemic morphine and intra-nucleus accumbens amphetamine. Pharmacol Biochem Behav 37:303-310.

Leyton M, Dagher A, Boileau I, Casey K, Baker GB, Diksic M, Gunn R, Young SN, Benkelfat C (2004) Decreasing amphetamine-induced dopamine release by acute phenylalanine/tyrosine depletion: A PET/[11C]raclopride study in healthy men. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 29:427-432.

Lichtenberg NT, Wassum KM (2017) Amygdala mu-opioid receptors mediate the motivating influence of cue-triggered reward expectations. The European journal of neuroscience 45:381-387.

Lichtenberg NT, Pennington ZT, Holley SM, Greenfield VY, Cepeda C, Levine MS, Wassum KM (2017) Basolateral Amygdala to Orbitofrontal Cortex Projections Enable Cue-Triggered Reward Expectations. The Journal of neuroscience : the official journal of the Society for Neuroscience 37:8374-8384.

Liddle PF (1987) The symptoms of chronic schizophrenia. A re-examination of the positive-negative dichotomy. Br J Psychiatry 151:145-151.

Lieberman JA, Kane JM, Alvir J (1987) Provocative tests with psychostimulant drugs in schizophrenia. Psychopharmacology (Berl) 91:415-433.

Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Keefe RS, Davis SM, Davis CE, Lebowitz BD, Severe J, Hsiao JK, Clinical Antipsychotic Trials of Intervention Effectiveness I (2005) Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med 353:1209-1223.

Lin EJ, Lee NJ, Slack K, Karl T, Duffy L, O'Brien E, Matsumoto I, Dedova I, Herzog H, Sainsbury A (2006) Distinct endocrine effects of chronic haloperidol or risperidone administration in male rats. Neuropharmacology 51:1129-1136.

Lin JY, Lin MZ, Steinbach P, Tsien RY (2009) Characterization of engineered channelrhodopsin variants with improved properties and kinetics. Biophys J 96:1803-1814.

Lindstrom LH, Gefvert O, Hagberg G, Lundberg T, Bergstrom M, Hartvig P, Langstrom B (1999) Increased dopamine synthesis rate in medial prefrontal cortex and striatum in schizophrenia indicated by L-(beta-11C) DOPA and PET. Biological psychiatry 46:681-688.

Lintas A, Chi N, Lauzon NM, Bishop SF, Gholizadeh S, Sun N, Tan H, Laviolette SR (2011) Identification of a dopamine receptor-mediated opiate reward memory switch in the basolateral amygdala-nucleus accumbens circuit. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:11172-11183.

Lipska BK, Jaskiw GE, Weinberger DR (1993) Postpubertal emergence of hyperresponsiveness to stress and to amphetamine after neonatal excitotoxic hippocampal damage: a potential animal model of schizophrenia. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 9:67-75.

Liu Y, Peter D, Roghani A, Schuldiner S, Prive GG, Eisenberg D, Brecha N, Edwards RH (1992) A cDNA that suppresses MPP+ toxicity encodes a vesicular amine transporter. Cell 70:539-551.

Ljungberg T, Ungerstedt U (1985) A rapid and simple behavioural screening method for simultaneous assessment of limbic and striatal blocking effects of neuroleptic drugs. Pharmacol Biochem Behav 23:479-485.

Loranger AW (1984) Sex difference in age at onset of schizophrenia. Archives of general psychiatry 41:157-161.

MacKenzie RG, Zigmond MJ (1985) Chronic neuroleptic treatment increases D-2 but not D-1 receptors in rat striatum. Eur J Pharmacol 113:159-165.

Maia TV, Frank MJ (2017) An Integrative Perspective on the Role of Dopamine in Schizophrenia. Biological psychiatry 81:52-66.

Malvaez M, Greenfield VY, Wang AS, Yorita AM, Feng L, Linker KE, Monbouquette HG, Wassum KM (2015) Basolateral amygdala rapid glutamate release encodes an outcome-specific representation vital for reward-predictive cues to selectively invigorate reward-seeking actions. Sci Rep 5:12511.

Mamo D, Kapur S, Keshavan M, Laruelle M, Taylor CC, Kothare PA, Barsoum P, McDonnell D (2008) D2 receptor occupancy of olanzapine pamoate depot using positron emission tomography: an open-label study in patients with schizophrenia. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33:298-304.

Mansbach RS, Geyer MA, Braff DL (1988) Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology (Berl) 94:507-514.

Mantsch JR, Saphier D, Goeders NE (1998) Corticosterone facilitates the acquisition of cocaine self-administration in rats: opposite effects of the type II glucocorticoid receptor agonist dexamethasone. The Journal of pharmacology and experimental therapeutics 287:72-80.

Mao Q, Tan YL, Luo XG, Tian L, Wang ZR, Tan SP, Chen S, Yang GG, An HM, Yang FD, Zhang XY (2016) Association of catechol-O-methyltransferase Val(108/158) Met genetic polymorphism with schizophrenia, P50 sensory gating, and negative symptoms in a Chinese population. Psychiatry research 242:271-276.

Maren S, Quirk GJ (2004) Neuronal signalling of fear memory. Nat Rev Neurosci 5:844-852.

Maren S, Phan KL, Liberzon I (2013) The contextual brain: implications for fear conditioning, extinction and psychopathology. Nat Rev Neurosci 14:417-428.

Marin C, Chase TN (1993) Dopamine D1 receptor stimulation but not dopamine D2 receptor stimulation attenuates haloperidol-induced behavioral supersensitivity and receptor up-regulation. Eur J Pharmacol 231:191-196.

Marin MT, Cruz FC, Planeta CS (2007) Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. Physiol Behav 90:29-35.

Marinelli M, Piazza PV (2002) Interaction between glucocorticoid hormones, stress and psychostimulant drugs. The European journal of neuroscience 16:387-394.

Marrow L, Statham A, Overton PG, Brain PF, Clark D (1999) The corticosteroid synthesis inhibitor metyrapone markedly enhances the behavioural effects of d-amphetamine. Addiction biology 4:89-92.

Mashhoon Y, Wells AM, Kantak KM (2010) Interaction of the rostral basolateral amygdala and prelimbic prefrontal cortex in regulating reinstatement of cocaine-seeking behavior. Pharmacol Biochem Behav 96:347-353.

Masterson FA, Crawford M (1982) The defense motivation system: A theory of avoidance behaviour. The behavioral and brain sciences 5:661-696.

Matsuno-Yagi A, Mukohata Y (1977) Two possible roles of bacteriorhodopsin; a comparative study of strains of Halobacterium halobium differing in pigmentation. Biochemical and biophysical research communications 78:237-243.

McAllister WR, McAllister DE (1971) Behavioral Measurement of Conditioned Fear. Aversive Conditioning and Learning:105-179.

McCreadie RG, Dingwall JM, Wiles DH, Heykants JJ (1980) Intermittent pimozide versus fluphenazine decanoate as maintenance therapy in chronic schizophrenia. Br J Psychiatry 137:510-517.

McCutcheon R, Beck K, Jauhar S, Howes OD (2018) Defining the Locus of Dopaminergic Dysfunction in Schizophrenia: A Meta-analysis and Test of the Mesolimbic Hypothesis. Schizophr Bull 44:1301-1311.

McCutcheon RA, Reis Marques T, Howes OD (2019a) Schizophrenia-An Overview. JAMA Psychiatry:1-10.

McCutcheon RA, Abi-Dargham A, Howes OD (2019b) Schizophrenia, Dopamine and the Striatum: From Biology to Symptoms. Trends Neurosci 42:205-220.

McDonald AJ (1982) Neurons of the lateral and basolateral amygdaloid nuclei: a Golgi study in the rat. J Comp Neurol 212:293-312.

McDonald AJ (1996) Glutamate and aspartate immunoreactive neurons of the rat basolateral amygdala: colocalization of excitatory amino acids and projections to the limbic circuit. J Comp Neurol 365:367-379.

McDonald AJ (1998) Cortical pathways to the mammalian amygdala. Prog Neurobiol 55:257-332.

McDonald AJ, Pearson JC (1989) Coexistence of GABA and peptide immunoreactivity in non-pyramidal neurons of the basolateral amygdala. Neurosci Lett 100:53-58.

McDonald AJ, Augustine JR (1993) Localization of GABA-like immunoreactivity in the monkey amygdala. Neuroscience 52:281-294.

McDonald AJ, Mascagni F (2002) Immunohistochemical characterization of somatostatin containing interneurons in the rat basolateral amygdala. Brain research 943:237-244.

McElvain JS, Schenk JO (1992) A multisubstrate mechanism of striatal dopamine uptake and its inhibition by cocaine. Biochem Pharmacol 43:2189-2199.

McLaughlin J, See RE (2003) Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology (Berl) 168:57-65.

McQuade RD, Duffy RA, Anderson CC, Crosby G, Coffin VL, Chipkin RE, Barnett A (1991) [3H]SCH 39166, a new D1-selective radioligand: in vitro and in vivo binding analyses. J Neurochem 57:2001-2010.

Meil WM, See RE (1997) Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. Behavioural brain research 87:139-148.

Meng ZH, Feldpaush DL, Merchant KM (1998) Clozapine and haloperidol block the induction of behavioral sensitization to amphetamine and associated genomic responses in rats. Brain Res Mol Brain Res 61:39-50.

Merchant KM, Dobie DJ, Filloux FM, Totzke M, Aravagiri M, Dorsa DM (1994) Effects of chronic haloperidol and clozapine treatment on neurotensin and c-fos mRNA in rat neostriatal subregions. The Journal of pharmacology and experimental therapeutics 271:460-471.

Meyer-Lindenberg A, Miletich RS, Kohn PD, Esposito G, Carson RE, Quarantelli M, Weinberger DR, Berman KF (2002) Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia. Nat Neurosci 5:267-271.

Michael AC, Ikeda M, Justice JB, Jr. (1987) Mechanisms contributing to the recovery of striatal releasable dopamine following MFB stimulation. Brain research 421:325-335.

Millan EZ, Kim HA, Janak PH (2017) Optogenetic activation of amygdala projections to nucleus accumbens can arrest conditioned and unconditioned alcohol consummatory behavior. Neuroscience 360:106-117.

Millan EZ, Reese RM, Grossman CD, Chaudhri N, Janak PH (2015) Nucleus Accumbens and Posterior Amygdala Mediate Cue-Triggered Alcohol Seeking and Suppress Behavior During the Omission of Alcohol-Predictive Cues. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 40:2555-2565.

Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. The Journal of pharmacology and experimental therapeutics 303:791-804.

Miller CA, Marshall JF (2005) Altered Fos expression in neural pathways underlying cue-elicited drug seeking in the rat. The European journal of neuroscience 21:1385-1393.

Miller G (2006) Optogenetics. Shining new light on neural circuits. Science 314:1674-1676.

Miracle AD, Brace MF, Huyck KD, Singler SA, Wellman CL (2006) Chronic stress impairs recall of extinction of conditioned fear. Neurobiol Learn Mem 85:213-218.

Mishara AL, Goldberg TE (2004) A meta-analysis and critical review of the effects of conventional neuroleptic treatment on cognition in schizophrenia: opening a closed book. Biological psychiatry 55:1013-1022.

Miyamoto S, Duncan GE, Marx CE, Lieberman JA (2005) Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. Mol Psychiatry 10:79-104.

Mizrahi R, Bagby RM, Zipursky RB, Kapur S (2005) How antipsychotics work: the patients' perspective. Progress in neuro-psychopharmacology & biological psychiatry 29:859-864.

Mizrahi R, Addington J, Rusjan PM, Suridjan I, Ng A, Boileau I, Pruessner JC, Remington G, Houle S, Wilson AA (2012) Increased stress-induced dopamine release in psychosis. Biological psychiatry 71:561-567.

MØller Nielsen I, Fjalland B, Pedersen V, Nymark M (1974) Pharmacology of neuroleptics upon repeated administration. Psychopharmacology (Berl) 34:95-104.

Montanaro N, Dall'Olio R, Gandolfi O, Vaccheri A (1982) Differential enhancement of behavioral sensitivity to apomorphine following chronic treatment of rats with (-)-sulpiride and haloperidol. European journal of pharmacology 81:1-9.

Morris ME (2000) Movement disorders in people with Parkinson disease: a model for physical therapy. Phys Ther 80:578-597.

Muench J, Hamer AM (2010) Adverse effects of antipsychotic medications. American family physician 81:617-622.

Mueser KT, McGurk SR (2003) Schizophrenia. The Lancet 363:2063-2072.

Muramoto K, Ono T, Nishijo H, Fukuda M (1993) Rat amygdaloid neuron responses during auditory discrimination. Neuroscience 52:621-636.

Murray RM, Quattrone D, Natesan S, van Os J, Nordentoft M, Howes O, Di Forti M, Taylor D (2016) Should psychiatrists be more cautious about the long-term prophylactic use of antipsychotics? Br J Psychiatry 209:361-365.

Nabavi S, Fox R, Proulx CD, Lin JY, Tsien RY, Malinow R (2014) Engineering a memory with LTD and LTP. Nature 511:348-352.

Nader K, Einarsson EO (2010) Memory reconsolidation: an update. Ann N Y Acad Sci 1191:27-41.

Naeem F, Kingdon D, Turkington D (2006) Cognitive behaviour therapy for schizophrenia: relationship between anxiety symptoms and therapy. Psychol Psychother 79:153-164.

Naeem M, White NM (2016) Parallel learning in an autoshaping paradigm. Behav Neurosci 130:376-392.

Nagel G, Brauner M, Liewald JF, Adeishvili N, Bamberg E, Gottschalk A (2005) Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses. Curr Biol 15:2279-2284.

Nagel G, Ollig D, Fuhrmann M, Kateriya S, Musti AM, Bamberg E, Hegemann P (2002) Channelrhodopsin-1: a light-gated proton channel in green algae. Science 296:2395-2398.

Nagel G, Szellas T, Huhn W, Kateriya S, Adeishvili N, Berthold P, Ollig D, Hegemann P, Bamberg E (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. Proc Natl Acad Sci U S A 100:13940-13945.

Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichmann R, Holden SS, Mertens KL, Anahtar M, Felix-Ortiz AC, Wickersham IR, Gray JM, Tye KM (2015) A circuit mechanism for differentiating positive and negative associations. Nature 520:675-678.

Neumeyer JL, Kula NS, Bergman J, Baldessarini RJ (2003) Receptor affinities of dopamine D1 receptor-selective novel phenylbenzazepines. Eur J Pharmacol 474:137-140.

Niemegeers CJ, Janssen PA (1979) A systematic study of the pharmacological activities of dopamine antagonists. Life Sci 24:2201-2216.

Nordstrom AL, Farde L, Wiesel FA, Forslund K, Pauli S, Halldin C, Uppfeldt G (1993) Central D2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. Biological psychiatry 33:227-235.

Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK (2004) Identification of separable cognitive factors in schizophrenia. Schizophr Res 72:29-39.

Nyberg S, Farde L, Halldin C, Dahl ML, Bertilsson L (1995) D2 dopamine receptor occupancy during low-dose treatment with haloperidol decanoate. The American journal of psychiatry 152:173-178.

Obuchowicz E (1999) Differential behavioural effect of quinpirole in neuroleptic-pretreated rats - role of alpha(1)-adrenoceptor. Eur J Pharmacol 384:101-109.

Oda Y, Tadokoro S, Takase M, Kanahara N, Watanabe H, Shirayama Y, Hashimoto K, Iyo M (2015) G protein-coupled receptor kinase 6/beta-arrestin 2 system in a rat model of dopamine supersensitivity psychosis. J Psychopharmacol 29:1308-1313.

Olausson P, Jentsch JD, Taylor JR (2004) Nicotine enhances responding with conditioned reinforcement. Psychopharmacology (Berl) 171:173-178.

Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J Comp Physiol Psychol 47:419-427.

Owen MJ, Craddock N, O'Donovan MC (2005) Schizophrenia: genes at last? Trends Genet 21:518-525.

Papenberg G, Karalija N, Salami A, Rieckmann A, Andersson M, Axelsson J, Riklund K, Lindenberger U, Lovden M, Nyberg L, Backman L (2019) Balance between Transmitter Availability and Dopamine D2 Receptors in Prefrontal Cortex Influences Memory Functioning. Cereb Cortex.

Pare D, Smith Y, Pare JF (1995) Intra-amygdaloid projections of the basolateral and basomedial nuclei in the cat: Phaseolus vulgaris-leucoagglutinin anterograde tracing at the light and electron microscopic level. Neuroscience 69:567-583.

Paulson PE, Robinson TE (1991) Sensitization to systemic amphetamine produces an enhanced locomotor response to a subsequent intra-accumbens amphetamine challenge in rats. Psychopharmacology (Berl) 104:140-141.

Paulson PE, Robinson TE (1995) Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: a microdialysis study in behaving rats. Synapse 19:56-65.

Pavlov IP (1927) Conditioned reflexes : an investigation of the physiological activity of the cerebral cortex. London: Oxford University Press.

Pellow S, File SE (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 24:525-529.

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149-167.

Piazza PV, Deminiere JM, Le Moal M, Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. Science 245:1511-1513.

Piazza PV, Deminiere JM, le Moal M, Simon H (1990) Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain research 514:22-26.

Piazza PV, Maccari S, Deminiere JM, Le Moal M, Mormede P, Simon H (1991) Corticosterone levels determine individual vulnerability to amphetamine self-administration. Proc Natl Acad Sci U S A 88:2088-2092.

Piazza PV, Rouge-Pont F, Deroche V, Maccari S, Simon H, Le Moal M (1996) Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. Proc Natl Acad Sci U S A 93:8716-8720.

Pijnenburg AJ, Honig WM, Van Rossum JM (1975) Inhibition of d-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. Psychopharmacologia 41:87-95.

Pijnenburg AJ, Honig WM, Van der Heyden JA, Van Rossum JM (1976) Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. Eur J Pharmacol 35:45-58.

Pilowsky LS, Busatto GF, Taylor M, Costa DC, Sharma T, Sigmundsson T, Ell PJ, Nohria V, Kerwin RW (1996) Dopamine D2 receptor occupancy in vivo by the novel atypical

antipsychotic olanzapine--a 123I IBZM single photon emission tomography (SPET) study. Psychopharmacology (Berl) 124:148-153.

Pinkham AE, Liu P, Lu H, Kriegsman M, Simpson C, Tamminga C (2015) Amygdala Hyperactivity at Rest in Paranoid Individuals With Schizophrenia. The American journal of psychiatry 172:784-792.

Pitkanen A, Savander V, LeDoux JE (1997) Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding functions of the amygdala. Trends Neurosci 20:517-523.

Primus RJ, Thurkauf A, Xu J, Yevich E, McInerney S, Shaw K, Tallman JF, Gallagher DW (1997) II. Localization and characterization of dopamine D4 binding sites in rat and human brain by use of the novel, D4 receptor-selective ligand [3H]NGD 94-1. The Journal of pharmacology and experimental therapeutics 282:1020-1027.

Pudiak CM, Bozarth MA (1997) Nitric oxide synthesis inhibition attenuates haloperidol-induced supersensitivity. J Psychiatry Neurosci 22:61-64.

Rademacher DJ, Kovacs B, Shen F, Napier TC, Meredith GE (2006) The neural substrates of amphetamine conditioned place preference: implications for the formation of conditioned stimulus-reward associations. The European journal of neuroscience 24:2089-2097.

Ramos M, Goni-Allo B, Aguirre N (2004) Studies on the role of dopamine D1 receptors in the development and expression of MDMA-induced behavioral sensitization in rats. Psychopharmacology (Berl) 177:100-110.

Read J, van Os J, Morrison AP, Ross CA (2005) Childhood trauma, psychosis and schizophrenia: a literature review with theoretical and clinical implications. Acta Psychiatr Scand 112:330-350.

Rebec GV, Segal DS (1979) Enhanced responsiveness to intraventricular infusion of amphetamine following its repeated systemic administration. Psychopharmacology (Berl) 62:101-102.

Rebec GV, Peirson EE, McPherson FA, Brugge K (1982) Differential sensitivity to amphetamine following long-term treatment with clozapine or haloperidol. Psychopharmacology (Berl) 77:360-366.

Reith J, Benkelfat C, Sherwin A, Yasuhara Y, Kuwabara H, Andermann F, Bachneff S, Cumming P, Diksic M, Dyve SE, Etienne P, Evans AC, Lal S, Shevell M, Savard G, Wong DF, Chouinard G, Gjedde A (1994) Elevated dopa decarboxylase activity in living brain of patients with psychosis. Proc Natl Acad Sci U S A 91:11651-11654.

Remington G, Seeman P, Shammi C, Mann S, Kapur S (2005) "Extended" antipsychotic dosing: rationale and pilot data. J Clin Psychopharmacol 25:611-613.

Remington G, Seeman P, Feingold A, Mann S, Shammi C, Kapur S (2011) "Extended" antipsychotic dosing in the maintenance treatment of schizophrenia: a double-blind, placebo-controlled trial. The Journal of clinical psychiatry 72:1042-1048.

Remington G, Mamo D, Labelle A, Reiss J, Shammi C, Mannaert E, Mann S, Kapur S (2006) A PET study evaluating dopamine D2 receptor occupancy for long-acting injectable risperidone. The American journal of psychiatry 163:396-401.

Reul JM, de Kloet ER (1986) Anatomical resolution of two types of corticosterone receptor sites in rat brain with in vitro autoradiography and computerized image analysis. Journal of steroid biochemistry 24:269-272.

Richtand NM, Welge JA, Logue AD, Keck PE, Jr., Strakowski SM, McNamara RK (2007) Dopamine and serotonin receptor binding and antipsychotic efficacy. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 32:1715-1726.

Robbins TW (1978) The acquisition of responding with conditioned reinforcement: effects of pipradrol, methylphenidate, d-amphetamine, and nomifensine. Psychopharmacology (Berl) 58:79-87.

Robbins TW, Watson BA, Gaskin M, Ennis C (1983) Contrasting interactions of pipradrol, d-amphetamine, cocaine, cocaine analogues, apomorphine and other drugs with conditioned reinforcement. Psychopharmacology (Berl) 80:113-119.

Robertson HA, Peterson MR, Murphy K, Robertson GS (1989) D1-dopamine receptor agonists selectively activate striatal c-fos independent of rotational behaviour. Brain research 503:346-349.

Robinson JB (1985) Stereoselectivity and isoenzyme selectivity of monoamine oxidase inhibitors. Enantiomers of amphetamine, N-methylamphetamine and deprenyl. Biochem Pharmacol 34:4105-4108.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247-291.

Robinson TE, Flagel SB (2009) Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences. Biological psychiatry 65:869-873.

Robinson TE, Angus AL, Becker JB (1985) Sensitization to stress: the enduring effects of prior stress on amphetamine-induced rotational behavior. Life Sci 37:1039-1042.

Robinson TE, Jurson PA, Bennett JA, Bentgen KM (1988) Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: a microdialysis study in freely moving rats. Brain research 462:211-222.

Robinson TE, Yager LM, Cogan ES, Saunders BT (2014) On the motivational properties of reward cues: Individual differences. Neuropharmacology 76 Pt B:450-459.

Rosengren E (1960) On the role of monoamine oxidase for the inactivation of dopamine in brain. Acta physiologica Scandinavica 49:370-375.

Rossi MA, Sukharnikova T, Hayrapetyan VY, Yang L, Yin HH (2013) Operant self-stimulation of dopamine neurons in the substantia nigra. PLoS One 8:e65799.

Rothman RB, Baumann MH (2003) Monoamine transporters and psychostimulant drugs. Eur J Pharmacol 479:23-40.

Rouge-Pont F, Piazza PV, Kharouby M, Le Moal M, Simon H (1993) Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals predisposed to amphetamine self-administration. A microdialysis study. Brain research 602:169-174.

Rouillard C, Bedard PJ (1988) Specific D1 and D2 dopamine agonists have synergistic effects in the 6-hydroxydopamine circling model in the rat. Neuropharmacology 27:1257-1264.

Rubio JM, Schoretsanitis G, John M, Tiihonen J, Taipale H, Guinart D, Malhotra AK, Correll CU, Kane JM (2020) Psychosis relapse during treatment with long-acting injectable antipsychotics in individuals with schizophrenia-spectrum disorders: an individual participant data meta-analysis. Lancet Psychiatry 7:749-761.

Rupniak N, Hong M, Mansfield S, Fleminger S, Dyer R, Jenner P, Marsden CD (1983) Elevation of circulating prolactin concentrations may not cause striatal dopamine receptor supersensitivity. Eur J Pharmacol 93:195-200.

Sah P, Faber ES, Lopez De Armentia M, Power J (2003) The amygdaloid complex: anatomy and physiology. Physiol Rev 83:803-834.

Sahlholm K, Zeberg H, Nilsson J, Ogren SO, Fuxe K, Arhem P (2016) The fast-off hypothesis revisited: A functional kinetic study of antipsychotic antagonism of the dopamine D2 receptor. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 26:467-476.

Salamone JD, Correa M (2012) The mysterious motivational functions of mesolimbic dopamine. Neuron 76:470-485.

Samaha AN (2014) Can antipsychotic treatment contribute to drug addiction in schizophrenia? Progress in neuro-psychopharmacology & biological psychiatry 52:9-16.

Samaha AN, Seeman P, Stewart J, Rajabi H, Kapur S (2007) "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:2979-2986.

Samaha AN, Reckless GE, Seeman P, Diwan M, Nobrega JN, Kapur S (2008) Less is more: antipsychotic drug effects are greater with transient rather than continuous delivery. Biological psychiatry 64:145-152.

Sandi C, Venero C, Guaza C (1996) Novelty-related rapid locomotor effects of corticosterone in rats. The European journal of neuroscience 8:794-800.

Sanghera MK, Rolls ET, Roper-Hall A (1979) Visual responses of neurons in the dorsolateral amygdala of the alert monkey. Exp Neurol 63:610-626.

Sauer B (1998) Inducible gene targeting in mice using the Cre/lox system. Methods 14:381-392.

Saunders BT, Richard JM, Margolis EB, Janak PH (2018) Dopamine neurons create Pavlovian conditioned stimuli with circuit-defined motivational properties. Nat Neurosci 21:1072-1083.

Sawaguchi T, Goldman-Rakic PS (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251:947-950.

Sayers AC, Burki HR, Ruch W, Asper H (1975) Neuroleptic-induced hypersensitivity of striatal dopamine receptors in the rat as a model of tardive dyskinesias. Effects of clozapine, haloperidol, loxapine and chlorpromazine. Psychopharmacologia 41:97-104.

Schaefer GJ, Michael RP (1984) Drug interactions on spontaneous locomotor activity in rats. Neuroleptics and amphetamine-induced hyperactivity. Neuropharmacology 23:909-914.

Schobert B, Lanyi JK (1982) Halorhodopsin is a light-driven chloride pump. The Journal of biological chemistry 257:10306-10313.

Schoenbaum G, Chiba AA, Gallagher M (2000) Changes in functional connectivity in orbitofrontal cortex and basolateral amygdala during learning and reversal training. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:5179-5189.

Schoenbaum G, Setlow B, Saddoris MP, Gallagher M (2003) Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. Neuron 39:855-867.

Schroeder JP, Packard MG (2002) Posttraining intra-basolateral amygdala scopolamine impairs food- and amphetamine-induced conditioned place preferences. Behav Neurosci 116:922-927.

Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80:1-27.

Schwarzkopf SB, Bruno JP, Mitra T (1993) Effects of haloperidol and SCH 23390 on acoustic startle and prepulse inhibition under basal and stimulated conditions. Progress in neuro-psychopharmacology & biological psychiatry 17:1023-1036.

Sciascia JM, Reese RM, Janak PH, Chaudhri N (2015) Alcohol-Seeking Triggered by Discrete Pavlovian Cues is Invigorated by Alcohol Contexts and Mediated by Glutamate Signaling in the Basolateral Amygdala. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 40:2801-2812.

See RE, Kruzich PJ, Grimm JW (2001) Dopamine, but not glutamate, receptor blockade in the basolateral amygdala attenuates conditioned reward in a rat model of relapse to cocaine-seeking behavior. Psychopharmacology (Berl) 154:301-310.

See RE, McLaughlin J, Fuchs RA (2003) Muscarinic receptor antagonism in the basolateral amygdala blocks acquisition of cocaine-stimulus association in a model of relapse to cocaine-seeking behavior in rats. Neuroscience 117:477-483.

Seeman P (1987) Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1:133-152.

Seeman P, McCormick PN, Kapur S (2007) Increased dopamine D2(High) receptors in amphetamine-sensitized rats, measured by the agonist [(3)H](+)PHNO. Synapse 61:263-267.

Seeman P, Lee T, Chau-Wong M, Wong K (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 261:717-719.

Seeman P, Weinshenker D, Quirion R, Srivastava LK, Bhardwaj SK, Grandy DK, Premont RT, Sotnikova TD, Boksa P, El-Ghundi M, O'Dowd B F, George SR, Perreault ML, Mannisto PT, Robinson S, Palmiter RD, Tallerico T (2005) Dopamine supersensitivity correlates with D2High states, implying many paths to psychosis. Proc Natl Acad Sci U S A 102:3513-3518.

Seo DO, Funderburk SC, Bhatti DL, Motard LE, Newbold D, Girven KS, McCall JG, Krashes M, Sparta DR, Bruchas MR (2016) A GABAergic Projection from the Centromedial Nuclei of the Amygdala to Ventromedial Prefrontal Cortex Modulates Reward Behavior. The Journal of neuroscience : the official journal of the Society for Neuroscience 36:10831-10842.

