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Augmenter la vitesse d'infusion de la cocaïne par voie intraveineuse induit des changements neurochimiques, neurobiologiques et comportementaux chez le rat: Implications pour la toxicomanie

par

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Augmenter la vitesse d'infusion de la cocaïne par voie intraveineuse induit des changements neurochimiques, neurobiologiques et comportementaux chez le rat: Implications pour la toxicomanie

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RÉSUMÉ

Plusieurs individus consomment la cocaïne, mais peu développent des troubles liés à l'usage de la drogue. Les facteurs pharmacocinétiques de la drogue et le patron de consommation sont importants dans ce processus. Les drogues et les voies d'administration qui produisent l'augmentation rapide des niveaux de drogue dans le cerveau faciliteraient cette transition. Similairement, l'accès intermittent à la cocaïne, qui induit des fluctuations de drogue dans le cerveau, serait impliqué aussi. Le travail présenté ici regarde de près comment la vitesse d'administration et la fréquence de consommation induisent des comportements et de la neuroplasticité caractéristiques à la toxicomanie chez le rat. Dans une première étude, nous avions déterminé les effets de la cocaïne sur les niveaux de drogue et de dopamine dans le striatum dorsal par microdialyse. La gamme de vitesses utilisée (5-90 secondes (s)) n'a pas modifié la concentration maximale de ces deux molécules, mais les infusions rapides ont fait que ces concentrations maximales soient atteintes plus rapidement. Ainsi, les différences induites par la vitesse d'administration sur le comportement et la neuroplasticité seraient dues à la vitesse à laquelle la drogue arrive au cerveau, et non à la quantité de drogue qu'y parvient. Nos travaux précédents montrent que les infusions rapides de cocaïne augmentent la motivation pour la drogue. Afin de mieux comprendre les mécanismes qui sous-tendent cette augmentation, nous avions examiné la neuroplasticité induite par l'infusion rapide de cocaïne chez des animaux montrant une motivation excessive pour la drogue sous ratio progressif. Premièrement, nous avions étudié les effets de la vitesse d'infusion sur l'expression corticostriatale de l'ARN messager (ARNm) du *brain-derived neurotrophic factor* (BDNF) et TrkB, son récepteur, chez des rats ayant un historique de consommation en continu (6 heures/session). Seulement les rats s'injectant des infusions rapides de drogue (infusées en 5 s) ont montré des altérations dans les niveaux de BDNF et TrkB, telles que l'augmentation des niveaux de l'ARNm de BDNF dans les cortex orbitofrontal, frontal et pariétal et la baisse des niveaux de l'ARNm de TrkB dans les cortex cingulé, frontal et pariétal et le striatum dorsal versus les rats s'auto-administrant des infusions plus lentes (infusées en 90 s). Ensuite, nous avions utilisé un modèle d'accès intermittent, où la concentration de cocaïne fluctue durant la session, pour

déterminer les effets de la vitesse d'infusion sur l'expression de l'ARN messager c-*fos*, un marqueur d'activité neuronale. Il a été récemment reporté dans la littérature que les consommateurs de cocaïne expérimentés ne consomment pas de façon continue, mais préfèrent espacer leur consommation, donc un modèle d'accès intermittent serait plus approprié pour l'étude de la toxicomanie. Ainsi, un premier groupe d'animaux a eu un accès intermittent à des infusions rapides de cocaïne, un modèle considéré pertinent à la toxicomanie, tandis que l'autre a eu accès à des infusions lentes en continu, qui permet une consommation importante sans provoquer des symptômes caractéristiques à la toxicomanie. Le groupe pertinent à la toxicomanie était plus motivé pour la drogue. De plus, ce groupe a montré une augmentation de l'expression de c-*fos* dans plusieurs structures corticostriatales. Ces résultats suggèrent que les infusions rapides et intermittentes de cocaïne facilitent le développement de neuroplasticité dans le cerveau, qui peut ensuite changer le comportement. De plus, le cortex orbitofrontal et le striatum dorsal seraient potentiellement impliqués. Ces deux structures sont interconnectées et sont engagées dans la toxicomanie. Nous avions déconnecté temporairement et pharmacologiquement ce circuit et avions observé une baisse significative de la motivation pour la drogue. En conclusion, les facteurs pharmacocinétiques sont importants dans le développement de modèles animaux adéquats de la toxicomanie. Une meilleure compréhension des mécanismes impliqués pourrait éventuellement améliorer le développement de pharmacothérapies pour la prévention et le traitement de la toxicomanie.

Mots clés : Accès intermittent, accès prolongé, cocaïne, dopamine, microdialyse, motivation, ratio progressif, toxicomanie, vitesse d'infusion

ABSTRACT

Many individuals will experiment with cocaine during their lifetime; yet, few will transition from occasional drug use to addiction. Pharmacokinetic factors of the drug and the way it is consumed are important in this process. Drugs and routes of administration that produce the rapid rise of drug levels in the brain are believed to facilitate this transition. Similarly, intermittent access to the drug, which provokes fluctuations of cocaine concentrations in the brain rather than maintaining continuous high levels, may also be involved. The work presented in this thesis examines how speed of drug delivery and frequency of intake may induce addiction-like symptoms in a rat model of cocaine self-administration and promote neuroplasticity. In a first study, microdialysis was used to determine the effects of rate of cocaine infusion on cocaine and dopamine levels in the dorsal striatum of awake, freely-moving rats. Over the range of infusion rates used (5-90 seconds), cocaine and dopamine concentrations did not significantly vary. Instead, rapid cocaine infusions lead to the faster rate of onset of both molecules in the brain, implying that differences in cocaine-induced behaviour and neuroplasticity are not due to how much drug reaches the brain but on how fast the drug does so. Previous work from our laboratory showed that rapid cocaine infusions increase motivation for cocaine, a symptom of addiction. Therefore, we examined potential changes in the brain caused by rapid cocaine infusions in animals showing excessive motivation for the drug as assessed under a progressive ratio schedule of reinforcement. First, we investigated the effects of cocaine infusion rate on corticostriatal expression of brain-derived neurotrophic factor (BDNF) and TrkB mRNA in rats with a history of chronic and continuous exposure to cocaine under long access conditions (6 hours/session) and found that only rats self-administering rapid cocaine injections had altered BDNF and TrkB mRNA levels. Next, new evidence suggested that experienced cocaine users do not consume the drug continuously, but rather space out each intoxicating event. Therefore, using an intermittent access model of self-administration, where brain cocaine levels fluctuate within the session, we examined the effects of rate of infusion on c-*fos* mRNA expression, a marker of neuronal activity. A first group of rats had intermittent access to rapid cocaine infusions, a model we consider as addiction-relevant, while the second had access to

slow and continuous cocaine, which promotes significant cocaine intake without promoting addiction-like behaviour. Animals in the addiction-relevant group presented greater motivation for cocaine and showed greater c-*fos* mRNA expression in various corticostriatal regions. Together, these results suggest that rapid and intermittent cocaine infusions facilitate the development of drug-induced neuroplasticity which may cause addiction-like behaviour, and particularly implicate the orbitofrontal cortex and the dorsal striatum. These two interconnected structures are both involved in addiction. Therefore, we temporarily and pharmacologically disconnected this circuit and found that it reduces motivation for cocaine. In conclusion, we show that pharmacokinetic factors are important when developing animal models of addiction, and that better understanding the mechanisms involved can potentially improve prevention and treatment therapies.

Keywords: Addiction, cocaine, dopamine, intermittent access, long access, microdialysis, motivation, rate of infusion, progressive ratio

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LISTE DES ABBRÉVIATIONS

AMPA, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

BDNF, brain-derived neurotrophic factor

BE, benzoylécgonine

Cmax, concentration maximale

DAT, transporteur de la dopamine

DS, striatum dorsal

EAAT, excitatory amino acid transporter

EME, écgonine méthyl ester

FR, ratio fixe

h, heure

IntA, accès intermittent

i.v., voie intraveineuse

i.p., voie intrapéritonéale

LgA, accès prolongé

LTD, long term depression

NC, norcocaine

NMDA, N-methyl-D-aspartate

OFC, cortex orbitofrontal

PR, ratio progressif

PrL, cortex prélimbique

s, seconde

ShA, accès limité

Tmax, temps requis pour atteindre la concentration maximale

TrkB, tropomyosin receptor kinase B

VGLUT, transporteur du glutamate

“La vraie sagesse est de savoir

que vous ne savez rien.”

- Socrates

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Introduction

CHAPITRE 1 – La toxicomanie

1.1 Description et statistiques

Les statistiques reportent qu'environ 250 millions d'individus (ou 5% de la population globale) ont consommé de la drogue au moins une fois en 2015 (UNODC, 2017). Malgré ce chiffre important, il faut noter que la majorité des consommateurs de drogues les utilisent de façon récréative. En effet, les études épidémiologiques suggèrent que très peu parmi ces derniers vont développer une addiction à leur drogue de choix [par exemple, 17% des consommateurs de cocaïne; (Anthony et Helzer, 2003; Anthony, Warner et Kessler, 1994)]. Par contre, il faut penser aux 29.5 millions d'individus parmi ces derniers qui souffrent de troubles liés à la drogue (UNODC, 2017). La toxicomanie est une maladie complexe. Elle est chronique et se caractérise par la recherche et la consommation de drogue compulsives et pathologiques, et qui persistent malgré des conséquences négatives (Camí et Farré, 2003).

Le fait de consommer une drogue n'est pas équivalent à avoir un trouble lié à l'usage de cette drogue. En effet, la toxicomanie consiste de nombreux symptômes, qui rendent ce trouble très complexe. Le "Diagnostic and Statistical Manual of Mental Disorders" (DSM-V) décrit 11 critères pour les troubles associés à la consommation d'une substance (APA, 2013). Les symptômes consistent d'une consommation de drogue en grandes quantités ou pour des périodes plus longues que prévues, le désir persistant ou infructueux pour réduire la consommation, ainsi qu'un temps et effort important pour chercher et obtenir la drogue ou récupérer de ses effets. De plus, l'individu ressent une envie intense de consommer (*craving*). Un autre symptôme qui marque la toxicomanie est le fait que la prise de drogue monopolise l'individu et donc fait diminuer l'attention

accordée aux activités sociales, occupationnelles et récréatives et rend l'individu incapable de remplir ses obligations majeures. Cette utilisation persiste malgré le fait que l'individu sache qu'il a un problème d'abus de drogues, que ses relations interpersonnelles et sociales sont affectées ou qu'il met sa vie en danger. Finalement, des symptômes de la tolérance ou de sevrage peuvent être ressentis.

1.2 Les facteurs impliqués dans la toxicomanie

Plusieurs facteurs peuvent rendre un individu plus à risque de développer la toxicomanie. Ces facteurs de risque incluent la variabilité individuelle et l'environnement. De plus, le développement de la toxicomanie peut aussi être facilité par les particularités de la drogue consommée, ainsi que certaines formes de neuroplasticité induites par la consommation de drogue. En effet, la vitesse et la fréquence à laquelle la drogue arrive à ses sites d'actions dans le cerveau peuvent influencer le risque de développer la toxicomanie. De plus, la consommation de drogues d'abus induit des changements neuronaux persistants au niveau moléculaire, cellulaire et structurel qui peuvent aussi être impliqués dans la toxicomanie. Ces aspects pharmacocinétiques et la neuroplasticité qu'ils induisent seront adressés dans cette thèse.

1.2.1 La variabilité individuelle

1.2.1.1 La prédisposition génétique

La transmission familiale des troubles liés à la consommation de drogues serait un facteur de risque important dans le développement de la toxicomanie (Cotton, 1979; Merikangas, Stolar, Stevens et et al., 1998). Par exemple, les membres de la famille des

individus ayant des troubles liés à l'usage des drogues seraient 8 fois plus à risque de développer des problèmes de consommation comparativement aux membres des familles des individus contrôles, qui n'ont pas un historique de troubles liés à la consommation de drogues (Merikangas, Stolar, et al., 1998). De plus, chez les individus adoptés, le risque de développer un trouble lié à l'usage des drogues augmente lorsque les parents biologiques sont consommateurs de drogues comparativement à d'autres individus élevés dans une situation adoptive similaire (Cadoret, Yates, Ed, Woodworth et Stewart, 1995).

Nombreuses études sur les familles (Bierut et al., 1998; Merikangas, Stolar, et al., 1998; Mirin, Weiss, Griffin et Michael, 1991), les jumeaux (Prescott et Kendler, 1999; Tsuang et al., 1996) et les individus adoptés (Cadoret, Troughton, O'Gorman et Heywood, 1986; Cadoret et al., 1995) suggèrent qu'une partie de l'implication de la transmission familiale dans le développement de la toxicomanie se baserait sur la génétique. Certaines variations génétiques rendraient les individus plus susceptibles à consommer la drogue et à développer des troubles liés à leur usage, par la suite. Par exemple, le polymorphisme nucléotidique (SNP) rs16969968 du gène qui code la sous-unité α5 du récepteur nicotinique cholinergique (CHRNA5) cause une substitution D398N, qui modifie le fonctionnement du récepteur (Bierut et al., 2008) et double le risque de développer des troubles liés à l'usage de la nicotine une fois exposé à la cigarette (Saccone et al., 2007). Similairement, une substitution A118G au niveau du gène codant le récepteur opioïde μ modifie les niveaux des sites de N-glycosylation, ce qui change la liaison entre le récepteur et la beta-endorphine, modifie ainsi ses propriétés et augmente le risque d'abus de l'héroïne et des autres opiacés (Bond et al., 1998). En effet, la littérature rapporte de

plus en plus de polymorphismes qui prédisposeraient aux troubles liés à l'usage des drogues, dont l'alcool (Lappalainen et al., 2002; Ray et Hutchison, 2004), la cocaïne (Ballon et al., 2005; Shumay et al., 2012), les opioïdes (Gerra et al., 2007; Szeto, Tang, Lee et Stadlin, 2001), le cannabis (Agrawal et al., 2009; Hopfer et al., 2007), la nicotine (Feng et al., 2004; Huang et al., 2008) et même la caféine (Cornelis et al., 2011; Josse, Da Costa, Campos et El-Sohemy, 2012). Cependant, certains facteurs génétiques peuvent aussi être protecteurs (Chen et al., 2002; Rao et al., 2000; Schoedel, Hoffmann, Rao, Sellers et Tyndale, 2004; Thomasson, Crabb, Edenberg et Li, 1993). Un exemple, serait une variation allélique dans les gènes de l'alcool déshydrogénase et de l'aldéhyde déshydrogénase chez la population asiatique qui encode une isoenzyme dont la fonctionnalité est réduite. Ceci provoque nombreux effets adverses suite à la consommation d'alcool par ces individus, et ainsi diminue leur risque d'abuser l'alcool (Thomasson et al., 1993). Un autre exemple chez les individus dépendants à la nicotine serait la présence d'un allèle défectueux du cytochrome P-450 2A6 (*2 et *4). Cette variation cause un métabolisme moins fonctionnel qui diminue le nombres de cigarettes fumées comparativement aux individus possédant une allèle normale (Rao et al., 2000).