Servonnet A, Samaha AN (2020) Antipsychotic-evoked dopamine supersensitivity. Neuropharmacology 163:107630.

Servonnet A, Uchida H, Samaha AN (2020a) Continuous versus extended antipsychotic dosing in schizophrenia: Less is more. Behavioural brain research 401:113076.

Servonnet A, Hernandez G, El Hage C, Rompre PP, Samaha AN (2020b) Optogenetic Activation of the Basolateral Amygdala Promotes Both Appetitive Conditioning and the Instrumental Pursuit of Reward Cues. The Journal of neuroscience : the official journal of the Society for Neuroscience 40:1732-1743.

Servonnet A, Minogianis EA, Bouchard C, Bedard AM, Levesque D, Rompre PP, Samaha AN (2017) Neurotensin in the nucleus accumbens reverses dopamine supersensitivity evoked by antipsychotic treatment. Neuropharmacology 123:10-21.

Sesack SR, Grace AA (2010) Cortico-Basal Ganglia reward network: microcircuitry. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 35:27-47.

Severson JA, Robinson HE, Simpson GM (1984) Neuroleptic-induced striatal dopamine receptor supersensitivity in mice: relationship to dose and drug. Psychopharmacology (Berl) 84:115-119.

Shapiro DA, Renock S, Arrington E, Chiodo LA, Liu LX, Sibley DR, Roth BL, Mailman R (2003) Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 28:1400-1411.

Shuto T, Kuroiwa M, Hamamura M, Yabuuchi K, Shimazoe T, Watanabe S, Nishi A, Yamamoto T (2006) Reversal of methamphetamine-induced behavioral sensitization by repeated administration of a dopamine D1 receptor agonist. Neuropharmacology 50:991-997.

Siegel BV, Jr., Trestman RL, O'Flaithbheartaigh S, Mitropoulou V, Amin F, Kirrane R, Silverman J, Schmeidler J, Keefe RS, Siever LJ (1996) D-amphetamine challenge effects on Wisconsin Card Sort Test. Performance in schizotypal personality disorder. Schizophr Res 20:29-32.

Silvestri S, Seeman MV, Negrete JC, Houle S, Shammi CM, Remington GJ, Kapur S, Zipursky RB, Wilson AA, Christensen BK, Seeman P (2000) Increased dopamine D2 receptor binding after long-term treatment with antipsychotics in humans: a clinical PET study. Psychopharmacology (Berl) 152:174-180.

Simpson GM, Angus JW (1970) A rating scale for extrapyramidal side effects. Acta Psychiatr Scand Suppl 212:11-19.

Simpson GM, Lee JH, Zoubok B, Gardos G (1979) A rating scale for tardive dyskinesia. Psychopharmacology (Berl) 64:171-179.

Sineshchekov OA, Jung KH, Spudich JL (2002) Two rhodopsins mediate phototaxis to low- and high-intensity light in Chlamydomonas reinhardtii. Proc Natl Acad Sci U S A 99:8689-8694.

Sinha R, Li CS (2007) Imaging stress- and cue-induced drug and alcohol craving: association with relapse and clinical implications. Drug Alcohol Rev 26:25-31.

Skinner BF (1938) The behavior of organisms; an experimental analysis. New York, London,: D. Appleton-Century Company.

Slifstein M, Kolachana B, Simpson EH, Tabares P, Cheng B, Duvall M, Frankle WG, Weinberger DR, Laruelle M, Abi-Dargham A (2008) COMT genotype predicts cortical-limbic D1 receptor availability measured with [11C]NNC112 and PET. Mol Psychiatry 13:821-827.

Smith RC, Davis JM (1975) Behavioral supersensitivity to apomorphine and amphetamine after chronic high dose haloperidol treatment. Psychopharmacology communications 1:285-293.

Smith RC, Davis JM (1976) Behavioral evidence for supersensitivity after chronic administration of haloperidol, clozapine, and thioridazine. Life Sci 19:725-731.

Smith Y, Pare D (1994) Intra-amygdaloid projections of the lateral nucleus in the cat: PHA-L anterograde labeling combined with postembedding GABA and glutamate immunocytochemistry. J Comp Neurol 342:232-248.

Sokoloff P, Giros B, Martres MP, Bouthenet ML, Schwartz JC (1990) Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 347:146-151.

Spiegel R, Fatemi SH (2003) Psychopharmacology : an introduction, 4th Edition. Chichester, West Sussex, England ; Hoboken, NJ: John Wiley & Sons.

Spudich JL, Yang CS, Jung KH, Spudich EN (2000) Retinylidene proteins: structures and functions from archaea to humans. Annu Rev Cell Dev Biol 16:365-392.

Stefanik MT, Kalivas PW (2013) Optogenetic dissection of basolateral amygdala projections during cue-induced reinstatement of cocaine seeking. Front Behav Neurosci 7:213.

Steinberg EE, Keiflin R, Boivin JR, Witten IB, Deisseroth K, Janak PH (2013) A causal link between prediction errors, dopamine neurons and learning. Nat Neurosci 16:966-973.

Steinberg EE, Boivin JR, Saunders BT, Witten IB, Deisseroth K, Janak PH (2014) Positive reinforcement mediated by midbrain dopamine neurons requires D1 and D2 receptor activation in the nucleus accumbens. PLoS One 9:e94771.

Stith RD, Person RJ, Dana RC (1976) Metyrapone inhibition of 3H-hydrocortisone uptake and binding in various brain regions of the pig. Neuroendocrinology 22:183-192.

Stolerman I (1992) Drugs of abuse: behavioural principles, methods and terms. Trends Pharmacol Sci 13:170-176.

Stringfield SJ, Higginbotham JA, Fuchs RA (2016) Requisite Role of Basolateral Amygdala Glucocorticoid Receptor Stimulation in Drug Context-Induced Cocaine-Seeking Behavior. Int J Neuropsychopharmacol 19.

Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, Tye KM, Kempadoo KA, Zhang F, Deisseroth K, Bonci A (2011) Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. Nature 475:377-380.

Sunahara RK, Guan HC, O'Dowd BF, Seeman P, Laurier LG, Ng G, George SR, Torchia J, Van Tol HH, Niznik HB (1991) Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. Nature 350:614-619.

Swanson LW (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain research bulletin 9:321-353.

Swartz MS, Wagner HR, Swanson JW, Stroup TS, McEvoy JP, Canive JM, Miller DD, Reimherr F, McGee M, Khan A, Van Dorn R, Rosenheck RA, Lieberman JA (2006) Substance use in persons with schizophrenia: baseline prevalence and correlates from the NIMH CATIE study. J Nerv Ment Dis 194:164-172.

Swerdlow NR, Geyer MA (1993) Clozapine and haloperidol in an animal model of sensorimotor gating deficits in schizophrenia. Pharmacol Biochem Behav 44:741-744.

Swerdlow NR, Keith VA, Braff DL, Geyer MA (1991) Effects of spiperone, raclopride, SCH 23390 and clozapine on apomorphine inhibition of sensorimotor gating of the startle response in the rat. The Journal of pharmacology and experimental therapeutics 256:530-536.

Swerdlow NR, Caine SB, Braff DL, Geyer MA (1992) The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. J Psychopharmacol 6:176-190.

Swerdlow NR, Braff DL, Taaid N, Geyer MA (1994) Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. Archives of general psychiatry 51:139-154.

Swerdlow NR, Koob GF, Cador M, Lorang M, Hauger RL (1993) Pituitary-adrenal axis responses to acute amphetamine in the rat. Pharmacol Biochem Behav 45:629-637.

Tadokoro S, Okamura N, Sekine Y, Kanahara N, Hashimoto K, Iyo M (2012) Chronic treatment with aripiprazole prevents development of dopamine supersensitivity and potentially supersensitivity psychosis. Schizophrenia bulletin 38:1012-1020.

Takeuchi H, Fervaha G, Uchida H, Suzuki T, Bies RR, Gronte D, Remington G (2014) Impact of once- versus twice-daily perphenazine dosing on clinical outcomes: an analysis of the CATIE data. The Journal of clinical psychiatry 75:506-511.

Taylor JR, Robbins TW (1984) Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. Psychopharmacology (Berl) 84:405-412.

Taylor JR, Horger BA (1999) Enhanced responding for conditioned reward produced by intra-accumbens amphetamine is potentiated after cocaine sensitization. Psychopharmacology (Berl) 142:31-40.

Taylor SF, Koeppe RA, Tandon R, Zubieta JK, Frey KA (2000) In vivo measurement of the vesicular monoamine transporter in schizophrenia. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 23:667-675.

Tenback DE, van Harten PN, Slooff CJ, van Os J, Group SS (2007) Worsening of psychosis in schizophrenia is longitudinally associated with tardive dyskinesia in the European Schizophrenia Outpatient Health Outcomes study. Compr Psychiatry 48:436-440.

Tenn CC, Fletcher PJ, Kapur S (2003) Amphetamine-sensitized animals show a sensorimotor gating and neurochemical abnormality similar to that of schizophrenia. Schizophr Res 64:103-114.

Tiberi M, Jarvie KR, Silvia C, Falardeau P, Gingrich JA, Godinot N, Bertrand L, Yang-Feng TL, Fremeau RT, Jr., Caron MG (1991) Cloning, molecular characterization, and chromosomal assignment of a gene encoding a second D1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D1A receptor. Proc Natl Acad Sci U S A 88:7491-7495.

Tonegawa S, Morrissey MD, Kitamura T (2018) The role of engram cells in the systems consolidation of memory. Nat Rev Neurosci 19:485-498.

Tovilla-Zarate C, Medellin BC, Fresan A, Lopez-Narvaez L, Castro TB, Juarez Rojop I, Ramirez-Bello J, Genis A, Nicolini H (2013) No association between catechol-o-methyltransferase Val108/158Met polymorphism and schizophrenia or its clinical symptomatology in a Mexican population. Mol Biol Rep 40:2053-2058.

Trono D (2000) Lentiviral vectors: turning a deadly foe into a therapeutic agent. Gene Ther 7:20-23.

Trulson ME, Cannon MS, Raese JD (1985) Identification of dopamine-containing cell bodies in the dorsal and median raphe nuclei of the rat brain using tyrosine hydroxylase immunochemistry. Brain research bulletin 15:229-234.

Tsai HC, Zhang F, Adamantidis A, Stuber GD, Bonci A, de Lecea L, Deisseroth K (2009) Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science 324:1080-1084.

Turrone P, Remington G, Kapur S, Nobrega JN (2003a) Differential effects of within-day continuous vs. transient dopamine D2 receptor occupancy in the development of vacuous chewing movements (VCMs) in rats. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 28:1433-1439.

Turrone P, Remington G, Kapur S, Nobrega JN (2003b) The relationship between dopamine D2 receptor occupancy and the vacuous chewing movement syndrome in rats. Psychopharmacology (Berl) 165:166-171.

Turrone P, Remington G, Kapur S, Nobrega JN (2005) Continuous but not intermittent olanzapine infusion induces vacuous chewing movements in rats. Biological psychiatry 57:406-411.

Tye KM, Janak PH (2007) Amygdala neurons differentially encode motivation and reinforcement. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:3937-3945.

Tye KM, Stuber GD, de Ridder B, Bonci A, Janak PH (2008) Rapid strengthening of thalamo-amygdala synapses mediates cue-reward learning. Nature 453:1253-1257.

Tye KM, Prakash R, Kim SY, Fenno LE, Grosenick L, Zarabi H, Thompson KR, Gradinaru V, Ramakrishnan C, Deisseroth K (2011) Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature 471:358-362.

Uchida H, Suzuki T (2014) Dose and dosing frequency of long-acting injectable antipsychotics: a systematic review of PET and SPECT data and clinical implications. J Clin Psychopharmacol 34:728-735.

Usiello A, Baik JH, Rouge-Pont F, Picetti R, Dierich A, LeMeur M, Piazza PV, Borrelli E (2000) Distinct functions of the two isoforms of dopamine D2 receptors. Nature 408:199-203.

Uwano T, Nishijo H, Ono T, Tamura R (1995) Neuronal responsiveness to various sensory stimuli, and associative learning in the rat amygdala. Neuroscience 68:339-361.

Vadnie CA, Ayers-Ringler J, Oliveros A, Abulseoud OA, Choi S, Hitschfeld MJ, Choi DS (2016) Antipsychotic-like effects of a neurotensin receptor type 1 agonist. Behavioural brain research 305:8-17.

van den Boss R, Cools AR, Ogren SO (1988) Differential effects of the selective D2-antagonist raclopride in the nucleus accumbens of the rat on spontaneous and d-amphetamine-induced activity. Psychopharmacology (Berl) 95:447-451.

van Rossum JM (1966) The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs. Arch Int Pharmacodyn Ther 160:492-494.

Van Tol HH, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, Civelli O (1991) Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 350:610-614.

Vanderschuren LJ, Schoffelmeer AN, Mulder AH, De Vries TJ (1999) Dopaminergic mechanisms mediating the long-term expression of locomotor sensitization following pre-exposure to morphine or amphetamine. Psychopharmacology (Berl) 143:244-253.

Varty GB, Higgins GA (1995) Examination of drug-induced and isolation-induced disruptions of prepulse inhibition as models to screen antipsychotic drugs. Psychopharmacology (Berl) 122:15-26.

Veldhuizen S, Urbanoski K, Cairney J (2007) Geographical variation in the prevalence of problematic substance use in Canada. Canadian journal of psychiatry Revue canadienne de psychiatrie 52:426-433.

Vertes RP (1991) A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. J Comp Neurol 313:643-668.

Vertes RP, Fortin WJ, Crane AM (1999) Projections of the median raphe nucleus in the rat. J Comp Neurol 407:555-582.

Vicente AM, Galvao-Ferreira P, Tecuapetla F, Costa RM (2016) Direct and indirect dorsolateral striatum pathways reinforce different action strategies. Curr Biol 26:R267-269.

Vonvoigtlander PF, Losey EG, Triezenberg HJ (1975) Increased sensitivity to dopaminergic agents after chronic neuroleptic treatment. The Journal of pharmacology and experimental therapeutics 193:88-94.

Waddington JL, Cross AJ, Gamble SJ, Bourne RC (1983) Spontaneous orofacial dyskinesia and dopaminergic function in rats after 6 months of neuroleptic treatment. Science 220:530-532.

Wadenberg ML, Hicks PB (1999) The conditioned avoidance response test re-evaluated: is it a sensitive test for the detection of potentially atypical antipsychotics? Neurosci Biobehav Rev 23:851-862.

Wadenberg ML, Soliman A, VanderSpek SC, Kapur S (2001) Dopamine D(2) receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical effects. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 25:633-641.

Waln O, Jankovic J (2013) An update on tardive dyskinesia: from phenomenology to treatment. Tremor Other Hyperkinet Mov (N Y) 3.

Walsh JJ, Christoffel DJ, Heifets BD, Ben-Dor GA, Selimbeyoglu A, Hung LW, Deisseroth K, Malenka RC (2018) 5-HT release in nucleus accumbens rescues social deficits in mouse autism model. Nature 560:589-594.

Wan FJ, Swerdlow NR (1993) Intra-accumbens infusion of quinpirole impairs sensorimotor gating of acoustic startle in rats. Psychopharmacology (Berl) 113:103-109.

Wang DV, Viereckel T, Zell V, Konradsson-Geuken A, Broker CJ, Talishinsky A, Yoo JH, Galinato MH, Arvidsson E, Kesner AJ, Hnasko TS, Wallen-Mackenzie A, Ikemoto S (2017) Disrupting Glutamate Co-transmission Does Not Affect Acquisition of Conditioned Behavior Reinforced by Dopamine Neuron Activation. Cell Rep 18:2584-2591.

Wang Y, Fang Y, Shen Y, Xu Q (2010) Analysis of association between the catechol-O-methyltransferase (COMT) gene and negative symptoms in chronic schizophrenia. Psychiatry research 179:147-150.

Washburn MS, Moises HC (1992) Electrophysiological and morphological properties of rat basolateral amygdaloid neurons in vitro. The Journal of neuroscience : the official journal of the Society for Neuroscience 12:4066-4079.

Weinberger DR (1987) Implications of normal brain development for the pathogenesis of schizophrenia. Archives of general psychiatry 44:660-669.

Werkman TR, Kruse CG, Nievelstein H, Long SK, Wadman WJ (2000) Neurotensin attenuates the quinpirole-induced inhibition of the firing rate of dopamine neurons in the rat substantia nigra pars compacta and the ventral tegmental area. Neuroscience 95:417-423.

White NM, Packard MG, Hiroi N (1991) Place conditioning with dopamine D1 and D2 agonists injected peripherally or into nucleus accumbens. Psychopharmacology (Berl) 103:271-276.

Wickens RH, Quartarone SE, Beninger RJ (2017) Inhibition of glycogen synthase kinase-3 by SB 216763 affects acquisition at lower doses than expression of amphetamine-conditioned place preference in rats. Behavioural pharmacology 28:262-271.

Wiesel FA, Farde L, Nordstrom AL, Sedvall G (1990) Central D1- and D2-receptor occupancy during antipsychotic drug treatment. Progress in neuro-psychopharmacology & biological psychiatry 14:759-767.

Wilmot CA, Szczepanik AM (1989) Effects of acute and chronic treatments with clozapine and haloperidol on serotonin (5-HT2) and dopamine (D2) receptors in the rat brain. Brain research 487:288-298.

Wise RA (1981) Intracranial self-stimulation: mapping against the lateral boundaries of the dopaminergic cells of the substantia nigra. Brain research 213:190-194.

Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5:483-494.

Wise RA, Rompre PP (1989) Brain dopamine and reward. Annu Rev Psychol 40:191-225.

Witten IB, Lin SC, Brodsky M, Prakash R, Diester I, Anikeeva P, Gradinaru V, Ramakrishnan C, Deisseroth K (2010) Cholinergic interneurons control local circuit activity and cocaine conditioning. Science 330:1677-1681.

Witten IB, Steinberg EE, Lee SY, Davidson TJ, Zalocusky KA, Brodsky M, Yizhar O, Cho SL, Gong S, Ramakrishnan C, Stuber GD, Tye KM, Janak PH, Deisseroth K (2011) Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. Neuron 72:721-733.

Wolff SB, Grundemann J, Tovote P, Krabbe S, Jacobson GA, Muller C, Herry C, Ehrlich I, Friedrich RW, Letzkus JJ, Luthi A (2014) Amygdala interneuron subtypes control fear learning through disinhibition. Nature 509:453-458.

Wolff T, Strecker M (1985) Lack of relationship between debrisoquine 4-hydroxylation and other cytochrome P-450 dependent reactions in rat and human liver. Biochem Pharmacol 34:2593-2598.

Wood PL, Kim HS, Marien MR (1987) Intracerebral dialysis: direct evidence for the utility of 3-MT measurements as an index of dopamine release. Life Sci 41:1-5.

Wu Z, Asokan A, Samulski RJ (2006) Adeno-associated virus serotypes: vector toolkit for human gene therapy. Mol Ther 14:316-327.

Xue YX, Chen YY, Zhang LB, Zhang LQ, Huang GD, Sun SC, Deng JH, Luo YX, Bao YP, Wu P, Han Y, Hope BT, Shaham Y, Shi J, Lu L (2017) Selective Inhibition of Amygdala Neuronal Ensembles Encoding Nicotine-Associated Memories Inhibits Nicotine Preference and Relapse. Biological psychiatry 82:781-793.

Yassa R, Jeste DV (1992) Gender differences in tardive dyskinesia: a critical review of the literature. Schizophr Bull 18:701-715.

Yun IA, Fields HL (2003) Basolateral amygdala lesions impair both cue- and cocaine-induced reinstatement in animals trained on a discriminative stimulus task. Neuroscience 121:747-757.

Yung AR, McGorry PD (1996) The prodromal phase of first-episode psychosis: past and current conceptualizations. Schizophr Bull 22:353-370.

Zhang F, Gradinaru V, Adamantidis AR, Durand R, Airan RD, de Lecea L, Deisseroth K (2010) Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. Nat Protoc 5:439-456.

Zhang F, Wang LP, Brauner M, Liewald JF, Kay K, Watzke N, Wood PG, Bamberg E, Nagel G, Gottschalk A, Deisseroth K (2007) Multimodal fast optical interrogation of neural circuitry. Nature 446:633-639.

Zhang F, Vierock J, Yizhar O, Fenno LE, Tsunoda S, Kianianmomeni A, Prigge M, Berndt A, Cushman J, Polle J, Magnuson J, Hegemann P, Deisseroth K (2011) The microbial opsin family of optogenetic tools. Cell 147:1446-1457.

Zhou QY, Grandy DK, Thambi L, Kushner JA, Van Tol HH, Cone R, Pribnow D, Salon J, Bunzow JR, Civelli O (1990) Cloning and expression of human and rat D1 dopamine receptors. Nature 347:76-80.

Zhuang X, Belluscio L, Hen R (2000) G(olf)alpha mediates dopamine D1 receptor signaling. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:RC91.

ANNEXES

1ST REVIEW: Antipsychotic-Evoked Dopamine Supersensitivity

Review published in Neuropharmacology
DOI: 10.1016/j.neuropharm.2019.05.007

Alice Servonnet[a] and Anne-Noël Samaha[b,c]

[a]Department of Neuroscience, Faculty of Medicine, Université de Montréal; [b]Department of Pharmacology and Physiology, Faculty of Medicine, Université de Montréal; [c]Groupe de recherche sur le système nerveux central, Faculty of Medicine, Université de Montréal. 2900 Edouard-Montpetit boulevard, Montreal (H3T 1J4), Quebec, Canada.

HIGHLIGHTS

Long-term antipsychotic treatment can evoke dopamine supersensitivity

This might impair treatment efficacy and worsen psychosis in schizophrenia patients

Dopamine supersensitivity is also linked to tolerance to antipsychotics in rats

D2-related changes are seen but are not perfect dopamine supersensitivity markers

Adequate dosing and treatment kinetics can prevent dopamine supersensitivity

Funding sources
This work was supported by grants from the National Science and Engineering Research Council of Canada (grant number 355923) and the Canada Foundation for Innovation to ANS (grant number 24326). ANS holds a salary award (grant number 28988) from the Fonds de la Recherche du Québec-Santé. These funding sources were not implicated in the writing of the article or in the decision to submit the article for publication.

Declaration of interest
ANS is a consultant for H. Lundbeck A/S. This had no influence on the manuscript's content. AS has no conflicts of interest to declare.

ABSTRACT

All antipsychotic medications attenuate the symptoms of psychosis by interacting with dopamine D2 receptors and reducing dopamine-mediated neurotransmission. However, long-term antipsychotic treatment can produce neuroadaptations that are thought to lead to dopamine supersensitivity. In patients with schizophrenia, this dopamine supersensitivity could compromise treatment efficacy, promote relapse to psychosis and trigger movement disorders. Such effects have been seen in patients treated with either typical or atypical antipsychotics. In non-human animals, chronic exposure to antipsychotic medications, using clinically pertinent doses and modes of administration, can also evoke dopamine supersensitivity. This is indicated by an augmented behavioural response to dopamine agonists and tolerance to the antipsychotic-like effects of ongoing treatment. Here, we first describe antipsychotic-evoked dopamine supersensitivity in patients with schizophrenia and in laboratory animals. We then review approaches to prevent or reverse antipsychotic-evoked dopamine supersensitivity, based on preclinical animal studies. This evidence suggests that using atypical antipsychotics and regular but intermittent (versus continuous) antipsychotic dosing/D2 receptor occupancy is significantly less likely to produce dopamine supersensitivity. Lastly, we discuss potential neurobiological mechanisms. These include changes at the D2 receptor, but also other changes within and outside of the dopamine system. We conclude that in parallel to the search for new antipsychotic molecules, we need to better understand how different dosing regimens with currently used medications influence long-term outcome. There is also a pressing need to better characterize the development and expression of dopamine supersensitivity in humans. This will help determine the treatment strategies least likely to evoke dopamine supersensitivity.

KEY WORDS

Antipsychotics, dopamine supersensitivity, psychosis, schizophrenia, rat

1. Introduction

Antipsychotic medications are used to treat psychosis symptoms in schizophrenia. Psychosis is generally thought to involve excessive dopamine signaling in the striatum [(Howes and Kapur, 2009), and see (Demjaha et al., 2012)]. Antipsychotics have their anti-psychotic effects by interacting with dopamine D2 receptors to decrease dopamine transmission in the striatum (Creese et al., 1976; Seeman et al., 1976; Farde et al., 1989; Richtand et al., 2007). However, long-term exposure to antipsychotic medications can produce compensatory changes in the brain believed to lead to supersensitivity to dopamine stimulation. In schizophrenia patients, antipsychotic-induced dopamine supersensitivity is thought to impair treatment efficacy, promote relapse to psychosis and also worsen psychotic symptoms (Chouinard et al., 1978; Fallon et al., 2012; Suzuki et al., 2015). In laboratory animals, antipsychotic-induced dopamine supersensitivity produces loss of antipsychotic efficacy (Samaha et al., 2007; Samaha et al., 2008; Gill et al., 2014) and an exaggerated behavioural response to dopamine agonists (Smith and Davis, 1975; Ericson et al., 1996; Samaha et al., 2007; Bedard et al., 2011). In the present review, we first describe the clinical implications of antipsychotic-evoked dopamine supersensitivity for the patient and the manifestations of this dopamine supersensitivity in laboratory animals. We then discuss how dopamine supersensitivity might be reversed or prevented, using approaches that preserve the long-term efficacy of antipsychotic treatment. Finally, we describe the neurobiological changes that have been associated with antipsychotic-evoked dopamine supersensitivity, within but also outside of the dopamine system.

2. Antipsychotic-evoked dopamine supersensitivity and its consequences

2.1. In humans

Antipsychotic medications can be necessary for people suffering from continued psychotic symptoms. However, for several decades now, it has become common to keep people with a diagnosis of schizophrenia on continuous antipsychotic medication for years (Murray et al., 2016). This practice is raising questions in the field (Samaha et al., 2008; Remington et al., 2011; Uchida and Suzuki, 2014; Chouinard et al., 2017). While stopping antipsychotic treatment altogether is generally linked to relapse to psychosis (Taylor et al., 2012), an important issue is whether chronic

and long-term occupancy of dopamine D2 receptors can change the brain in ways that compromise outcome. The evidence suggests that it does. In some patients, chronic antipsychotic exposure evokes neuroadaptations that are thought to lead to a state of supersensitivity to dopamine receptor stimulation. This phenomenon was first described in the late 1970's by Guy Chouinard and colleagues (Chouinard et al., 1978; Chouinard and Chouinard, 2008; Chouinard et al., 2017). They proposed that chronic suppression of dopamine-mediated neurotransmission by antipsychotic leads to compensatory overactivity and a dopamine supersensitive state. This in turn would promote both relapse to psychotic symptoms—via tolerance to previously observed anti-psychotic effects of medication but also via enhanced stress-induced psychotic relapse—and tardive dyskinesia (see FIG. A1.1A). Chouinard et al. also outlined criteria to identify dopamine supersensitivity evoked by antipsychotic medication, after at least 3 months of treatment (Chouinard and Jones, 1980; Chouinard et al., 2017). These include i) acute worsening of psychosis symptoms when treatment is discontinued/dose is reduced/medication is switched, ii) increased vulnerability to stress while on medication, iii) tolerance to the therapeutic effects of antipsychotic treatment, and/or iv) the presence of tardive dyskinesia (Chouinard et al., 1986). Of note, at present, clear evidence of antipsychotic-evoked dopamine supersensitivity as involving a sensitized response to dopamine receptor stimulation is based in great part on data from studies using laboratory animals (see next section). Definitive proof that chronic exposure to antipsychotic drugs can sensitize the dopamine system of humans is still lacking. Therefore, in humans, the concept of a 'dopamine supersensitive state' involving antipsychotic-induced sensitization of the dopamine system remains theoretical. This being said, here we use the term 'dopamine supersensitivity' to remain consistent with a literature that has been expanding since the 1970's (Vonvoigtlander et al., 1975; Chouinard et al., 1978). As will be discussed in section 4.2, there is also good reason to believe that systems other than dopamine could mediate the behavioural symptoms of dopamine supersensitivity.

Treatment failure and worsening of psychosis can involve many factors (illness progression, not taking one's medication, stressful life changes), but there are reasons to believe that antipsychotic treatment itself is also a cause. First, tardive dyskinesia and dopamine supersensitivity are linked. Tardive dyskinesia is not a symptom of schizophrenia, rather it involves antipsychotic-induced brain changes that could be linked to dopamine supersensitivity (Casey, 1991; Waln and Jankovic, 2013). Tardive dyskinesia is one of the best behavioural predictors of dopamine supersensitivity (Chouinard and Chouinard, 2008). Using tardive dyskinesia to identify dopamine supersensitivity

in patients, it has been reported that dopamine supersensitivity could be involved in psychotic relapse in 30-40% of patients with schizophrenia (Fallon and Dursun, 2011; Fallon et al., 2012). In addition, up to 70% of treatment-resistant patients show evidence of dopamine supersensitivity (Suzuki et al., 2015). A study including 8620 patients also found that worsening of psychotic symptoms and onset of tardive dyskinesia coincide in time, and that the greater the degree of psychotic worsening, the greater the risk of developing tardive dyskinesia (Tenback et al., 2007). Just like antipsychotic-evoked dopamine supersensitivity, tardive dyskinesia can also persist following treatment withdrawal (Muench and Hamer, 2010). Both relapse to psychosis and tardive dyskinesia can also be controlled, at least temporarily, by increasing antipsychotic dosing (Chouinard et al., 1978). All of these observations support the idea that common neurobiological mechanisms underlie the two phenomena. Second, the idea that antipsychotic treatment can increase the response to dopamine receptor stimulation is also supported by evidence that antipsychotic-treated schizophrenia patients are more sensitive to the psychotogenic effects of amphetamine relative to untreated patients (Lieberman et al., 1987). Finally, chronic treatment with D2 receptor antagonists can trigger both tardive dyskinesia and withdrawal psychosis in people with no psychiatric diagnoses (Lu et al., 2002; Roy-Desruisseaux et al., 2011; Seeman, 2014). These observations suggest that antipsychotic treatment can change the brain in ways that promote psychosis and that this potentially involves a state of dopamine supersensitivity. Still, it must be considered that some clinical studies report no signs of antipsychotic-evoked dopamine supersensitivity in schizophrenia. For instance, schizophrenia patients withdrawn from treatment with long-acting injectable paliperidone palmitate do not show more severe positive symptoms or tardive dyskinesia relative to patients that are maintained on treatment (Emsley et al., 2018).

2.2 In non-human animals

In animals, two behavioural paradigms commonly used to measure antipsychotic/anti-dopaminergic effects are suppression of conditioned avoidance responding (Wadenberg and Hicks, 1999) and suppression of the psychomotor response to dopamine receptor agonists (Ljungberg and Ungerstedt, 1985). There is no clear relation between the avoidance response to an aversive stimulus in laboratory animals and psychosis in humans (Li et al., 2007). However, the conditioned avoidance responding model has very high predictive validity for antipsychotic properties (Wadenberg and Hicks, 1999). All antipsychotic medications that are effective in the clinic

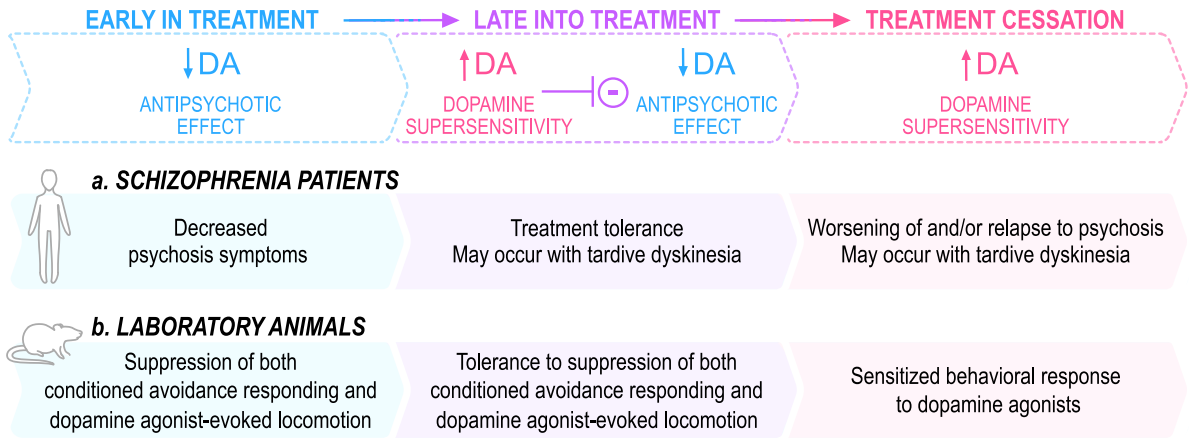

FIG. A1.1 — Behavioural features of antipsychotic-evoked dopamine supersensitivity in people with schizophrenia and in laboratory animals. (***A***) Early in treatment, antipsychotic medications effectively treat schizophrenia symptoms, especially psychotic symptoms such as psychosis, through a decrease in dopamine transmission. However, in the later stages of treatment, antipsychotic medications can induce neuroadaptations that are proposed to lead to dopamine supersensitivity. These neuroadaptations are thought to produce treatment tolerance, to persist after treatment withdrawal and to promote relapse to and/or worsening of psychosis. Antipsychotic-evoked dopamine supersensitivity can also co-occur with tardive dyskinesia. (***B***) In laboratory animals, common indices of antipsychotic-like effects are the suppression of the conditioned avoidance response to an aversive cue and the suppression of the locomotor effects of dopamine agonists. Initially, antipsychotic medications produce these antipsychotic-like effects. However, with longer-term exposure, antipsychotic medications no longer suppress conditioned avoidance responding and locomotor activity (*i.e.*, the animals show treatment tolerance), and this is linked to the emergence of dopamine supersensitivity. This dopamine supersensitivity can persist after antipsychotic treatment cessation, as indicated by sensitization to the behavioural effects of dopamine agonists.

selectively disrupt conditioned avoidance responding at doses that do not change unconditioned escape responses, and there is a positive correlation between this effect and antipsychotic potency in the clinic (Kuribara and Tadokoro, 1981; Arnt, 1982; Wadenberg et al., 2001). As regards suppression of dopamine agonist-induced psychomotor activity, this is not an effect exclusive to antipsychotic compounds. However, it is a reliable and often used test to measure the antidopaminergic properties of antipsychotic drugs (Ljungberg and Ungerstedt, 1985; Arnt, 1995).

Studies using laboratory animals show that long-term antipsychotic treatment can produce dopamine supersensitivity (see FIG. A1.1B). Many studies show dopamine supersensitivity after antipsychotic treatment cessation. Thus, compared to antipsychotic-naïve animals, animals previously exposed to antipsychotics (> 2 weeks) are supersensitive to the psychomotor activating effects of dopamine agonists, including apomorphine (Smith and Davis, 1975; Montanaro et al.,

1982; Carvalho et al., 2009), amphetamine (Smith and Davis, 1975; Vonvoigtlander et al., 1975; Montanaro et al., 1981; Ericson et al., 1996; Samaha et al., 2007; Samaha et al., 2008; Bedard et al., 2011, 2013) and cocaine (Kosten, 1997; Pudiak and Bozarth, 1997; Fukushiro et al., 2008). Previous work has also shown that the psychomotor response to an intra-accumbens infusion of dopamine is potentiated following chronic exposure to either haloperidol, sulpiride or clozapine (Halperin et al., 1989). However, excessively high antipsychotic doses were used [see (Kapur et al., 2003)]. Using clinically-relevant doses, we have shown that the psychomotor response to an intra-accumbens infusion of amphetamine is similar in dopamine supersensitive versus antipsychotic-naïve rats (El Hage et al., 2015). This suggests that the expression of dopamine supersensitivity also involves stimulation of dopamine receptors outside of the nucleus accumbens. Recent work in female rats also highlights that there could be sex differences in the emergence of antipsychotic-induced dopamine supersensitivity, and that further investigations are needed, including characterization of both low and higher antipsychotic doses (Madularu et al., 2014; Madularu et al., 2016). This issue notwithstanding, there is strong evidence that upon antipsychotic/anti-dopaminergic treatment cessation, the dopamine supersensitive state is fully uncovered, as indicated by an exaggerated behavioural response to dopamine agonists. Of note, an exaggerated behavioral response to dopamine agonists is not a diagnostic criterion of antipsychotic-evoked dopamine supersensitivity in humans. Instead, in preclinical studies, acute administration of a dopamine agonist is used as a pharmacological model of the increased dopamine neurotransmission that is linked to psychosis in humans. In this way, dopamine agonists are used to probe the functional consequences of increased dopamine neurotransmission in dopamine supersensitive animals.

There is also evidence of 'breakthrough' dopamine supersensitivity during ongoing antipsychotic treatment (FIG. A1.1B). Initially (≤ ~1 week of treatment), antipsychotic drugs suppress both the psychomotor response to dopamine agonists and the conditioned avoidance response produced by an aversive cue (Asper et al., 1973; MØller Nielsen et al., 1974; Samaha et al., 2007). However, with continued exposure (≥ ~1 week), ongoing antipsychotic treatment can lose efficacy in both paradigms (Asper et al., 1973; MØller Nielsen et al., 1974; Samaha et al., 2007; Amato et al., 2018). Compounds with antipsychotic-like effects are also ineffective in rats previously chronically treated with antipsychotics, and this is linked to the development of dopamine supersensitivity (Gill et al., 2014). Gill et al.'s (2014) findings are also important because they show antipsychotic-

induced dopamine supersensitivity in a neurodevelopmental animal model of schizophrenia. This extends the large number of studies showing antipsychotic-induced dopamine supersensitivity in otherwise neurologically intact animals. Furthermore, as observed in humans (Fallon and Dursun, 2011; Fallon et al., 2012), studies using rodents support a link between antipsychotic-evoked dopamine supersensitivity and tardive dyskinesia. Indeed, in rodents, antipsychotic treatments that promote dopamine supersensitivity also increase the likelihood of vacuous chewing movements, a tardive dyskinesia-like feature (Turrone et al., 2003b, a, 2005). Thus, the studies reviewed above highlight two key elements. First, in preclinical studies, dopamine supersensitivity involves tolerance to some effects (tolerance to antipsychotic-induced suppression of conditioned avoidance responding during ongoing treatment), and sensitization to other effects (an enhanced psychomotor response to dopamine agonists after antipsychotic treatment cessation; FIG. A1.1B). In other words, tolerance to some effects and sensitization to other effects can be evoked by the exact same treatment regimen, in the exact same subject. And both of the effects described are relevant to dopamine supersensitivity. A second key conclusion is that while short-term antipsychotic treatment produces antipsychotic-like effects in animals, treatment failure can be observed with longer-term treatment (≥ ~1 week) and this is potentially linked to dopamine supersensitivity.

In non-human animals, antipsychotic-induced dopamine supersensitivity also augments the reward-enhancing effects of dopamine agonists. This could have implications for co-morbid substance use disorders in people with schizophrenia [reviewed in Samaha (2014)]. Over 40% of people with schizophrenia also have a substance use disorder, with a 2-3 times greater risk in men than women (Hunt et al., 2018). In comparison, the prevalence of substance use disorders in the general population in Canada and the United States is ~10-20 % (Anthony et al., 1996; Veldhuizen et al., 2007). Some people with schizophrenia might use drugs to alleviate symptoms of their illness (Khantzian, 1985) or the side effects of antipsychotic treatment (Schneier and Siris, 1987). The high prevalence of drug use might also involve antipsychotic-evoked dopamine supersensitivity, as this can enhance the rewarding properties of drug of abuse (Samaha, 2014). For instance, daily haloperidol injection increases cocaine intake in rats, while antipsychotic-naïve animals maintain constant and lower levels of cocaine intake (Roberts and Vickers, 1987). Withdrawal from chronic antipsychotic treatment also enhances cocaine self-administration behaviour in squirrel monkeys (Howell and Byrd, 1992). More recent work suggests that antipsychotic treatment regimens might potentiate reward function only if they produce dopamine supersensitivity. When rats were given

different chronic antipsychotic treatment regimens, only the treatment regimens that produced dopamine supersensitivity also produced sensitization to the reward-enhancing properties of amphetamine [*i.e.*, amphetamine-induced potentiation of conditioned reward; (Bedard et al., 2011, 2013)]. Antipsychotic treatment can also influence the response to drug cues in schizophrenia patients. For instance, cocaine cues elicit greater drug craving in patients treated with typical antipsychotics relative to risperidone or olanzapine-treated patients (Smelson et al., 2002; Smelson et al., 2006). More work on this issue is needed. For instance, it is not known how antipsychotic-induced dopamine supersensitivity might contribute to changes in the response to drug cues in humans. However, the data in the animal studies described above (Bedard et al., 2011, 2013) suggest that perhaps by inducing dopamine supersensitivity, some antipsychotic treatment regimens could contribute to drug seeking and taking behaviours in vulnerable people with schizophrenia (Samaha, 2014).

3. Variables that influence the development and expression of dopamine supersensitivity

A better understanding of the variables that modulate antipsychotic-evoked dopamine supersensitivity could lead to the development of new strategies to prevent or reverse this supersensitivity. For any given person with schizophrenia, antipsychotic treatment can be a remarkably dynamic process over the course of the illness, with switching between antipsychotics and combinations of treatments. This makes it difficult to determine in humans the types of treatment regimens that are more or less likely to produce dopamine supersensitivity. Animal models are particularly useful in this context because they can help establish causal relationships between different treatment regimens and the development of dopamine supersensitivity. In animal models, the type of antipsychotic medication (typical versus atypical), the dose and the temporal kinetics of treatment (continuous versus regular but intermittent) are all decisive in determining the risk of dopamine supersensitivity. We review these findings here. We also discuss implications for the clinic.

3.1. Typical versus atypical antipsychotic drugs

It is estimated that 13-39% of people with schizophrenia treated with either typical or atypical antipsychotics have dopamine supersensitivity, while virtually no antipsychotic-naïve patients do (Woerner et al., 1991; Fallon et al., 2012). Both typical and atypical antipsychotic drugs can produce dopamine supersensitivity, but supersensitivity might persist longer after cessation of treatment with typical antipsychotics. Animals previously exposed to the typical antipsychotics haloperidol (Smith and Davis, 1975; Vonvoigtlander et al., 1975; Montanaro et al., 1982; Fleminger et al., 1983; Marin and Chase, 1993; Samaha et al., 2007; Fukushiro et al., 2008; Tadokoro et al., 2012; Bedard et al., 2013), sulpiride (Montanaro et al., 1982; Fleminger et al., 1983), trifluoperazine (Clow et al., 1980) or thioridazine (Vonvoigtlander et al., 1975; Clow et al., 1980; Calza et al., 1990) are supersensitive to the behavioural effects of dopamine agonists. In contrast, animals previously exposed to the atypical antipsychotics olanzapine (Samaha et al., 2007; Bedard et al., 2013), aripiprazole (Tadokoro et al., 2012), risperidone (Carvalho et al., 2009) or ziprasidone (Fukushiro et al., 2008) generally show a normal locomotor response to dopamine agonists, suggesting no dopamine supersensitivity. Other studies have examined dopamine supersensitivity during ongoing treatment with typical versus atypical antipsychotics. This has been achieved by assessing tolerance to ongoing treatment, as tolerance to antipsychotic effects is a diagnostic criterion for dopamine supersensitivity in humans (Chouinard et al., 1986; Moncrieff, 2006; Chouinard and Chouinard, 2008; Iyo et al., 2013; Chouinard et al., 2017). In rats, there is evidence that tolerance to antipsychotic effects develops during ongoing treatment with typical (haloperidol) or atypical (olanzapine) antipsychotics, as shown by decreased antipsychotic-induced suppression of both amphetamine-induced locomotor activity and conditioned avoidance responding (Samaha et al., 2007; Amato et al., 2018). Another symptom of antipsychotic-induced dopamine supersensitivity in humans is tardive dyskinesia. Tardive dyskinesia is related to antipsychotic-induced vacuous chewing movements in rats, and these can also emerge during ongoing chronic treatment with either haloperidol or olanzapine (Turrone et al., 2003b, a, 2005). Thus, dopamine supersensitivity symptoms can ‘breakthrough’ ongoing treatment with either typical or atypical antipsychotics, but dopamine-mediated behaviours might more readily return to normal after discontinuation of atypical versus typical antipsychotics.

Some work also suggests that atypical antipsychotics can both prevent and reverse dopamine supersensitivity. Co-administration of the atypical antipsychotic ziprasidone (Fukushiro et al., 2008) or risperidone (Carvalho et al., 2009) with haloperidol is reported to prevent the development

of haloperidol-induced dopamine supersensitivity (*i.e.* the potentiated psychomotor response to amphetamine or cocaine). Chronic treatment with aripiprazole can also reverse the exaggerated psychomotor response to methamphetamine evoked by prior chronic treatment with haloperidol (Tadokoro et al., 2012). However, these studies did not provide measurements of antipsychotic levels/D2 receptor occupancy in the brain. This makes it difficult to determine whether effects are due to peripheral, pharmacokinetic interactions versus central pharmacological actions.

Typical versus atypical antipsychotics might produce different outcomes through several mechanisms. First, the two drug classes might produce different degrees of dopaminergic disruption. Compared to typical antipsychotics, atypical antipsychotics bind to D2 receptors more loosely (Seeman et al., 1997). Endogenous dopamine might therefore more easily displace atypical antipsychotics from the receptor, allowing more endogenous dopaminergic signaling (Seeman et al., 1997). In turn, this could attenuate compensatory upregulation within the dopamine system during chronic antipsychotic treatment, making dopamine supersensitivity less persistent over time. Second, atypical antipsychotics also have higher affinities at several serotonin receptors (Meltzer et al., 1989) compared to typical antipsychotics, and this might temper dopamine supersensitivity. As research on these issues unfolds, the possibility that atypical antipsychotics are less likely to produce dopamine supersensitivity should be considered and investigated further in the clinic.

3.2. The pharmacokinetics of antipsychotic treatment

When considering the response to antipsychotic medications, as for all pharmacologically active compounds, the pharmacokinetics determine the pharmacodynamics. In the context of dopamine supersensitivity, there are two critical pharmacokinetic variables to consider; the temporal kinetics of treatment (how often antipsychotic/D2 occupancy levels rise and fall over the day), and dose (how much antipsychotic/D2 occupancy is achieved in the brain). In this section we review how manipulations of the within-day kinetics of treatment influences the development of dopamine supersensitivity and antipsychotic efficacy over time. We first compare the long-standing clinical strategy of maintaining continuously high brain levels of antipsychotic/D2 occupancy to a more extended, intermittent dosing strategy. We then discuss the influence of antipsychotic dosing on the risk of dopamine supersensitivity.

3.2.1. The within-day kinetics of antipsychotic treatment

From their discovery nearly 70 years ago, antipsychotic treatment regimens have been given to produce continuously high concentrations of drug. The assumption is that high and continuous levels of antipsychotic/D2 occupancy are necessary to maintain efficacy and reduce the risk of relapse. This idea contributed to the early introduction of depot antipsychotic injections, to the recommendation that antipsychotic medications be given several times a day if they have shorter half-lives, and to the more recent rush to develop and market extended release formulations for antipsychotics. Such approaches produce steady state levels of medication/D2 occupancy (Farde et al., 1989; Remington et al., 2006; Mamo et al., 2008). As illustrated in FIG. A1.2A, this can be modeled in rats by administering antipsychotic drugs via a subcutaneously implanted osmotic minipump (Kapur et al., 2003; Samaha et al., 2007) or via a long-acting injectable formulation (Turrone et al., 2003a). Using these continuous-treatment approaches, clinically-representative doses [*i.e.*, doses producing 65-80% of striatal D2 receptor occupancy (Wadenberg et al., 2000; Natesan et al., 2006)] promote dopamine supersensitivity to the behavioural effects of dopamine agonists (Ericson et al., 1996; Samaha et al., 2007; Samaha et al., 2008; Bedard et al., 2011, 2013), tolerance to the effects of antipsychotics in tests of antipsychotic-like efficacy (Samaha et al., 2007; Samaha et al., 2008; Amato et al., 2018), and increased probability of vacuous chewing movements (Turrone et al., 2003b, a, 2005) in laboratory rats. A different outcome emerges if clinically-representative antipsychotic doses are given regularly, but intermittently such that striatal D2 occupancy is high for a few hours following antipsychotic administration, and then decreases until the next injection [see FIG. A1.2B; Kapur et al. (2003)]. When rats are given this within-day transient antipsychotic treatment regimen, they are less likely to show vacuous chewing movements (Turrone et al., 2003b, 2005) and they also do not show a potentiated behavioral response to dopamine agonists after cessation of antipsychotic treatment (Ericson et al., 1996; Samaha et al., 2008; Bedard et al., 2011; Servonnet et al., 2017). These observations concord with a clinical study showing that schizophrenia patients with tardive dyskinesia have higher D2 occupancy levels (estimated from antipsychotic blood concentrations) at through compared to schizophrenia patients with no sign of tardive dyskinesia, whereas D2 occupancy at peak is similar between the two groups (Yoshida et al., 2014). Furthermore, when antipsychotic drugs are given intermittently at clinically relevant doses (achieving within-day transient peaks in D2 receptor occupancy), antipsychotic-like effects in animal models are potentiated over time (Li et al., 2007;

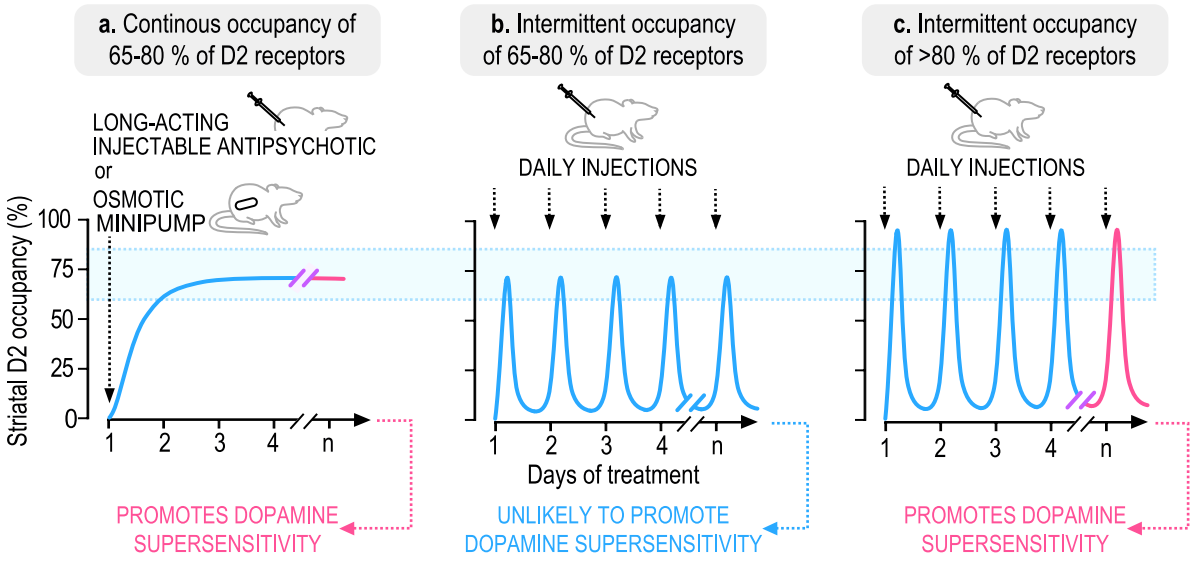

FIG. A1.2 — Both the temporal kinetics and dose of antipsychotic treatment determine the development of dopamine supersensitivity. In patients with schizophrenia and in laboratory animals, most antipsychotic drugs produce therapeutic effects when they occupy > 65 % of striatal D2 receptors, and antipsychotics are also more likely to produce extrapyramidal effects with > 80 % occupancy (65-80 % occupancy, indicated with blue shading in the panels). It is standard practice to prescribe daily antipsychotic dosing. If there is treatment compliance, this would produce virtually continuous D2 receptor occupancy at a 65-80 % level. (***A***) This can be modelled in laboratory animals by administering long-acting injectable antipsychotics or by giving antipsychotics through an osmotic minipump implanted subcutaneously. Such continuous dosing/D2 receptor occupancy promotes dopamine supersensitivity. (***B***) Alternatively, when antipsychotic drugs are given via daily systemic injections to rats, this produces peaks and troughs in striatal D2 receptor occupancy. If peak striatal levels remain between 65-80% this is less likely to produce dopamine supersensitivity, and antipsychotic-like effects are maintained. (***C***) However, when antipsychotic drugs are given via daily systemic injections, but at high doses (producing >80 % of occupancy at peak), this also promotes dopamine supersensitivity. See section 3.2 for further discussion of continuous versus intermittent dosing strategies.

Samaha et al., 2008). This contrasts with treatment tolerance induced by continuous exposure (Samaha et al., 2008). Therefore, when using clinically representative doses, continuous antipsychotic administration could favor neurobiological changes leading to treatment tolerance, whereas regular but intermittent administration could favor neurobiological changes that enhance antipsychotic effects over time. These findings suggest that less is more, and that within-day transient treatment might not only be more effective over time than continuous treatment, but it might also prevent the development of dopamine supersensitivity.

The findings above challenge the assumption that sustained D2 occupancy is required to maintain antipsychotic response, and recent work in patients supports this idea. While some patients may need continuous dosing to control symptoms, there is evidence that intermittent dosing strategies can be effective. Initially when such strategies were investigated, they involved alternating between periods of treatment and long drug-free gaps lasting up to months. Such strategies were not successful, as they increased rates of relapse (Jolley et al., 1990; Schooler, 1991; Jolley and Hirsch, 1993; Gaebel, 1994; Peuskens, 1996; Carpenter et al., 1999; Gaebel et al., 2002). However, intermittent but more regular dosing strategies have yielded very promising results. In a double-blind study lasting 9 months, McCreadie et al. (1980) show that long-acting injectable fluphenazine or taking oral pimozide on 4 consecutive days/week are equally effective in preventing psychosis relapse. In a double-blind study lasting 6 months, Remington et al. (2011) show that taking oral antipsychotic every day or every 2 days is equally effective in treating schizophrenia symptoms. A PET study on people with schizophrenia that were stabilized on haloperidol decanoate also showed that continuous occupancy of $\geq 65\%$ D2 receptors was not necessary to prevent relapse to psychosis (Nyberg et al., 1995). This idea is further supported by the work of Uchida and Suzuki (2014), who reviewed a total of 14 studies and concluded that long-acting injectable antipsychotics can remain effective for several months, such that they can be administered at dosing intervals longer than those recommended in product monographs (Uchida and Suzuki, 2014).

Thus, regular but intermittent dosing decreases antipsychotic exposure, and it can provide clinical benefits comparable to daily dosing. Moreover, the results indicate that sustained D2 occupancy, 24 hours a day is not necessary [see also (Remington et al., 2005)]. Not taking one's medication has a negative impact on outcome and this is why the clinical recommendations are to take medication regularly and to not miss doses. However, in the face of findings showing that at least some patients might benefit from regular but intermittent dosing, we also agree with Bosches and Manschreck (2002) when they ask, "Why do we persist in dosing patients on a daily schedule in spite of data that suggest that this may not be necessary or even desirable?" [p. 204, (Boshes and Manschreck, 2002)]. This being said, it must be considered that taking medication on a regular but intermittent schedule could be more difficult for some patients than daily dosing. Still, prescribing practices should be evidence based, and the evidence suggests that intermittent but regular dosing can be sufficient to maintain efficacy and to also reduce the risk of dopamine supersensitivity.

3.2.2. Antipsychotic dose

High doses of antipsychotic promote dopamine supersensitivity, both with intermittent and continuous administration (see FIG. A1.2). For instance, in people with first-episode schizophrenia, movement disorders, which can be linked to dopamine supersensitivity, are seen when antipsychotic-induced striatal D2 receptor occupancy ≥ 78% (Kapur et al., 2000). A similar link between striatal D2 receptor occupancy and dopamine supersensitivity is seen in non-human animals. As shown in FIG. A1.2C, if antipsychotic medication is given intermittently but with high doses such that peak levels of D2 occupancy are above 80 %, this produces an exaggerated psychomotor response to dopamine agonists following antipsychotic treatment cessation, indicating dopamine supersensitivity (Smith and Davis, 1975; Montanaro et al., 1982; Fukushiro et al., 2008; Carvalho et al., 2009). Continuous exposure to higher antipsychotic doses also increases the risk of developing dopamine supersensitivity, as indicated by a loss of antipsychotic-like efficacy during ongoing treatment, increased risk of vacuous chewing movements and a greater psychomotor response to amphetamine following treatment cessation (Turrone et al., 2003b, a; Samaha et al., 2007). When striatal D2 occupancy by antipsychotics is between 60-80%, there is no added therapeutic benefit of increasing dose any further (Kapur et al., 2000; Tsuboi et al., 2015). Instead, >80% striatal D2 occupancy increases the likelihood of dopamine supersensitivity. Given these observations, it is recommended to gradually reduce the dose of antipsychotic given in the acute phase, and to treat patients with the minimal therapeutic dose during the maintenance phase (Chouinard et al., 2017). Indeed, during the maintenance phase of schizophrenia treatment, using an antipsychotic dose equivalent to that used during the acute phase or giving ~ 50% of this initial dose is equally effective in preventing relapse (Uchida et al., 2011).The same principle applies to chronically treated patients that show signs of dopamine supersensitivity. When dopamine supersensitivity develops, previously efficacious doses of antipsychotic can no longer produce adequate therapeutic effects, and further increases in dose are needed to produce such effects (Chouinard et al., 1978; Chouinard and Jones, 1980; Chouinard, 1991). However, instead of increasing the dose, it is also recommended to progressively use the minimal therapeutic dose [for discussion see (Chouinard et al., 2017)].

4. Neurobiological mechanisms underlying antipsychotic-evoked dopamine supersensitivity

4.1. Changes within the dopamine system

4.1.1. Dopamine D2 receptors

Dopamine supersensitivity has been linked to increases in both the density and function of D2 receptors, and these changes have been characterized most extensively in the striatum. FIG. A1.3 illustrates how striatal D2 receptors change during chronic antipsychotic treatment and following treatment cessation. The model presented in FIG. A1.3 is based largely on findings in laboratory animals showing that chronic antipsychotic treatment increases striatal D2 receptor levels in laboratory animals (Burt et al., 1977; Fleminger et al., 1983; Severson et al., 1984; MacKenzie and Zigmond, 1985; Wilmot and Szczepanik, 1989; Jiang et al., 1990; Marin and Chase, 1993; Merchant et al., 1994; Huang et al., 1997; Samaha et al., 2007; Samaha et al., 2008; Ginovart and Kapur, 2012; Tadokoro et al., 2012; Oda et al., 2015b). Similar findings have been reported in humans (Silvestri et al., 2000; Kestler et al., 2001). Importantly, patients with a history of chronic antipsychotic treatment have more striatal D2 receptors available than antipsychotic-naïve patients with schizophrenia, and the latter have normal levels of D2 receptor availability (Silvestri et al., 2000; Howes et al., 2012). D2 receptor upregulation might not be linked to changes in D2 internalization, because levels of proteins implicated in receptor internalization (G protein-coupled receptor kinase 6 and β-arrestin 2) are unchanged following chronic antipsychotic treatment (Oda et al., 2015b). Mutations in the genes that code for these proteins are also no more frequent in schizophrenia patients that have symptoms of antipsychotic-induced dopamine supersensitivity relative to patients that do not (Oda et al., 2015a).