1.2.1.2 Les traits de personnalité particuliers

Certains traits de personnalité peuvent aussi augmenter la vulnérabilité aux troubles liés à l'usage des drogues d'abus. En effet, les personnes qui montrent des traits associés à une grande recherche de nouveauté et de sensations, l'impulsivité, l'agressivité et la recherche du risque seraient plus aptes à expérimenter avec les drogues d'abus et seraient donc plus à risque de devenir toxicomanes (Kosten, Ball et Rounsaville, 1994; Krueger, 1999). Par exemple, plusieurs études suggèrent que la

dysfonction cognitive et les traits de personnalité anxieux, impulsifs et compulsifs seraient des endophénotypes pour les troubles liés à l'usage des psychostimulants (Ersche et al., 2013; Ersche, Turton, Pradhan, Bullmore et Robbins, 2010; Karen D. Ersche et al., 2012). Ces études rapportent que les individus ayant un trouble lié à l'usage des stimulants, ainsi que leurs frères et sœurs n'ayant pas un historique de consommation de drogue, montreraient tous les deux plus de déficiences en leurs fonctions cognitives et exécutives et plus de traits anxieux-impulsifs que des sujets contrôles sains. De plus, ces déficiences sont plus prononcées chez les individus avec des troubles liés à l'usage de la drogue que leurs membres de familles, suggérant qu'elles s'empirent avec la consommation chronique de la drogue (Ersche et al., 2013; Ersche et al., 2010; Karen D. Ersche et al., 2012).

1.2.1.3 L'âge

La littérature suggère que les troubles liés à l'usage des drogues apparaissent plus rapidement lorsque la consommation commence à un jeune âge (Anthony et Petronis, 1995; Chen, O'Brien et Anthony, 2005; Chen, Storr et Anthony, 2009). Cette conséquence de l'initiation précoce de la consommation est vraie pour plusieurs substances, dont les cigarettes [nicotine; (Breslau, Fenn et Peterson, 1993)], l'alcool (Grant et Dawson, 1997; Nelson et Wittchen, 1998) et le cannabis (Chen et al., 2005). Ainsi, les personnes qui ont fumé leur première cigarette entre 14 et 16 ans sont 1.6 fois plus à risque de développer des troubles de dépendance que ceux qui ont commencé plus tard (Breslau et al., 1993).

Il faut aussi mentionner que l'âge d'apparition de la consommation d'une drogue peu aussi prédire l'abus subséquent d'une autre drogue. Par exemple, l'âge du début de

la consommation d'alcool influence la possibilité de commencer la consommation de marijuana (Yamaguchi et Kandel, 1984). Chez les mâles qui ont continué à boire l'alcool régulièrement depuis leur première consommation, un 39% supplémentaire aura aussi initié la prise de marijuana à l'âge de 25 ans parmi ceux qui ont débuté leur consommation d'alcool à 15 ans versus ceux qui ont commencé à boire à 21 ans (Yamaguchi et Kandel, 1984). Similairement, plus un individu est jeune lors du début de sa première consommation de cigarettes, plus le risque de développer des troubles liés à l'usage de l'alcool et des drogues d'abus est augmenté (Lewinsohn, Rohde et Brown, 1999).

1.2.1.4 Le sexe

Des différences entre les sexes seraient aussi un facteur important dans la prédisposition à développer un trouble lié à l'usage des drogues d'abus. Par exemple, les femmes boivent moins d'alcool que les hommes et ont moins de problèmes associés avec sa consommation (Nolen-Hoeksema, 2004). Des statistiques provenant du *National Longitudinal Alcohol Epidemiologic Survey*, une étude basée sur 42 862 Américains âgés de 18 ans et plus, rapportent que les mâles sont plus à risque que les femmes à répondre à tous les critères du DSM-IV pour les troubles liés à la consommation d'alcool [hommes: 18.55% vs. femmes: 8.43%; (Grant, 1997)]. Les femmes consomment moins d'alcool, sont moins susceptibles que les hommes de boire quotidiennement et de consommer de façon excessive en « binges », atteignent des taux d'alcoolémie plus élevés, sont plus sensibles aux effets physiologiques de l'alcool [(Mumenthaler, Taylor, O'Hara et Yesavage, 1999) et révisé dans (Fattore, Altea et Fratta, 2008)] et développent plus rapidement des dommages au cerveau [atrophie; (Mann et al., 2005)] que les hommes. Aussi, tandis que la consommation d'alcool semble être nécessaire à la progression vers

l'usage de drogues illicites chez les hommes, cette transition impliquerait plutôt l'abus de la nicotine chez les femmes (Fattore et al., 2008). Cependant, parmi les hommes et les femmes qui sont vulnérables aux troubles liés à l'usage des drogues, les femmes progressent plus rapidement entre leur première consommation de drogue et la toxicomanie (Anglin, Hser et McGlothlin, 1987; Brady et Randall, 1999). De plus, elles expérimentent avec les drogues d'abus à un plus jeune âge que les hommes. Par exemple, plus d'adolescentes rapportent des troubles liés à l'usage de la cocaïne, une consommation plus fréquente et plus de symptômes d'intoxication à des faibles doses de drogues que les adolescents. Toutefois, cette distinction entre les sexes disparait à l'âge adulte (Chen et Kandel, 2002). Similairement, les femmes sont plus susceptibles à initier l'usage de la méthamphétamine à un plus jeune âge que les hommes, mais elles démontrent des améliorations plus importantes que les hommes lorsqu'elles reçoivent un traitement pour leurs troubles liés à l'usage de cette drogue (Hser, Evans et Huang, 2005). De plus, les femmes éprouvent plus de difficulté à cesser l'utilisation de la drogue et sont plus vulnérables à la rechute que les hommes. Par exemple, les femmes sont plus à risque de développer des troubles liés à l'usage de la nicotine [femmes : 30.2% vs. hommes 26.9%; (Kandel, Chen, Warner, Kessler et Grant, 1997)], signalent une plus grande difficulté à cesser de fumer (Carpenter, Upadhyaya, LaRowe, Saladin et Brady, 2006) et sont plus susceptibles à la rechute [57% plus à risque; (McWhorter, Boyd et Mattson, 1990; Ward, Klesges, Zbikowski, Bliss et Garvey, 1997)] que les hommes.

Ces disparités pourraient être dues à différents facteurs. Premièrement, il y a les différences hormonales entre les sexes. Par exemple, les hormones ovariennes pourraient influencer la vulnérabilité aux troubles liés à la consommation de la cocaïne.

Les femmes en phase folliculaire de leur cycle menstruel (caractérisée par des taux d'estradiol élevés et des taux de progestérone bas) ressentent plus d'effets subjectifs positifs tels qu'un « high » et une « stimulation » lorsque la cocaïne est fumée qu'en phase lutéale (où les niveaux d'estradiol et de progestérone sont tous les deux élevés) (Evans, Haney et Foltin, 2002; Sofuoglu, Dudish-Poulsen, Nelson, Pentel et Hatsukami, 1999). Par contre, cet effet semble dépendre de la voie d'administration et aucune différence est détectée lorsque la cocaïne est prise par la voie intranasale (Collins, Evans, Foltin et Haney, 2007). Similairement, une étude chez les rats suggère que les androgènes augmentent l'activité de l'enzyme hépatique d'alcool déshydrogénase qui est responsable de l'élimination de l'alcool. Ainsi, comme l'élimination est nécessaire pour éviter les conséquences négatives de la consommation d'alcool, il se peut que le fait que cette enzyme est plus active chez les hommes favorise le développement des troubles liés à son usage car ils ressentent moins d'effets, tandis qu'elle diminue la consommation chez les femmes qui veulent éviter les conséquences négatives de l'intoxication (Lakoza et Barkov, 1980). Un deuxième facteur impliquerait des différences dans la morphologie du cerveau et dans la neurotransmission. Par exemple, une étude employant la tomographie par émission de positrons en 3-D et le radioligand [^{11}C] FLB 457 qui possède une haute affinité pour les récepteurs extrastriataux de la famille D2 démontre que les récepteurs D2 du cortex frontal des femmes montrent plus de potentiels de liaison que ceux des hommes (Kaasinen, Någren, Hietala, Farde et Rinne, 2001). Ces résultats pourraient, en partie, expliquer les différences observées dans la vulnérabilité aux drogues d'abus entre les sexes. Finalement, il ne faut pas oublier les différences pharmacocinétiques et pharmacodynamiques qui existent entre les sexes, et qui peuvent

influencer la consommation de drogue ainsi que la vulnérabilité aux troubles liés à leur usage. Des différences entre les sexes existent au niveau de la biodisponibilité, de la distribution et de la clairance des drogues. Par exemple, la filtration glomérulaire est supérieure chez les hommes que les femmes (Gross, Friedman, Azevedo, Silveiro et Pecis, 1992), ce qui influence le temps que la drogue reste dans le corps. Similairement, la majorité des psychostimulants sont lipophiles. Ainsi, des différences entre le ratio muscle:gras chez les hommes et les femmes feraient en sorte que ces drogues restent plus longtemps dans le corps des femmes, qui elles possèdent plus de tissu adipeux que les hommes (Fattore et al., 2008).

1.2.1.5 La comorbidité avec d'autres troubles psychiatriques

Les personnes souffrant de troubles psychiatriques démontrent une incidence plus élevée d'usage de drogues et de troubles associés à cette utilisation (Grant et Harford, 1995; Merikangas, Mehta, et al., 1998; Regier et al., 1990; Swendsen et Merikangas, 2000). Les résultats du *National Epidemiologic Survey on Alcohol and Related Conditions – III*, une étude conçue pour mesurer les troubles liés à l'usage des drogues, de l'alcool et de la nicotine, ainsi que des troubles psychiatriques en fonction du DSM-V chez des résidents américains non institutionnalisés de 18 ans et plus a signalé plusieurs associations significatives entre les troubles liés à l'usage de la drogue et le trouble dépressif majeur, la dysthymie, le trouble bipolaire, le trouble de stress post-traumatique, le trouble de la personnalité antisociale, le désordre anxieux généralisé, la phobie sociale, le trouble de la panique et les troubles de la personnalité schizotypiques (Grant, Saha, Ruan et et al., 2016). Cette comorbidité entre la toxicomanie et les troubles psychiatriques peut être alimentée par le besoin de s'auto-médiquer, afin d'alléger les symptômes

négatifs provenant du trouble psychiatrique. Ainsi, les individus qui souffrent du trouble d'anxiété sociale éprouvent souvent aussi des troubles liés à l'usage de l'alcool, car ils boivent pour se sentir mieux dans leur peau lors de situations sociales (Morris, Stewart et Ham, 2005). De même, les vétérans de guerre diagnostiqués avec un trouble de stress post-traumatique souffrent aussi souvent d'un trouble lié à l'usage des drogues ou de l'alcool (Back et al., 2014; Carlson et al., 2010). Une autre raison expliquant pourquoi les deux types de troubles apparaissent souvent ensemble est le fait que la consommation chronique de drogue induise des symptômes qui persistent et qui peuvent déclencher le trouble psychiatrique. Par exemple, la consommation d'alcool par les femmes augmente depuis les dernières trois décennies, et ceci met les femmes plus à risque d'avoir des rapports sexuels non protégés, de contracter une maladie transmise sexuellement ou d'être victime d'une agression sexuelle ou d'un viol (Hingson, Heeren, Winter et Wechsler, 2005), qui à la suite peut causer un trouble de stress post-traumatique ou une dépression (Rothbaum, Foa, Riggs, Murdock et Walsh, 1992). Un autre cas serait la consommation de méthamphétamine qui produit une dépression lorsque l'effet de la drogue se dissipe, et cette dépression est un symptôme qui peut s'approfondir et s'empirer avec le temps et la consommation chronique (Darke et al., 2008).

1.2.2 Les facteurs environnementaux

Un deuxième facteur qui peut favoriser le développement de la toxicomanie est l'environnement. L'environnement dans lequel la drogue est administrée, ainsi que les stimuli qui sont associés avec la drogue, peuvent moduler les effets comportementaux et subjectifs de la drogue. Par exemple, les lieux d'injection, le matériel utilisé pour la consommation (e.g. seringues et pipes), les individus avec qui la consommation se fait

et les comportements qui suivent la consommation sont tous des stimuli associés avec la consommation de drogues. Ces stimuli sont si forts qu'ils peuvent réinitier la consommation même après sa discontinuation (Childress, Ehrman, McLellan et O'Brien, 1988; Childress et al., 1993). En effet, suite à quelques prises de drogues, des indices environnementaux neutres peuvent acquérir un état motivationnel positif équivalent à la drogue elle-même. Ainsi, les appariements des stimuli environnementaux avec le stimulus non-conditionné (la consommation directe de la drogue), transformeront ces stimuli environnementaux en stimuli conditionnés qui auront eux aussi la capacité d'évoquer une réponse similaire à la consommation de drogue (Siegel, 2005; Stewart, De Wit et Eikelboom, 1984). Les stimuli associés aux drogues d'abus favorisent de développement de la toxicomanie, car ils motivent la recherche et la prise de drogue (Ehrman, Robbins, Childress et O'Brien, 1992) et peuvent aussi provoquer la perte de contrôle et l'incapacité de résister au désir de consommer celle-ci (O'Brien, Childress, Ehrman et Robbins, 1998). De plus, cette envie intense met l'individu à risque de rechute, malgré une longue période d'abstinence et l'absence de symptômes de sevrage (De Wit et Stewart, 1981), et explique pourquoi la thérapie pour les troubles liés à l'usage des drogues doit aussi cibler la diminution des réponses conditionnées (O'Brien et al., 1998).

L'environnement familial, les expériences de la vie et le stress (Caprioli, Celentano, Paolone et Badiani, 2007; E. Goeders, 2003; Kreibich et al., 2009; Sinha, 2008) peuvent aussi favoriser le développement de troubles liés à l'usage des drogues. Nombreuses études suggèrent que le stress lié au combat (Triffleman, Marmar, Delucchi et Ronfeldt, 1995), aux événements violents traumatisants (Clark, Masson, Delucchi, Hall et Sees, 2001), à l'insatisfaction conjugale (José, Van Oers, Van De Mheen, Garretsen

et Mackenbach, 2000), aux traumatismes induits lors de l'enfance (Triffleman et al., 1995) et au stress professionnel (Fennell, Rodin et Kantor, 1981; José et al., 2000) peut prédisposer certains individus à initier un comportement de prise de drogue et favoriser des troubles liés à l'usage des drogues.

1.2.3 Les caractéristiques de la drogue

Les facteurs pharmacocinétiques de la drogue, les formulations de la substance et la voie d'administration utilisée pour la consommation influencent le risque de développer la toxicomanie. Par exemple, le métabolisme d'une drogue modifie ses effets. Le métabolisme de la cocaïne se fait très rapidement et produit principalement deux métabolites inactifs, la benzoylécgonine et l'écgonine méthyl ester, qui n'ont aucun effet subséquent (Jones, 1984). Au contraire, la cocaïne peut aussi être métabolisée par les microsomes hépatiques en norcocaine, un métabolite aussi actif que la cocaïne et qui est impliqué dans l'hépatotoxicité (Kloss, Rosen et Rauckman, 1984). Il est aussi possible que le métabolite soit plus actif que la substance parentale, tel qu'est le cas avec le nordiazepam, un métabolite du prazepam (Caccia et Garattini, 1990).

La formulation de la drogue influence aussi la pharmacocinétique de celle-ci. Ceci se voit particulièrement bien avec la cocaïne qui est disponible sous nombreuses formes et qui est consommée par différentes voies d'administration. Par exemple, la cocaïne HCl (sel hydrochlorique) est une formulation hydrosoluble qui favorise sa consommation via les voies intranasale, orale ou intraveineuse (Warner, 1993). Par contre, elle est aussi caractérisée par un point de fusion très bas, qui cause sa destruction complète lorsqu'elle est brulée, et donc ne lui permet pas d'être fumée (Warner, 1993). Cependant, cet inconvénient a permis la création de la cocaïne *freebase* ou crack, une forme basique de

la drogue qui peut être consommée par inhalation, car elle résiste à la pyrolyse (Jones, 1990). La formulation influence aussi la quantité de drogue qui arrive au cerveau. Les feuilles de coca ne contiennent qu'environ 0.5-1% cocaïne. La prise orale des feuilles de coca réduit la biodisponibilité de la drogue à 20-40% (Jones, 1984; Verebey et Gold, 1988), tandis que la consommation de la cocaïne HCl par voie intraveineuse mène 100% de la cocaïne consommée directement dans le système circulatoire et donc plus de drogue rentre dans le cerveau (Verebey et Gold, 1988).