The relationship between D2 receptor upregulation and antipsychotic-induced dopamine supersensitivity is complex. Work is needed to determine whether in humans, D2 receptor changes are causally linked to dopamine supersensitivity. In non-human animals, there is evidence that changes in D2 receptor density and dopamine supersensitivity can be dissociable. Intermittent antipsychotic treatment regimens that do not produce dopamine supersensitivity (*i.e.*, no loss of efficacy during ongoing treatment and no exaggerated behavioral response to amphetamine) do not elevate striatal levels of D2 receptors (Samaha et al., 2008). However, treatment failure can be seen

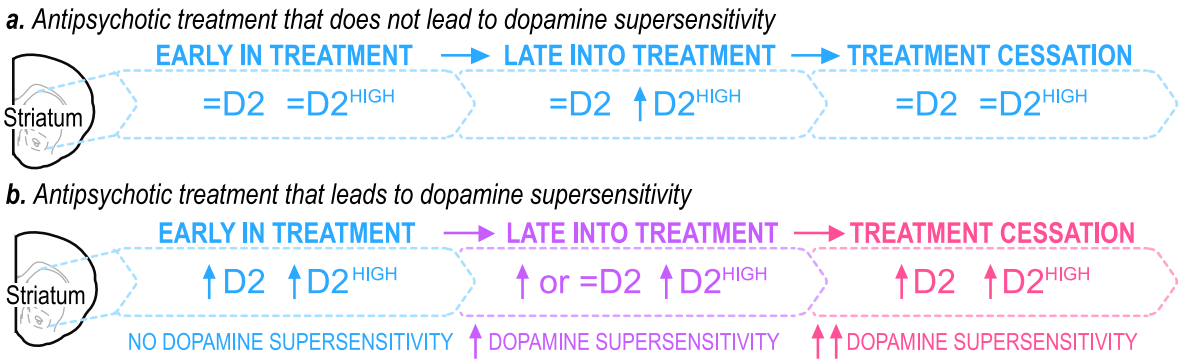

FIG. A1.3 — Changes in striatal D2/D2^{HIGH} receptor levels and antipsychotic-evoked dopamine supersensitivity. (***A***) Chronic antipsychotic treatment regimens that do not produce dopamine supersensitivity elevate striatal levels of D2^{HIGH} receptors late into treatment, but receptor levels are unchanged early in treatment. Under these conditions, D2/D2^{HIGH} receptor levels return to normal after treatment cessation. (***B***) When an antipsychotic treatment regimen produces dopamine supersensitivity, striatal levels of D2/D2^{HIGH} receptors can be increased early during antipsychotic treatment, when dopamine supersensitivity is not yet expressed. This upregulation persists during long-term treatment and after treatment cessation.

without significant changes in striatal D2 receptor levels, and there can also be D2 receptor upregulation early during a continuous antipsychotic treatment regimen, at a time when there is no behavioural evidence of dopamine supersensitivity (Samaha et al., 2007). Thus, behavioural sensitivity to dopamine is not always predicted by striatal D2 receptor upregulation [also see (Pierce et al., 1991; Flores et al., 1996)]. Instead, one possibility is that an increase in striatal D2 receptor density is a consequence of exposure to high antipsychotic doses. For instance, exposure to high-dose antipsychotic increases striatal D2 receptor density, while exposure to either doses that achieve less than 80% striatal D2 occupancy or to a treatment regimen that produces transient (versus continuous) occupancy does not (Ginovart et al., 2009). It is noteworthy that even if there is an increase in striatal D2 receptor density, D2 occupancy by antipsychotics remains high during chronic treatment, in both humans and non-human animals (Kapur et al., 2003; Turrone et al., 2003b; Samaha et al., 2007; Amato et al., 2018). Thus, it is unlikely that antipsychotics lose efficacy over time because of low levels of D2 occupancy resulting from D2 receptor upregulation.

Beyond changes in striatal D2 receptor number, antipsychotic-induced dopamine supersensitivity is also linked to increased striatal D2 receptor function. D2 receptors are metabotropic and they are in a functional, high affinity state for dopamine when they are coupled to Gi/o proteins (referred to as D2^{HIGH}). In contrast, D2 receptors are in a functionally inert, low affinity state for dopamine

when they are uncoupled to Gi/o proteins ($D2^{LOW}$). Thus, the proportion of $D2^{HIGH}$ receptors can significantly influence D2-mediated signaling. As shown in FIG. A1.3, chronic exposure to antipsychotics increases striatal levels of $D2^{HIGH}$ receptors in non-human animals (Seeman et al., 2005; Samaha et al., 2007; Samaha et al., 2008; Seeman, 2008a, b). The $D2^{HIGH}$ increase is more pronounced when the antipsychotic treatment regimen (continuous exposure to haloperidol via subcutaneous osmotic minipump) produces behavioural dopamine supersensitivity, as indicated by an exaggerated psychomotor response to amphetamine (Samaha et al., 2008). $D2^{HIGH}$ receptor elevation and antipsychotic-induced dopamine supersensitivity also follow a similar time course (Samaha et al., 2007). However, higher doses of antipsychotic (haloperidol via subcutaneous osmotic minipump) can increase $D2^{HIGH}$ sites early during treatment, before any behavioural evidence of dopamine supersensitivity (Samaha et al., 2007). In addition, intermittent antipsychotic exposure, as achieved via daily subcutaneous injections, can still increase striatal $D2^{HIGH}$ sites, in the absence of any behavioural signs of dopamine supersensitivity (Seeman et al., 2005; Samaha et al., 2008). There is still no conclusive evidence of elevated $D2^{HIGH}$ receptors in patients with schizophrenia. A PET imaging study of medication-free and medication-naïve schizophrenia patients did not find increased $D2^{HIGH}$ receptors (Graff-Guerrero et al., 2009). However, it is also possible that $D2^{HIGH}$ receptors are difficult to quantify with this method, because they are occupied by endogenous dopamine and/or because the tracer used measures $D2^{HIGH}$, $D2^{LOW}$ and D3 receptors (Graff-Guerrero et al., 2009). The link between changes in $D2^{HIGH}$ sites and the behavioural manifestations of dopamine supersensitivity also needs further study. This issue notwithstanding, it is possible that $D2^{HIGH}$ sites must increase beyond a certain threshold before dopamine supersensitivity is observed (Samaha et al., 2008). Future work assessing D2-mediated intra-cellular signaling could also shed light on this issue.

Antipsychotic-induced dopamine supersensitivity could involve changes in D2-mediated activity on both sides of the synapse. Dopamine supersensitive rats show enhanced amphetamine-induced expression of the immediate early genes c-fos and Nur77 in the striatum (Bedard et al., 2011, 2013). This suggests increased post-synaptic signaling when dopamine levels are increased. Antipsychotic-treated animals also show greater presynaptic D2 receptor-mediated inhibition of excitatory postsynaptic potentials (EPSP) in caudate-putamen neurons (Calabresi et al., 1992). Changes in presynaptic D2 receptor activity might be specific to the caudate-putamen, as the activity of presynaptic D2 receptors seems unaltered in the nucleus accumbens (Chesi et al., 1995).

The idea that dopamine supersensitivity is linked to increased presynaptic D2-mediated activity is further supported by the observation that initially (2 days into treatment), antipsychotic treatment (continuous exposure via subcutaneous minipump) increases basal dopamine overflow, but with more extended treatment (6-12 days), dopamine overflow decreases as dopamine supersensitivity develops (Samaha et al., 2007; Amato et al., 2018). The effects on dopamine overflow are discussed further in section 4.1.3 below.

4.1.2. D1 receptors

Chronic antipsychotic treatment generally does not change striatal D1 receptor density in humans (Kestler et al., 2001) or laboratory animals (Fleminger et al., 1983; MacKenzie and Zigmond, 1985; Jiang et al., 1990; Marin and Chase, 1993). One exception is the work of Huang et al. (1997), showing striatal D1 receptor upregulation following chronic clozapine but not haloperidol treatment in rats. As this matter is resolved, the available evidence suggests that D1 receptor-mediated signaling could be involved in dopamine supersensitivity. In rats supersensitive to the psychomotor effects of apomorphine following antipsychotic treatment cessation, chronic injections of a D1 agonist reverse both the supersensitivity and the upregulated striatal D2 receptors (Marin and Chase, 1993). Surprisingly, chronic injections of a D2 agonist do not influence the expression of dopamine supersensitivity (Marin and Chase, 1993). However, the findings of Marin and Chase (1993) must be interpreted with caution. Their rats were treated with an antipsychotic dose that was ~20 times higher than what would be clinically relevant [based on Kapur et al. (2003)], and the study did not include antipsychotic-naïve rats for comparison.

4.1.3. Dopamine release and re-uptake

Initially, antipsychotics enhance both dopamine overflow and turnover in the nucleus accumbens and caudate-putamen [(Ericson et al., 1996; Samaha et al., 2007; Amato et al., 2018) but see Amato et al. (2011)]. This likely involves occupancy of presynaptic D2 receptors. It could also involve diminished negative feedback onto dopamine neurons, as dopamine transporter density in the ventral tegmental area and the substantia nigra is decreased at this stage of treatment (Amato et al., 2018). However, dopamine release and re-uptake change with more long-term treatment, when dopamine supersensitivity and loss of antipsychotic-like efficacy are seen. First, dopamine transporter density returns to normal (antipsychotic-naïve) levels (Amato et al., 2018). In the

nucleus accumbens and caudate-putamen, baseline or amphetamine-stimulated dopamine overflow also return to normal levels or are diminished (Samaha et al., 2007; Amato et al., 2011; Amato et al., 2018). Similarly, striatal dopamine turnover, which is initially enhanced, normalizes with long-term antipsychotic treatment (Ericson et al., 1996). The decrease or normalization of striatal dopamine levels could involve upregulation of presynaptic D2 receptors, as discussed above. Amato et al. (2018) hypothesized that the increased dopamine release/up-take early in treatment underlies the therapeutic effects of antipsychotics, while tolerance to this dopaminergic effect underlies treatment failure. In support of this, they found that infusion of a selective dopamine transporter inhibitor (GBR12909) into the caudate-putamen rescues antipsychotic-like effects. However, the effects of GBR12909 in antipsychotic-naïve animals were not reported. This makes it difficult to determine whether the GBR12909 effects are relevant to antipsychotic response. Still, the findings concord with others. For instance, See and Murray (1992) show that acute treatment with raclopride increases extracellular levels of dopamine in the caudate-putamen, and that rats chronically treated with haloperidol are tolerant to this effect. Thus, initially, antipsychotics increase striatal dopamine release and uptake. With chronic antipsychotic treatment, there is tolerance to this effect and this coincides with the emergence of dopamine supersensitivity and treatment failure.

Antipsychotic-induced changes in dopamine release/re-uptake do not always correlate with dopamine supersensitivity. Dopamine supersensitivity persists after haloperidol treatment cessation, as indicated by potentiated amphetamine-induced locomotion (Smith and Davis, 1975; Rebec et al., 1982; Meng et al., 1998; Samaha et al., 2007; Bedard et al., 2013). However, amphetamine-induced dopamine release in the nucleus accumbens or caudate-putamen is unchanged after haloperidol treatment cessation (Compton and Johnson, 1988; Ichikawa and Meltzer, 1992). Striatal levels of the dopamine transporter are also unchanged (Ase et al., 1999). Conversely, after cessation of chronic treatment with atypical antipsychotics, dopamine supersensitivity is less likely to persist (Samaha et al., 2007; Bedard et al., 2013), yet amphetamine-evoked dopamine release in the caudate-putamen (but not the nucleus accumbens) can still be potentiated (Compton and Johnson, 1988; Ichikawa and Meltzer, 1992). Thus, dopamine release and re-uptake can change over the course of antipsychotic treatment and cessation and more research is required to determine how this might be linked to dopamine supersensitivity.

4.2. Contributions of non-dopaminergic systems

There are several reasons to look beyond dopamine. First, antipsychotic drugs interact with multiple neurotransmitter systems including dopamine, serotonin, noradrenaline and acetylcholine (Arnt and Skarsfeldt, 1998). Second, psychosis could also be mediated by neurotransmitter systems other than dopamine, such as glutamate and GABA (Olney and Farber, 1995). Third, in both humans and non-human animals, chronic antipsychotic treatment can evoke behavioural supersensitivity to non-selective dopamine agonists that enhance monoamine signaling in general, this includes amphetamine and cocaine (Smith and Davis, 1975; Vonvoigtlander et al., 1975; Kosten, 1997). Fourth, serotonin (Kapur and Remington, 1996), neurotensin (Binder et al., 2001) and glutamate (Javitt, 2007) all interact with the dopamine system and influence dopamine-dependent behaviours. Below we review evidence that serotonin, glutamate, neurotensin and other systems could mediate antipsychotic-evoked dopamine supersensitivity.

4.2.1. Serotonin

Several observations suggest that serotonin-mediated activity could be involved in antipsychotic-induced dopamine supersensitivity. First, many antipsychotic medications, especially of the atypical class, have high affinities at several serotonin receptor types (Meltzer et al., 1989). Second, early work shows that when animals are given an antipsychotic treatment regimen that produces dopamine supersensitivity (4-6 months of trifluoperazine given via the drinking water), they are also behaviourally sensitized to compounds that increase serotonin activity (Dawbarn et al., 1981). Finally, outside of the context of antipsychotics, dopamine supersensitivity is linked to enhanced functional interactions between serotonin 5-HT2 receptors and dopamine. For example, in cocaine-sensitized or dopamine-depleted rats, there is an enhanced influence of 5-HT2A receptor activity on dopamine overflow in the nucleus accumbens, striatal gene expression, and psychomotor activity (Yan et al., 2000; Bishop et al., 2004; Brown and Gerfen, 2006). In dopamine-denervated rats, injecting a 5-HT2A receptor antagonist into the caudate-putamen normalizes the potentiated psychomotor response to dopamine receptor stimulation seen in these rats (Bishop et al., 2005). Based on this literature, we determined whether 5-HT2A receptor activity influences the expression of antipsychotic-evoked dopamine supersensitivity in a previous study (Charron et al., 2015). We found that 5-HT2 or 5-HT2A receptor antagonists normalize the potentiated psychomotor response to amphetamine in dopamine-supersensitive rats at doses that do not influence amphetamine-

induced locomotion in control rats (Charron et al., 2015). This could involve the observation that 5-HT2A receptor activation promotes striatal dopamine release (Porras et al., 2002). Of note, chronic antipsychotic treatment does not alter extracellular levels of serotonin or its metabolite 5-HIAA in the striatum (Ichikawa and Meltzer, 1990; Amato et al., 2011). Striatal serotonin metabolism/turnover is also unchanged (Dawbarn et al., 1981; Ase et al., 1999). Finally, animals treated with an antipsychotic regimen that produces dopamine supersensitivity (daily s.c. injections of high dose haloperidol) do not show changes in striatal levels of the serotonin transporter (Ase et al., 1999).

Chronic antipsychotic treatment changes 5-HT2A receptor density in the brain and effects can be different with typical versus atypical antipsychotics. Chronic treatment with atypical antipsychotic drugs decreases striatal levels of 5-HT2A receptors (O'Dell et al., 1990; Steward et al., 2004). In contrast, chronic treatment with typical antipsychotic drugs increases 5-HT2A receptor density in the caudate-putamen (Wilmot and Szczepanik, 1989). Both increases (Wilmot and Szczepanik, 1989) and decreases (Wilmot and Szczepanik, 1989) have been reported in the nucleus accumbens. Still, other studies report that chronic treatment with either typical or atypical antipsychotics does not change striatal levels of 5-HT2A receptors (Wilmot and Szczepanik, 1989; O'Dell et al., 1990; Steward et al., 2004). The discrepancies could be due to differences in the doses and extent of antipsychotic treatment. However, when antipsychotic treatment explicitly leads to dopamine supersensitivity, there is altered 5-HT2A receptor density in corticostriatal regions. Dopamine supersensitive rats (treated with a typical antipsychotic) have decreased 5-HT2A receptor density in the prelimbic cortex (Charron et al., 2015). This is consistent with post-mortem studies in antipsychotic-treated schizophrenia patients (Burnet et al., 1996). In contrast, dopamine supersensitive rats have increased 5-HT2A receptor density in the caudate-putamen (Charron et al., 2015). If these 5-HT2A receptor-related changes in the cortex and striatum mediate dopamine supersensitivity, this could involve changes in the functional interactions between 5-HT2A receptors and other neurotransmitter systems in corticostriatal-dependent networks.

4.2.2. Glutamate

Antipsychotic-induced dopamine supersensitivity could involve glutamate hypoactivity in the striatum. Compared to antipsychotic-naïve animals, animals exposed to antipsychotics and that also show dopamine supersensitivity (*i.e.*, as indicated by an augmented psychomotor response to

apomorphine) are hyposensitive to the locomotor effects of NMDA, AMPA or kainic acid infused in the caudate-putamen [(Ossowska, 1995), also see (Freed et al., 1989)]. Chronic antipsychotic treatment produces subtle changes in AMPA and NMDA levels in the striatum. There are no significant changes in the overall density of striatal NMDA or AMPA receptors, or NMDA receptor 1 or 2 subunits (Johnson et al., 1994; Meshul et al., 1996; Scarr et al., 2002; Hanaoka et al., 2003; Oda et al., 2017). However, there can be cell type-specific effects. In the caudate-putamen, NMDA receptor subunit 1 density is increased in dendritic spines but decreased in glia after chronic antipsychotic exposure (Rodriguez and Pickel, 1999). Also, while chronic antipsychotic treatment does not change the total density of AMPA receptors in the striatum, the density of AMPA receptors in a high affinity state is increased (McCoy et al., 1996). It remains to be determined how these glutamate-related changes might contribute to antipsychotic-evoked dopamine supersensitivity. In the meantime, as mentioned earlier, striatal D2 receptor activation more effectively inhibits EPSPs after chronic antipsychotic treatment (Calabresi et al., 1992). This suggests that prolonged exposure to antipsychotics, and perhaps the emergence of dopamine supersensitivity, involves changes in glutamate-mediated signaling in the striatum.

4.2.3. Neurotensin

Neurotensin is a neuropeptide that can oppose dopamine effects through activation of type 1 neurotensin receptors (NTS1). Activation of NTS1 receptors decreases D2 receptor affinity for dopamine (Agnati et al., 1983; von Euler et al., 1990; Li et al., 1995). This could involve internalization of D2 receptors, through pathways dependent on both protein kinase C and β-arrestin 1 (Thibault et al., 2011). NTS1 receptor activation can also evoke D2 internalization via formation of D2-NTS1 complexes (Koschatzky et al., 2011; Borroto-Escuela et al., 2013). Given these neurotensin-D2 interactions, neurotensin agonists have been investigated as potential antipsychotic drugs (Boules et al., 2007). Striatal neurotensin might also regulate the expression of dopamine supersensitivity, as it attenuates dopamine-dependent behaviours. Indeed, infusing neurotensin into the nucleus accumbens decreases the psychomotor response to both dopamine agonists given systemically (Ervin et al., 1981; Robledo et al., 1993; Feifel et al., 1997) and dopamine infused into the nucleus accumbens (Kalivas et al., 1984). We also found previously that when dopamine supersensitive rats receive a single infusion of neurotensin into the nucleus accumbens (10 µg/hemisphere), this normalizes the potentiated amphetamine-induced locomotion

seen in these rats (Servonnet et al., 2017). In contrast, the same treatment does not influence amphetamine-induced locomotion in antipsychotic-naïve rats or in rats exposed to antipsychotics intermittently via daily injection, a regimen that does not produce dopamine supersensitivity (Servonnet et al., 2017). Thus, in dopamine supersensitive rats, the anti-dopaminergic effects of neurotensin in the nucleus accumbens are potentiated (Servonnet et al., 2017). It is possible then, that the increased response to neurotensin can be exploited pharmacologically to attenuate the behavioural manifestations of dopamine supersensitivity.

Antipsychotic-treated rats have increased striatal neurotensin protein (Govoni et al., 1980; Goedert et al., 1985; Bissette et al., 1988; Frey et al., 1988; Kilts et al., 1988; Myers et al., 1992; See et al., 1995; Kinkead et al., 2000) and mRNA levels (Merchant et al., 1992; Merchant et al., 1994; Servonnet et al., 2017). In rats showing antipsychotic-induced dopamine supersensitivity specifically, neurotensin mRNA expression is increased in both the caudate-putamen and nucleus accumbens (Servonnet et al., 2017). In contrast, when rats are given an antipsychotic treatment regimen that does not produce dopamine supersensitivity, neurotensin mRNA levels are increased only in the nucleus accumbens. As striatal neurotensin has anti-dopaminergic effects, the increased neurotensin-mediated activity could be a compensatory neuroadaptation in dopamine supersensitive animals. In contrast, dopamine-supersensitive rats have unchanged overall levels of NTS1 receptors in the striatum (Kinkead et al., 2000; Servonnet et al., 2017).

4.2.4. Other systems

GABA, acetylcholine, noradrenergic and nitric oxide systems could also be involved in dopamine supersensitivity. First, GABA signaling in the striatum might be disrupted in antipsychotic-evoked dopamine supersensitivity. For instance, infusing a mixture of GABAA and GABAB agonists into the nucleus accumbens suppresses amphetamine-induced locomotion in antipsychotic-naïve rats, but the same manipulation does not influence the exaggerated psychomotor response to amphetamine in dopamine supersensitive rats (El Hage et al., 2015). One possibility is that the accumbens is not necessary for the expression of dopamine supersensitivity. Alternatively, the effects of GABA receptor stimulation could be altered in dopamine supersensitive animals. In support of this, in dopamine supersensitive rats (but not antipsychotic-exposed rats that did not develop dopamine supersensitivity), the GABA/glutamate ratio in the striatum is increased relative to controls (Oda et al., 2017). This could involve enhanced activity of the enzymes glutamic acid

decarboxylase 1 and 2, which convert glutamate to GABA (Oda et al., 2017). In parallel, co-administration of a nitric oxide synthesis inhibitor (Pudiak and Bozarth, 1997), a wide-spectrum muscarinic receptor antagonist or a selective muscarinic receptor type 1 antagonist (Carvey et al., 1986; Butkerait and Friedman, 1988) during chronic antipsychotic treatment also prevents the development of dopamine supersensitivity (*i.e.*, prevents the exaggerated psychomotor response to dopamine agonists induced by the antipsychotic treatment alone). Once dopamine supersensitivity is established, administration of an α1 receptor antagonist can also reverse the potentiated psychomotor response to a dopamine agonist (Obuchowicz, 1999). Finally, antipsychotic-evoked dopamine supersensitivity may also involve structural changes in the brain. For instance, chronic antipsychotic treatment potentiates neurogenesis (Kippin et al., 2005). This could contribute to antipsychotic-induced increases in striatal volume (Andersson et al., 2002). Interestingly, antipsychotic-induced increases in striatal volume are more pronounced in animals that have tardive dyskinesia-like features (Chakos et al., 1998). Such features are linked to a dopamine supersensitive state. However, the link between such structural changes and the emergence of antipsychotic-evoked dopamine supersensitivity remains to be determined. Thus, in addition to the dopamine system, several biological systems seem implicated in the development and expression of antipsychotic-induced dopamine supersensitivity.

5. Concluding remarks and perspectives

Antipsychotic drugs can produce neuroadaptations that are believed to lead to a dopamine supersensitive state. We reviewed the changes at dopamine D2 receptors and dopamine transporters that are linked to dopamine supersensitivity, and we also discussed the involvement of biological systems beyond dopamine. However, the neurobiological mechanisms that cause the development and expression of dopamine supersensitivity have yet to be identified fully. Animal models will be particularly valuable in this context as they allow us to establish causal links between antipsychotic treatment and the development of dopamine supersensitivity. Studies using laboratory animals should continue to use antipsychotic treatment regimens that most faithfully mimic the regimens used in the clinic. Using acute dosing and/or very high doses can give partial or even irrelevant information. It will also be critical to better characterize the factors that influence the development and expression of antipsychotic-evoked dopamine supersensitivity in patients with schizophrenia.

These factors can include the type of antipsychotic treatment regimen, stress and pre-existing symptom severity or quality. We also reviewed the evidence from the animal literature that raises questions about whether atypical versus typical antipsychotics—when adequately dosed—might be less likely to produce dopamine supersensitivity on the long term. Finally, we discussed findings from animal studies showing that regular but intermittent dosing avoids the development of dopamine supersensitivity. This links to evidence that similar intermittent dosing approaches can be used effectively in at least some people with schizophrenia. Together, the evidence convincingly suggests that sustained antipsychotic treatment/D2 receptor occupancy is not always necessary to maintain clinical response, and that regular but intermittent treatment strategies should be investigated further in the clinic. These issues should be considered at the very outset of antipsychotic treatment. In conclusion, we believe that the evidence reviewed here highlights two things. First, animal models can be exceptionally useful in modeling antipsychotic-induced dopamine supersensitivity and in investigating neurobiological mechanisms. Second, in parallel to the search for new antipsychotic molecules, we need to better understand the medications currently used. Treatment regimens can then be designed in ways that attenuate the symptoms of schizophrenia while minimizing the risk of neuroadaptations that promote dopamine supersensitivity.

REFERENCES – ANNEX 1

Agnati LF, Fuxe K, Benfenati F, Battistini N (1983) Neurotensin in vitro markedly reduces the affinity in subcortical limbic 3H-N-propylnorapomorphine binding sites. Acta physiologica Scandinavica 119:459-461.

Amato D, Natesan S, Yavich L, Kapur S, Muller CP (2011) Dynamic regulation of dopamine and serotonin responses to salient stimuli during chronic haloperidol treatment. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 14:1327-1339.

Amato D, Canneva F, Cumming P, Maschauer S, Groos D, Dahlmanns JK, Gromer TW, Chiofalo L, Dahlmanns M, Zheng F, Kornhuber J, Prante O, Alzheimer C, von Horsten S, Muller CP (2018) A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failure reversal: the role of the dopamine transporter. Molecular psychiatry.

Andersson C, Hamer RM, Lawler CP, Mailman RB, Lieberman JA (2002) Striatal volume changes in the rat following long-term administration of typical and atypical antipsychotic drugs. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 27:143-151.

Anthony JC, Warner LA, Kessler RC (1996) Comparative Epidemiology of Dependence on Tobacco, Alcohol, Controlled Substances, and Inhalants: Basic Findings From the National Comorbidity Survey. Experimental and Clinical Psychopharmacology 2:244-268.

Arnt J (1982) Pharmacological specificity of conditioned avoidance response inhibition in rats: inhibition by neuroleptics and correlation to dopamine receptor blockade. Acta pharmacologica et toxicologica 51:321-329.

Arnt J (1995) Differential effects of classical and newer antipsychotics on the hypermotility induced by two dose levels of D-amphetamine. European journal of pharmacology 283:55-62.

Arnt J, Skarsfeldt T (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 18:63-101.

Ase AR, Amdiss F, Hebert C, Huang N, van Gelder NM, Reader TA (1999) Effects of antipsychotic drugs on dopamine and serotonin contents and metabolites, dopamine and serotonin transporters, and serotonin1A receptors. Journal of neural transmission 106:75-105.

Asper H, Baggiolini M, Burki HR, Lauener H, Ruch W, Stille G (1973) Tolerance phenomena with neuroleptics catalepsy, apomorphine stereotypies and striatal dopamine metabolism in the rat after single and repeated administration of loxapine and haloperidol. European journal of pharmacology 22:287-294.

Bedard AM, Maheux J, Levesque D, Samaha AN (2011) Continuous, but not intermittent, antipsychotic drug delivery intensifies the pursuit of reward cues. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 36:1248-1259.

Bedard AM, Maheux J, Levesque D, Samaha AN (2013) Prior haloperidol, but not olanzapine, exposure augments the pursuit of reward cues: implications for substance abuse in schizophrenia. Schizophrenia bulletin 39:692-702.

Binder EB, Kinkead B, Owens MJ, Nemeroff CB (2001) Neurotensin and dopamine interactions. Pharmacological reviews 53:453-486.

Bishop C, Daut GS, Walker PD (2005) Serotonin 5-HT2A but not 5-HT2C receptor antagonism reduces hyperlocomotor activity induced in dopamine-depleted rats by striatal administration of the D1 agonist SKF 82958. Neuropharmacology 49:350-358.

Bishop C, Tessmer JL, Ullrich T, Rice KC, Walker PD (2004) Serotonin 5-HT2A receptors underlie increased motor behaviors induced in dopamine-depleted rats by intrastriatal 5-HT2A/2C agonism. The Journal of pharmacology and experimental therapeutics 310:687-694.

Bissette G, Dauer WT, Kilts CD, O'Connor L, Nemeroff CB (1988) The effect of the stereoisomers of butaclamol on neurotensin content in discrete regions of the rat brain. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 1:329-335.

Borroto-Escuela DO, Ravani A, Tarakanov AO, Brito I, Narvaez M, Romero-Fernandez W, Corrales F, Agnati LF, Tanganelli S, Ferraro L, Fuxe K (2013) Dopamine D2 receptor signaling dynamics of dopamine D2-neurotensin 1 receptor heteromers. Biochemical and biophysical research communications 435:140-146.

Boshes RA, Manschreck TC (2002) Review of antipsychotic medication administration: a proposal of intermittent dosing. Schizophrenia bulletin 28:203-222.

Boules M, Shaw A, Fredrickson P, Richelson E (2007) Neurotensin agonists: potential in the treatment of schizophrenia. CNS drugs 21:13-23.

Brown P, Gerfen CR (2006) Plasticity within striatal direct pathway neurons after neonatal dopamine depletion is mediated through a novel functional coupling of serotonin 5-HT2 receptors to the ERK 1/2 map kinase pathway. The Journal of comparative neurology 498:415-430.

Burnet PW, Eastwood SL, Harrison PJ (1996) 5-HT1A and 5-HT2A receptor mRNAs and binding site densities are differentially altered in schizophrenia. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 15:442-455.

Burt DR, Creese I, Snyder SH (1977) Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. Science 196:326-328.

Butkerait P, Friedman E (1988) Scopolamine modulates apomorphine-induced behavior in rats treated with haloperidol or SCH 23390. European journal of pharmacology 148:269-272.

Calabresi P, De Murtas M, Mercuri NB, Bernardi G (1992) Chronic neuroleptic treatment: D2 dopamine receptor supersensitivity and striatal glutamatergic transmission. Annals of neurology 31:366-373.

Calza L, Giardino L, Piazza PV, Amato G (1990) Thioridazine chronic administration: a behavioural and autoradiographic study. Neuroscience letters 109:206-211.

Carpenter WT, Jr., Buchanan RW, Kirkpatrick B, Lann HD, Breier AF, Summerfelt AT (1999) Comparative effectiveness of fluphenazine decanoate injections every 2 weeks versus every 6 weeks. The American journal of psychiatry 156:412-418.

Carvalho RC, Fukushiro DF, Helfer DC, Callegaro-Filho D, Trombin TF, Zanlorenci LH, Sanday L, Silva RH, Frussa-Filho R (2009) Long-term haloperidol treatment (but not risperidone) enhances addiction-related behaviors in mice: role of dopamine D2 receptors. Addiction biology 14:283-293.

Carvey PM, Kao LC, Tanner CM, Goetz CG, Klawans HL (1986) The effect of antimuscarinic agents on haloperidol induced behavioral hypersensitivity. European journal of pharmacology 120:193-199.

Casey DE (1991) Neuroleptic drug-induced extrapyramidal syndromes and tardive dyskinesia. Schizophrenia research 4:109-120.

Chakos MH, Shirakawa O, Lieberman J, Lee H, Bilder R, Tamminga CA (1998) Striatal enlargement in rats chronically treated with neuroleptic. Biological psychiatry 44:675-684.

Charron A, Hage CE, Servonnet A, Samaha AN (2015) 5-HT2 receptors modulate the expression of antipsychotic-induced dopamine supersensitivity. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 25:2381-2393.

Chesi AJ, Feasey-Truger KJ, Alzheimer C, ten Bruggencate G (1995) Dopamine autoreceptor sensitivity is unchanged in rat nucleus accumbens after chronic haloperidol treatment: an in vivo and in vitro voltammetric study. The European journal of neuroscience 7:2450-2457.

Chouinard G (1991) Severe cases of neuroleptic-induced supersensitivity psychosis. Diagnostic criteria for the disorder and its treatment. Schizophrenia research 5:21-33.

Chouinard G, Jones BD (1980) Neuroleptic-induced supersensitivity psychosis: clinical and pharmacologic characteristics. The American journal of psychiatry 137:16-21.

Chouinard G, Chouinard VA (2008) Atypical antipsychotics: CATIE study, drug-induced movement disorder and resulting iatrogenic psychiatric-like symptoms, supersensitivity rebound psychosis and withdrawal discontinuation syndromes. Psychother Psychosom 77:69-77.

Chouinard G, Jones BD, Annable L (1978) Neuroleptic-induced supersensitivity psychosis. The American journal of psychiatry 135:1409-1410.

Chouinard G, Annable L, Ross-Chouinard A (1986) Supersensitivity psychosis and tardive dyskinesia: a survey in schizophrenic outpatients. Psychopharmacol Bull 22:891-896.

Chouinard G, Samaha AN, Chouinard VA, Peretti CS, Kanahara N, Takase M, Iyo M (2017) Antipsychotic-Induced Dopamine Supersensitivity Psychosis: Pharmacology, Criteria, and Therapy. Psychother Psychosom 86:189-219.

Clow A, Theodorou A, Jenner P, Marsden CD (1980) Cerebral dopamine function in rats following withdrawal from one year of continuous neuroleptic administration. European journal of pharmacology 63:145-157.

Compton DR, Johnson KM (1988) Effects of acute and chronic clozapine and haloperidol on in vitro release of acetylcholine and dopamine from striatum and nucleus accumbens. The Journal of pharmacology and experimental therapeutics 248:521-530.

Creese I, Burt DR, Snyder SH (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192:481-483.

Dawbarn D, Long SK, Pycock CJ (1981) Increased central 5-hydroxytryptamine receptor mechanisms in rats after chronic neuroleptic treatment. British journal of pharmacology 73:149-156.

Demjaha A, Murray RM, McGuire PK, Kapur S, Howes OD (2012) Dopamine synthesis capacity in patients with treatment-resistant schizophrenia. The American journal of psychiatry 169:1203-1210.

El Hage C, Bedard AM, Samaha AN (2015) Antipsychotic treatment leading to dopamine supersensitivity persistently alters nucleus accumbens function. Neuropharmacology 99:715-725.

Emsley R, Nuamah I, Gopal S, Hough D, Fleischhacker WW (2018) Relapse After Antipsychotic Discontinuation in Schizophrenia as a Withdrawal Phenomenon vs Illness Recurrence: A Post Hoc Analysis of a Randomized Placebo-Controlled Study. The Journal of clinical psychiatry 79.

Ericson H, Radesater AC, Servin E, Magnusson O, Mohringe B (1996) Effects of intermittent and continuous subchronic administration of raclopride on motor activity, dopamine turnover and receptor occupancy in the rat. Pharmacology & toxicology 79:277-286.

Ervin GN, Birkemo LS, Nemeroff CB, Prange AJ, Jr. (1981) Neurotensin blocks certain amphetamine-induced behaviours. Nature 291:73-76.

Fallon P, Dursun SM (2011) A naturalistic controlled study of relapsing schizophrenic patients with tardive dyskinesia and supersensitivity psychosis. Journal of psychopharmacology 25:755-762.

Fallon P, Dursun S, Deakin B (2012) Drug-induced supersensitivity psychosis revisited: characteristics of relapse in treatment-compliant patients. Therapeutic advances in psychopharmacology 2:13-22.

Farde L, Wiesel FA, Nordstrom AL, Sedvall G (1989) D1- and D2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. Psychopharmacology 99 Suppl:S28-31.

Feifel D, Minor KL, Dulawa S, Swerdlow NR (1997) The effects of intra-accumbens neurotensin on sensorimotor gating. Brain research 760:80-84.

Fleminger S, Rupniak NM, Hall MD, Jenner P, Marsden CD (1983) Changes in apomorphine-induced stereotypy as a result of subacute neuroleptic treatment correlates with increased D-2 receptors, but not with increases in D-1 receptors. Biochemical pharmacology 32:2921-2927.

Flores G, Barbeau D, Quirion R, Srivastava LK (1996) Decreased binding of dopamine D3 receptors in limbic subregions after neonatal bilateral lesion of rat hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 16:2020-2026.

Freed WJ, Cannon-Spoor HE, Rodgers CR (1989) Attenuation of the behavioral response to quisqualic acid and glutamic acid diethyl ester by chronic haloperidol administration. Life sciences 44:1303-1308.

Frey P, Lis M, Coward DM (1988) Neurotensin concentrations in rat striatum and nucleus accumbens: Further studies of their regulation. Neurochemistry international 12:33-38.

Fukushiro DF, Carvalho Rde C, Ricardo VP, Alvarez Jdo N, Ribeiro LT, Frussa-Filho R (2008) Haloperidol (but not ziprasidone) withdrawal potentiates sensitization to the hyperlocomotor effect of cocaine in mice. Brain research bulletin 77:124-128.

Gaebel W (1994) Intermittent medication--an alternative? Acta psychiatrica Scandinavica Supplementum 382:33-38.

Gaebel W, Janner M, Frommann N, Pietzcker A, Kopcke W, Linden M, Muller P, Muller-Spahn F, Tegeler J (2002) First vs multiple episode schizophrenia: two-year outcome of intermittent and maintenance medication strategies. Schizophrenia research 53:145-159.

Gill KM, Cook JM, Poe MM, Grace AA (2014) Prior antipsychotic drug treatment prevents response to novel antipsychotic agent in the methylazoxymethanol acetate model of schizophrenia. Schizophrenia bulletin 40:341-350.

Ginovart N, Kapur S (2012) Role of dopamine D(2) receptors for antipsychotic activity. Handbook of experimental pharmacology:27-52.

Ginovart N, Wilson AA, Hussey D, Houle S, Kapur S (2009) D2-receptor upregulation is dependent upon temporal course of D2-occupancy: a longitudinal [11C]-raclopride PET study in cats. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 34:662-671.

Goedert M, Iversen SD, Emson PC (1985) The effects of chronic neuroleptic treatment on neurotensin-like immunoreactivity in the rat central nervous system. Brain research 335:334-336.

Govoni S, Hong JS, Yang HY, Costa E (1980) Increase of neurotensin content elicited by neuroleptics in nucleus accumbens. The Journal of pharmacology and experimental therapeutics 215:413-417.

Graff-Guerrero A, Mizrahi R, Agid O, Marcon H, Barsoum P, Rusjan P, Wilson AA, Zipursky R, Kapur S (2009) The dopamine D2 receptors in high-affinity state and D3 receptors in schizophrenia: a clinical [11C]-(+)-PHNO PET study. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 34:1078-1086.

Halperin R, Guerin JJ, Jr., Davis KL (1989) Regional differences in the induction of behavioral supersensitivity by prolonged treatment with atypical neuroleptics. Psychopharmacology 98:386-391.

Hanaoka T, Toyoda H, Mizuno T, Kikuyama H, Morimoto K, Takahata R, Matsumura H, Yoneda H (2003) Alterations in NMDA receptor subunit levels in the brain regions of rats chronically administered typical or atypical antipsychotic drugs. Neurochemical research 28:919-924.

Howell LL, Byrd LD (1992) Enhanced sensitivity to the behavioral effects of cocaine after chronic administration of D2-selective dopamine antagonists in the squirrel monkey. The Journal of pharmacology and experimental therapeutics 262:907-915.

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophrenia bulletin 35:549-562.

Howes OD, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A, Kapur S (2012) The nature of dopamine dysfunction in schizophrenia and what this means for treatment. Archives of general psychiatry 69:776-786.

Huang N, Ase AR, Hebert C, van Gelder NM, Reader TA (1997) Effects of chronic neuroleptic treatments on dopamine D1 and D2 receptors: homogenate binding and autoradiographic studies. Neurochemistry international 30:277-290.

Hunt GE, Large MM, Cleary M, Lai HMX, Saunders JB (2018) Prevalence of comorbid substance use in schizophrenia spectrum disorders in community and clinical settings, 1990-2017: Systematic review and meta-analysis. Drug Alcohol Depend 191:234-258.

Ichikawa J, Meltzer HY (1990) Apomorphine does not reverse reduced basal dopamine release in rat striatum and nucleus accumbens after chronic haloperidol treatment. Brain research 507:138-142.

Ichikawa J, Meltzer HY (1992) The effect of chronic atypical antipsychotic drugs and haloperidol on amphetamine-induced dopamine release in vivo. Brain research 574:98-104.

Iyo M, Tadokoro S, Kanahara N, Hashimoto T, Niitsu T, Watanabe H, Hashimoto K (2013) Optimal extent of dopamine D2 receptor occupancy by antipsychotics for treatment of dopamine supersensitivity psychosis and late-onset psychosis. Journal of clinical psychopharmacology 33:398-404.

Javitt D (2007) Glutamate and schizophrenia: phencyclidine, N-methyl-d-aspartate receptors, and dopamine–glutamate interactions. International review of neurobiology 78:69-108.

Jiang LH, Kasser RJ, Altar CA, Wang RY (1990) One year of continuous treatment with haloperidol or clozapine fails to induce a hypersensitive response of caudate putamen neurons to dopamine D1 and D2 receptor agonists. The Journal of pharmacology and experimental therapeutics 253:1198-1205.

Johnson AE, Liminga U, Liden A, Lindefors N, Gunne LM, Wiesel FA (1994) Chronic treatment with a classical neuroleptic alters excitatory amino acid and GABAergic neurotransmission in specific regions of the rat brain. Neuroscience 63:1003-1020.

Jolley AG, Hirsch SR (1993) Continuous versus intermittent neuroleptic therapy in schizophrenia. Drug Saf 8:331-339.

Jolley AG, Hirsch SR, Morrison E, McRink A, Wilson L (1990) Trial of brief intermittent neuroleptic prophylaxis for selected schizophrenic outpatients: clinical and social outcome at two years. Bmj 301:837-842.

Kalivas PW, Nemeroff CB, Prange AJ, Jr. (1984) Neurotensin microinjection into the nucleus accumbens antagonizes dopamine-induced increase in locomotion and rearing. Neuroscience 11:919-930.

Kapur S, Remington G (1996) Serotonin-dopamine interaction and its relevance to schizophrenia. The American journal of psychiatry 153:466-476.

Kapur S, VanderSpek SC, Brownlee BA, Nobrega JN (2003) Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. The Journal of pharmacology and experimental therapeutics 305:625-631.

Kapur S, Zipursky R, Jones C, Remington G, Houle S (2000) Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. The American journal of psychiatry 157:514-520.

Kestler LP, Walker E, Vega EM (2001) Dopamine receptors in the brains of schizophrenia patients: a meta-analysis of the findings. Behavioural pharmacology 12:355-371.

Khantzian EJ (1985) The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence. The American journal of psychiatry 142:1259-1264.

Kilts CD, Anderson CM, Bissette G, Ely TD, Nemeroff CB (1988) Differential effects of antipsychotic drugs on the neurotensin concentration of discrete rat brain nuclei. Biochemical pharmacology 37:1547-1554.

Kinkead B, Shahid S, Owens MJ, Nemeroff CB (2000) Effects of acute and subchronic administration of typical and atypical antipsychotic drugs on the neurotensin system of the rat brain. The Journal of pharmacology and experimental therapeutics 295:67-73.

Kippin TE, Kapur S, van der Kooy D (2005) Dopamine specifically inhibits forebrain neural stem cell proliferation, suggesting a novel effect of antipsychotic drugs. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:5815-5823.

Koschatzky S, Tschammer N, Gmeiner P (2011) Cross-receptor interactions between dopamine D2L and neurotensin NTS1 receptors modulate binding affinities of dopaminergics. ACS chemical neuroscience 2:308-316.

Kosten TA (1997) Enhanced neurobehavioral effects of cocaine with chronic neuroleptic exposure in rats. Schizophrenia bulletin 23:203-213.

Kuribara H, Tadokoro S (1981) Correlation between antiavoidance activities of antipsychotic drugs in rats and daily clinical doses. Pharmacology, biochemistry, and behavior 14:181-192.

Li M, Fletcher PJ, Kapur S (2007) Time course of the antipsychotic effect and the underlying behavioral mechanisms. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 32:263-272.

Li XM, Ferraro L, Tanganelli S, O'Connor WT, Hasselrot U, Ungerstedt U, Fuxe K (1995) Neurotensin peptides antagonistically regulate postsynaptic dopamine D2 receptors in rat nucleus accumbens: a receptor binding and microdialysis study. Journal of neural transmission General section 102:125-137.

Lieberman JA, Kane JM, Alvir J (1987) Provocative tests with psychostimulant drugs in schizophrenia. Psychopharmacology 91:415-433.

Ljungberg T, Ungerstedt U (1985) A rapid and simple behavioural screening method for simultaneous assessment of limbic and striatal blocking effects of neuroleptic drugs. Pharmacology, biochemistry, and behavior 23:479-485.

Lu ML, Pan JJ, Teng HW, Su KP, Shen WW (2002) Metoclopramide-induced supersensitivity psychosis. Ann Pharmacother 36:1387-1390.

MacKenzie RG, Zigmond MJ (1985) Chronic neuroleptic treatment increases D-2 but not D-1 receptors in rat striatum. European journal of pharmacology 113:159-165.

Madularu D, Shams WM, Brake WG (2014) Estrogen potentiates the behavioral and nucleus accumbens dopamine response to continuous haloperidol treatment in female rats. The European journal of neuroscience 39:257-265.

Madularu D, Kulkarni P, Yee JR, Kenkel WM, Shams WM, Ferris CF, Brake WG (2016) High estrogen and chronic haloperidol lead to greater amphetamine-induced BOLD activation in awake, amphetamine-sensitized female rats. Hormones and behavior 82:56-63.

Mamo D, Kapur S, Keshavan M, Laruelle M, Taylor CC, Kothare PA, Barsoum P, McDonnell D (2008) D2 receptor occupancy of olanzapine pamoate depot using positron emission tomography: an open-label study in patients with schizophrenia. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33:298-304.

Marin C, Chase TN (1993) Dopamine D1 receptor stimulation but not dopamine D2 receptor stimulation attenuates haloperidol-induced behavioral supersensitivity and receptor up-regulation. European journal of pharmacology 231:191-196.

McCoy L, Cox C, Richfield EK (1996) Chronic treatment with typical and atypical antipsychotics increases the AMPA-preferring form of AMPA receptor in rat brain. European journal of pharmacology 318:41-45.

McCreadie RG, Dingwall JM, Wiles DH, Heykants JJ (1980) Intermittent pimozide versus fluphenazine decanoate as maintenance therapy in chronic schizophrenia. The British journal of psychiatry : the journal of mental science 137:510-517.

Meltzer HY, Matsubara S, Lee JC (1989) The ratios of serotonin2 and dopamine2 affinities differentiate atypical and typical antipsychotic drugs. Psychopharmacol Bull 25:390-392.

Meng ZH, Feldpaush DL, Merchant KM (1998) Clozapine and haloperidol block the induction of behavioral sensitization to amphetamine and associated genomic responses in rats. Brain research Molecular brain research 61:39-50.

Merchant KM, Dobner PR, Dorsa DM (1992) Differential effects of haloperidol and clozapine on neurotensin gene transcription in rat neostriatum. The Journal of neuroscience : the official journal of the Society for Neuroscience 12:652-663.

Merchant KM, Dobie DJ, Filloux FM, Totzke M, Aravagiri M, Dorsa DM (1994) Effects of chronic haloperidol and clozapine treatment on neurotensin and c-fos mRNA in rat neostriatal subregions. The Journal of pharmacology and experimental therapeutics 271:460-471.

Meshul CK, Bunker GL, Mason JN, Allen C, Janowsky A (1996) Effects of subchronic clozapine and haloperidol on striatal glutamatergic synapses. Journal of neurochemistry 67:1965-1973.

MØller Nielsen I, Fjalland B, Pedersen V, Nymark M (1974) Pharmacology of neuroleptics upon repeated administration. Psychopharmacology 34:95-104.

Moncrieff J (2006) Does antipsychotic withdrawal provoke psychosis? Review of the literature on rapid onset psychosis (supersensitivity psychosis) and withdrawal-related relapse. Acta psychiatrica Scandinavica 114:3-13.

Montanaro N, Dall'Olio R, Gandolfi O, Vaccheri A (1981) Behavioral supersensitivity to methamphetamine following chronic treatment with (--)-sulpiride in the rat. Bollettino della Societa italiana di biologia sperimentale 57:401-406.

Montanaro N, Dall'Olio R, Gandolfi O, Vaccheri A (1982) Differential enhancement of behavioral sensitivity to apomorphine following chronic treatment of rats with (-)-sulpiride and haloperidol. European journal of pharmacology 81:1-9.

Muench J, Hamer AM (2010) Adverse effects of antipsychotic medications. American family physician 81:617-622.

Murray RM, Quattrone D, Natesan S, van Os J, Nordentoft M, Howes O, Di Forti M, Taylor D (2016) Should psychiatrists be more cautious about the long-term prophylactic use of

antipsychotics? The British journal of psychiatry : the journal of mental science 209:361-365.

Myers B, Levant B, Bissette G, Nemeroff CB (1992) Pharmacological specificity of the increase in neurotensin concentrations after antipsychotic drug treatment. Brain research 575:325-328.

Natesan S, Reckless GE, Nobrega JN, Fletcher PJ, Kapur S (2006) Dissociation between in vivo occupancy and functional antagonism of dopamine D2 receptors: comparing aripiprazole to other antipsychotics in animal models. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 31:1854-1863.

Nyberg S, Farde L, Halldin C, Dahl ML, Bertilsson L (1995) D2 dopamine receptor occupancy during low-dose treatment with haloperidol decanoate. The American journal of psychiatry 152:173-178.

O'Dell SJ, La Hoste GJ, Widmark CB, Shapiro RM, Potkin SG, Marshall JF (1990) Chronic treatment with clozapine or haloperidol differentially regulates dopamine and serotonin receptors in rat brain. Synapse 6:146-153.

Obuchowicz E (1999) Differential behavioural effect of quinpirole in neuroleptic-pretreated rats - role of alpha(1)-adrenoceptor. European journal of pharmacology 384:101-109.

Oda Y, Kanahara N, Kimura H, Watanabe H, Hashimoto K, Iyo M (2015a) Genetic association between G protein-coupled receptor kinase 6/beta-arrestin 2 and dopamine supersensitivity psychosis in schizophrenia. Neuropsychiatric disease and treatment 11:1845-1851.

Oda Y, Tadokoro S, Takase M, Kanahara N, Watanabe H, Shirayama Y, Hashimoto K, Iyo M (2015b) G protein-coupled receptor kinase 6/beta-arrestin 2 system in a rat model of dopamine supersensitivity psychosis. Journal of psychopharmacology 29:1308-1313.

Oda Y, Fujita Y, Oishi K, Nakata Y, Takase M, Niitsu T, Kanahara N, Shirayama Y, Hashimoto K, Iyo M (2017) Alterations in glutamatergic signaling in the brain of dopamine supersensitivity psychosis and non-supersensitivity psychosis model rats. Psychopharmacology 234:3027-3036.

Olney JW, Farber NB (1995) Glutamate receptor dysfunction and schizophrenia. Archives of general psychiatry 52:998-1007.

Ossowska K (1995) The subsensitivity of striatal glutamate receptors induced by chronic haloperidol in rats. European journal of pharmacology 294:685-691.

Peuskens J (1996) Proper psychosocial rehabilitation for stabilised patients with schizophrenia: the role of new therapies. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 6 Suppl 2:S7-12.

Pierce RC, Rowlett JK, Bardo MT, Rebec GV (1991) Chronic ascorbate potentiates the effects of chronic haloperidol on behavioral supersensitivity but not D2 dopamine receptor binding. Neuroscience 45:373-378.

Porras G, Di Matteo V, Fracasso C, Lucas G, De Deurwaerdere P, Caccia S, Esposito E, Spampinato U (2002) 5-HT2A and 5-HT2C/2B receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 26:311-324.

Pudiak CM, Bozarth MA (1997) Nitric oxide synthesis inhibition attenuates haloperidol-induced supersensitivity. Journal of psychiatry & neuroscience : JPN 22:61-64.

Rebec GV, Peirson EE, McPherson FA, Brugge K (1982) Differential sensitivity to amphetamine following long-term treatment with clozapine or haloperidol. Psychopharmacology 77:360-366.

Remington G, Seeman P, Shammi C, Mann S, Kapur S (2005) "Extended" antipsychotic dosing: rationale and pilot data. Journal of clinical psychopharmacology 25:611-613.

Remington G, Seeman P, Feingold A, Mann S, Shammi C, Kapur S (2011) "Extended" antipsychotic dosing in the maintenance treatment of schizophrenia: a double-blind, placebo-controlled trial. The Journal of clinical psychiatry 72:1042-1048.

Remington G, Mamo D, Labelle A, Reiss J, Shammi C, Mannaert E, Mann S, Kapur S (2006) A PET study evaluating dopamine D2 receptor occupancy for long-acting injectable risperidone. The American journal of psychiatry 163:396-401.

Richtand NM, Welge JA, Logue AD, Keck PE, Jr., Strakowski SM, McNamara RK (2007) Dopamine and serotonin receptor binding and antipsychotic efficacy. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 32:1715-1726.

Roberts DC, Vickers G (1987) The effect of haloperidol on cocaine self-administration is augmented with repeated administrations. Psychopharmacology 93:526-528.

Robledo P, Maldonado R, Koob GF (1993) Neurotensin injected into the nucleus accumbens blocks the psychostimulant effects of cocaine but does not attenuate cocaine self-administration in the rat. Brain research 622:105-112.

Rodriguez JJ, Pickel VM (1999) Enhancement of N-methyl-D-aspartate (NMDA) immunoreactivity in residual dendritic spines in the caudate-putamen nucleus after chronic haloperidol administration. Synapse 33:289-303.

Roy-Desruisseaux J, Landry J, Bocti C, Tessier D, Hottin P, Trudel JF (2011) Domperidone-induced tardive dyskinesia and withdrawal psychosis in an elderly woman with dementia. Ann Pharmacother 45:e51.

Samaha AN (2014) Can antipsychotic treatment contribute to drug addiction in schizophrenia? Progress in neuro-psychopharmacology & biological psychiatry 52:9-16.

Samaha AN, Seeman P, Stewart J, Rajabi H, Kapur S (2007) "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:2979-2986.

Samaha AN, Reckless GE, Seeman P, Diwan M, Nobrega JN, Kapur S (2008) Less is more: antipsychotic drug effects are greater with transient rather than continuous delivery. Biological psychiatry 64:145-152.

Scarr E, Parkin FM, Pavey G, Dean B (2002) Decreased density of [3H]TCP binding following antipsychotic drug withdrawal in rats. Life sciences 70:2699-2705.

Schneier FR, Siris SG (1987) A review of psychoactive substance use and abuse in schizophrenia: Patterns of drug choice. Journal of Nervous and Mental Disease 175:641-652.

Schooler NR (1991) Maintenance medication for schizophrenia: strategies for dose reduction. Schizophrenia bulletin 17:311-324.

See RE, Murray CE (1992) Changes in striatal dopamine release and metabolism during and after subchronic haloperidol administration in rats. Neuroscience letters 142:100-104.

See RE, Lynch AM, Aravagiri M, Nemeroff CB, Owens MJ (1995) Chronic haloperidol-induced changes in regional dopamine release and metabolism and neurotensin content in rats. Brain research 704:202-209.

Seeman P (2008a) Dopamine D2(High) receptors moderately elevated by sertindole. Synapse 62:389-393.

Seeman P (2008b) Dopamine D2(High) receptors moderately elevated by bifeprunox and aripiprazole. Synapse 62:902-908.

Seeman P (2014) Yes, Breast is best but taper domperidone when stopping (e-letter). Br J Gen Pract.

Seeman P, Corbett R, Van Tol HH (1997) Atypical neuroleptics have low affinity for dopamine D2 receptors or are selective for D4 receptors. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 16:93-110; discussion 111-135.

Seeman P, Lee T, Chau-Wong M, Wong K (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 261:717-719.

Seeman P, Weinshenker D, Quirion R, Srivastava LK, Bhardwaj SK, Grandy DK, Premont RT, Sotnikova TD, Boksa P, El-Ghundi M, O'Dowd B F, George SR, Perreault ML, Mannisto PT, Robinson S, Palmiter RD, Tallerico T (2005) Dopamine supersensitivity correlates with

D2High states, implying many paths to psychosis. Proceedings of the National Academy of Sciences of the United States of America 102:3513-3518.

Servonnet A, Minogianis EA, Bouchard C, Bedard AM, Levesque D, Rompre PP, Samaha AN (2017) Neurotensin in the nucleus accumbens reverses dopamine supersensitivity evoked by antipsychotic treatment. Neuropharmacology 123:10-21.

Severson JA, Robinson HE, Simpson GM (1984) Neuroleptic-induced striatal dopamine receptor supersensitivity in mice: relationship to dose and drug. Psychopharmacology 84:115-119.

Silvestri S, Seeman MV, Negrete JC, Houle S, Shammi CM, Remington GJ, Kapur S, Zipursky RB, Wilson AA, Christensen BK, Seeman P (2000) Increased dopamine D2 receptor binding after long-term treatment with antipsychotics in humans: a clinical PET study. Psychopharmacology 152:174-180.

Smelson DA, Losonczy MF, Davis CW, Kaune M, Williams J, Ziedonis D (2002) Risperidone decreases craving and relapses in individuals with schizophrenia and cocaine dependence. Canadian journal of psychiatry Revue canadienne de psychiatrie 47:671-675.

Smelson DA, Ziedonis D, Williams J, Losonczy MF, Williams J, Steinberg ML, Kaune M (2006) The efficacy of olanzapine for decreasing cue-elicited craving in individuals with schizophrenia and cocaine dependence: a preliminary report. Journal of clinical psychopharmacology 26:9-12.