La voie d'administration de la cocaïne influence aussi le risque de toxicomanie pour une drogue, car elle module la vitesse d'apparition des effets de la drogue, ainsi que leur intensité et leur durée d'action. En effet, plus une drogue arrive rapidement au cerveau, plus la drogue risque d'être abusée [(Ferri et Gossop, 1999; Gossop, Griffiths, Powis et Strang, 1992; Gossop, Griffiths, Powis et Strang, 1994; Hatsukami et Fischman, 1996) et révisé dans (Allain, Minogianis, Roberts et Samaha, 2015)]. Dans le cas de la cocaïne, la voie intraveineuse et l'inhalation sont les voies qui mènent de hautes concentrations de drogue au cerveau très rapidement, contrairement aux voies orale et intranasale (Hatsukami et Fischman, 1996; Jones, 1990). Les effets de la vitesse d'infusion de la cocaïne sur la consommation, la motivation pour la drogue et la neuroplasticité induite dans les régions de la récompense et de la motivation du cerveau seront décrits lors de cette thèse.

1.2.4 La neuroplasticité induite par la consommation de drogue

Un aspect important de la recherche en toxicomanie est d'identifier les changements persistants induits par les drogues d'abus dans le cerveau. La théorie de l'attribution pathologique d'une valeur motivationnelle à un stimulus ou <<the incentive

sensitization theory>> postule que la consommation des drogues d'abus rend le système de récompense hypersensible (Robinson et Berridge, 1993). L'hypersensibilisation du système dopaminergique impliquerait de la neuroplasticité dans les régions de la récompense du cerveau. Ces neuroadaptations rendraient la drogue plus désirable et favoriseraient des comportements de recherche et de prise de drogue compulsifs (Anagnostaras et Robinson, 1996; Robinson et Berridge, 1993). Similairement, la neuroplasticité induite au niveau du système glutamatergique faciliterait le développement de la toxicomanie (Kalivas, 2004, 2009; Kalivas, LaLumiere, Knackstedt et Shen, 2009; Kalivas et O'Brien, 2008). Ces changements peuvent être moléculaires ou structurels. Des neuroadaptations moléculaires induites par l'administration chronique de drogues, par exemple, se caractérisent par des changements dans l'expression de plusieurs facteurs de transcription (tels que ΔfosB et CREB) dans les régions du cerveau qui sont impliquées dans la récompense et la motivation (Larson et al., 2011; McClung et al., 2004; Nestler, 2001, 2004b). Au niveau structurel, plusieurs études montrent les changements provoqués dans l'organisation des dendrites des neurones moyens épineux à la suite de l'administration chronique de drogue. Les neurones épineux sont impliqués dans la signalisation excitatrice du cerveau (Shepherd, 1996). L'administration répétée de cocaïne et d'amphétamine augmente l'arborisation dendritique et la densité épineuse des dendrites sur les neurones moyens épineux du noyau accumbens et des cellules pyramidales du cortex préfrontal (Robinson, Gorny, Mitton et Kolb, 2001; Robinson et Kolb, 1997, 1999a). À l'opposé, l'administration chronique de morphine diminue l'arborisation et la densité épineuse des neurones moyens épineux dans le noyau accumbens et les cortex frontal et pariétal (Robinson et Kolb, 1999b). De plus, la

morphine diminue la taille des neurones dopaminergiques mésolimbiques (Sklair-Tavron et al., 1996). Ceci n'est qu'un survol, et nombreuses neuroadaptations seront discutées dans cette thèse.

CHAPITRE 2 – La cocaïne

2.1 Les statistiques et l'historique

Dix-sept millions de personnes mondialement ont consommé de la cocaïne en 2015 et, fait troublant, l'Amérique du Nord est le plus grand marché de cocaïne dans le monde (UNODC, 2017). Depuis 2013, la prévalence annuelle d'usage de cocaïne augmente continuellement et, en 2015, a été reportée comme étant 1.75% aux États-Unis et 1.5% au Canada (UNODC, 2017). Plus proche de nous, la quantification du métabolite inactif de la cocaïne, la benzoylécgonine, dans les eaux d'égouts (une analyse qui permet l'étude des tendances de consommation de cocaïne) indique que les taux à Montréal sont plus élevés que la moyenne globale (450 mg versus la moyenne de 200 mg/jour/1000 habitants) (UNODC, 2017).

Par contre, la consommation de cocaïne n'a pas commencé récemment. Cette drogue, qui provient de la feuille des arbrisseaux *Erythroxylon coca* et *Eythroxylon novogranatense*, possède une longue histoire. Des gourdes en lime utilisées par les mâcheurs de coca ont été retrouvés dans les anciens sites d'enterrement datant de 8850-4650 av. J.-C. (Stolberg, 2011). Initialement, cette plante était prise oralement pour augmenter l'énergie, diminuer la fatigue et calmer la faim. De plus, jusqu'à la fin du 19^{e} siècle, elle était prescrite par les médecins, car ils croyaient qu'elle favorisait une bonne santé (Stolberg, 2011). Cependant, la cocaïne n'a été utilisée pour ses propriétés anesthésiantes qu'à partir de 1860, lorsque Albert Niemann a isolé le composé actif de la feuille de coca. De nos jours, l'usage médical de la cocaïne est limité à cause de son potentiel addictif et la toxicité qu'elle peut induire. Par contre, la cocaïne est utilisée

comme un anesthésiant local pour les chirurgies des muqueuses de la cavité orale, laryngale et nasale (Catterall et Mackie, 2015; Stolberg, 2011).

2.2 La pharmacologie de la cocaïne

2.2.1 La cocaïne est un psychostimulant et un sympathomimétique

La cocaïne appartient à la famille des psychostimulants, car elle induit une forte activation psychomotrice (Meyer et Quenzer, 2005). Cette stimulation psychomotrice est accompagnée de l'augmentation de la vigilance, de l'éveil, de la confiance, du bien-être, de la concentration, de l'anxiété et de l'excitation comportementale, ainsi que l'intensification des sentiments de plaisir (Gawin, 1991; Meyer et Quenzer, 2005).

De plus, la cocaïne est un sympathomimétique. Le système nerveux sympathique fait partie du système nerveux autonome, le système qui gère l'activité normale des organes viscéraux et des fonctions automatiques du corps. Ainsi, en jouant sur ce système, la cocaïne augmente la pression artérielle et le rythme cardiaque (Resnick, Kestenbaum et Schwartz, 1977), induit l'hyperthermie (Callaway et Clark, 1994; Crandall, Vongpatanasin et Victor, 2002) et provoque la libération des hormones stéroïdiennes corticolibérine, corticotrophine et cortisol (Baumann et al., 1995; Heesch et al., 1995; Sarnyai, Shaham et Heinrichs, 2001). Ensemble, ces changements psychologiques et physiologiques expliquent pourquoi les consommateurs qui se rendent en urgence se plaignent de troubles cardiaques, neurologiques, pulmonaires et psychiatriques, et se mettent à risque de mort soudaine (Warner, 1993).

2.2.2 Le mécanisme d'action de la cocaïne

La cocaïne est un inhibiteur de la recapture pure de nombreux neurotransmetteurs, soit la dopamine, la noradrénaline et la sérotonine (Heikkila, Orlansky et Cohen, 1975; Koe, 1976). Elle agit au niveau de la synapse, en se liant à leurs transporteurs spécifiques et en bloquant leur recapture. Ceci produit une augmentation de la concentration de ces neurotransmetteurs dans la synapse et induit l'augmentation de la transmission dans les neurones impliqués (Heikkila et al., 1975).

De plus, la cocaïne possède des propriétés anesthésiques locales. Cette drogue produit ces effets en bloquant les canaux sodiques voltage-dépendants situés sur la membrane cellulaire des neurones. Ces canaux sont nécessaires à la production des potentiels d'actions. En se liant aux canaux sodiques, elle inhibe l'initiation et la conduction des influx nerveux, et provoque la perte temporaire de sensation au site d'application (Crumb et Clarkson, 1990; Scholz, 2002).

2.2.3 La pharmacocinétique de la cocaïne

La pharmacocinétique de la cocaïne varie énormément en fonction de la forme et de la voie d'administration de la drogue (Porrino, 1993). La vitesse et le taux d'absorption de la cocaïne, sa distribution, son métabolisme et son élimination modulent l'activité et la durée de vie de cette drogue dans le système, pouvant ainsi influencer le développement de la toxicomanie. Ainsi, la vitesse d'administration de la cocaïne est importante, car elle module la vitesse d'apparition des effets de la drogue, l'intensité de ces effets et la durée d'action. En effet, plus les effets ressentis suite à la prise de drogue sont grands et immédiats, plus la drogue risque d'être abusée (Hatsukami et Fischman, 1996). Par

exemple, lorsque la cocaïne est prise par la voie intraveineuse ou en fumant du crack, deux voies d'administration qui mènent la drogue rapidement au cerveau, le risque de développer une toxicomanie est plus grande que lorsque cette drogue est consommée par des voies plus lentes, telles que les voies intranasale et orale (Hatsukami et Fischman, 1996; Jones, 1990).

Les différentes formulations disponibles pour la cocaïne permettent sa consommation via injection par la voie intraveineuse, absorption par les membranes muqueuses ou en la fumant. Depuis longtemps, les peuples de l'Amérique du Sud brûlent les feuilles de coca et ensuite les consomment en les mâchant. Cette voie d'administration orale ne permet d'obtenir qu'une faible concentration de drogue (≤1%) par une absorption lente et soutenue à travers les membranes muqueuses de la bouche et du tractus gastro-intestinale (Fischman et Schuster, 1984). Par cette voie, la concentration plasmatique veineuse maximale de la cocaïne est atteinte très lentement; entre 1 et 6 heures après le début de la consommation (Jufer, Walsh et Cone, 1998; van Dyke, Jatlow, Ungerer, Barash et Byck, 1978). En effet, la biodisponibilité de la drogue est diminuée à 20-40% suite à l'effet de premier passage hépatique (Jones, 1984; Verebey et Gold, 1988), ce qui permet d'éviter les effets toxiques normalement causés par les formes purifiées de la drogue (Warner, 1993). La cocaïne HCl (sel hydrochlorique) est une deuxième forme de la drogue, qui possède une configuration poudreuse, fine et hydrosoluble (Warner, 1993), et qui peut être prise par les voies intranasale, orale ou intraveineuse. La voie intranasale est souvent la méthode utilisée lors de l'initiation à la cocaïne (Dunn et Laranjeira, 1999). La concentration plasmatique veineuse maximale ou de cocaïne radiomarquée est atteinte en 20 à 60 minutes (Javaid, Fischman, Schuster,

Dekirmenjian et Davis, 1978; Jeffcoat, Perez-Reyes, Hill, Sadler et Cook, 1989; Lukas et al., 1996; Resnick et al., 1977; Van Dyke, Barash, Jatlow et Byck, 1976; van Dyke et al., 1978). De plus, les effets subjectifs et physiologiques culminent entre 15-40 minutes après la consommation et persistent pour plus d'une heure (Hatsukami et Fischman, 1996). La biodisponibilité de la drogue suite à sa consommation par la voie intranasale n'est que de 20-30% (Verebey et Gold, 1988). De plus, il faut mentionner que la consommation chronique de la cocaïne par cette voie produit la constriction des vaisseaux des muqueuses, ce qui diminue la quantité et la vitesse à laquelle la drogue est absorbée avec le temps (Verebey et Gold, 1988). La voie intraveineuse utilise aussi la formulation de cocaïne HCl. L'injection rapide d'une grande dose de cocaïne qui mène 100% de la drogue dans la circulation, et donc très rapidement au cerveau, accorde un grand potentiel d'abus à cette voie (Verebey et Gold, 1988). La concentration plasmatique veineuse maximale ou de cocaïne radiomarquée suite à la prise de la drogue par la voie intraveineuse est atteinte très rapidement; entre 2-15 minutes après le début de la consommation (Evans, Cone et Henningfield, 1996; Javaid et al., 1978; Jeffcoat et al., 1989; Jenkins, Oyler et Cone, 1995; Resnick et al., 1977), tandis que concentration plasmatique artérielle maximale est atteinte en 15 secondes (Evans et al., 1996). Lorsque la cocaïne est injectée, les effets subjectifs et physiologiques apparaissent très rapidement (en 30 secondes) et atteignent leur maximum en quelques minutes [1-5 minutes; (Evans et al., 1996; Hatsukami et Fischman, 1996; Jones, 1984)]. Un désavantage de cette formulation est qu'elle possède un point de fusion très bas, ce qui fait qu'elle ne résiste pas à la pyrolyse (Warner, 1993). Ainsi, des formes basiques de la cocaïne ont été créées pour la consommation par inhalation, le *freebase* et le *crack*, car

elles sont des formulations plus volatiles et plus résistantes. La cocaïne *freebase* est préparée en dissolvant la cocaïne HCl dans l'eau et en ajoutant une base (ammoniac) et un solvant (éther), qui est ensuite évaporé pour produire une forme cristallisée de la drogue. À l'opposé, le crack est formé en chauffant un mélange de cocaïne HCl, d'eau et du bicarbonate de soude (Warner, 1993). Par le fait qu'elle soit fumée, des hautes concentrations de drogue atteignent le cerveau très rapidement (Verebey et Gold, 1988). Fumer du crack augmente rapidement les concentrations plasmatiques veineuses maximales ou de la cocaïne radiomarquée [en 2-10 minutes; (Evans et al., 1996; Jeffcoat et al., 1989; Jenkins et al., 1995)], ainsi que les concentrations plasmatiques artérielles maximales [en 0.25 minutes; (Evans et al., 1996)] et produit des effets subjectifs similaires à ceux de la voie intraveineuse (Evans et al., 1996; Jones, 1990).