Smith RC, Davis JM (1975) Behavioral supersensitivity to apomorphine and amphetamine after chronic high dose haloperidol treatment. Psychopharmacology communications 1:285-293.

Steward LJ, Kennedy MD, Morris BJ, Pratt JA (2004) The atypical antipsychotic drug clozapine enhances chronic PCP-induced regulation of prefrontal cortex 5-HT2A receptors. Neuropharmacology 47:527-537.

Suzuki T, Kanahara N, Yamanaka H, Takase M, Kimura H, Watanabe H, Iyo M (2015) Dopamine supersensitivity psychosis as a pivotal factor in treatment-resistant schizophrenia. Psychiatry research 227:278-282.

Tadokoro S, Okamura N, Sekine Y, Kanahara N, Hashimoto K, Iyo M (2012) Chronic treatment with aripiprazole prevents development of dopamine supersensitivity and potentially supersensitivity psychosis. Schizophrenia bulletin 38:1012-1020.

Taylor M, Cavanagh J, Hodgson R, Tiihonen J (2012) Examining the effectiveness of antipsychotic medication in first-episode psychosis. Journal of psychopharmacology 26:27-32.

Tenback DE, van Harten PN, Slooff CJ, van Os J, Group SS (2007) Worsening of psychosis in schizophrenia is longitudinally associated with tardive dyskinesia in the European Schizophrenia Outpatient Health Outcomes study. Compr Psychiatry 48:436-440.

Thibault D, Albert PR, Pineyro G, Trudeau LE (2011) Neurotensin triggers dopamine D2 receptor desensitization through a protein kinase C and beta-arrestin1-dependent mechanism. The Journal of biological chemistry 286:9174-9184.

Tsuboi T, Suzuki T, Bies RR, Remington G, Pollock BG, Mimura M, Uchida H (2015) Challenging the need for sustained blockade of dopamine D(2) receptor estimated from antipsychotic plasma levels in the maintenance treatment of schizophrenia: A single-blind, randomized, controlled study. Schizophrenia research 164:149-154.

Turrone P, Remington G, Kapur S, Nobrega JN (2003a) The relationship between dopamine D2 receptor occupancy and the vacuous chewing movement syndrome in rats. Psychopharmacology 165:166-171.

Turrone P, Remington G, Kapur S, Nobrega JN (2003b) Differential effects of within-day continuous vs. transient dopamine D2 receptor occupancy in the development of vacuous chewing movements (VCMs) in rats. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 28:1433-1439.

Turrone P, Remington G, Kapur S, Nobrega JN (2005) Continuous but not intermittent olanzapine infusion induces vacuous chewing movements in rats. Biological psychiatry 57:406-411.

Uchida H, Suzuki T (2014) Dose and dosing frequency of long-acting injectable antipsychotics: a systematic review of PET and SPECT data and clinical implications. Journal of clinical psychopharmacology 34:728-735.

Uchida H, Suzuki T, Takeuchi H, Arenovich T, Mamo DC (2011) Low dose vs standard dose of antipsychotics for relapse prevention in schizophrenia: meta-analysis. Schizophrenia bulletin 37:788-799.

Veldhuizen S, Urbanoski K, Cairney J (2007) Geographical variation in the prevalence of problematic substance use in Canada. Canadian journal of psychiatry Revue canadienne de psychiatrie 52:426-433.

von Euler G, Mailleux P, Vanderhaeghen JJ, Fuxe K (1990) Neurotensin reduces the affinity of dopamine D2 receptors in membranes from post mortem human caudate-putamen. Neuroscience letters 109:325-330.

Vonvoigtlander PF, Losey EG, Triezenberg HJ (1975) Increased sensitivity to dopaminergic agents after chronic neuroleptic treatment. The Journal of pharmacology and experimental therapeutics 193:88-94.

Wadenberg ML, Hicks PB (1999) The conditioned avoidance response test re-evaluated: is it a sensitive test for the detection of potentially atypical antipsychotics? Neuroscience and biobehavioral reviews 23:851-862.

Wadenberg ML, Soliman A, VanderSpek SC, Kapur S (2001) Dopamine D(2) receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical

effects. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 25:633-641.

Wadenberg ML, Kapur S, Soliman A, Jones C, Vaccarino F (2000) Dopamine D2 receptor occupancy predicts catalepsy and the suppression of conditioned avoidance response behavior in rats. Psychopharmacology 150:422-429.

Waln O, Jankovic J (2013) An update on tardive dyskinesia: from phenomenology to treatment. Tremor Other Hyperkinet Mov (N Y) 3.

Wilmot CA, Szczepanik AM (1989) Effects of acute and chronic treatments with clozapine and haloperidol on serotonin (5-HT2) and dopamine (D2) receptors in the rat brain. Brain research 487:288-298.

Woerner MG, Kane JM, Lieberman JA, Alvir J, Bergmann KJ, Borenstein M, Schooler NR, Mukherjee S, Rotrosen J, Rubinstein M, et al. (1991) The prevalence of tardive dyskinesia. Journal of clinical psychopharmacology 11:34-42.

Yan Q, Reith ME, Yan S (2000) Enhanced accumbal dopamine release following 5-HT(2A) receptor stimulation in rats pretreated with intermittent cocaine. Brain research 863:254-258.

Yoshida K, Bies RR, Suzuki T, Remington G, Pollock BG, Mizuno Y, Mimura M, Uchida H (2014) Tardive dyskinesia in relation to estimated dopamine D2 receptor occupancy in patients with schizophrenia: analysis of the CATIE data. Schizophrenia research 153:184-188.

2ND REVIEW: Continuous versus extended antipsychotic dosing in schizophrenia: Less is more

Review published in Behavioural Brain Research
DOI: 10.1016/j.bbr.2020.113076

Alice Servonnet[a], Hiroyuki Uchida[b] and Anne-Noël Samaha[c,d]

[a]Department of Neurosciences, Faculty of Medicine, Université de Montréal, Montreal, Canada.

[b]Department of Neuropsychiatry, Keio University School of Medicine, Tokyo, Japan.

[c]Department of Pharmacology and Physiology, Faculty of Medicine, Université de Montréal, Montreal, Canada.

[d]Groupe de recherche sur le système nerveux central, Faculty of Medicine, Université de Montréal, Montreal, Canada.

Declarations of interest: HU has received grants from Eisai, Otsuka Pharmaceutical, ainippon-Sumitomo Pharma, and Meiji-Seika Pharmaceutical; speaker's honoraria from Otsuka Pharmaceutical, Dainippon-Sumitomo Pharma, Eisai, and Meiji-Seika Pharma; and advisory panel payments from Dainippon-Sumitomo Pharma within the past three years.

ABSTRACT

Antipsychotic drugs temper psychotic symptoms by interacting with dopamine D2 receptors to reduce dopamine neurotransmission. Currently, the standard of care involves antipsychotic treatment protocols that achieve steady-state levels of medication. Maintaining patients on continuous treatment is thought to be necessary to keep them stabilised. However, continuous antipsychotic exposure increases the risk of adverse effects over time. These effects include metabolic and cardiovascular disorders, extrapyramidal complications, and dopamine receptor supersensitivity, the latter of which could potentially promote both treatment tolerance and psychosis relapse. In the present review, we describe evidence showing that continuous exposure to antipsychotic drugs can not only worsen long-term outcome, but—past acute phase treatment—it is also *unnecessary* to effectively manage schizophrenia symptoms. We also describe evidence that regular but extended dosing, allowing predictable periods of lower antipsychotic levels/D2 occupancy, is both safe and effective in patients, and it greatly reduces drug exposure overall. Studies in laboratory animals show that compared to continuous antipsychotic exposure, regular but extended dosing actually has superior antipsychotic-like efficacy, and it also substantially reduces the likelihood of both motor side effects and dopamine receptor supersensitivity. We propose that regular, but extended dosing should be considered in the long-term treatment of people with schizophrenia, because the available evidence suggests it can be just as effective as continuous treatment, while decreasing overall drug exposure and potentially reducing harmful side effects.

KEY WORDS

Antipsychotic drugs; Extended dosing; Continuous dosing; Schizophrenia

ABBREVIATIONS

LAI, long-acting injectable; PET, positron emission tomography; CATIE, Clinical Antipsychotic Trials in Intervention Effectiveness.

1. Introduction

A dogma in the treatment of schizophrenia is that maintaining steady-state levels of antipsychotic drug is necessary to effectively manage symptoms. Here we review literature that challenges this dogma. The standard of care for people with schizophrenia involves antipsychotic treatment regimens that achieve sustained antipsychotic levels, at doses typically producing greater than 65 % occupancy of striatal D2 receptors. This is effective in treating acute psychosis and in preventing relapse. However, maintaining continuous medication can do more harm than good, because it increases the incidence of deleterious effects (Whitaker, 2004). Unwanted effects of antipsychotic medication include metabolic and cardiac complications, extrapyramidal disruptions, and dopamine receptor supersensitivity, the latter of which can both reduce antipsychotic efficacy and worsen psychosis (Chouinard *et al.*, 1978; Muench and Hamer, 2010; Murray *et al.*, 2016). This greatly impairs the health and quality of life of schizophrenia patients. We will highlight here recent studies in humans and laboratory animals that confront the idea that continuous exposure to antipsychotic drugs is necessary to maintain antipsychotic efficacy. These studies have compared antipsychotic treatment regimens that achieve sustained brain levels, to regimens achieving regular but extended antipsychotic dosing. Regular, extended dosing is achieved by increasing the time interval between doses. Importantly, medication is still taken at consistent and predictable intervals, achieving regular peaks and troughs in medication levels, but at intervals short enough so that schizophrenia symptoms do not worsen (*e.g.*, taking oral antipsychotic drug every other day instead of everyday). As reviewed previously by Remington *et al.* (Remington *et al.*, 2014), the evidence shows that regular but extended antipsychotic intake can be a safe and effective approach to treat schizophrenia patients. Extended dosing has also been examined in laboratory rats by giving antipsychotic medications via daily systemic injection, to produce regular peaks and troughs in medication levels/D2 receptor occupancy. As we will highlight in Section 4, preclinical studies in rodents show that regular, extended dosing is actually more efficacious behaviourally than continuous dosing, while reducing the risk of side effects. Thus, we lay out below that the temporal kinetics of treatment (*i.e.*, continuous versus regular but extended antipsychotic exposure) are decisive in predicting outcome. The frequency with which drug concentrations reach threshold levels throughout the course of treatment determines both treatment efficacy and the risk of unwanted side effects. We conclude then, that the temporal kinetics of treatment should be

considered in the clinic, and that extended rather than continuous antipsychotic dosing could increase efficacy and potentially reduce side effects.

2. Maintaining continuous antipsychotic drug levels: more harm than good

Antipsychotic drugs exert their antipsychotic effect by reducing D2 receptor-mediated signalling. Evidence obtained from brain positron emission tomography (PET) studies demonstrates that blockade of greater than 65 % of striatal dopamine D2 receptors with antipsychotic drugs increases the likelihood of a therapeutic response in the acute phase of schizophrenia treatment (Fig. A2.1) (Farde *et al.*, 1992; Kapur *et al.*, 2000b; Uchida *et al.*, 2011). This notion has led to the conventional dosing strategy, which advocates using orally-administered or long-acting injectable (LAI) antipsychotics in dosing regimens that produce steady-state delivery of antipsychotic drugs to the brain and continuous blockade of over 65 % of dopamine D2 receptors (Figs. A2.2A-B). Antipsychotic drugs are also associated with many dose-dependent side effects, including extrapyramidal side effects (Uchida *et al.*, 2011), sudden cardiac death (Ray *et al.*, 2009; Schneeweiss and Avorn, 2009), and cognitive impairments (Sakurai *et al.*, 2012). Among these side effects, extrapyramidal side effects and cognitive impairments occur more likely once dopamine D2 receptor blockade exceeds approximately 80 % (Fig. A2.1) (Uchida *et al.*, 2011; Sakurai *et al.*, 2012). Based on these findings, the therapeutic window of 65-80 % D2 occupancy has been proposed for younger adults, and it is also used to determine dose and dosing frequency of new antipsychotic drugs.

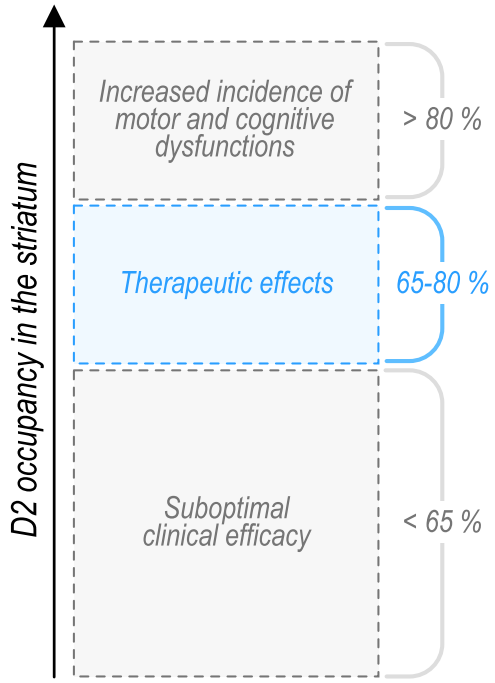

FIG. A2.1 — The relationship between dopamine D2 receptor occupancy in the striatum by antipsychotic medication and clinical outcome. In the acute phase of schizophrenia treatment, most antipsychotics show therapeutic efficacy without an increased risk of extrapyramidal effects and cognitive deficits at doses that occupy ~65 to 80 % of striatal D2 receptors. Clozapine and quetiapine are notable exceptions to this (see Section 3.1 in text).

However, this therapeutic window of D2 occupancy is based on data from patients who were receiving acute phase treatment. Recent work shows that lower levels of antipsychotic-induced D2 receptor occupancy are effective during the maintenance phase of antipsychotic treatment (Uchida *et al.*, 2008; Ikai *et al.*, 2012; Mizuno *et al.*, 2012; Moriguchi *et al.*, 2013). Thus, therapeutic levels of D2 occupancy during the different stages of psychosis and schizophrenia must still be refined, as continuous antipsychotic treatment might not be necessary or even desirable in the maintenance phase of treatment.

2.1 Continuous antipsychotic treatment and dopamine receptor supersensitivity in schizophrenia

Conventional antipsychotic dosing strategies, which achieve steady-state antipsychotic levels, can also produce dopamine receptor supersensitivity in patients (Chouinard *et al.*, 1978). This is indicated by a rapid relapse to psychosis upon drug discontinuation or dose reduction, emergence of new or more severe symptoms of psychosis, tolerance to previously observed therapeutic effects and drug-induced movement disorders (Chouinard *et al.*, 1978; Chouinard, 2004; Fallon and Dursun, 2011; Chouinard *et al.*, 2017; Servonnet and Samaha, 2020). Thus, dopamine supersensitivity can be detected through the presence of tardive dyskinesia (Miller and Chouinard, 1993) and supersensitivity psychosis (Chouinard *et al.*, 1978; Chouinard *et al.*, 1982). In line with this idea, a recent meta-analysis covering a total cohort of 5130 individuals found tardive dyskinesia to be the strongest predictor of psychosis relapse in patients with confirmed adherence to antipsychotic treatment (*i.e.*, patients given LAI antipsychotics) (Rubio *et al.*, 2020). The prevalence rate of dopamine supersensitivity psychosis is estimated to be 30 % among schizophrenia patients, and even higher (70 %) in treatment-resistant patients (Kimura *et al.*, 2014; Suzuki *et al.*, 2015; Takase *et al.*, 2015). However, the nature of the relationship between antipsychotic-induced dopamine supersensitivity psychosis and treatment resistance is not yet clear, because treatment resistance is commonly observed from illness onset, before long-term antipsychotic exposure (Demjaha *et al.*, 2017). Using the presence of tardive dyskinesia to detect dopamine receptor supersensitivity, it is reported that supersensitivity psychosis could contribute to relapse in 30-40% of patients with schizophrenia (Fallon and Dursun, 2011; Fallon *et al.*, 2012). Some studies report no evidence of dopamine supersensitivity in schizophrenia patients withdrawn from antipsychotic treatment (Emsley *et al.*, 2018). However, as the authors of that study noted

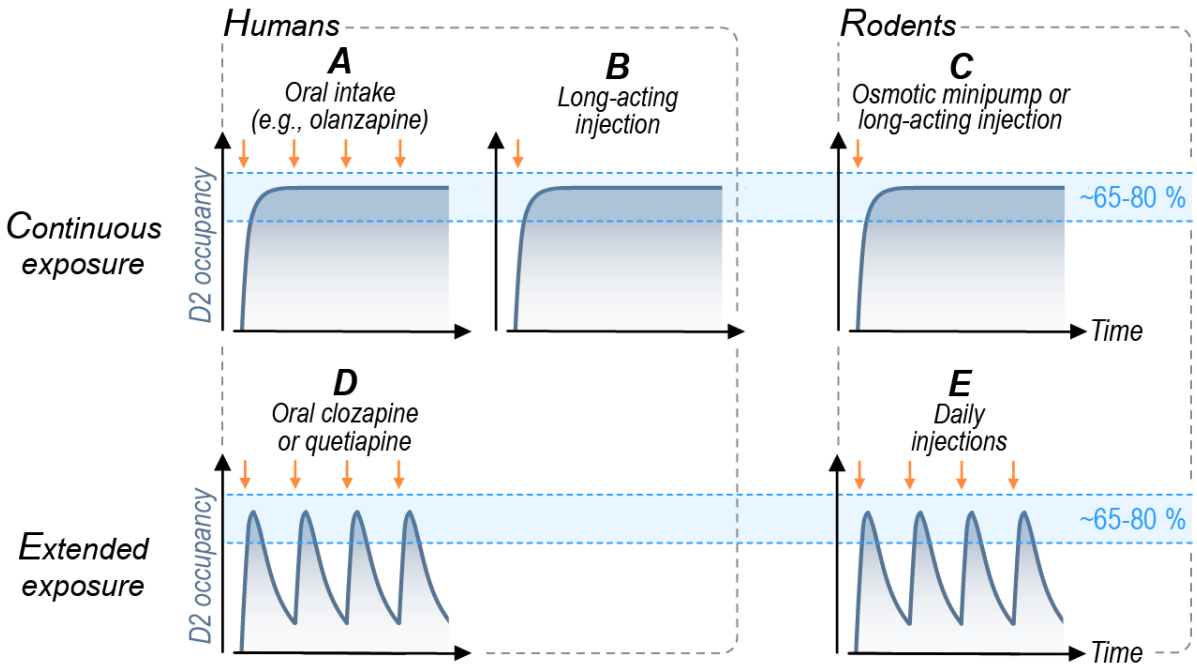

FIG. A2.2 — The relationship between antipsychotic treatment regimen and the temporal kinetics of striatal dopamine D2 receptor occupancy in humans and laboratory rats. The therapeutic range of 65-80 % is shaded in blue. In the clinic, standard treatment regimens for schizophrenia involve (***A***) daily oral intake of antipsychotic drugs or (***B***) administration of long-acting injectable antipsychotics. These dosing strategies typically achieve continuous levels of striatal D2 occupancy, above 65 %. (***C***) In rats, administration of antipsychotic drugs via a subcutaneously implanted osmotic minipump, or via intra-muscular administration of a long-acting injectable antipsychotic also produces continuous striatal D2 receptor occupancy above 65 %. (***D***) Because of their fast pharmacokinetic profile, regular oral intake of clozapine or quetiapine does not achieve continuous levels of D2 occupancy above 65 %, instead producing peaks and troughs in D2 occupancy, akin to a regular, but extended dosing protocol. (***E***) When rats receive antipsychotic drugs via regular, daily injection, this achieves transient D2 receptor occupancy > 65 %. In both ***C*** and ***E***, doses can be used that achieve clinically-relevant levels of D2 occupancy (i.e., 65-80 %).

'(…) *because patients were treated with a LAI antipsychotic, withdrawal from antipsychotic treatment was gradual, potentially protecting patients from developing withdrawal or discontinuation symptoms*.' [p. e6; (Emsley *et al.*, 2018)]. Moreover, even people with no psychiatric diagnoses can develop both tardive dyskinesia and withdrawal psychosis when given chronic treatment with dopamine D2 receptor antagonists (Lu *et al.*, 2002; Roy-Desruisseaux *et al.*, 2011; Seeman, 2014). Thus, the available evidence supports the notion that sustained antipsychotic treatment can cause dopaminergic dysfunction and that this can manifest as tardive dyskinesia and worsening of psychosis.

2.2 Continuous antipsychotic exposure promotes treatment failure, motor side effects and dopamine receptor supersensitivity in rats

In rats, continuous antipsychotic treatment produces aversive effects that are similar to those that can be seen in antipsychotic-treated schizophrenia patients. As illustrated in Fig. A2.2C, some treatment regimens used in preclinical animal studies can mimic the continuous levels of antipsychotic exposure typical in schizophrenia patients. These regimens include administration of a LAI antipsychotic (Turrone *et al.*, 2003b) or administration of antipsychotic drug via a surgically implanted, subcutaneous osmotic minipump (Kapur *et al.*, 2003). Just like in humans, continuous antipsychotic treatment produces tolerance to antipsychotic-like effects in rodents. Antipsychotic-like effects are commonly evaluated in rats using two well validated tests: suppression of conditioned avoidance responding (Wadenberg and Hicks, 1999) and of the psychomotor activating effects of a dopamine agonist (Ljungberg and Ungerstedt, 1985). Antipsychotic medications reliably disrupt conditioned avoidance responding (Wadenberg *et al.*, 2001). While it is not clear how conditioned avoidance of an aversive stimulus in rats relates to psychosis in humans (Li *et al.*, 2007), the conditioned avoidance responding model shows high predictive validity for antipsychotic activity (Wadenberg and Hicks, 1999). As regards disruption of the psychomotor response to dopamine agonists, compounds other than antipsychotic drugs can also have this effect. Nonetheless, suppression of the psychomotor response to a dopamine agonist is a reliable and often used test to probe the antidopaminergic effects of antipsychotic drugs.

In rats receiving continuous antipsychotic treatment, the antipsychotic initially suppresses both conditioned avoidance responding and the psychomotor effects of dopamine agonists, but it loses efficacy later in treatment, even though striatal D2 occupancy remains above 65 % (Samaha *et al.*, 2007; Samaha *et al.*, 2008; Amato *et al.*, 2018). Also, just like in humans, continuous antipsychotic treatment in rats produces signs related to tardive dyskinesia (*i.e.* vacuous chewing movements) (Turrone *et al.*, 2003b, a). Rats receiving continuous antipsychotic treatment also develop a dopamine supersensitive state, as can occur in some patients with schizophrenia (Chouinard *et al.*, 1978; Chouinard and Jones, 1980; Fallon and Dursun, 2011; Kimura *et al.*, 2014; Suzuki *et al.*, 2015; Chouinard *et al.*, 2017). Dopamine supersensitivity in rats manifests among others as an exaggerated response to dopamine receptor stimulation. This exaggerated response is the most commonly used index of antipsychotic-induced dopamine supersensitivity in laboratory rodents.

In rats, antipsychotic-induced dopamine supersensitivity produces robust sensitization to the psychomotor-activating effects of dopamine agonists, but also to their reward-enhancing effects (Gianutsos *et al.*, 1974; Sayers *et al.*, 1975; Vonvoigtlander *et al.*, 1975; Smith and Davis, 1976; Ericson *et al.*, 1996; Samaha *et al.*, 2007; Samaha *et al.*, 2008; Bedard *et al.*, 2011; Gill *et al.*, 2014). Antipsychotic-evoked dopamine supersensitivity is often studied in otherwise neurologically intact animals, but it can also be seen in well-established animal models of schizophrenia symptoms (Gill *et al.*, 2014). Antipsychotic-evoked dopamine supersensitivity is also reported to render rats unresponsive to novel agents that otherwise restore electrophysiological and behavioural abnormalities in a rat model of schizophrenia-like symptoms (Gill *et al.*, 2014). The implication is that this could explain at least in part why new compounds fail in clinical trials involving antipsychotic-experienced schizophrenia patients (Gill *et al.*, 2014). Studies in rats also show that once dopamine supersensitivity has developed, it can 'break through' ongoing antipsychotic treatment (Samaha *et al.*, 2007; Samaha *et al.*, 2008; Amato *et al.*, 2018), and this is potentially linked to both functional tolerance to antipsychotics (Samaha *et al.*, 2007; Samaha *et al.*, 2008) and the emergence of vacuous chewing movements (Turrone *et al.*, 2003b, a).

3. Extended dosing in schizophrenia

Regular antipsychotic dosing remains the standard of care in the clinic, because this produces continuous levels of dopamine D2 receptor blockade that are thought to maximize therapeutic response in the acute treatment phase. However, different dosing schedules have been tested to reduce dose-dependent antipsychotic side effects as well as medication cost in the maintenance phase. An initial strategy involved targeted antipsychotic dosing, where antipsychotic treatment is resumed at the earliest signs of psychotic relapse following discontinuation. This dosing method has not been a success, because it increases the risk of relapse and rehospitalization compared to regular, continuous dosing (Jolley *et al.*, 1990; Schooler, 1991; Jolley and Hirsch, 1993; Gaebel, 1994; Gaebel *et al.*, 2002). Indeed, it is extremely difficult to effectively detect prodromal symptoms or the early signs of relapse (Remington and Kapur, 2010; Saito *et al.*, 2020). However, recent findings suggest that the long-term outcomes of targeted treatment might warrant further exploration. Wunderink *et al.* (2007) completed an initial study comparing targeted versus maintenance treatment for 18 months, followed by a second study assessing outcome 5 years later

(Wunderink *et al.*, 2013). In the initial study, targeted treatment was associated with a greater risk of relapse than maintenance treatment (Wunderink *et al.*, 2007), in accordance with previous reports (Jolley *et al.*, 1990; Schooler, 1991; Jolley and Hirsch, 1993; Gaebel, 1994; Gaebel *et al.*, 2002). However, in the 5-year follow-up period (during which the initial treatment strategy was not necessarily maintained in each individual), patients in the initial targeted treatment group were taking lower doses of antipsychotic medications on average, and they also showed better social functioning over time relative to patients in the initial maintenance treatment group, while symptomatic remission did not differ between groups (Wunderink *et al.*, 2013). Because patients were not required to stay in their initial treatment group beyond the first 18 months, the findings suggest that perhaps some patients might benefit from targeted treatment on the long term (Wunderink *et al.*, 2013).

Persons with schizophrenia can have a low medication compliance rate, regularly skipping medications. However, this is not the same as the extended, but regular dosing strategies discussed here. A key difference being that with such strategies, dosing occurs at predictable intervals, and these intervals are short (*e.g.*, taking medication every other day, instead of every day). In contrast, medication non-adherence can mean that patients remain off medication for periods too long to effectively manage symptoms. Another issue concerns the percentage of patients with schizophrenia that are receiving continuous antipsychotic medication. A recent meta-analysis suggests that ~70 % of schizophrenia patients comply with their treatment (Yaegashi *et al.*, 2020). This suggests that many patients are receiving continuous antipsychotic exposure.

Another approach to reduce overall drug exposure is extended, but regular antipsychotic dosing, in which antipsychotic drugs are given regularly but with longer intervals than usual (*e.g.*, every 2 days for oral antipsychotic drugs instead of everyday). Thereby, unlike the targeted approach, extended dosing involves regular, predictable periods of low drug exposure (*i.e.*, < 65 % of D2 occupancy) *but not drug-free periods per se*. Below we lay out the evidence that extended dosing schedules producing regular but transient D2 occupancy above 65 % are as effective as conventional, continuous dosing schedules in preventing relapse.

3.1 Continuous D2 receptor blockade is not necessary to maintain clinical efficacy

Atypical antipsychotic drugs generally dissociate more rapidly from D2 receptors [*i.e.*, have a faster k_{off}) than typicals do (Seeman, 2002) [but see (Sahlholm *et al.*, 2016)], but atypicals are still efficacious in treating schizophrenia symptoms. This has led to the hypothesis that antipsychotic efficacy could be achieved even with extended antipsychotic dosing. Interestingly, the fast k_{off} property of atypicals could explain why they are generally less likely than typical antipsychotic drugs to produce dopamine-dependent side effects. For instance, compared to typical antipsychotics, clozapine is unlikely to produce tardive dyskinesia and can in fact improve tardive dyskinesia caused by other antipsychotic agents (Pardis *et al.*, 2019). Other atypical antipsychotic drugs—including the D2 antagonists risperidone, olanzapine and quetiapine and the D2 partial agonist aripiprazole—are also thought to be less likely to produce tardive dyskinesia when compared to typicals (Dolder and Jeste, 2003; Correll *et al.*, 2004; Miller *et al.*, 2007; Carbon *et al.*, 2017). Furthermore, preclinical studies demonstrate that dopamine supersensitivity is less pronounced and shorter lasting with atypical versus typical antipsychotic drugs (Samaha *et al.*, 2007; Tadokoro *et al.*, 2012; Bedard *et al.*, 2013; Amato *et al.*, 2018). A faster k_{off} might reduce the risk of dopamine-related side effects by allowing a greater degree of endogenous dopamine neurotransmission via D2 receptors, despite presence of the antipsychotic.

Antipsychotic medications with a fast pharmacokinetic profile have also been informative in thinking about extended dosing. Indeed, clozapine and quetiapine not only have some of the fastest dissociation rates from D2 receptors amongst all antipsychotics (Seeman, 2002), they also show a fast pharmacokinetic profile (*i.e.*, rapid absorption and rapid elimination half-life [Jann *et al.*, 1993; Goren and Levin, 1998]), leading to peaks in D2 occupancy which then decline within hours after antipsychotic administration (Fig. A2.2D). Despite this more transient action, clozapine and quetiapine are still clinically efficacious. For instance, quetiapine can be taken 2-3 times per day (Goren and Levin, 1998; DeVane and Nemeroff, 2001) and occupies 60-65 % of striatal D2 receptors 3 h after administration, and 0-20 % after 12 h (Kapur *et al.*, 2000c). Despite this transient exposure, the antipsychotic still improves schizophrenia symptoms (Kapur *et al.*, 2000c). Similarly, clozapine is generally taken once a day (Meyer and Stahl, 2019) and occupies ≤ 65 % of striatal D2 receptors 6-14 h after administration [*e.g.*, 33-65% occupancy (Wiesel *et al.*, 1990; Farde *et al.*, 1992; Tauscher *et al.*, 2002a)]. Preclinical studies also show that clozapine-induced D2 receptor occupancy declines within hours after administration. In non-human primates, clozapine (0.2-5 mg/kg, i.v.) can occupy up to 83 % of striatal D2 receptors immediately after

administration, and occupancy declines to ~40-60 % 5 h later (Suhara *et al.*, 2002). Similarly, in rats, clozapine (15 mg/kg, s.c.) occupies 60 % of striatal D2 receptors 1 h after administration, and occupancy effectively disappears 24 h later (Kapur *et al.*, 2003). In summary, clozapine and quetiapine interact more loosely with D2 receptors and also possess a fast pharmacokinetic profile, and both characteristics could contribute to the lower incidence of extrapyramidal effects with these drugs relative to other antipsychotic drugs (typical or atypical), including haloperidol, chlorpromazine, sulpiride, risperidone and olanzapine (Leucht *et al.*, 2009; Rummel-Kluge *et al.*, 2012; Suttajit *et al.*, 2013; Martino *et al.*, 2018).

Because continuous occupancy of D2 receptors does not seem required to maintain antipsychotic efficacy, some studies have investigated the effects of extending the interval between oral antipsychotic doses. In a pilot study including 11 patients, Remington *et al.* (2005) found that antipsychotic efficacy is maintained for at least 1-6 months with regular, but extended dosing (*i.e.*, every second or third day). Later, Remington *et al.* (2011) replicated this finding in a larger study (N = 35) comparing the efficacy of alternate-day versus daily dosing with risperidone or olanzapine in a double-blind, randomized placebo-controlled trial. They followed the patients for 6 months and found no increase in the risk of symptom exacerbation, relapse, or re-hospitalization in the extended dosing group (Remington *et al.*, 2011). Similarly, Takeuchi and colleagues (Takeuchi *et al.*, 2014) used the dataset from the Clinical Antipsychotic Trials in Intervention Effectiveness (CATIE) to compare the therapeutic efficacy of once- versus twice-daily administration of perphenazine, which has a plasma half-life of 8-12 h. They found no differences in effectiveness or side effects (Takeuchi *et al.*, 2014). Although levels of antipsychotic-induced dopamine D2 receptor blockade were not measured in these studies, it would be reasonable to assume that the individuals receiving extended dosing treatment had lower levels of D2 receptor blockade at trough [see also (Tauscher *et al.*, 2002b)].

Previous studies with LAI antipsychotics also show that transient D2 occupancy above 65 % as well as longer intervals between treatment administrations does not compromise antipsychotic efficacy. For example, Nyberg *et al.* (1995) demonstrated in a [11C]raclopride PET study that eight stabilized outpatients with schizophrenia maintained a clinical response when administered haloperidol decanoate every 4 weeks, despite mean D2 blockade levels decreasing from 73 % (range: 60-82 %) at week 1 to 53 % (range: 20-74 %) at week 4. Another PET study led to the same

conclusion where seven subjects with schizophrenia received LAI risperidone monthly, rather than bi-weekly, for one year (Uchida *et al.*, 2008). Although more than half of subjects showed < 65 % D2 receptor blockade measured with [11C]raclopride PET at trough, none relapsed over the follow-up period. In a randomized, double-blind placebo-controlled trial (N = 50), Carpenter *et al.* (1999) found that fluphenazine decanoate is still effective when administered every 6 weeks instead of every 2 weeks, indicating that extended dosing intervals do not compromise the efficacy of LAI antipsychotics (Carpenter *et al.*, 1999).

Consistent with this notion, a series of cross-sectional studies reported dopamine D2 receptor blockade estimated from antipsychotic blood concentrations among patients with clinically stable schizophrenia and found that sustained blockade of dopamine D2 receptor over 65 % may not be necessary for relapse prevention (Ikai *et al.*, 2012; Mizuno *et al.*, 2012; Moriguchi *et al.*, 2013). In these studies, estimated levels of dopamine D2 receptor blockade by antipsychotic were < 65 % at trough in approximately half of the patients (Ikai *et al.*, 2012; Mizuno *et al.*, 2012; Moriguchi *et al.*, 2013). The same group also conducted a single-blind, 52-week randomized controlled trial (N = 68), in which clinically stable patients with schizophrenia receiving risperidone or olanzapine were randomly assigned to either a continuous D2 blockade group (*i.e.*, D2 receptor blockade of > 65 % at trough, estimated from antipsychotic blood concentrations) or a non-continuous blockade group (*i.e.*, an estimated peak level of > 65 % with an estimated trough level of < 65 %) (Tsuboi *et al.*, 2015). Twenty-six (76.5 %) subjects in the continuous blockade group and thirty-one (91.2 %) subjects in the non-continuous blockade groups completed the study. There were no significant group differences in any of the assessment scales for symptomatology or side effects. The association between peak/trough dopamine D2 receptor blockade with antipsychotic drugs, estimated from antipsychotic blood concentrations, and tardive dyskinesia was examined, using the dataset from the CATIE (Yoshida *et al.*, 2014). As a result, estimated dopamine D2 receptor blockade levels at trough were significantly higher in subjects who developed involuntary movements during the study period (N = 23) than in subjects who did not (N = 195) (71.7 ± 14.4 % versus 64.3 ± 19.3 %, $p < 0.05$), with no significant group differences in the estimated peak levels of D2 receptor blockade (75.4 ± 8.7 % versus 72.1 ± 9.9 %, $p = 0.07$). Thus, greater dopamine D2 receptor blockade with antipsychotics at trough can also increase the risk of tardive dyskinesia.

3.2 The case with long-acting injectable antipsychotics

Evidence has consistently shown the effectiveness of LAI antipsychotics in preventing relapse in patients with schizophrenia (Lähteenvuo *et al.*, 2018). In parallel, we do not know enough about the potential long-term disadvantages of treatment with atypical LAI antipsychotic drugs in humans. Indeed, long-term follow-up data with clinical assessments especially for side effects are critically lacking (Uchida and Suzuki, 2014). In addition, further investigations are clearly needed to determine appropriate dosing intervals of LAI antipsychotic drugs (Uchida and Suzuki, 2014) and target drug concentrations (Schoretsanitis *et al.*, 2019). In fact, LAI risperidone that is indicated for bi-weekly administration could be given monthly (Gharabawi *et al.*, 2007; Uchida *et al.*, 2008), although the data are still preliminary. Because data on appropriate dosing interval is insufficient, patients must be carefully selected for treatment with LAI antipsychotics, based on patient treatment adherence and preferences (Takahashi *et al.*, 2020), as well as dosing frequency of LAI treatment when it is administered. Moreover, potential biological changes such as dopamine supersensitivity due to this formulation should also be acknowledged. Indeed, as reviewed below in animal models (Section 4), compared to regular but extended dosing, continuous dosing can promote robust dopamine receptor supersensitivity. In this context, recent work shows that extended dosing of LAI antipsychotics, beyond the intervals indicated in product monographs is worth considering (Gharabawi *et al.*, 2007; Uchida *et al.*, 2008). Some studies claim that treatment with LAI antipsychotics is beneficial for patients with a history of dopamine supersensitivity psychosis (Kimura *et al.*, 2014; Kimura *et al.*, 2016). However, these results should be interpreted with much caution. First, the studies did not include a placebo condition and experimenters were also not blind to treatment condition (Kimura *et al.*, 2014; Kimura *et al.*, 2016). Second, the findings are sharply at odds with controlled animal studies showing that continuous dopamine D2 receptor blockade at clinically representative levels actually promotes dopamine supersensitivity (Ericson *et al.*, 1996; Samaha *et al.*, 2008; Amato *et al.*, 2018).

Thus, clinical data suggest that continuous D2 occupancy is not required for the maintenance of antipsychotic response. New data from methodologically robust clinical trials highlight the many benefits of extended, but regular antipsychotic dosing. As discussed more in depth later (Section 6), this must now be confirmed in larger-scale, randomized controlled trials with longer follow-up periods.

4. Benefits of extended relative to continuous dosing: lessons from rats

Work in laboratory animals shows that extended dosing actually produces a superior behavioural profile relative to continuous dosing. Extended antipsychotic dosing can be modelled in rats by injecting them daily with antipsychotic drugs. As illustrated in Fig. A2.2E, this achieves > 65 % of striatal D2 occupancy for a few hours, followed by a decline in occupancy until the next day's injection, leading to peaks and troughs in striatal D2 occupancy (Kapur *et al.*, 2003; Turrone *et al.*, 2003a, 2005). There is a vast literature on the effects of repeated antipsychotic injections in laboratory animals. In many of these studies, high and clinically unrepresentative doses of antipsychotic drugs were used (Kapur *et al.*, 2003), and this promotes unwanted side effects (Smith and Davis, 1975; Montanaro *et al.*, 1982; Turrone *et al.*, 2003a; Fukushiro *et al.*, 2008). Here we focus specifically on studies where clinically relevant antipsychotic doses were used, that is doses achieving 65-80 % striatal D2 receptor occupancy at peak (Kapur *et al.*, 2003).

4.1. Continuous antipsychotic treatment loses antipsychotic-like efficacy over time, while extended treatment remains effective

As mentioned in Section 2.2, a common index of antipsychotic-like efficacy in rats is the suppression of the conditioned avoidance response to an aversive cue. In rats, both continuous and extended dosing regimens initially suppress this conditioned response, but over time continuous dosing loses efficacy whereas extended dosing actually becomes more effective (Figs. A2.3A-B) (Li *et al.*, 2007; Samaha *et al.*, 2008; Mead and Li, 2010). It appears then that continuous versus extended dosing promotes neuroadaptations that lead to opposite outcomes: tolerance versus sensitization to antipsychotic-like effects, respectively. Remarkably, this occurs even when a 20-fold lower dose is administered using extended dosing, such that greater efficacy is produced with lesser drug (0.5 mg/kg haloperidol for continuous treatment (Samaha *et al.*, 2008), versus 0.025-0.05 mg/kg haloperidol for extended treatment [Li *et al.*, 2007; Samaha *et al.*, 2008)]. Interestingly, rats previously given extended treatment still show a suppressed conditioned avoidance response on days where they do not receive an antipsychotic injection (Fig. A2.3B), and responding returns to control levels after repeated testing/additional drug-free days (Li *et al.*, 2007). This suggests that extended antipsychotic treatment can produce persistent antipsychotic-like effects, thus reducing the need for daily drug administration.

Studies using suppression of the locomotor response to dopamine agonists as an index of antipsychotic-like efficacy also show that extended antipsychotic dosing is more efficacious than continuous dosing. As seen with the conditioned avoidance responding paradigm, both continuous and extended antipsychotic dosing initially suppress the locomotor-stimulating effects of dopamine agonists (Figs. A2.3C-D) (Samaha *et al.*, 2008). This reflects the anti-dopaminergic effects of antipsychotics. However, with continued treatment, continuous dosing failed in maintaining this

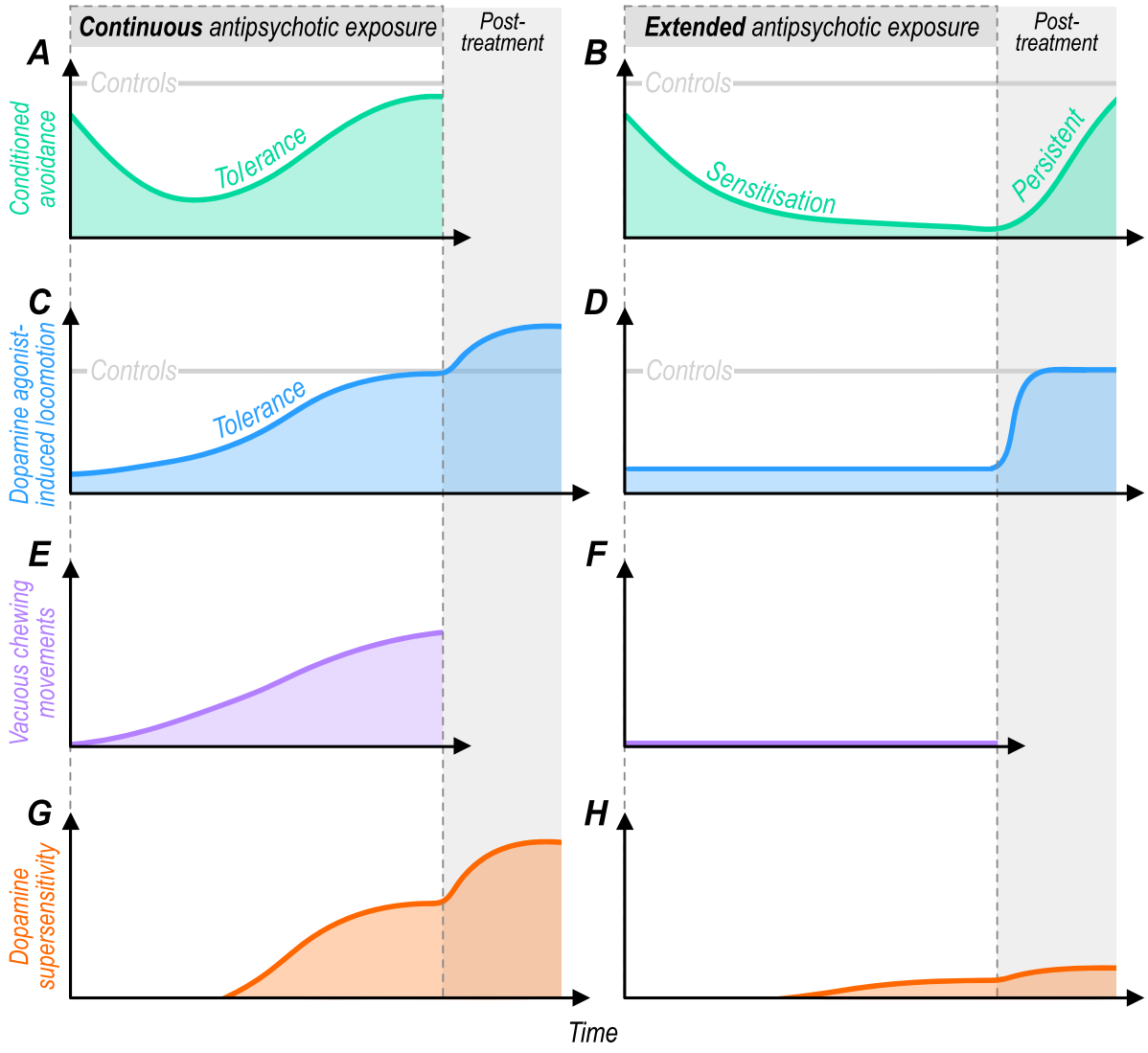

FIG. A2.3 — In rats, continuous exposure to antipsychotic drugs promotes treatment tolerance, movement disorders and a state of dopamine receptor supersensitivity, while extended exposure becomes more efficacious over time, and also reduces the risk of both movement disorders and dopamine supersensitivity. (*A*) Continuous antipsychotic treatment progressively loses (*see next page*) →

(*FIG. A2.3*) → efficacy in the suppression of conditioned avoidance responding task (i.e., rats develop tolerance to this antipsychotic effect). In contrast, (***B***) extended antipsychotic treatment gains efficacy over time (i.e., rats develop sensitization to this antipsychotic effect) and also maintains efficacy even after treatment cessation. Similarly, (***C***) continuous antipsychotic treatment initially suppresses the locomotor response to dopamine agonists—indicating anti-dopaminergic efficacy—but fails over time. After discontinuation of continuous antipsychotic treatment, rats show an exaggerated psychomotor response to dopamine agonists, indicating a dopamine supersensitive state. In contrast, (***D***) extended antipsychotic treatment maintains anti-dopaminergic efficacy. After discontinuation of extended antipsychotic treatment, rats generally show a normal psychomotor response to dopamine agonists, indicating no dopamine supersensitivity. (***E***) Over time, continuous antipsychotic treatment promotes high levels of vacuous chewing movements, a sign related to tardive dyskinesia. (***F***) Extended antipsychotic treatment reduces the risk of vacuous chewing movements. (***G***) Continuous antipsychotic exposure promotes a dopamine supersensitive state that can breakthrough ongoing antipsychotic treatment, and that is fully unmasked after treatment cessation. In contrast, (***H***) extended antipsychotic exposure reduces the risk of dopamine supersensitivity.

anti-dopaminergic effect, whereas extended dosing maintained efficacy—even though the latter involved a 10-fold lower dose (0.5 mg/kg haloperidol for continuous treatment, versus 0.05 mg/kg haloperidol for extended treatment) (Samaha *et al.*, 2008). Thus, using two different behavioural paradigms (suppression of conditioned avoidance responding and of the locomotor response to dopamine agonists), extended antipsychotic dosing proves more effective than continuous dosing, in spite of lesser drug exposure.

4.2 Extended versus continuous antipsychotic dosing reduces the likelihood of motor side effects

Extended dosing considerably reduces the risk of motor disturbances in rats (see Figs. A2.3E-F). For instance, after 2 months of treatment, 33 to 54 % of rats receiving continuous haloperidol or olanzapine (at doses achieving between ~65-90 % of D2 occupancy) show high levels of vacuous chewing movements (≥ 8 vacuous chewing movements/2 minutes) (Turrone *et al.*, 2003a, 2005). In parallel, rats receiving similar doses but using an extended treatment approach do not develop vacuous chewing movements after 2 months of treatment, even at doses that achieve ~70-90 % of D2 occupancy at peak. The temporal kinetics of treatment also interact with antipsychotic drug-type to determine outcome. At a dose achieving very high D2 occupancy (~95 %) at peak, extended dosing with the atypical antipsychotic olanzapine does not produce significant vacuous chewing movements in rats (Turrone *et al.*, 2005), whereas extended dosing with the typical antipsychotic

haloperidol does, at least in some rats (17 %) (Turrone *et al.*, 2003a). This incidence remains lower than that seen when animals are treated continuously with haloperidol or olanzapine (33-54 %), at doses achieving lower D2 occupancy (~65-90 %) (Turrone *et al.*, 2003a, 2005). While preclinical studies using doses achieving > 90 % D2 occupancy are less clinically pertinent, the studies above demonstrate that extended antipsychotic administration—even with high doses—is much less likely to produce motor side effects in laboratory animals relative to continuous dosing. As additional converging evidence for this, with doses achieving 65-80 % D2 occupancy at peak, neither acute (Wadenberg *et al.*, 2000; Wadenberg *et al.*, 2001) nor repeated (Samaha *et al.*, 2008) injections of antipsychotic produce catalepsy, another manifestation of motor impairment related to extrapyramidal effects. Thus, when antipsychotic medication disrupts dopamine neurotransmission continuously, this promotes neuroplastic changes that lead to motor side effects, but if disruption of dopamine neurotransmission is only transient, this can prevent such neuroplasticity.

4.3. Extended antipsychotic exposure is unlikely to promote dopamine supersensitivity

A notable added benefit of extended versus continuous antipsychotic dosing is that extended dosing reduces the risk of dopamine supersensitivity (see Figs. A2.3G-H). After treatment cessation, rats previously treated continuously have a significantly enhanced psychomotor response to dopamine agonists, while animals previously treated using an extended approach show either no change or a modest increase compared to antipsychotic-naïve control rats (Figs. A2.3C-D) (Ericson *et al.*, 1996; Samaha *et al.*, 2008; Bedard *et al.*, 2011, 2013; Servonnet *et al.*, 2017).

In summary, continuous antipsychotic dosing promotes treatment tolerance and dopamine supersensitivity, whereas extended dosing can gain efficacy over time and is unlikely to produce dopamine supersensitivity—all the while reducing total exposure to medication.

5. Neurobiological effects of continuous versus extended antipsychotic dosing

The neurobiological mechanisms underlying the different behavioural profiles produced by continuous versus extended antipsychotic dosing remain largely unknown, but there are at least some clues. For example, the D2 receptor is a critical target for antipsychotic efficacy (Creese *et al.*, 1976; Seeman *et al.*, 1976; Farde *et al.*, 1989; Richtand *et al.*, 2007), and some studies have

assessed the effects of continuous versus extended antipsychotic dosing on D2 receptor number and function. In rats, continuous but not extended antipsychotic exposure increases the number of D2 receptors in the striatum (Samaha *et al.*, 2008; Ginovart *et al.*, 2009). D2 receptors are functional and in a high affinity state for dopamine when coupled to Gi/o proteins (referred to as '$D2^{HIGH}$'). Both continuous and extended antipsychotic dosing can enhance the number of $D2^{HIGH}$ receptors, but this effect is greatest with continuous treatment (Samaha *et al.*, 2008). This suggests that continuous versus extended antipsychotic treatment might differentially influence D2-mediated intracellular function. In support, following chronic treatment (17 days), extended but not continuous haloperidol dosing increases mRNA levels for the immediate early gene c-*fos* in the caudate-putamen (Samaha *et al.*, 2008). These gene effects were consistent with effects on behaviour, as extended but not continuous dosing also increased behavioural antipsychotic efficacy over time (Samaha *et al.*, 2008). The positive correlation between c-*fos* mRNA induction and behavioural efficacy suggests that gene regulation could be a step in a cascade of intracellular events that contribute to or maintain response to antipsychotics over time (Li *et al.*, 2007; Samaha *et al.*, 2008; Mead and Li, 2010). This remains to be investigated further.

The mode of antipsychotic treatment also influences the neurobiological response to dopamine agonists. For instance, rats previously given continuous—but not extended—haloperidol dosing show enhanced d-amphetamine-induced gene regulation in the caudate-putamen, as indicated by increased mRNA levels for the immediate early genes c-*fos* and Nur77 (Bedard *et al.*, 2011). The nature of the relationship between the ability of continuous antipsychotic dosing to enhance d-amphetamine-induced gene regulation on the one hand and to potentiate behavioural d-amphetamine effects on the other, is not known. As further research resolves this issue, the available evidence suggests that continuous antipsychotic treatment changes the behavioural and neurobiological impact of dopamine stimulation, and that with extended treatment, these changes either do not occur or are less pronounced (Figs. A2.3G-H).

Lastly, continuous versus extended antipsychotic exposure exert distinct influences on neurobiological systems that modulate dopamine function indirectly, namely neurotensin. Neurotensin is a neuropeptide that can oppose dopamine effects through activation of type 1 neurotensin receptors (Binder *et al.*, 2001). Activation of these receptors decreases the affinity of D2 receptors for dopamine (Agnati *et al.*, 1983; von Euler *et al.*, 1990; Li *et al.*, 1995), and this is

thought to involve D2 receptor internalization (Koschatzky *et al.*, 2011; Thibault *et al.*, 2011; Borroto-Escuela *et al.*, 2013). The temporal kinetics of treatment determine the effects of antipsychotic treatment on neurotensin and neurotensin receptor expression. Continuous but not extended antipsychotic treatment enhances neurotensin expression in the caudate-putamen, whereas only extended treatment increases neurotensin type 1 receptor level in the same region [(Servonnet *et al.*, 2017); see also (Kinkead *et al.*, 2000)]. Most importantly, neurotensin injected into the nucleus accumbens attenuates the locomotor response to dopamine agonists (Ervin *et al.*, 1981), and this effect is potentiated in rats with a history of continuous versus extended haloperidol dosing (Servonnet *et al.*, 2017). Because only rats exposed to continuous (versus extended) haloperidol develop a dopamine supersensitive state (Samaha *et al.*, 2008; Bedard *et al.*, 2011; Servonnet *et al.*, 2017), the findings suggest that antipsychotic-evoked dopamine supersensitivity is accompanied by an enhanced ability of nucleus accumbens neurotensin to modulate dopamine-dependent behaviour. The implication is that in individuals with established antipsychotic-induced dopamine supersensitivity, the increased responsiveness to neurotensin can be exploited pharmacologically to reverse the expression of this supersensitivity.

6. Current limitations and future directions

Further investigations are warranted into the long-term effects of extended dosing strategies. For instance, it remains unclear whether extended antipsychotic dosing is less likely to produce side effects than continuous exposure in humans, as is the case in laboratory animals (see Section 4). It is tempting to predict that by reducing antipsychotic drug exposure, extended dosing strategies will reduce the likelihood of adverse side effects, as compared to continuous dosing. However, this still needs to be determined conclusively, because some findings suggest that merely reducing overall antipsychotic drug exposure does not necessarily reduce side effects. For instance, studies in non-human primates (Linn *et al.*, 2001) and schizophrenia patients (Achalia *et al.*, 2014) show that intermittent antipsychotic exposure involving drug-free periods lasting months can exacerbate antipsychotic-induced movement disorders. However, extended dosing is different from this. It involves regular intervals of low drug exposure (*i.e.*, < 65 % of D2 occupancy), rather than long drug-free periods.

Also, evidence regarding the efficacy of extended dosing strategies in humans (as described in Section 3) comes from a small number of studies. However, because the available evidence is promising, future studies should expand on the existing literature and address specific issues. First, future studies should include greater sample sizes. Second, antipsychotic efficacy and risk of adverse effects need to be assessed for longer follow-up periods. Third, the feasibility/efficacy of extended dosing strategies should be investigated with many different antipsychotic drugs, because most studies have only used risperidone or olanzapine. Fourth, extended dosing has been studied in a limited number of double-blind, randomized controlled trials so far (Carpenter *et al.*, 1999; Remington *et al.*, 2011; Takeuchi *et al.*, 2014). Hence, the promising findings discussed in Section 3 need to be extended with more methodologically rigorous trials. Furthermore, it will be important to determine how extended antipsychotic dosing strategies influence the kinetics of D2 receptor occupancy in humans, because so far this has not been addressed or been estimated from plasma levels of antipsychotic drugs. Plasma concentrations do not always predict brain concentrations/D2 receptor occupancy by antipsychotic (Tauscher *et al.*, 2002b; Kurose *et al.*, 2020). Hence, future studies should measure dopamine D2 receptor occupancy levels directly, using brain imaging techniques. In addition, in theory, dosing intervals in an extended dosing regimen can also be irregular, ranging from 2 to 4 days or even longer. However, the feasibility and efficacy of extended but irregular dosing is yet to be assessed. Lastly, extended dosing is unlikely a "one-size-fits-all" approach. Empirical data are needed to identify patients best suited for extended, but regular antipsychotic dosing.

There are also important limitations to the preclinical studies discussed in Section 4. First, only one type of extended dosing approach has been studied in laboratory animals, *i.e.*, within-day transient exposure, achieved with daily antipsychotic injection (see Fig. A2.2D). It will be important in future work to characterize this more systematically in laboratory animals. For instance, one can reasonably predict that if D2 receptor occupancy by antipsychotic is low enough for long enough, this will compromise antidopaminergic effects over time. Second, it is not obvious what the extended dosing strategy used in the rat studies in Section 4 might correspond to in humans, not the least of which because antipsychotic pharmacokinetics can be quite different across the two species (Kapur *et al.*, 2000a; Kapur *et al.*, 2003). Third, it is unclear how the neuronal changes described in Section 5 contribute to the different behavioural effects of continuous versus extended antipsychotic treatment, as the studies above have generally established correlational but not causal

links. Because of the clinical implications of antipsychotic-induced dopamine supersensitivity, particular effort should be devoted to identify why continuous antipsychotic exposure is more likely to produce this supersensitivity compared to extended exposure. Fourth, in parallel to dopamine supersensitivity and motor disturbances, antipsychotic treatment can produce many other deleterious effects, including cardiovascular and metabolic disorders (Muench and Hamer, 2010; De Hert *et al.*, 2011). Future work should determine how extended versus continuous exposure influences the incidence of such effects. Fifth, there are important caveats in the preclinical models used to compare extended versus continuous antipsychotic treatment. For instance, we are not aware of a single study examining this in female laboratory animals, as this work has been done exclusively in males so far. This is an urgent knowledge gap to start filling, as schizophrenia and antipsychotic treatment are relevant to both women and men. Additionally, to our knowledge, the effects of extended antipsychotic exposure have been studied only in otherwise neurologically intact rodents. Using neurologically intact animals is useful to establish cause-and-effect relationships between the temporal kinetics of antipsychotic treatment and changes in brain and behaviour. However, it remains undetermined how extended versus continuous antipsychotic exposure fare in animal models of schizophrenia-like symptoms. Sixth, because extended dosing strategies have shown great promise in studies carried out on patients with schizophrenia (see Section 3), one important avenue for future research is determining the extent to which extended antipsychotic exposure can reverse the unwanted effects previously produced by continuous antipsychotic treatment. These unwanted effects would include treatment tolerance, extrapyramidal motor side-effects and dopamine supersensitivity. Lastly, the behavioural and neurochemical effects of continuous versus extended antipsychotic dosing have most often been compared using haloperidol [*e.g.*, (Turrone *et al.*, 2003a; Samaha *et al.*, 2008; Bedard *et al.*, 2011)]. Little is known about similar effects using atypical antipsychotic drugs, and future research can address this.