La cocaïne est un alcaloïde bicyclique, dont les groupes fonctionnels d'esters carboxyméthylique et benzoate et le groupe N-méthyl sont susceptibles à la biotransformation par les estérases plasmatiques et hépatiques (Inaba, 1989; Jones, 1984). Les métabolites principaux sont la benzoylécgonine (BE) et l'écgonine méthyl ester (EME) (Inaba, Stewart et Kalow, 1978; Jones, 1984; Stewart, Inaba, Tang et Kalow, 1977). Ces deux produits d'hydrolyse sont des métabolites inactifs. Ils sont donc incapables de bloquer la recapture de la dopamine et ne produisent aucun effet pharmacologique (Jatlow, 1988). Un troisième métabolite de la cocaïne est la norcocaine (NC), un produit de la N-déméthylation de la cocaïne par des microsomes hépatiques (cytochrome P450). La norcocaine est un métabolite actif de cette drogue (Hawks, Kopin, Colburn et Thoa, 1974; Misra, Pontani et Vadlamani, 1979; Stewart, Inaba, Lucassen et Kalow, 1979). L'injection quotidienne de la norcocaine (i.p.; 10 mg/kg/infusion) pendant

une semaine inhibe la recapture de la norépinephrine tritiée (^{3}H-NE) par les synaptosomes chez des singes de manière équivalente à l'inhibition produite par la cocaïne, tandis que les autres métabolites (dont BE et EME) inhibent la recapture de cette amine que de façon limitée (Hawks et al., 1974). Similairement, l'inhibition de la recapture de sérotonine dans le cortex cérébral produite par la cocaïne et la norcocaine ne varie pas chez la souris, tandis que l'inhibition de la recapture de la dopamine dans le striatum ne diminue que très peu à la suite de l'administration de la norcocaine vs. celle de la cocaïne (Reith, Meisler, Sershen et Lajtha, 1986). Au niveau physiologique, la cocaïne et la norcocaine chez le rat produisent des effets cardiovasculaires (augmentation du rythme cardiaque) et convulsivants similaires suite à leur administration par voie intraveineuse ou intracisternale (Misra, Nayak, Bloch et Mulé, 1975) et leur administration par voie i.v. produit de la tachycardie et de l'hyperventilation chez le singe rhésus (Borne et al., 1977; Wilson, Bedford, Kibbe et Sam, 1978). Au niveau comportemental, la norcocaine en i.v. est auto administrée par les singes (Bedford, Borne et Wilson, 1980) et les chiens (Risner et Jones, 1980), indiquant que cette molécule possède des propriétés renforçatrices et suggérant qu'elle pourrait contribuer, en partie, aux effets de la cocaïne. De plus, la cocaïne et la norcocaine par voie i.p. diminuent significativement de façon dose-dépendante la consommation de nourriture chez le rat comparativement à une infusion de salin (Bedford et al., 1980). Par contre, ces deux molécules se différencient au niveau des effets produits sur la locomotion. Malgré que les deux substances produisent de la stéréotypie lorsqu'elles sont administrées par infusion intracérébrale chez la souris versus l'infusion du véhicule (Reith, Meisler et Lajtha, 1985), l'administration par voie i.p. ou i.v. de la cocaïne stimule la locomotion

spontanée, tandis que celle de la norcocaine la diminue (Reith et Lajtha, 1986; Reith et al., 1985).

La demi-vie de la cocaïne et de ses métabolites peut varier significativement. Inversement à la cocaïne qui possède une demi-vie très courte [0.5-1.5 heures; (Jatlow, 1988)], nombreux métabolites persistent beaucoup plus longtemps chez l'humain [EME : 3.6 heures et BE : 7.5 heures, mais pas NC : 0.4-1.6 heures; (Ambre, 1985; Ambre, Ruo, Nelson et Belknap, 1988; Misra et al., 1979)]. Environ 85-90% de la dose de cocaïne administrée est retrouvée, par la suite dans l'urine, avec 1-5% qui se trouve dans son état initial, tandis que 75-90% est éliminé sous les formes de EME et BE.

CHAPITRE 3 – Les systèmes impliqués dans la toxicomanie

La toxicomanie est une maladie complexe. Il est donc attendu que les mécanismes qui sous-tendent cette maladie soient nombreux et diverses. Les effets renforçateurs produits par la cocaïne, ainsi que les autres drogues d'abus, sont fortement liés au fait que ces drogues augmentent les concentrations de dopamine dans les régions terminales des neurones dopaminergiques, et surtout au niveau du système dopaminergique mésolimbique (Di Chiara et Imperato, 1988; Pontieri, Tanda et Di Chiara, 1995). Cette hausse de la transmission dopaminergique est importante pour le développement de comportements de recherche et de prise de drogue (Berridge et Robinson, 1998; Di Chiara, 1999; Wise, 2004). Cependant, la dopamine n'est pas seule à médier le développement de la toxicomanie. Le système glutamatergique y participe aussi. Ceci se fait en partie par les effets directs ou indirects du système glutamatergique sur le système dopaminergique via les voies méso-corticolimbiques (Tzschentke, 2001; Tzschentke et Schmidt, 2000), mais aussi par des changements induits par les drogues d'abus dans la transmission glutamatergique et l'activité corticale normale (Kalivas, 2004). Malgré que d'autres systèmes de neurotransmetteurs possèdent des rôles dans la toxicomanie [par ex. la sérotonine (White, 1998) et révisé dans (Filip, Frankowska, Zaniewska, Golda et Przegaliñski, 2005)], seulement ces deux systèmes seront discutés ici, pour leur pertinence dans les projets décrits dans cette thèse.

3.1. La dopamine

3.1.1. La synthèse de la dopamine

La dopamine est un neurotransmetteur qui appartient à la famille des monoamines et plus précisément des catécholamines (Meyer et Quenzer, 2005). La synthèse de la dopamine débute avec la conversion de la tyrosine en l-3,4-dihydroxyphénylalanine (L-DOPA) par l'enzyme tyrosine hydroxylase (Figure 1). La L-DOPA est ensuite décarboxylée par l'enzyme décarboxylase des acides aminés aromatiques pour former la dopamine. La synthèse de la dopamine est bien régulée afin d'assurer que la dopamine, ainsi que les deux autres catécholamines synthétisées par ce système (la noradrénaline et l'adrénaline), soient toujours disponibles. Le contrôle de ce système débute avec la régulation de la tyrosine hydroxylase, l'enzyme limitante de la synthèse de la dopamine. Cette enzyme est régulée par les quantités de dopamine et de noradrénaline présentes dans la terminaison nerveuse. La présence de hautes concentrations de ces deux neurotransmetteurs engendre un mécanisme de « feedback » négatif qui inhibe la tyrosine hydroxylase et la synthèse subséquente des catécholamines. Ensuite, la synthèse de dopamine peut aussi être provoquée par la présence élevée de la L-DOPA. La synthèse des catécholamines est suivie par leur transport et

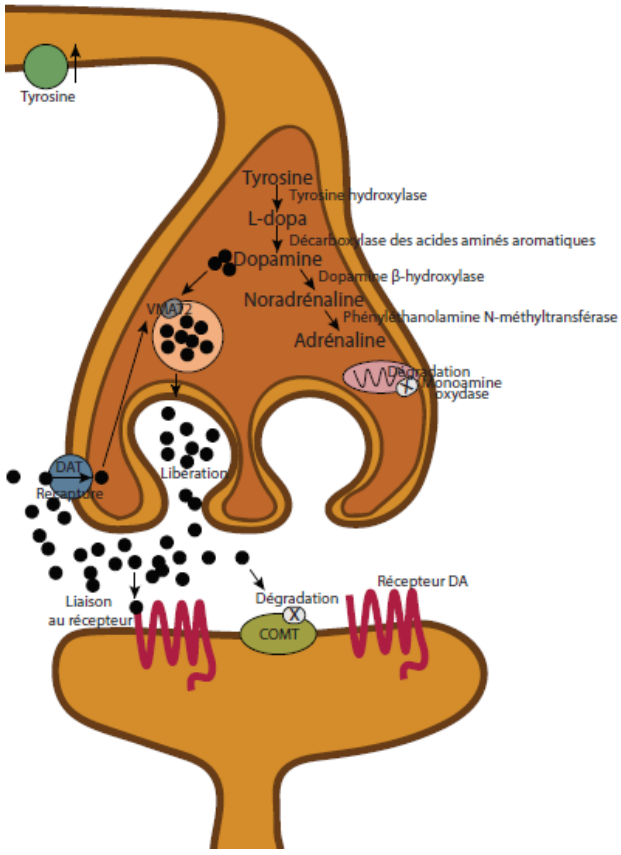

Figure 1. Synthèse, libération et dégradation de la dopamine

leur stockage dans des vésicules synaptiques par le transporteur vésiculaire des monoamines 2 (VMAT2). Finalement, la libération des catécholamines est accomplie par le passage d'un influx nerveux dans la terminaison nerveuse. Ceci permet la fusion des vésicules avec la membrane et leur libération dans la fente synaptique par exocytose. Des autorécepteurs localisés sur les corps cellulaires, dendrites et terminaisons des neurones servent de système de contrôle. Ils maintiennent l'homéostasie en diminuant le flux de calcium généré par l'influx nerveux, ce qui prévient la fusion des vésicules et évite la libération des neurotransmetteurs (Daubner, Le et Wang, 2011; Elsworth et Roth, 1997; Meyer et Quenzer, 2005; Molinoff et Axelrod, 1971).

3.1.2 La recapture de la dopamine par les transporteurs de la dopamine (DAT)

La recapture de la dopamine est nécessaire pour contrôler la neurotransmission dopaminergique et maintenir l'homéostasie du système nerveux central (Giros, Jaber, Jones, Wightman et Caron, 1996; Iversen, 1971). La recapture de la dopamine se fait principalement par les DATs (Amara et Kuhar, 1993; Uhl et Johnson, 1994). Les DATs sont des protéines plasmatiques pré-synaptiques retrouvées sur les terminaisons nerveuses dopaminergiques (Amara et Kuhar, 1993; Norregaard et Gether, 2001). Ils sont des transporteurs dépendants du cotransport Na^+/Cl^- (Chen et Reith, 2000; Norregaard et Gether, 2001; Zhang et al., 2009). Une fois que la dopamine a été recapturée, elle est soit restockée dans les vésicules, soit elle est métabolisée par la monoamine oxydase et l'aldéhyde déshydrogénase ou par la catécholamine-O-méthyltransférase (COMT) en acide dihydroxyphénylacétique (DOPAC) et acide homovanilique (HVA), respectivement, et éliminée (Eisenhofer, Kopin et Goldstein, 2004; Kopin, 1985).

3.1.2.1 Le rôle du DAT dans la toxicomanie à la cocaïne

Le DAT est une cible importante des psychostimulants, dont la cocaïne (Heikkila et al., 1975; Ritz, Lamb et Kuhar, 1987; Ross et Renyi, 1966; Taylor et Ho, 1978). En se liant au DAT, la cocaïne inhibe la recapture de la dopamine, favorise son accumulation dans la synapse et prolonge la neurotransmission dopaminergique. Le DAT serait responsable des effets renforçateurs produit par la cocaïne (Ritz et al., 1987). Chez l'humain, une étude de tomographie par émission de positrons a permis de démontrer que la consommation de doses de cocaïne couramment utilisées par les personnes ayant un trouble lié à l'usage de la cocaïne (0.3-0.6 mg/kg) inhibe entre de 60-77% des DATs dans le striatum, et que les sentiments subjectifs ressentis corrèlent avec le taux d'occupation des DATs (Volkow et al., 1997).

Il faut noter que le DAT n'est pas le seul transporteur qui joue un rôle dans la toxicomanie. Premièrement, la cocaïne bloque aussi les transporteurs de la sérotonine et de la norépinephrine (Kuhar, Ritz et Boja, 1991; Ritz et al., 1987). De plus, les études sur les souris transgéniques DAT-/- (souris «knock-out») et double «knock-out» pour les transporteurs de la sérotonine et/ou de la norépinephrine suggèrent un rôle de ces transporteurs dans le maintien des propriétés renforçatrices de la cocaïne (Rocha, 2003; Rocha et al., 1998). Ceci suggère que la cocaïne produirait ses effets en jouant sur de nombreux systèmes.

3.1.3 Les récepteurs dopaminergiques

Les récepteurs dopaminergiques sont des récepteurs métabotropes et appartiennent à la famille des récepteurs transmembranaires couplés à la protéine G

(Jaber, Robinson, Missale et Caron, 1996). Cinq récepteurs dopaminergiques différents ont été clonés jusqu'à maintenant. Ils se divisent en deux familles : les récepteurs qui ressemblent à D1 et les récepteurs qui ressemblent à D2, selon leurs effets sur la protéine G et la synthèse du messager secondaire AMPc (Kebabian et Calne, 1979; Sibley et Monsma Jr, 1992).

Les récepteurs D1 et D5 font partie des récepteurs dopaminergiques de la famille D1. Ces récepteurs stimulent l'adénylate cyclase en interagissant avec la protéine G stimulatrice (G_s), permettant ainsi la conversion de l'ATP en AMP cyclique (Jaber et al., 1996). L'AMP cyclique lie l'enzyme protéine kinase A (PKA), qui ensuite phosphoryle plusieurs protéines impliquées dans la transduction du signal et la modulation de l'expression des gènes (Neve, Seamans et Trantham-Davidson, 2004). Un des substrats activés par PKA est DARPP-32 (*dopamine and cyclic AMP-regulated phosphoprotein*) (Beaulieu et Gainetdinov, 2011; Svenningsson et al., 2004), qui suite à sa phosphorylation sur Thr34 inhibe la protéine phosphatase 1 [PP1; (Greengard, Allen et Nairn, 1999; Hemmings Jr, Greengard, Tung et Cohen, 1984)]. L'activation de DARPP-32 active aussi le *mammalian target of rapamycin complex 1* (mTORC1), qui ensuite induit la phosphorylation de la protéine ribosomale S6 (Santini et al., 2012; Santini, Heiman, Greengard, Valjent et Fisone, 2009). PKA/DARPP-32 activent aussi les *extracellular signal-regulated kinases 1 and 2* ([Erk1/2; (Fiorentini et al., 2011; Fiorentini, Savoia, Savoldi, Barbon et Missale, 2013; Santini et al., 2012; Santini et al., 2007)]. L'activation de ces protéines est importante dans la régulation en aval de la transcription et la traduction, et joue un rôle dans la plasticité synaptique du cerveau (Costa-Mattioli, Sossin, Klann et Sonenberg, 2009; Thomas et Huganir, 2004). Ces récepteurs induisent

aussi la transcription de gènes à la suite de l'activation du facteur de transcription cyclic-AMP response element-binding protein [CREB; (Liu et Graybiel, 1996)]. Également, la famille des récepteurs D1 module les niveaux de calcium intracellulaire via différents mécanismes (Kisilevsky et al., 2008; Swapna, Bondy et Morikawa, 2016). Les canaux potassiques sont atténués via l'activation des cascades PKA/DARPP-32 induite par les récepteurs de la famille D1, tandis que ces derniers sont augmentés par l'inhibition de cette cascade par les récepteurs de la famille D2 ainsi que la libération des sous-unités βγ de la protéine G [$G_{\beta\gamma}$; (Greif, Lin, Liu et Freedman, 1995; Neve et al., 2004)]. Les récepteurs D1 modulent aussi les effets dopaminergiques sur la pompe Na^+/K^+ ATPase, qui est essentielle au maintien du gradient électrochimique nécessaire à l'excitabilité cellulaire neuronale et musculaire, ainsi que le transport à travers les membranes épithéliales (Blom et al., 2012). Cependant, l'activation de récepteurs D2 serait aussi potentiellement requis (Bertorello, Hopfield, Aperia et Greengard, 1990; Hazelwood, Free, Cabrera, Skinbjerg et Sibley, 2008). Finalement, les récepteurs de la famille D1 interagissent avec les récepteurs glutamatergiques NMDA et AMPA et augmentent leurs réponses à travers le cerveau (Flores-Hernandez et al., 2002; Gonzalez-Islas et Hablitz, 2003; Levine et al., 1996; Seamans, Durstewitz, Christie, Stevens et Sejnowski, 2001; Tseng et O'Donnell, 2004; Zheng, Zhang, Bunney et Shi, 1999)

Malgré leur homologie, l'emplacement des récepteurs D1 et D5 dans le cerveau est très différent. Le récepteur D1 est hautement exprimé dans le caudé-putamen, le noyau accumbens et le tubercule olfactif, mais aussi dans le cortex cérébral, l'hypothalamus et le thalamus (Dearry et al., 1990; Mansour et al., 1990; Meador-Woodruff et al., 1996; Monsma, Mahan, McVittie, Gerfen et Sibley, 1990; Sibley et

Monsma, 1992), tandis que le récepteur D5 se retrouve plutôt au niveau de l'hippocampe et de l'hypothalamus, et moins dans le striatum et le cortex frontal (Ciliax et al., 2000; Sibley et Monsma, 1992; Tiberi et al., 1991).

La famille des récepteurs dopaminergiques qui ressemblent à D2 inclue les récepteurs D2, D3 et D4. Ces derniers se distinguent des récepteurs de la famille D1, car ils interagissent avec la protéine G inhibitrice (G_i) pour empêcher la formation d'adénylate cyclase, diminuant ainsi la formation d'AMP cyclique et la phosphorylation des substrats de PKA (Jaber et al., 1996; Kebabian et Calne, 1979; Neve et al., 2004). De plus, les récepteurs de la famille D2 régulent nombreuses autres voies de signalisation, dont les phospholipases, les canaux ioniques et les MAP kinases. Plusieurs de ces cascades sont modulées par les sous-unités $G_{\beta\gamma}$ libérées lors de l'activation du récepteur (Neve et al., 2004). Par exemple, ils activent les cascades impliquant les *mitogen-activated protein kinases* (MAPK) par $G_{\beta\gamma}$ (Choi, Jeong, Park et Baik, 1999; Cussac, Newman-Tancredi, Pasteau et Millan, 1999; Faure, Voyno-Yasenetskaya et Bourne, 1994; Luo, Kokkonen, Wang, Neve et Roth, 1998; Oak, Lavine et Van Tol, 2001). Les récepteurs de la famille D2 diminuent aussi l'activité des canaux calciques de types L, N et P/Q, possiblement par un mécanisme impliquant $G_{\beta\gamma}$. Ceci impliquerait une stimulation de l'hydrolyse du phosphatidylinositol par la phospholipase C qui induit la synthèse de l'inositol 1,4,5-triphosphate et qui, par la suite, mobilise les stocks de calcium intracellulaire (Hernández-López et al., 2000; Seabrook, Kemp, et al., 1994; Seabrook, Knowles, et al., 1994).

Le récepteur D2 est le plus abondant des trois. Il s'exprime principalement dans le caudé-putamen, le noyau accumbens, le tubercule olfactif, l'aire tegmentale ventrale et la substance noire (Bunzow et al., 1988; Mansour et al., 1990; Meador-Woodruff et al.,

1996; Sibley et Monsma, 1992). Le récepteur D3 se retrouve dans le noyau accumbens, le tubercule olfactif, les îles de Calleja et l'hypothalamus (Meador-Woodruff et al., 1996; Sibley et Monsma, 1992; Sokoloff, Giros, Martres, Bouthenet et Schwartz, 1990), tandis que le récepteur D4 est localisé surtout dans le cortex frontal, le mésencéphale, l'amygdale et le bulbe rachidien, et de façon limitée dans le striatum (Sibley et Monsma, 1992; Van Tol et al., 1991).

3.1.4 Les voies dopaminergiques et leurs fonctions

Les voies dopaminergiques sont situés principalement dans la substantia nigra pars compacta, l'aire tegmentale ventrale et l'hypothalamus, proches de la base du mésencéphale [Figure 2; (Meyer et Quenzer, 2005)]. Elles se divisent en trois groupes :

3.1.4.1 La voie nigro-striée

La voie nigro-striée part de la substance noire et envoie ses projections via le faisceau médial du télencéphale vers le striatum dorsal et le globus pallidus, qui ensemble forment les noyaux basaux (Andén et al., 1964; Woodburne, Crosby et McCotter, 1946). La dopamine de la voie nigro-striée est impliquée dans la locomotion et le contrôle du mouvement (Barbeau, 1974). L'importance du fonctionnement adéquat de cette voie apparait surtout dans le Parkinsonisme et la maladie de Huntington, où sa perturbation est associée avec une déplétion de dopamine, ce qui engendre nombreux troubles moteurs (Bernheimer, Birkmayer, Hornykiewicz, Jellinger et Seitelberger, 1973; Kish, Shannak et Hornykiewicz, 1988). De plus, la déplétion de la dopamine induite par une lésion 6-hydroxydopamine (6-OHDA) ou des antagonistes comme la réserpine

diminue la locomotion. Cependant, ces effets sont contrecarrés par l'administration de la L-DOPA ou d'autres agonistes dopaminergiques (Carlsson, 1959; Ungerstedt, 1968).

3.1.4.2 Les voies mésocorticale et mésolimbique

Les corps cellulaires de ces deux voies se situent dans l'aire tegmentale ventrale. La voie mésolimbique projette ses fibres vers les structures limbiques qui incluent le noyau accumbens, l'amygdale, le septum, le tubercule olfactif et le cortex frontal, en traversant le faisceau médial du télencéphale, tandis que la voie mésocorticale envoie ses projections au cortex préfrontal, orbitofrontal, cingulaire et périrhinal (Camí et Farré, 2003). La dopamine est importante dans l'apprentissage, la cognition, les émotions, la mémoire, la faim, le stress et la motivation (Brozoski, Brown, Rosvold et Goldman, 1979; Salamone, Cousins et Snyder, 1997; Salamone, Correa, Mingote et Weber, 2005; Schultz, 2007; Simon, Taghzouti et Le Moal, 1986; Wise, 2004). Donc un débalancement en dopamine dans les voies méso-corticolimbiques pourrait engendrer des déficits à tous ces niveaux.

De plus, ces voies sont très importantes dans l'étude de la toxicomanie, car elles sont les cibles des drogues d'abus. Premièrement, le système dopaminergique est impliqué dans le renforcement et la récompense (Koob, 1992; Pierce et Kumaresan, 2006; Wise, 2004; Wise et Bozarth, 1987). De nombreuses études ont rapporté que les drogues d'abus augmentent les niveaux de dopamine extracellulaire dans ces voies, et surtout au niveau du noyau accumbens (Di Chiara et Imperato, 1988; Pontieri et al., 1995; Pontieri, Tanda, Orzi et Di Chiara, 1996). De plus, les lésions (Corrigall, Franklin, Coen et Clarke, 1992; Lyness, Friedle et Moore, 1979; Roberts, Koob, Klonoff et Fibiger, 1980; Roberts et Koob, 1982) et les antagonistes dopaminergiques (Caine et Koob, 1994;

Corrigall et Coen, 1991; Rassnick, Pulvirenti et Koob, 1992; Rolinski et Scheel-Krüger, 1973) ciblant ces voies diminuent le renforcement des drogues d'abus et réduisent les comportements liés à leur consommation, tels que l'auto administration de drogues et l'activité locomotrice.

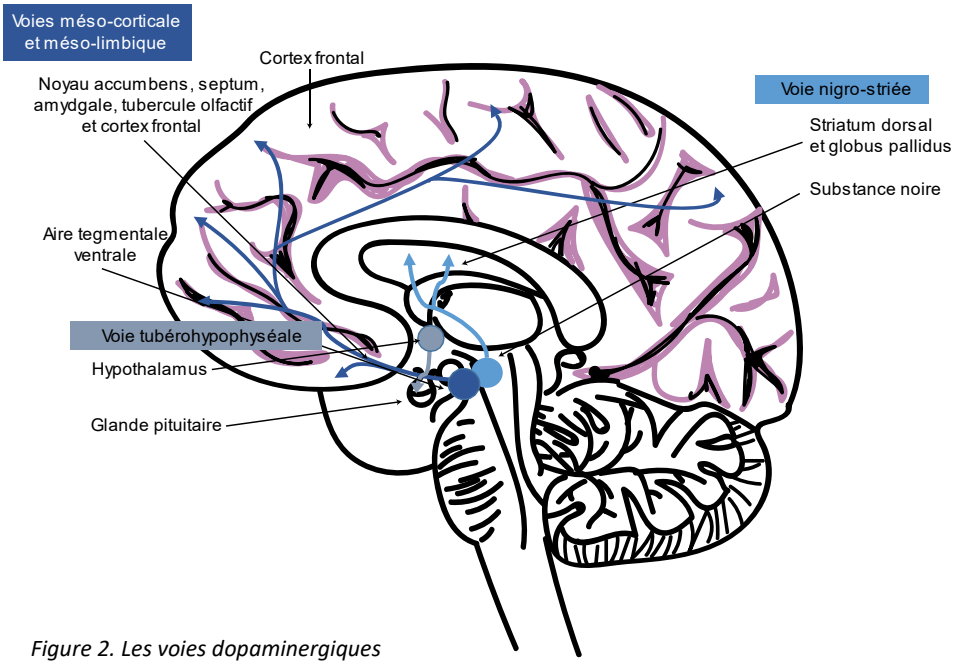

Figure 2. Les voies dopaminergiques

3.1.4.3 La voie tubérohypophyséale

Dans cette troisième voie, la dopamine agit sur le système endocrinien via la régulation de la sécrétion de la prolactine et de l'hormone de croissance. Cette voie regroupe des neurones dopaminergiques qui connectent l'hypothalamus à la glande pituitaire (Ayano, 2016; Ben-Jonathan et Hnasko, 2001).

3.2 Le glutamate

3.2.1 La synthèse du glutamate

Le glutamate est le principal neurotransmetteur excitateur dans le cerveau (Meldrum, 2000; Meyer et Quenzer, 2005). En effet, il est la forme ionisée de l'acide aminé acide glutamique. Le glutamate, contrairement aux autres neurotransmetteurs, possède de nombreuses fonctions biochimiques dont la synthèse de protéines, le métabolisme cellulaire et il est précurseur du neurotransmetteur inhibiteur GABA (Fonnum, 1984; Meyer et Quenzer, 2005). Ceci explique sont abondance à travers le cerveau, mais surtout au niveau des neurones glutamatergiques, ainsi que sa synthèse via plusieurs réactions chimiques différentes. La grande partie du glutamate provient du métabolisme du glucose (Beloff-Chain, Catanzaro, Masi et Pocchiari, 1955; Gaitonde, Dahl et Elliott, 1965). Cependant, le précurseur immédiat de la synthèse du glutamate dans les neurones est la glutamine, qui est transformée par l'enzyme glutaminase, une enzyme mitochondriale, dans les terminaisons présynaptiques (Bradford et Ward, 1976; Hamberger, Chiang, Nylén, Scheff et Cotman, 1979; Shank et Aprison, 1977). Un résumé de la synthèse du glutamate se retrouve dans la figure 3.

3.2.2 L'entreposage du glutamate

Une fois synthétisé, l'entreposage du glutamate dans les vésicules se fait par les transporteurs vésiculaires du glutamate (VGLUT). Trois sous-types de ce transporteur ont été identifiés : VGLUT1, 2 et 3 (Fremeau et al., 2002; Fremeau et al., 2001; Gras et al., 2002; Herzog et al., 2001). L'activité de ces transporteurs dépend de gradient électrochimique protonique généré par le H+-ATPase vésiculaire (Cidon et Sihra, 1989;

Disbrow, Gershten et Ruth, 1982). Les VGLUT 1 et 2 sont les transporteurs les plus abondants et possèdent une distribution complémentaire à travers le cerveau, avec les VGLUT-1 localisés principalement dans le cortex cérébral, le cervelet et l'hippocampe et les VGLUT-2 situés dans le thalamus, hypothalamus et le rhombencéphale (Fremeau et al., 2001; Kaneko, Fujiyama et Hioki, 2002). Le VGLUT-3 est moins exprimé et se retrouve sur les neurones qui permettent la libération d'autres neurotransmetteurs, tels que les neurones glutamatergiques du striatum et les interneurones gabaergiques du cortex et de l'hippocampe (Fremeau et al., 2002). Ces transporteurs permettent la libération majoritaire du neurotransmetteur dans la fente synaptique, mais la libération du glutamate peut aussi se faire par exocytose (Bezzi et al., 2004; Pasti, Zonta, Pozzan, Vicini et Carmignoto, 2001) ou via l'antiport cystéine-glutamate (Baker, Xi, Shen, Swanson et Kalivas, 2002).

Comme le glutamate induit l'excitation neuronale, il est nécessaire que les concentrations extracellulaire soient gardées à un minimum et que cette molécule soit stockée dans des vésicules, afin de prévenir les dommages causés par l'excitation neuronale excessive, qui peut aller jusqu'à provoquer la mort cellulaire (Lewerenz et Maher, 2015; Rothman et Olney, 1986). Une fois les molécules de glutamate libérées dans la fente synaptique, une autre famille de transporteurs, les transporteurs des acides aminés excitateurs (excitatory amino acid transporters [EAATs]), est responsable d'enlever le glutamate de la fente et d'inhiber ses actions (Danbolt, 2001; Seal et Amara, 1999). Ces transporteurs sont sur la membrane plasmatique et sont tous Na+-dépendants, donc ils nécessitent le gradient transmembranaire pour le transport du glutamate (Balcar, 2002; Danbolt, 2001; Seal et Amara, 1999). Cinq EAATs ont été

clonés du système nerveux central mammalien : EAAT1 à 5. Les EAAT-1 et -2 sont principalement localisés sur les astrocytes, tandis que les EAAT-3 à 5 se retrouvent sur les neurones (Seal et Amara, 1999).

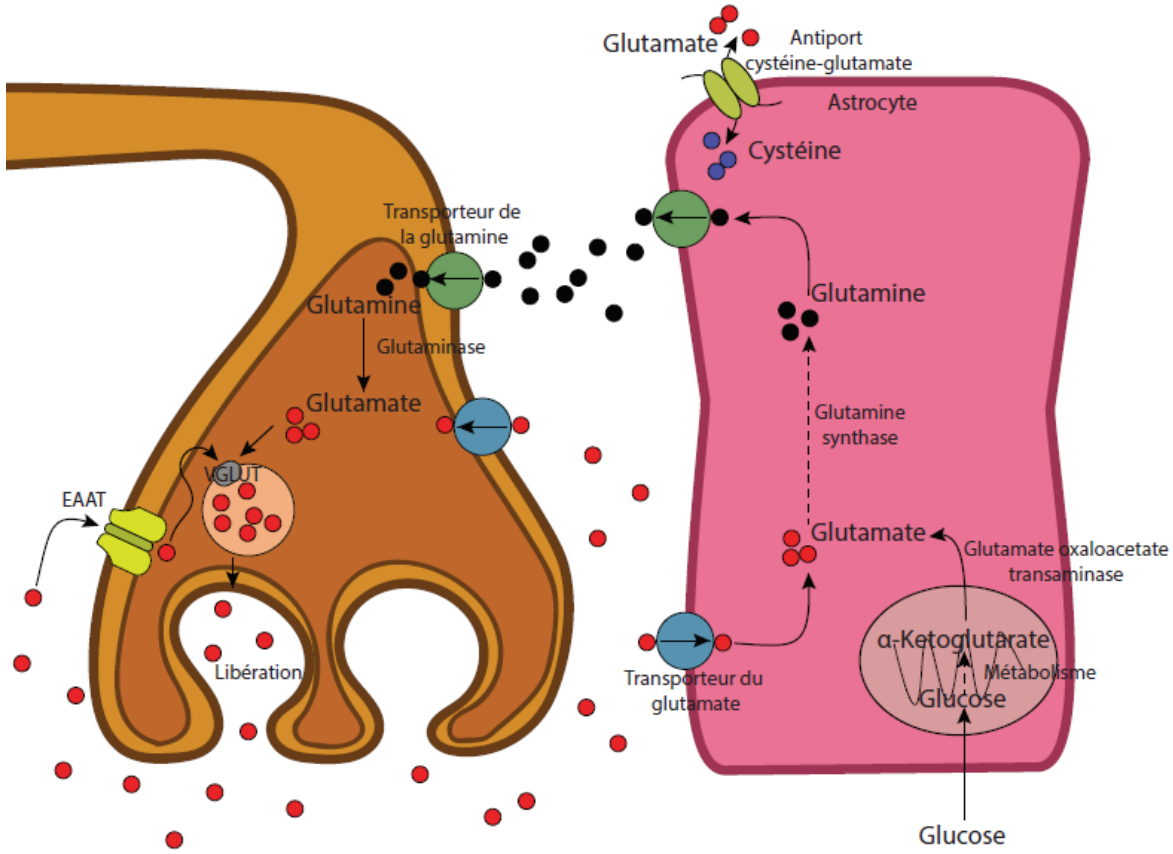

Figure 3. Synthèse et stockage du glutamate.