7. Concluding remarks

For decades now, standard clinical practice in the management of schizophrenia symptoms has been to maintain steady-state levels of D2 receptor occupancy, because this is thought to be essential to maintain efficacy. However, continuous blockade of more than 65 % of D2 receptors promotes neuroadaptations leading to treatment failure, motor disturbances and supersensitivity to

dopamine receptor stimulation. In contrast, regular but transient peaks in antipsychotic levels/D2 receptor occupancy, as can be achieved with extended dosing strategies, are sufficient to maintain treatment efficacy and also reduce the risk of motor side effects and dopamine supersensitivity. Remarkably, the superior profile of extended versus continuous dosing strategies is seen even when extended treatment achieves much lower antipsychotic drug exposure overall. Most research comparing outcome with continuous versus extended antipsychotic exposure has been in preclinical models. However, we also reviewed findings showing that in patients, extended dosing strategies are effective in preventing psychosis relapse. Moreover, extended dosing strategies hold great promise as they could reduce dose-dependent antipsychotic side effects. This prediction can be evaluated conclusively in future work. Given the literature that we have discussed here, we also view with apprehensiveness the current commercial push to market continuous-release formulations, including LAI antipsychotics. Since the available clinical evidence, especially regarding safety, is still in shortage, large-scale methodologically rigorous randomized controlled trials with LAI antipsychotics, involving longer follow-up periods are clearly warranted. As such work unfolds, we propose that while continuous antipsychotic treatment might benefit some patients, at some treatment stages (and it remains to be determined exactly who these patients might be), the available evidence suggests that extended dosing strategies achieving transient peaks in antipsychotic levels are at least as efficacious as continuous dosing, while also being less harmful and therefore more ethical.

FUNDING

ANS is supported by a salary grant from the Fonds de Recherche du Québec – Santé (grant number 28988). This funding source was not involved in the writing of the article and in the decision to submit the article for publication.

REFERENCES - ANNEX 2

Achalia RM, Chaturvedi SK, Desai G, Rao GN, Prakash O (2014) Prevalence and risk factors associated with tardive dyskinesia among Indian patients with schizophrenia. Asian J Psychiatr 9:31-35.

Agnati LF, Fuxe K, Benfenati F, Battistini N (1983) Neurotensin in vitro markedly reduces the affinity in subcortical limbic 3H-N-propylnorapomorphine binding sites. Acta physiologica Scandinavica 119:459-461.

Amato D, Canneva F, Cumming P, Maschauer S, Groos D, Dahlmanns JK, Gromer TW, Chiofalo L, Dahlmanns M, Zheng F, Kornhuber J, Prante O, Alzheimer C, von Horsten S, Muller CP (2018) A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failure reversal: the role of the dopamine transporter. Mol Psychiatry.

Bedard AM, Maheux J, Levesque D, Samaha AN (2011) Continuous, but not intermittent, antipsychotic drug delivery intensifies the pursuit of reward cues. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 36:1248-1259.

Bedard AM, Maheux J, Levesque D, Samaha AN (2013) Prior haloperidol, but not olanzapine, exposure augments the pursuit of reward cues: implications for substance abuse in schizophrenia. Schizophr Bull 39:692-702.

Binder EB, Kinkead B, Owens MJ, Nemeroff CB (2001) Neurotensin and dopamine interactions. Pharmacological reviews 53:453-486.

Borroto-Escuela DO, Ravani A, Tarakanov AO, Brito I, Narvaez M, Romero-Fernandez W, Corrales F, Agnati LF, Tanganelli S, Ferraro L, Fuxe K (2013) Dopamine D2 receptor signaling dynamics of dopamine D2-neurotensin 1 receptor heteromers. Biochemical and biophysical research communications 435:140-146.

Carbon M, Hsieh CH, Kane JM, Correll CU (2017) Tardive Dyskinesia Prevalence in the Period of Second-Generation Antipsychotic Use: A Meta-Analysis. The Journal of clinical psychiatry 78:e264-e278.

Carpenter WT, Jr., Buchanan RW, Kirkpatrick B, Lann HD, Breier AF, Summerfelt AT (1999) Comparative effectiveness of fluphenazine decanoate injections every 2 weeks versus every 6 weeks. The American journal of psychiatry 156:412-418.

Chouinard G (2004) New nomenclature for drug-induced movement disorders including tardive dyskinesia. The Journal of clinical psychiatry 65 Suppl 9:9-15.

Chouinard G, Jones BD (1980) Neuroleptic-induced supersensitivity psychosis: clinical and pharmacologic characteristics. The American journal of psychiatry 137:16-21.

Chouinard G, Jones BD, Annable L (1978) Neuroleptic-induced supersensitivity psychosis. The American journal of psychiatry 135:1409-1410.

Chouinard G, Creese I, Boisvert D, Annable L, Bradwejn J, Jones B (1982) High neuroleptic plasma levels in patients manifesting supersensitivity psychosis. Biological psychiatry 17:849-852.

Chouinard G, Samaha AN, Chouinard VA, Peretti CS, Kanahara N, Takase M, Iyo M (2017) Antipsychotic-Induced Dopamine Supersensitivity Psychosis: Pharmacology, Criteria, and Therapy. Psychother Psychosom 86:189-219.

Correll CU, Leucht S, Kane JM (2004) Lower risk for tardive dyskinesia associated with second-generation antipsychotics: a systematic review of 1-year studies. The American journal of psychiatry 161:414-425.

Creese I, Burt DR, Snyder SH (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192:481-483.

De Hert M, Detraux J, van Winkel R, Yu W, Correll CU (2011) Metabolic and cardiovascular adverse effects associated with antipsychotic drugs. Nat Rev Endocrinol 8:114-126.

Demjaha A, Lappin JM, Stahl D, Patel MX, MacCabe JH, Howes OD, Heslin M, Reininghaus UA, Donoghue K, Lomas B, Charalambides M, Onyejiaka A, Fearon P, Jones P, Doody G, Morgan C, Dazzan P, Murray RM (2017) Antipsychotic treatment resistance in first-episode psychosis: prevalence, subtypes and predictors. Psychol Med 47:1981-1989.

DeVane CL, Nemeroff CB (2001) Clinical pharmacokinetics of quetiapine: an atypical antipsychotic. Clin Pharmacokinet 40:509-522.

Dolder CR, Jeste DV (2003) Incidence of tardive dyskinesia with typical versus atypical antipsychotics in very high risk patients. Biological psychiatry 53:1142-1145.

Emsley R, Nuamah I, Gopal S, Hough D, Fleischhacker WW (2018) Relapse After Antipsychotic Discontinuation in Schizophrenia as a Withdrawal Phenomenon vs Illness Recurrence: A Post Hoc Analysis of a Randomized Placebo-Controlled Study. The Journal of clinical psychiatry 79.

Ericson H, Radesater AC, Servin E, Magnusson O, Mohringe B (1996) Effects of intermittent and continuous subchronic administration of raclopride on motor activity, dopamine turnover and receptor occupancy in the rat. Pharmacology & toxicology 79:277-286.

Ervin GN, Birkemo LS, Nemeroff CB, Prange AJ, Jr. (1981) Neurotensin blocks certain amphetamine-induced behaviours. Nature 291:73-76.

Fallon P, Dursun SM (2011) A naturalistic controlled study of relapsing schizophrenic patients with tardive dyskinesia and supersensitivity psychosis. J Psychopharmacol 25:755-762.

Fallon P, Dursun S, Deakin B (2012) Drug-induced supersensitivity psychosis revisited: characteristics of relapse in treatment-compliant patients. Therapeutic advances in psychopharmacology 2:13-22.

Farde L, Wiesel FA, Nordstrom AL, Sedvall G (1989) D1- and D2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. Psychopharmacology (Berl) 99 Suppl:S28-31.

Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G (1992) Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Arch Gen Psychiatry 49:538-544.

Fukushiro DF, Carvalho Rde C, Ricardo VP, Alvarez Jdo N, Ribeiro LT, Frussa-Filho R (2008) Haloperidol (but not ziprasidone) withdrawal potentiates sensitization to the hyperlocomotor effect of cocaine in mice. Brain research bulletin 77:124-128.

Gaebel W (1994) Intermittent medication--an alternative? Acta Psychiatr Scand Suppl 382:33-38.

Gaebel W, Janner M, Frommann N, Pietzcker A, Kopcke W, Linden M, Muller P, Muller-Spahn F, Tegeler J (2002) First vs multiple episode schizophrenia: two-year outcome of intermittent and maintenance medication strategies. Schizophr Res 53:145-159.

Gharabawi GM, Gearhart NC, Lasser RA, Mahmoud RA, Zhu Y, Mannaert E, Naessens I, Bossie CA, Kujawa M, Simpson GM (2007) Maintenance therapy with once-monthly administration of long-acting injectable risperidone in patients with schizophrenia or schizoaffective disorder: a pilot study of an extended dosing interval. Ann Gen Psychiatry 6:3.

Gianutsos G, Drawbaugh RB, Hynes MD, Lal H (1974) Behavioral evidence for dopaminergic supersensitivity after chronic haloperidol. Life Sci 14:887-898.

Gill KM, Cook JM, Poe MM, Grace AA (2014) Prior antipsychotic drug treatment prevents response to novel antipsychotic agent in the methylazoxymethanol acetate model of schizophrenia. Schizophr Bull 40:341-350.

Ginovart N, Wilson AA, Hussey D, Houle S, Kapur S (2009) D2-receptor upregulation is dependent upon temporal course of D2-occupancy: a longitudinal [11C]-raclopride PET study in cats. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 34:662-671.

Goren JL, Levin GM (1998) Quetiapine, an atypical antipsychotic. Pharmacotherapy 18:1183-1194.

Ikai S, Remington G, Suzuki T, Takeuchi H, Tsuboi T, Den R, Hirano J, Tsunoda K, Nishimoto M, Watanabe K, Mimura M, Mamo D, Uchida H (2012) A cross-sectional study of plasma risperidone levels with risperidone long-acting injectable: implications for dopamine D2 receptor occupancy during maintenance treatment in schizophrenia. The Journal of clinical psychiatry 73:1147-1152.

Jann MW, Grimsley SR, Gray EC, Chang WH (1993) Pharmacokinetics and pharmacodynamics of clozapine. Clin Pharmacokinet 24:161-176.

Jolley AG, Hirsch SR (1993) Continuous versus intermittent neuroleptic therapy in schizophrenia. Drug Saf 8:331-339.

Jolley AG, Hirsch SR, Morrison E, McRink A, Wilson L (1990) Trial of brief intermittent neuroleptic prophylaxis for selected schizophrenic outpatients: clinical and social outcome at two years. Bmj 301:837-842.

Kapur S, Wadenberg ML, Remington G (2000a) Are animal studies of antipsychotics appropriately dosed? Lessons from the bedside to the bench. Canadian journal of psychiatry Revue canadienne de psychiatrie 45:241-246.

Kapur S, VanderSpek SC, Brownlee BA, Nobrega JN (2003) Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. The Journal of pharmacology and experimental therapeutics 305:625-631.

Kapur S, Zipursky R, Jones C, Remington G, Houle S (2000b) Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry 157:514-520.

Kapur S, Zipursky R, Jones C, Shammi CS, Remington G, Seeman P (2000c) A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D2 receptor occupancy. Archives of general psychiatry 57:553-559.

Kimura H, Kanahara N, Sasaki T, Komatsu N, Ishige M, Muneoka K, Ino H, Yoshimura K, Yamanaka H, Suzuki T, Komatsu H, Watanabe H, Shimizu E, Iyo M (2016) Risperidone long-acting injectable in the treatment of treatment-resistant schizophrenia with dopamine supersensitivity psychosis: Results of a 2-year prospective study, including an additional 1-year follow-up. J Psychopharmacol 30:795-802.

Kimura H, Kanahara N, Komatsu N, Ishige M, Muneoka K, Yoshimura M, Yamanaka H, Suzuki T, Komatsu H, Sasaki T, Hashimoto T, Hasegawa T, Shiina A, Ishikawa M, Sekine Y, Shiraishi T, Watanabe H, Shimizu E, Hashimoto K, Iyo M (2014) A prospective comparative study of risperidone long-acting injectable for treatment-resistant schizophrenia with dopamine supersensitivity psychosis. Schizophr Res 155:52-58.

Kinkead B, Shahid S, Owens MJ, Nemeroff CB (2000) Effects of acute and subchronic administration of typical and atypical antipsychotic drugs on the neurotensin system of the rat brain. The Journal of pharmacology and experimental therapeutics 295:67-73.

Koschatzky S, Tschammer N, Gmeiner P (2011) Cross-receptor interactions between dopamine D2L and neurotensin NTS1 receptors modulate binding affinities of dopaminergics. ACS Chem Neurosci 2:308-316.

Kurose S, Mimura Y, Uchida H, Takahata K, Kim E, Suzuki T, Mimura M, Takeuchi H (2020) Dissociation in Pharmacokinetic Attenuation Between Central Dopamine D2 Receptor Occupancy and Peripheral Blood Concentration of Antipsychotics: A Systematic Review. The Journal of clinical psychiatry 81.

Lähteenvuo M, Tanskanen A, Taipale H, Hoti F, Vattulainen P, Vieta E, Tiihonen J (2018) Real-world Effectiveness of Pharmacologic Treatments for the Prevention of Rehospitalization

in a Finnish Nationwide Cohort of Patients With Bipolar Disorder. JAMA Psychiatry 75:347-355.

Leucht S, Corves C, Arbter D, Engel RR, Li C, Davis JM (2009) Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. Lancet 373:31-41.

Li M, Fletcher PJ, Kapur S (2007) Time course of the antipsychotic effect and the underlying behavioral mechanisms. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 32:263-272.

Li XM, Ferraro L, Tanganelli S, O'Connor WT, Hasselrot U, Ungerstedt U, Fuxe K (1995) Neurotensin peptides antagonistically regulate postsynaptic dopamine D2 receptors in rat nucleus accumbens: a receptor binding and microdialysis study. Journal of neural transmission General section 102:125-137.

Linn GS, Lifshitz K, O'Keeffe RT, Lee K, Camp-Lifshitz J (2001) Increased incidence of dyskinesias and other behavioral effects of re-exposure to neuroleptic treatment in social colonies of Cebus apella monkeys. Psychopharmacology (Berl) 153:285-294.

Ljungberg T, Ungerstedt U (1985) A rapid and simple behavioural screening method for simultaneous assessment of limbic and striatal blocking effects of neuroleptic drugs. Pharmacol Biochem Behav 23:479-485.

Lu ML, Pan JJ, Teng HW, Su KP, Shen WW (2002) Metoclopramide-induced supersensitivity psychosis. Ann Pharmacother 36:1387-1390.

Martino D, Karnik V, Osland S, Barnes TRE, Pringsheim TM (2018) Movement Disorders Associated With Antipsychotic Medication in People With Schizophrenia: An Overview of Cochrane Reviews and Meta-Analysis. Canadian journal of psychiatry Revue canadienne de psychiatrie:706743718777392.

Mead A, Li M (2010) Avoidance-suppressing effect of antipsychotic drugs is progressively potentiated after repeated administration: an interoceptive drug state mechanism. J Psychopharmacol 24:1045-1053.

Meyer ME, Stahl SS (2019) The Clozapine Handbook: Stahl's Handbooks. Cambridge University Press.

Miller DD, Eudicone JM, Pikalov A, Kim E (2007) Comparative assessment of the incidence and severity of tardive dyskinesia in patients receiving aripiprazole or haloperidol for the treatment of schizophrenia: a post hoc analysis. The Journal of clinical psychiatry 68:1901-1906.

Miller R, Chouinard G (1993) Loss of striatal cholinergic neurons as a basis for tardive and L-dopa-induced dyskinesias, neuroleptic-induced supersensitivity psychosis and refractory schizophrenia. Biological psychiatry 34:713-738.

Mizuno Y, Bies RR, Remington G, Mamo DC, Suzuki T, Pollock BG, Tsuboi T, Watanabe K, Mimura M, Uchida H (2012) Dopamine D2 receptor occupancy with risperidone or olanzapine during maintenance treatment of schizophrenia: a cross-sectional study. Progress in neuro-psychopharmacology & biological psychiatry 37:182-187.

Montanaro N, Dall'Olio R, Gandolfi O, Vaccheri A (1982) Differential enhancement of behavioral sensitivity to apomorphine following chronic treatment of rats with (-)-sulpiride and haloperidol. Eur J Pharmacol 81:1-9.

Moriguchi S, Bies RR, Remington G, Suzuki T, Mamo DC, Watanabe K, Mimura M, Pollock BG, Uchida H (2013) Estimated dopamine D(2) receptor occupancy and remission in schizophrenia: analysis of the CATIE data. J Clin Psychopharmacol 33:682-685.

Muench J, Hamer AM (2010) Adverse effects of antipsychotic medications. American family physician 81:617-622.

Murray RM, Quattrone D, Natesan S, van Os J, Nordentoft M, Howes O, Di Forti M, Taylor D (2016) Should psychiatrists be more cautious about the long-term prophylactic use of antipsychotics? Br J Psychiatry 209:361-365.

Nyberg S, Farde L, Halldin C, Dahl ML, Bertilsson L (1995) D2 dopamine receptor occupancy during low-dose treatment with haloperidol decanoate. The American journal of psychiatry 152:173-178.

Pardis P, Remington G, Panda R, Lemez M, Agid O (2019) Clozapine and tardive dyskinesia in patients with schizophrenia: A systematic review. J Psychopharmacol 33:1187-1198.

Ray WA, Chung CP, Murray KT, Hall K, Stein CM (2009) Atypical antipsychotic drugs and the risk of sudden cardiac death. New England Journal of Medicine 360:225-235.

Remington G, Kapur S (2010) Antipsychotic dosing: how much but also how often? Schizophr Bull 36:900-903.

Remington G, Seeman P, Shammi C, Mann S, Kapur S (2005) "Extended" antipsychotic dosing: rationale and pilot data. J Clin Psychopharmacol 25:611-613.

Remington G, Fervaha G, Foussias G, Agid O, Turrone P (2014) Antipsychotic dosing: found in translation. J Psychiatry Neurosci 39:223-231.

Remington G, Seeman P, Feingold A, Mann S, Shammi C, Kapur S (2011) "Extended" antipsychotic dosing in the maintenance treatment of schizophrenia: a double-blind, placebo-controlled trial. The Journal of clinical psychiatry 72:1042-1048.

Richtand NM, Welge JA, Logue AD, Keck PE, Jr., Strakowski SM, McNamara RK (2007) Dopamine and serotonin receptor binding and antipsychotic efficacy. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 32:1715-1726.

Roy-Desruisseaux J, Landry J, Bocti C, Tessier D, Hottin P, Trudel JF (2011) Domperidone-induced tardive dyskinesia and withdrawal psychosis in an elderly woman with dementia. Ann Pharmacother 45:e51.

Rubio JM, Schoretsanitis G, John M, Tiihonen J, Taipale H, Guinart D, Malhotra AK, Correll CU, Kane JM (2020) Psychosis relapse during treatment with long-acting injectable antipsychotics in individuals with schizophrenia-spectrum disorders: an individual participant data meta-analysis. Lancet Psychiatry 7:749-761.

Rummel-Kluge C, Komossa K, Schwarz S, Hunger H, Schmid F, Kissling W, Davis JM, Leucht S (2012) Second-generation antipsychotic drugs and extrapyramidal side effects: a systematic review and meta-analysis of head-to-head comparisons. Schizophr Bull 38:167-177.

Sahlholm K, Zeberg H, Nilsson J, Ogren SO, Fuxe K, Arhem P (2016) The fast-off hypothesis revisited: A functional kinetic study of antipsychotic antagonism of the dopamine D2 receptor. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 26:467-476.

Saito Y, Sakurai H, Kane JM, Schooler NR, Suzuki T, Mimura M, Uchida H (2020) Predicting relapse with residual symptoms in schizophrenia: A secondary analysis of the PROACTIVE trial. Schizophr Res 215:173-180.

Sakurai H, Bies R, Stroup S, Keefe R, Rajji T, Suzuki T, Mamo D, Pollock B, Watanabe K, Mimura M, Uchida H (2012) Dopamine D2 receptor occupancy and cognition in schizophrenia: analysis of the CATIE data. Schizophr Bull 39:564-574.

Samaha AN, Seeman P, Stewart J, Rajabi H, Kapur S (2007) "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:2979-2986.

Samaha AN, Reckless GE, Seeman P, Diwan M, Nobrega JN, Kapur S (2008) Less is more: antipsychotic drug effects are greater with transient rather than continuous delivery. Biological psychiatry 64:145-152.

Sayers AC, Burki HR, Ruch W, Asper H (1975) Neuroleptic-induced hypersensitivity of striatal dopamine receptors in the rat as a model of tardive dyskinesias. Effects of clozapine, haloperidol, loxapine and chlorpromazine. Psychopharmacologia 41:97-104.

Schneeweiss S, Avorn J (2009) Antipsychotic agents and sudden cardiac death--how should we manage the risk? New England Journal of Medicine 360:295-296.

Schooler NR (1991) Maintenance medication for schizophrenia: strategies for dose reduction. Schizophr Bull 17:311-324.

Schoretsanitis G, Villasante-Tezanos AG, de Leon J (2019) Studies of Half-Lives of Long-Acting Antipsychotics are Needed. Pharmacopsychiatry 52:45-46.

Seeman P (2002) Atypical antipsychotics: mechanism of action. Canadian journal of psychiatry Revue canadienne de psychiatrie 47:27-38.

Seeman P (2014) Yes, Breast is best but taper domperidone when stopping (e-letter). Br J Gen Pract.

Seeman P, Lee T, Chau-Wong M, Wong K (1976) Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 261:717-719.

Servonnet A, Samaha AN (2020) Antipsychotic-evoked dopamine supersensitivity. Neuropharmacology 163:107630.

Servonnet A, Minogianis EA, Bouchard C, Bedard AM, Levesque D, Rompre PP, Samaha AN (2017) Neurotensin in the nucleus accumbens reverses dopamine supersensitivity evoked by antipsychotic treatment. Neuropharmacology 123:10-21.

Smith RC, Davis JM (1975) Behavioral supersensitivity to apomorphine and amphetamine after chronic high dose haloperidol treatment. Psychopharmacology communications 1:285-293.

Smith RC, Davis JM (1976) Behavioral evidence for supersensitivity after chronic administration of haloperidol, clozapine, and thioridazine. Life Sci 19:725-731.

Suhara T, Okauchi T, Sudo Y, Takano A, Kawabe K, Maeda J, Kapur S (2002) Clozapine can induce high dopamine D(2) receptor occupancy in vivo. Psychopharmacology (Berl) 160:107-112.

Suttajit S, Srisurapanont M, Xia J, Suttajit S, Maneeton B, Maneeton N (2013) Quetiapine versus typical antipsychotic medications for schizophrenia. Cochrane Database Syst Rev:CD007815.

Suzuki T, Kanahara N, Yamanaka H, Takase M, Kimura H, Watanabe H, Iyo M (2015) Dopamine supersensitivity psychosis as a pivotal factor in treatment-resistant schizophrenia. Psychiatry research 227:278-282.

Tadokoro S, Okamura N, Sekine Y, Kanahara N, Hashimoto K, Iyo M (2012) Chronic treatment with aripiprazole prevents development of dopamine supersensitivity and potentially supersensitivity psychosis. Schizophr Bull 38:1012-1020.

Takahashi K, Yamazawa R, Suzuki T, Mimura M, Uchida H (2020) Gap between patients with schizophrenia and their psychiatrists on the needs to psychopharmacological treatment: A cross-sectional study. Neuropsychopharmacol Rep.

Takase M, Kanahara N, Oda Y, Kimura H, Watanabe H, Iyo M (2015) Dopamine supersensitivity psychosis and dopamine partial agonist: a retrospective survey of failure of switching to aripiprazole in schizophrenia. J Psychopharmacol 29:383-389.

Takeuchi H, Fervaha G, Uchida H, Suzuki T, Bies RR, Gronte D, Remington G (2014) Impact of once- versus twice-daily perphenazine dosing on clinical outcomes: an analysis of the CATIE data. The Journal of clinical psychiatry 75:506-511.

Tauscher J, Kufferle B, Asenbaum S, Tauscher-Wisniewski S, Kasper S (2002a) Striatal dopamine-2 receptor occupancy as measured with [123I]iodobenzamide and SPECT predicted the occurrence of EPS in patients treated with atypical antipsychotics and haloperidol. Psychopharmacology (Berl) 162:42-49.

Tauscher J, Jones C, Remington G, Zipursky RB, Kapur S (2002b) Significant dissociation of brain and plasma kinetics with antipsychotics. Mol Psychiatry 7:317-321.

Thibault D, Albert PR, Pineyro G, Trudeau LE (2011) Neurotensin triggers dopamine D2 receptor desensitization through a protein kinase C and beta-arrestin1-dependent mechanism. The Journal of biological chemistry 286:9174-9184.

Tsuboi T, Suzuki T, Bies RR, Remington G, Pollock BG, Mimura M, Uchida H (2015) Challenging the need for sustained blockade of dopamine D(2) receptor estimated from antipsychotic plasma levels in the maintenance treatment of schizophrenia: A single-blind, randomized, controlled study. Schizophr Res 164:149-154.

Turrone P, Remington G, Kapur S, Nobrega JN (2003a) Differential effects of within-day continuous vs. transient dopamine D2 receptor occupancy in the development of vacuous chewing movements (VCMs) in rats. Neuropsychopharmacology 28:1433-1439.

Turrone P, Remington G, Kapur S, Nobrega JN (2003b) The relationship between dopamine D2 receptor occupancy and the vacuous chewing movement syndrome in rats. Psychopharmacology (Berl) 165:166-171.

Turrone P, Remington G, Kapur S, Nobrega JN (2005) Continuous but not intermittent olanzapine infusion induces vacuous chewing movements in rats. Biological psychiatry 57:406-411.

Uchida H, Suzuki T (2014) Dose and dosing frequency of long-acting injectable antipsychotics: a systematic review of PET and SPECT data and clinical implications. J Clin Psychopharmacol 34:728-735.

Uchida H, Takeuchi H, Graff-Guerrero A, Suzuki T, Watanabe K, Mamo DC (2011) Dopamine D2 receptor occupancy and clinical effects: a systematic review and pooled analysis. J Clin Psychopharmacol 31:497-502.

Uchida H, Mamo DC, Kapur S, Labelle A, Shammi C, Mannaert EJ, Mann SW, Remington G (2008) Monthly administration of long-acting injectable risperidone and striatal dopamine D2 receptor occupancy for the management of schizophrenia. The Journal of clinical psychiatry 69:1281-1286.

von Euler G, Mailleux P, Vanderhaeghen JJ, Fuxe K (1990) Neurotensin reduces the affinity of dopamine D2 receptors in membranes from post mortem human caudate-putamen. Neurosci Lett 109:325-330.

Vonvoigtlander PF, Losey EG, Triezenberg HJ (1975) Increased sensitivity to dopaminergic agents after chronic neuroleptic treatment. The Journal of pharmacology and experimental therapeutics 193:88-94.

Wadenberg ML, Hicks PB (1999) The conditioned avoidance response test re-evaluated: is it a sensitive test for the detection of potentially atypical antipsychotics? Neurosci Biobehav Rev 23:851-862.

Wadenberg ML, Soliman A, VanderSpek SC, Kapur S (2001) Dopamine D(2) receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical effects. Neuropsychopharmacology 25:633-641.

Wadenberg ML, Kapur S, Soliman A, Jones C, Vaccarino F (2000) Dopamine D2 receptor occupancy predicts catalepsy and the suppression of conditioned avoidance response behavior in rats. Psychopharmacology (Berl) 150:422-429.

Whitaker R (2004) The case against antipsychotic drugs: a 50-year record of doing more harm than good. Med Hypotheses 62:5-13.

Wiesel FA, Farde L, Nordstrom AL, Sedvall G (1990) Central D1- and D2-receptor occupancy during antipsychotic drug treatment. Progress in neuro-psychopharmacology & biological psychiatry 14:759-767.

Wunderink L, Nieboer RM, Wiersma D, Sytema S, Nienhuis FJ (2013) Recovery in remitted first-episode psychosis at 7 years of follow-up of an early dose reduction/discontinuation or maintenance treatment strategy: long-term follow-up of a 2-year randomized clinical trial. JAMA Psychiatry 70:913-920.

Wunderink L, Nienhuis FJ, Sytema S, Slooff CJ, Knegtering R, Wiersma D (2007) Guided discontinuation versus maintenance treatment in remitted first-episode psychosis: relapse rates and functional outcome. The Journal of clinical psychiatry 68:654-661.

Yaegashi H, Kirino S, Remington G, Misawa F, Takeuchi H (2020) Adherence to Oral Antipsychotics Measured by Electronic Adherence Monitoring in Schizophrenia: A Systematic Review and Meta-analysis. CNS drugs 34:579-598.

Yoshida K, Bies RR, Suzuki T, Remington G, Pollock BG, Mizuno Y, Mimura M, Uchida H (2014) Tardive dyskinesia in relation to estimated dopamine D2 receptor occupancy in patients with schizophrenia: analysis of the CATIE data. Schizophr Res 153:184-188.