3.2.3 Les récepteurs glutamatergiques

À la suite de la libération du glutamate dans la fente synaptique, ce neurotransmetteur produit ses effets en agissant sur les récepteurs ionotropes (neurotransmission excitatrice rapide) ou métabotropes (neurotransmission plus lente via leur couplage à la protéine G et modulation de messagers secondaires intracellulaires)

(Meyer et Quenzer, 2005; Shigeri, Seal et Shimamoto, 2004). Il y a trois familles de récepteurs ionotropes, chacune nommée pour son agoniste de prédilection, soit les récepteurs AMPA (a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), kaïnate ou NMDA (N-methyl-D-aspartate). Aussi, huit sous-types de récepteurs glutamatergiques métabotropes ont été identifiés (mGluR1-8) [révisé dans (Hollmann et Heinemann, 1994; Nakanishi et al., 1998)].

3.2.4 Les voies glutamatergiques et leurs fonctions

Le glutamate possède plusieurs fonctions physiologiques et comportementales, mais les rôles les plus importants sont au niveau de la plasticité synaptique, l'apprentissage et la mémoire (Meyer et Quenzer, 2005). Il y a cinq voies glutamatergiques, dont la voie cortico-corticale, la voie thalamo-corticale, la voie cortico-thalamique, la voie cortico-tronc cérébrale et la voie corticostriatale [Figure 4; (Schwartz, Sachdeva et Stahl, 2012)]. Malgré l'importance de toutes ces voies dans l'homéostasie du glutamate, cette section se concentrera seulement sur la voie corticostriatale, qui est une voie bien étudiée pour ses implications dans la toxicomanie (Kalivas, 2004, 2009; Kalivas et al., 2009).

3.2.4.1 La voie corticostriatale

La voie corticostriatale en consiste des projections du cortex préfrontal vers le striatum dorsal (voie corticostriatale) et le noyau accumbens (voie cortico-accumbale). Cette voie est importante dans le développement et l'expression des comportements caractéristiques de la toxicomanie (Kalivas et al., 2009). Les travaux de Pierce, Bell, Duffy et Kalivas (1996) sont parmi les premiers à impliquer la neurotransmission

glutamatergique, plutôt que dopaminergique, dans la sensibilisation psychomotrice. La sensibilisation psychomotrice se manifeste par l'activation progressive et persistante de la locomotion des animaux à la suite d'une exposition répétée à la drogue (Anagnostaras et Robinson, 1996; Robinson et Becker, 1986; Stewart et Badiani, 1993). Pierce et al. (1996) ont démontré que l'administration répétée de cocaïne augmente la neurotransmission glutamatergique dans le *core* du noyau accumbens seulement chez des rats qui avaient développé une sensibilisation psychomotrice à la suite de l'administration quotidienne d'une infusion de cocaïne par voie intrapéritonéale pendant 3-4 semaines. De plus, seulement les rats qui ont montré une sensibilisation locomotrice après l'administration répétée de cocaïne ont augmenté leur locomotion à la suite d'une microinfusion d'AMPA dans le noyau accumbens administrée après 21 jours de retrait, lorsqu'ils ont été comparés aux rats non-sensibilisés et aux rats contrôles qui avaient reçu du salin. Aussi, ils ont montré que l'augmentation de dopamine détectée par microdialyse après la perfusion d'AMPA dans le noyau accumbens ne varie pas entre ces 3 groupes, mais injecter de la cocaïne augmente la concentration extracellulaire de glutamate seulement dans le *core* des animaux sensibilisés. Finalement, le prétraitement du *core* du noyau accumbens avec l'antagoniste non-NMDA CNQX a diminué la locomotion induite par la cocaïne uniquement chez les rats du groupe montrant une sensibilisation psychomotrice. Similairement, des lésions dans les projections du cortex préfrontal au noyau accumbens diminuent la sensibilisation psychomotrice (Wolf, Dahlin, Hu, Xue et White, 1995).

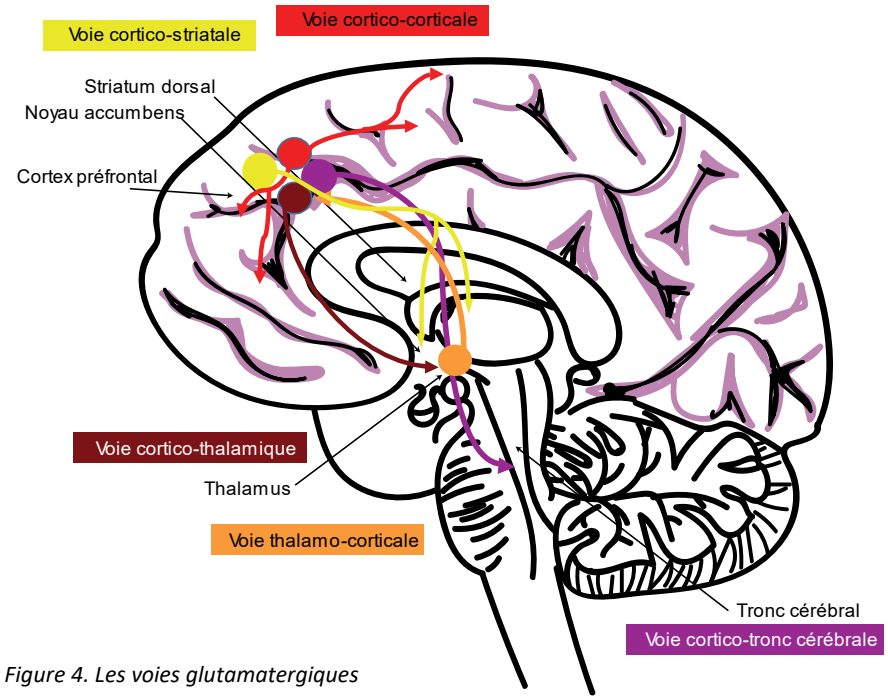

Figure 4. Les voies glutamatergiques

Le glutamate est aussi potentiellement impliqué dans autres comportements associés à la toxicomanie. Par exemple, dans le modèle animal de rechute, l'inhibition du récepteur AMPA dans le *core* du noyau accumbens suggère que la neurotransmission glutamatergique provoquerait le rétablissement du comportement de recherche et de prise de drogue induit par la drogue elle-même ou un indice associé à la drogue (Cornish et Kalivas, 2000; Di Ciano et Everitt, 2001). Aussi, l'inactivation du cortex orbitofrontal (Fuchs, Evans, Parker et See, 2004) et de ses projections au striatum dorsal (Fuchs, Branham et See, 2006) diminue le risque du rétablissement de la recherche de drogue induite par un indice associé à la cocaïne. Finalement, la libération de glutamate dans le *core* du noyau accumbens lors du rétablissement de la recherche de cocaïne résulte des afférentes glutamatergiques provenant du cortex préfrontal (McFarland, Lapish et Kalivas, 2003). De plus, dans le modèle animal du test de préférence conditionnée (CPP),

les travaux de Layer, Uretsky et Wallace (1993) et de Kaddis, Uretsky et Wallace (1995) montrent que l'inhibition du récepteur AMPA par l'antagoniste DNQX diminue le phénomène de préférence de place conditionné induit par la cocaïne et l'amphétamine.

CHAPITRE 4 – Les modèles animaux de la toxicomanie

Les modèles animaux sont nécessaires à l'étude de la toxicomanie. Malgré qu'il y ait encore beaucoup de travail à faire pour mieux comprendre ce trouble et qu'il n'y a pas de modèle animal parfait, les modèles animaux ont permis l'étude de nombreux symptômes caractéristiques de la toxicomanie, dont l'augmentation de la consommation (Ahmed et Koob, 1998; Ahmed et Koob, 1999; Ben-Shahar, Ahmed, Koob et Ettenberg, 2004a; Mantsch, Yuferov, Mathieu-Kia, Ho et Kreek, 2004; Wee, Mandyam, Lekic et Koob, 2008), la motivation excessive pour la drogue (Ben-Shahar, Posthumus, Waldroup et Ettenberg, 2008; Deroche-Gamonet, Belin et Piazza, 2004; Paterson et Markou, 2003; Zimmer, Oleson et Roberts, 2012), la recherche et la prise de drogues en dépit de conséquences négatives (Deroche-Gamonet et al., 2004; Pelloux, Everitt et Dickinson, 2007; Vanderschuren et Everitt, 2004), le *craving* (Grimm, Hope, Wise et Shaham, 2001; Markou et al., 1993) et la vulnérabilité à la rechute malgré de longues périodes d'abstinence (Ahmed et Koob, 1998; Deroche-Gamonet et al., 2004; Knackstedt et Kalivas, 2007).

Dans la section qui suit, les modèles animaux utilisés dans cette thèse seront discutés, soit l'auto administration de la cocaïne par voie intraveineuse (4.1) et la mesure de l'activité locomotrice (4.2).

4.1 L'auto administration de drogue par voie intraveineuse

L'auto administration d'une drogue par voie intraveineuse (i.v.) est une technique de conditionnement opérant, qui se fait par apprentissage instrumental. L'utilisation du

modèle d'auto administration date des années 60s, lorsque Weeks (1962) a montré que les rats s'auto administrent la morphine en appuyant sur un levier. Cette action permet l'infusion de la drogue par voie intraveineuse via un cathéter implanté dans la veine jugulaire. Depuis, l'auto administration de plusieurs drogues abusées par l'humain se fait aussi par les rongeurs et les singes (Schuster et Thompson, 1969; Yokel, 1987). Chez l'animal, l'auto administration de drogue nécessite l'implantation d'un cathéter dans la veine jugulaire, afin de permettre l'infusion de la drogue directement dans le système circulatoire. L'animal est ensuite placé dans une cage opérante où il doit effectuer une tâche opérante (par ex. appuyer sur un levier ou rentrer son museau dans un réceptacle) pour recevoir l'infusion de drogue. L'avantage de ce modèle est que plusieurs paramètres peuvent être manipulés, dans le but d'étudier différents aspects de la toxicomanie. Par exemple, dans les études décrites dans cette thèse, l'horaire de renforcement a été manipulé. Nos études débutaient toujours avec un ratio fixe (FR), qui se caractérise par la nécessité d'appuyer un nombre de fois fixe sur le levier actif afin d'obtenir une infusion de drogue (Panlilio et Goldberg, 2007). Cet horaire de renforcement est utile dans l'identification des drogues qui possèdent un risque d'abus élevé, car il permet d'évaluer le patron de consommation des drogues en question (Arnold et Roberts, 1997). Par contre, le FR peut être insensible aux changements dans le renforcement produits par la drogue, de manière à ce qu'on manque les changements dans le taux de consommation [il sert plutôt d'indice qualitatif que quantitatif; (Arnold et Roberts, 1997)]. Donc, l'efficacité de renforcement de la drogue à maintenir un comportement est mieux mesurée sous un horaire de renforcement à ratio progressif (PR). Avec le PR, le nombre d'appuis nécessaire à l'obtention de la prochaine infusion de drogue augmente exponentiellement

avec chaque infusion successive, et ceci jusqu'à ce que l'animal cesse d'appuyer. Le dernier ratio atteint avant l'abandon de l'animal est considéré comme étant son point de rupture ou *breakpoint*, et sert à mesurer sa motivation à obtenir la drogue. Similairement, le nombre d'infusions auto administrées et le nombre d'appuis sur le levier actif avant l'abandon servent aussi d'indice du travail que l'animal est prêt à faire pour obtenir sa dose. En effet, la motivation pour la drogue est un symptôme caractéristique de la toxicomanie, qui représente le fait qu'un individu passera beaucoup de temps à chercher la drogue et dépensera beaucoup d'énergie pour l'obtenir. Ainsi, le PR permet d'étudier la persistance du comportement de consommation, plutôt que le taux de réponse face à la drogue. Ceci est avantageux, car l'auto administration de drogues d'abus change souvent la fréquence de la réponse opérante. Par contre, il faut faire attention à des états comme la stéréotypie, où ces modifications ne sont pas toujours reliées à l'efficacité renforçatrice de la drogue administrée (Panlilio et Goldberg, 2007; Richardson et Roberts, 1996).

Le modèle d'auto administration de la drogue est très avantageux, car il permet la modélisation de plusieurs aspects différents de la toxicomanie observés chez l'humain (consommation, motivation, rechute, usage malgré les conséquences négatives) et peut être jumelé à différentes autres techniques, telle que la microdialyse qui permet d'étudier la pharmacocinétique de la drogue simultanément (O'connor, Chapman, Butler et Mead, 2011). De plus, ce modèle possède une grande validité, car c'est l'animal lui-même qui décide la dose de drogue qu'il va consommer pendant une session, plutôt que l'expérimentateur (Panlilio et Goldberg, 2007). Cependant, comme tout modèle, le modèle d'auto administration de la drogue par le rat pour l'étude de la toxicomanie

possède aussi certaines limites. Premièrement, le potentiel d'abus d'une drogue dépend d'une multitude de facteurs biologiques, environnementaux, sociaux, etc. qui peuvent difficilement tous être mesurés dans un même modèle (Katz et Goldberg, 1988; O'Brien, 2008). De plus, pas tous les symptômes d'une consommation de drogues ou de la toxicomanie, tels que l'euphorie, la psychose paranoïde et la stimulation de l'humeur, peuvent être observés chez l'animal. Il y a aussi des différences entre le système nerveux central du rat et de l'humain, ainsi que leur profile métabolique, auxquelles il faut faire preuve de prudence lors de la juxtaposition chez l'humain des résultats retrouvés dans le rat (Epstein, Preston et Jasinski, 2006; O'connor et al., 2011). Finalement, cette méthode est couteuse en termes de temps et de ressources, car, malheureusement, la durée de vie des cathéters n'est que de quelques mois, ce qui limite la durée et la complexité des études (Panlilio et Goldberg, 2007).

4.2 Activité locomotrice induite par la drogue

La cocaïne est un psychostimulant, qui augmente l'activité locomotrice (Meyer et Quenzer, 2005). La sensibilisation aux effets des drogues d'abus, selon la théorie de l'attribution pathologique d'une valeur motivationnelle à un stimulus ou *incentive sensitization theory* de Robinson et Berridge (1993) (décrite au chapitre 1), est facilement observée en laboratoire par la sensibilisation locomotrice créée suite à l'administration répétée de drogue. Initialement, l'évaluation des effets des drogues sur l'activation psychomotrice était considérée importante dans les études sur la toxicomanie, car les systèmes neuronaux impliqués dans la sensibilisation psychomotrice se chevauchent avec les systèmes impliqués dans la récompense et la motivation, soit le système méso-

corticolimbique (Wise et Bozarth, 1987). Des études plus récentes suggèrent qu'il y aurait plutôt une ségrégation fonctionnelle des effets récompensants et locomoteurs des psychostimulants au niveau du striatum ventral. Cependant, ces effets seraient anatomiquement dissociables à l'intérieur de cette structure, avec le *core* du noyau accumbens responsable des effets stimulants locomoteurs de la cocaïne et de l'amphétamine, tandis que le *shell* médian contribuerait aux effets gratifiants des psychostimulants (Sellings et Clarke, 2003; Sellings, McQuade et Clarke, 2006).

La sensibilisation psychomotrice est l'augmentation progressive et persistante de l'activité locomotrice à la suite d'une exposition répétée à la drogue (Anagnostaras et Robinson, 1996; Robinson et Becker, 1986; Stewart et Badiani, 1993). Différents aspects de la locomotion peuvent être évalués, tels que l'augmentation de la locomotion horizontale, le reniflement, les mouvements de la tête, ainsi que des mouvements verticaux (Robinson et Becker, 1986). Par contre, des doses plus élevées de drogue peuvent causer une baisse de la locomotion et l'apparition d'un comportement stéréotypé, qui est caractérisé par l'augmentation des actions répétitives, dont les mouvements de la tête et le toilettage (Robinson et Becker, 1986). L'activité locomotrice induite par la cocaïne est mesurée dans les cages de locomotion ou directement dans les cages opérantes. Le sol de ces cages est équipé de paires de photocellules infrarouges. Ainsi, en bougeant, le nombre de fois que l'animal traverse un faisceau lumineux est comptabilisé, ce qui permet de mesurer la locomotion des animaux suite aux différents traitements. Malgré la simplicité de cette technique et la capacité fiable de détecter des changements du comportement locomoteur après la consommation de drogues, cette méthode possède aussi quelques limites. Premièrement, nos cages

permettent seulement la mesure de la locomotion horizontale, et non pas des mouvement verticaux, tels que l'élévation de l'animal sur ses pattes arrière, le reniflement, les mouvements de la tête, etc. De plus, la mesure de la locomotion peut être un peu biaisée par l'espace restreint des cages opérantes. Finalement, il faut aussi mentionner que les photocellules sont incapables de différencier la stéréotypie d'une baisse de la locomotion, ce qui rend l'observation directe par l'expérimentateur ou la prise en vidéo de la session nécessaire à l'analyse des résultats.

CHAPITRE 5 – Facteurs de croissance et gènes précoces comme marqueurs de la neuroplasticité induite par la cocaïne

5.1 Le BDNF dans l'étude de la neuroplasticité induite par la cocaïne et les drogues d'abus

5.1.1 Description du BDNF et de son récepteur TrkB

Brain-derived neurotrophic factor (BDNF) est un facteur de croissance qui appartient à la famille des neurotrophines des facteurs neurotrophiques (Barde, Edgar et Thoenen, 1982). Les neurotrophines produisent leurs effets biologiques en jouant principalement sur la famille des récepteurs de la tyrosine kinase (Trk). Le BDNF agit spécifiquement sur le récepteur TrkB avec haute affinité (Bibel et Barde, 2000; Klein et al., 1991; Patapoutian et Reichardt, 2001), par lequel il médie majoritairement ses fonctions synaptiques, mais aussi se lie avec faible affinité aux récepteurs de la neurotrophine p75, un membre de la famille des facteurs de nécrose tumorale (TNF) [(Chao, 1994; Rodriguez-Tebar, Dechant et Barde, 1990) et le tout révisé dans (Chao et Hempstead, 1995; Dechant, Rodríguez-Tébar et Barde, 1994; Reichardt, 2006)]. La liaison de BDNF au récepteur TrkB produit la dimérisation du récepteur, l'autophosphorylation des résidus de tyrosine sur le domaine catalytique et induit ainsi l'activation du récepteur (Huang et Reichardt, 2003). Par la suite, l'activation du récepteur TrkB déclenche l'activation de plusieurs cascades de signalisation intracellulaire, qui incluent les voies de la phosphoinositide 3-kinase, de la MAP/ERK kinase et de la phospholipase C-γ (Heerssen et Segal, 2002; Kaplan et Miller, 2000; Patapoutian et Reichardt, 2001).

En conditions normales, le BDNF (l'ARNm ou la protéine) s'exprime dans plusieurs régions du système nerveux central des souris (Hofer, Pagliusi, Hohn, Leibrock et Barde, 1990), des rats (Conner, Lauterborn, Yan, Gall et Varon, 1997; Ernfors, Ibanez, Ebendal, Olson et Persson, 1990; Ernfors, Wetmore, Olson et Persson, 1990), des singes (Zhang et al., 2007) et de l'humain (Murer et al., 1999), mais principalement dans l'hippocampe, l'aire tegmentale ventrale, l'amygdale et le cortex frontal. Au contraire, son récepteur TrkB est largement exprimé partout dans le cerveau (Merlio, Ernfors, Jaber et Persson, 1992; Numan et Seroogy, 1999).

Le BDNF et l'activation des cascades de signalisation qu'il induit sont impliqués dans de nombreuses fonctions, dont le développement et la survie des neurones (Huang et Reichardt, 2001; Jones, Fariñas, Backus et Reichardt, 1994; Kalcheim, Barde, Thoenen et Le Douarin, 1987; Schwartz, Borghesani, Levy, Pomeroy et Segal, 1997), la neurotransmission synaptique et l'excitabilité neuronale (Patterson et al., 1996; Poo, 2001), l'organisation cytosquelettique (Dijkhuizen et Ghosh, 2005; Horch, Krüttgen, Portbury et Katz, 1999; McAllister, Lo et Katz, 1995; Segal, Pomeroy et Stiles, 1995), la mémoire (Alonso et al., 2002; Bekinschtein et al., 2008; Cirulli, Berry, Chiarotti et Alleva, 2004; Mizuno, Yamada, He, Nakajima et Nabeshima, 2003; Mizuno, Yamada, Olariu, Nawa et Nabeshima, 2000; Mizuno, Yamada, Takei, et al., 2003) et l'apprentissage (Hall, Thomas et Everitt, 2000; Linnarsson, Björklund et Ernfors, 1997; Tyler, Alonso, Bramham et Pozzo-Miller, 2002). Mais depuis quelques années, son rôle dans la toxicomanie est de plus en plus étudié [révisé dans (Bolaños et Nestler, 2004; Ghitza et al., 2010; Koskela et al., 2017; Li et Wolf, 2015; Russo, Mazei-Robison, Ables et Nestler, 2009)].

5.1.2 La cocaïne, le BDNF et le TrkB

L'administration aiguë de la cocaïne peut produire des changements dans l'expression des niveaux de BDNF dans le cerveau (Fumagalli, Di Pasquale, Caffino, Racagni et Riva, 2007; Le Foll, Diaz et Sokoloff, 2005). Cependant, ces effets sont plus nombreux et significatifs lors de la consommation chronique de cette drogue. L'administration répétée de la cocaïne (5 infusions consécutives (1/jour) versus 1 injection par i.p.) augmente les niveaux d'ARN messager, mais diminue les niveaux de protéine de BDNF dans le cortex préfrontal, et augmente les niveaux de la protéine précurseur de BDNF (pro-BDNF) dans le striatum (Fumagalli et al., 2007). Similairement, l'auto administration de cocaïne, mais non de sucrose, lors des sessions prolongées (4 heures/session pendant ~3 semaines) augmente les niveaux de la protéine BDNF (Graham et al., 2007) et de TrkB (Graham et al., 2009) dans le *shell* du noyau accumbens, mais pas dans le *core* et le caudé-putamen. Le BDNF jouerait potentiellement aussi un rôle dans la plasticité synaptique qui favorise la rechute à la suite de l'administration chronique de la cocaïne. Par exemple, chez des rats qui s'auto administrent la cocaïne ou du sucrose pendant 10 sessions (6 heures/session), il y une augmentation progressive des taux de la protéine BDNF dans l'aire tegmentale ventrale, le noyau accumbens et l'amygdale, et ceci en fonction du temps de retrait depuis la dernière dose (1, 30 et 90 jours), seulement chez ceux qui ont consommé la cocaïne (Grimm et al., 2003). Il y a aussi une augmentation de l'expression de l'ARN messager de BDNF et une baisse de TrkB (région CA2 seulement) 10 jours après le retrait de la cocaïne dans l'hippocampe de rats ayant reçu 5 injections consécutives de drogue en i.p. [1 injection/jour; (Filip et al., 2006)]. Ces résultats sont constants avec ceux de Pu, Liu et Poo (2006) qui indiquent

qu'après 5-7 jours d'injections quotidiennes de cocaïne, il y a une élévation des niveaux de protéine BDNF dans l'aire tegmentale ventrale des rats après 10-15 jours de retrait.

Similairement, des changements dans la signalisation médiée par le BDNF peuvent aussi induire des comportements de recherche et de prise de drogue. La délétion de TrkB dans les neurones du noyau accumbens chez la souris provoque une baisse de la courbe dose-réponse de l'auto administration de cocaïne et diminue la consommation totale (Graham et al., 2009). L'infusion intracérébrale de BDNF dans le *shell* du noyau accumbens augmente la motivation pour la cocaïne mesurée sous ratio progressif (Graham et al., 2007). Au contraire, la dérégulation du BDNF endogène par le traitement avec un anticorps anti-BDNF induit une baisse dans la courbe dose-réponse de l'auto administration de la cocaïne et diminue le risque de rechute induite par la cocaïne ou le stress (Graham et al., 2007).

5.2 Le C-*fos* dans l'étude de la neuroplasticité induite par la cocaïne et les drogues d'abus

5.2.1 Description de c-*fos*

Le c-*fos* est un proto-oncogène, i.e. un gène qui peut potentiellement devenir un oncogène et causer une tumeur à la suite de mutations ou sa surexpression. Ce gène a été identifié à partir de son homologie avec v-*fos*, l'oncogène rétroviral provenant du virus de l'ostéosarcome murin Finkel-Biskis-Jinkin (FBJ) (Curran et Teich, 1982). En effet, la forme normale (c-*fos*) et la forme virale peuvent induire des tumeurs osseuses *in vivo* et altérer les fibroblastes *in vitro* (Curran, Peters, Van Beveren, Teich et Verma, 1982; Verma, 1986). La transcription du c-*fos* est induite très rapidement par des mitogènes et des agents de différentiation spécifique (Verma, 1986). Le fait que le c-*fos* permet de

modifier rapidement l'expression de gènes en réponse à des changements dans le milieu extracellulaire explique pourquoi ce dernier appartient à la famille des gènes précoces immédiats (Curran et Morgan, 1995; Sheng et Greenberg, 1990).

Le c-*fos*, ainsi que les autres membres de la famille Fos (FosB, Fra-1 et Fra-2) sont des facteurs de transcription qui à la suite de leur dimérisation avec un membre de la famille Jun, permettent l'activation du complexe transcriptionnel de la protéine activatrice-1 (AP-1). Cette forme active peut se lier aux sites AP-1 dans les régions régulatrices de différents gènes et ainsi permettre leur transcription (Curran et Franza Jr, 1988; Sassone-Corsi, Sisson et Verma, 1988; Sheng et Greenberg, 1990). Le c-*fos* agit comme un facteur de transcription. Il est impliqué dans de nombreuses fonctions à travers le corps, telles que la croissance et la différentiation cellulaire, le développement (Distel, Ro, Rosen, Groves et Spiegelman, 1987; Müller et Wagner, 1984; Nishikura et Murray, 1987; Rüther, Garber, Komitowski, Müller et Wagner, 1987), ainsi que dans la plasticité neuronale (Curran et Morgan, 1987).

De nos jours, les gènes Fos sont aussi utilisés comme marqueurs de l'activité neuronale au niveau du cerveau (Dragunow et Faull, 1989; Kovács, 1998), car la transcription du c-*fos* en conditions basales est très faible et celle-ci est facilement induite par une variété de stimuli (Kovács, 1998), dont les substances d'abus (Hyman et Malenka, 2001; Nestler, 2004a). En effet, l'induction de la transcription de l'ARNm de c-*fos* débute dans les 5 minutes après la stimulation et atteint son pic en 30-60 minutes, tandis que la protéine atteint ses niveaux maximaux en 1-3 heures (Kovács, 1998; Morgan et Curran, 1991).

5.2.2 La cocaïne et le c-*fos*

L'administration aiguë de la cocaïne induit l'expression rapide et transitoire de l'ARN messager du c-*fos* et de la protéine Fos dans le striatum (Graybiel, Moratalla et Robertson, 1990; Hope, Kosofsky, Hyman et Nestler, 1992; Young, Porrino et Iadarola, 1991). À L'opposé, l'administration chronique de cocaïne semble induire une tolérance à la réponse c-*fos* induite par la cocaïne (Daunais, Roberts et McGinty, 1993; Daunais, Roberts et McGinty, 1995; Hope et al., 1992; Moratalla, Elibol, Vallejo et Graybiel, 1996). Cet effet est contrecarré progressivement par des périodes de sevrage plus longues (Moratalla et al., 1996). De plus, l'administration chronique de la cocaïne induirait ΔFosB (Hope et al., 1992; McClung et Nestler, 2003), une variante d'épissage de FosB, dans le noyau accumbens (Hope et al., 1994). La surexpression de cette variante a été impliquée dans les effets renforçateurs et locomoteurs induits par la cocaïne (Colby, Whisler, Steffen, Nestler et Self, 2003; Kelz et al., 1999).

Certaines études suggèrent que les changements observés au niveau de la régulation de c-*fos* seraient induits par des facteurs pharmacocinétiques. Par exemple, la quantité de cocaïne auto administrée, déterminée par l'exposition aiguë ou chronique à la drogue (1 versus 6 jours) produit des changements dans les niveaux de la protéine c-*fos*. Ces altérations sont caractérisées par l'augmentation ou la diminution de l'expression de cette protéine dans différentes structures du cerveau (e.g. l'exposition chronique à la cocaïne, lorsque comparée à une exposition aiguë, diminue le c-*fos* dans le *shell* du noyau accumbens, mais l'augmente dans l'aire tegmentale ventral (Zahm et al., 2009). De plus, la vitesse d'infusion de la cocaïne modifie l'expression de l'ARN messager de c-*fos* en traitement aiguë. En effet, les infusions rapides de cocaïne (en 5

versus 100 secondes) augmentent l'expression de l'ARN messager de c-*fos* dans les cortex préfrontal médian et orbitofrontal, et dans le striatum (Ferrario et al., 2008; Samaha, Mallet, Ferguson, Gonon et Robinson, 2004).

CHAPITRE 6 – Données récentes, objectifs et hypothèses

La toxicomanie est une maladie complexe, dont plusieurs facteurs favorisent son développement. Une grande partie de la recherche se concentre sur le rôle des facteurs biologiques, génétiques et environnementaux dans la transition d'un usage occasionnel vers la toxicomanie. Par contre, un bon modèle animal de la toxicomanie tient compte de facteurs pharmacocinétiques [voir (Allain et al., 2015)]. Deux facteurs pharmacocinétiques de la consommation de la cocaïne seront évalués dans cette thèse, soit les effets de 1) la vitesse d'administration de la drogue et 2) la fréquence de consommation.

6.1 Les effets de la vitesse d'infusion de la cocaïne sur la Cmax et le Tmax de la cocaïne et de la dopamine dans le striatum.

Les drogues, les formulations et les voies d'administration qui permettent l'atteinte de taux élevés de drogue rapidement au cerveau faciliteraient le développement de la toxicomanie. Les individus qui consomment la cocaïne par des voies rapides (voie intraveineuse ou inhalation) sont plus à risque de développer la toxicomanie, comparativement à ceux qui utilisent des voies plus lentes [voie intranasale; (Ferri et Gossop, 1999; Gossop et al., 1992; Gossop et al., 1994; Hatsukami et Fischman, 1996; Van Dyke et Byck, 1982)]. De plus, plusieurs consommateurs de la cocaïne passent de la voie intranasale aux voies plus rapides, au fur et à mesure que la toxicomanie se développe (Dunn et Laranjeira, 1999; Gorelick, 1992).

Au niveau préclinique, les résultats sont similaires. Chez les singes, l'infusion rapide de la cocaïne par voie intraveineuse augmente la consommation (Balster et

Schuster, 1973; Kato, Wakasa et Yanagita, 1987; Panlilio et al., 1998) et la motivation pour la drogue (Woolverton et Wang, 2004). Chez les rats, l'administration de cocaïne infusée rapidement favorise de nombreux comportements caractéristiques de la toxicomanie. L'auto administration d'infusions rapides de cocaïne par voie intraveineuse produit de grands changements comportementaux et neurobiologiques, et ceci malgré une petite variation dans la vitesse d'infusion (i.e. en 4-5 versus 16-100 secondes). Les infusions rapides augmentent la consommation de drogue (Allain, Roberts, Levesque et Samaha, 2017; Bouayad-Gervais, Minogianis, Lévesque et Samaha, 2014; Minogianis, Lévesque et Samaha, 2013; Wakabayashi, Weiss, Pickup et Robinson, 2010), la motivation pour la drogue (Allain et al., 2017; Minogianis et al., 2013) et la vulnérabilité à la rechute (Wakabayashi et al., 2010). Aussi, les infusions rapides facilitent le développement de la sensibilisation psychomotrice induite par la cocaïne (Allain et al., 2017; Samaha, Li et Robinson, 2002; Samaha et al., 2004). Finalement, elles causent aussi de nombreux changements neurobiologiques (Allain et al., 2017; Brown et Kiyatkin, 2005; Ferrario et al., 2008; Samaha et al., 2004).

Ces données suggèrent que même des petites différences dans la vitesse d'administration de la cocaïne par i.v. peuvent influencer le comportement. Effectivement, il est important de comprendre comment des petites différences dans la vitesse d'infusion de la cocaïne par i.v. entre 5 et 90-100 secondes peuvent influencer le risque de toxicomanie. Étudier les effets produits par cette gamme de vitesse (5-90 s) est importante pour les raisons suivantes : 1) les individus expérimentés à la cocaïne rapportent qu'ils s'injectent la drogue entre 3 et 10 secondes (Zernig et al., 2003); 2) la différence temporelle pour le début des effets subjectifs produits par la cocaïne par voie

intraveineuse versus la voie intranasale est similaire à cette gamme de vitesses (i.v. : 2-9 secondes (Zernig et al., 2003); i.n. : 30-120 secondes (Jones, 1984; Weiss R.D., 1993); et 3) l'administration intraveineuse de la cocaïne entre 2 et 60 secondes chez l'humain, permet d'observer des différences dans les mesures subjectives des effets de la drogue, tels que le *rush* et le *high* (Abreu, Bigelow, Fleisher et Walsh, 2001; Nelson et al., 2006). Cependant, nous ne savons pas si cela est dû à la vitesse à laquelle la drogue arrive à ses sites d'actions dans le cerveau, aux concentrations maximales atteintes ou si les deux possibilités seraient potentiellement impliquées. Cette question reste sans réponse, car les concentrations de cocaïne dans le cerveau à la suite d'infusions de la drogue à différentes vitesses par voie intraveineuse ont seulement été modélisées (Allain et al., 2015; Allain et al., 2017), mais pas mesurées. En effet, une étude de modélisation pharmacocinétique suggère qu'injecter la cocaïne entre 5 et 100 secondes ne change pas les concentrations maximales de drogue dans le cerveau (Cmax), mais change plutôt le temps nécessaire pour atteindre ce pic (Tmax) (Samaha et al., 2002).

Nous avons donc émis l'hypothèse que le fait de varier la vitesse d'infusion de la cocaïne par voie intraveineuse entre 5 et 90 secondes ne produit pas de changements importants dans la concentration maximale de la drogue, mais plutôt modifie le temps nécessaire pour atteindre ces pics (Article 1). Premièrement, nous nous basons sur la modélisation pharmacocinétique décrite ci-dessus (Samaha et al., 2002). Deuxièmement, Ferrario et al. (2008) avaient démontré qu'augmenter la vitesse d'infusion de la cocaïne entre 100 et 5 secondes n'influençait pas les niveaux maximaux de dopamine induit par la drogue, mais la concentration de cocaïne augmentait plus rapidement. Sachant que les concentrations de dopamine et de cocaïne sont fortement

corrélés (Nicolaysen, Pan et Justice Jr, 1988; Shou, Ferrario, Schultz, Robinson et Kennedy, 2006), il est probable que les concentrations de cocaïne varient de la même façon.

6.2 Les changements neurobiologiques induits par la vitesse d'infusion de la cocaïne provoquent les changements comportementaux observés : effets de la vitesse d'infusion de la cocaïne sur la régulation du facteur de transcription BDNF.

Les changements observés dans le comportement suite aux variations dans la vitesse d'infusion de la cocaïne seraient potentiellement dus aux changements neurobiologiques induits par la vitesse à laquelle la drogue arrive au cerveau (Samaha et Robinson, 2005). En effet, l'induction et la persistance de ces neuroadaptations seraient possiblement la cause du développement de comportements pathologiques qui sont caractéristiques de la toxicomanie [voir (Kalivas et O'Brien, 2008)], tels que la recherche et prise de drogue compulsive, la motivation excessive pour obtenir la drogue, ainsi que la rechute induite par les indices associés à la drogue ou la drogue elle-même. L'administration d'infusions rapides de drogue par i.v. cause nombreux changements dans le cerveau. Par exemple, chez le rat, l'administration rapide de la cocaïne modifie la régulation de gènes précoces immédiats *c-fos* et *arc* (Ferrario et al., 2008; Samaha et al., 2004)] et des récepteurs métabotropes glutamatergiques 2/3 [(mGluR2/3); (Allain et al., 2017)], et induit des changements plus rapides dans les niveaux de dopamine extracellulaire (Ferrario et al., 2008) et l'inhibition du transporteur de la dopamine (Samaha et al., 2004). Finalement, les infusions rapides de la cocaïne par voie intraveineuse (Brown et Kiyatkin, 2005) ou l'administration de la cocaïne par la voie

intraveineuse versus la voie intrapéritonéale (Porrino, 1993) augmentent l'activité métabolique de plusieurs régions méso-corticostriatales, signe de leur recrutement. De plus, l'administration d'infusions rapide de cocaïne induisent une sensibilisation psychomotrice plus importante [(Allain et al., 2017; Samaha et al., 2002; Samaha et al., 2004); malgré que la sensibilisation peut aussi être évoquée par des injections lentes de cocaïne via les voies intrapéritonéale, sous-cutanée et orale (Churchill, Swanson, Urbina et Kalivas, 1999; Dow-Edwards, Fico, Osman, Gamagaris et Hutchings, 1989; Li, Acerbo et Robinson, 2004)].

Lors de la recherche de neuroplasticité induite par la consommation de la cocaïne ou autres drogues d'abus, il faut être capable de différencier les neuroadaptations impliquées dans la toxicomanie de celles qui ne correspondent qu'à des changements liés à la simple utilisation de la drogue. Cette distinction est nécessaire non seulement pour pouvoir bien distinguer la neuroplasticité qui facilite le développement de la toxicomanie, mais aussi pour pouvoir éventuellement trouver de bonnes cibles pour prévenir ou traiter cette maladie. Ainsi, deux études présentées dans cette thèse (Articles 2 et 3) ont comme but d'identifier des neuroadaptations produites par la consommation répétée d'infusions rapides de cocaïne chez des animaux qui ont démontré une motivation excessive pour la drogue sous ratio progressif. Dans un premier cas, nous avons examiné les changements produits par l'administration chronique d'infusions rapides de cocaïne (en 5 versus 90 secondes) chez des rats qui avaient eu un accès prolongé à la drogue (LgA; 6 heures/session) sur l'expression de l'ARNm du facteur de croissance *Brain-derived neurotrophic factor* (BDNF), ainsi que son récepteur *Tropomyosin receptor kinase B* (TrkB). La consommation de la cocaïne altère la

signalisation intracellulaire et l'activité des facteurs de transcription (Lu, 2003; Patapoutian et Reichardt, 2001), ce qui induit des changements persistants dans la plasticité synaptique (McGinty, Whitfield Jr et Berglind, 2010). Puisque 1) la consommation chronique de la cocaïne modifie les niveaux de l'ARN messager et de la protéine de BDNF (Fumagalli et al., 2007; Grimm et al., 2003; Im, Hollander, Bali et Kenny, 2010) et 2) l'altération de la signalisation médiée par BDNF dans les régions corticostriatales module des comportements de recherche et de prise de drogue (Graham et al., 2007; Graham et al., 2009; Im et al., 2010; McGinty et al., 2010), nous avons émis l'hypothèse que l'administration d'infusions rapides de cocaïne (en 5 versus 90 s) induirait des changements dans la régulation de l'ARNm de BDNF et de son récepteur TrkB et que ces changements seraient différents de ceux qui sont provoqués par des infusions plus lentes de drogue (Article 2).

6.3 Les effets de la fréquence de consommation de la cocaïne et de la vitesse d'infusion sur la régulation de l'ARN messager du facteur de transcription c-*fos*.

Pendant longtemps, la littérature suggérait que l'exposition chronique du cerveau à de grandes quantités de cocaïne de façon continue était nécessaire pour reproduire des comportements caractéristiques de la toxicomanie (Ahmed et Koob, 1998). Ainsi, la consommation de cocaïne était évaluée suite à des sessions d'accès prolongé à la drogue [6 h/session; Long access (LgA); (Ahmed et Koob, 1998)]. En effet, l'administration de la cocaïne en LgA, comparativement aux sessions d'accès limité à la drogue [1-2 h/session; Short access (ShA)], augmente la consommation de cocaïne avec le temps (Ahmed et Koob, 1998; Ahmed et Koob, 1999; Ben-Shahar, Ahmed, Koob et

Ettenberg, 2004b; Mantsch et al., 2004; Wee et al., 2008), la motivation pour la drogue sous ratio progressif (Ben-Shahar et al., 2008; Paterson et Markou, 2003), ainsi que le risque du rétablissement de recherche induit par la drogue elle-même suite à une longue période de sevrage (Ferrario et al., 2005; Knackstedt et Kalivas, 2007). Au niveau neurobiologique, la consommation de cocaïne en LgA, mais non en ShA, diminue les taux d'ARNm du récepteur dopaminergique D2 dans les cortex préfrontal médian et orbitofrontal, ainsi que la protéine du récepteur D2 dans le cortex préfrontal médian après 4 et 44 jours de sevrage (Briand et al., 2008). Similairement, l'accès prolongé à la drogue modifie l'expression des récepteurs glutamatergiques dans le cortex préfrontal médian en diminuant l'expression des sous-unités GluA du récepteur AMPA (Sun, Zelek-Molik et McGinty, 2013) et des sous-unités N2Ra et N2Rb du récepteur NMDA (Ben-Shahar et al., 2009). Les rats en LgA, et non ceux en ShA, montrent aussi des changements structurels au niveau des neurones moyens épineux du noyau accumbens. La densité des épines dendritiques sur ces neurones est augmentée après un mois d'abstinence (Ferrario et al., 2005). Ces changements comportementaux et neurobiologiques suggèrent que beaucoup de drogue prise de façon continue est nécessaire au développement de symptômes similaires à ceux qui caractérisent la toxicomanie.

Plus récemment, un sondage effectué auprès de consommateurs de la cocaïne rapporte que les usagés expérimentés et les usagés occasionnels consomment de quantités similaires de cocaïne, prises dans environ la même plage de temps. Cependant, les personnes ayant un trouble lié à l'usage de la cocaïne obtiennent moins d'utilisations que les usagers occasionnels. Ceci suggère que les consommateurs expérimentés consomment de plus grandes doses de drogue espacées par des

intervalles de temps plus longues entre chaque consommation (Beveridge et al., 2012), ce qui produit des fluctuations des niveaux de cocaïne dans leur cerveau. Le modèle d'accès intermittent (IntA) à la drogue, développé par Zimmer *et al*., permet de créer des pics et des baisses de cocaïne dans le cerveau de rats qui s'auto administrent la drogue (Zimmer, Dobrin et Roberts, 2011; Zimmer et al., 2012). Durant la session IntA, la cocaïne est disponible pendant des périodes de 5 minutes, qui sont séparées par des temps morts de 25 minutes où la drogue est indisponible. Ceci forme un cycle, qui est répété douze fois durant la session. Considérant que la demi-vie de la cocaïne dans le cerveau du rat est d'environ une vingtaine de minutes (Hurd, Kehr et Ungerstedt, 1988; Nayak, Misra et Mule, 1976), ceci permet la baisse de la concentration de cocaïne avant la prochaine période d'accès à la drogue. Ce qui est intéressant avec ce patron de consommation est que les rats IntA montrent nombreux comportements qui caractérisent la toxicomanie, et ceci malgré le fait qu'ils consomment moins de drogue que les rats LgA. Malgré une exposition moins importante à la drogue, l'IntA, comme le LgA, permet le développement de sensibilisation psychomotrice (Allain et al., 2017), la consommation malgré des conséquences néfastes (Singer, Fadanelli, Kawa et Robinson, 2018) et le risque du rétablissement du comportement de recherche induit par la des indices associés à la drogue (Kawa, Bentzley et Robinson, 2016; Singer et al., 2018) ou la drogue elle-même (Kawa et al., 2016). De plus, l'accès intermittent favorise l'augmentation de la consommation avec le temps (Allain, Bouayad-Gervais et Samaha, 2018; Kawa et al., 2016; Pitchers, Wood, Skrzynski, Robinson et Sarter, 2017), comme chez les rats LgA. Cependant, les rats IntA sont plus motivés pour la drogue que les rats LgA (Allain et al., 2018; Kawa et al., 2016; Zimmer et al., 2012). Finalement, l'IntA produit aussi de la

neuroplasticité au niveau du transporteur dopaminergique, décrit par le développement de la sensibilisation aux effets bloquants de la cocaïne sur le transporteur dopaminergique, tandis que le LgA induit la tolérance à ces mêmes effets (Calipari, Ferris, Zimmer, Roberts et Jones, 2013).

Ceci dit, dans la deuxième étude examinant les changements induits par la vitesse d'infusion de la cocaïne, nous avions voulu comparer les changements dans l'expression de l'ARNm du gène précoce c-*fos*, un marqueur d'activation neuronale, chez un groupe de rats plutôt pertinent à la toxicomanie (IntA avec infusions de drogue en 5 secondes) versus un groupe de rats qui montre une consommation importante sans présenter des comportements caractéristiques à la toxicomanie (LgA avec infusions de drogue en 90 secondes) (Article 3). Alors, nous avions émis l'hypothèse que les animaux qui consomment des infusions rapides et intermittentes de cocaïne seraient plus motivés pour la drogue et montreraient des changements différents au niveau de l'expression de l'ARNm c-*fos* dans les régions corticostriatales comparativement aux animaux qui auraient reçu un accès continu à la cocaïne infusée plus lentement. Nous émettons cette hypothèse parce que 1) les rats IntA sont plus motivés pour la drogue que les rats LgA (Allain et al., 2018; Kawa et al., 2016; Zimmer et al., 2012) et 2) les infusions rapides de cocaïne (en 5 versus 100 secondes) augmentent l'expression de l'ARN messager de c-*fos* dans les cortex préfrontal médian et orbitofrontal, et dans le striatum (Ferrario et al., 2008; Samaha et al., 2004).

6.4 Rôle potentiel du cortex orbitofrontal, du striatum dorsal et du circuit OFC-DS dans la motivation excessive pour la cocaïne à la suite d'infusions rapides et intermittentes de la drogue.

Les différentes observations obtenues lors de ces derniers travaux portant sur la neuroplasticité induite au niveau de la régulation des gènes BDNF, TrkB et c-*fos,* nous ont conduit à nous concentrer sur le cortex orbitofrontal et le striatum dorsal comme cibles qui contrôlent la motivation excessive pour la drogue. L'OFC est impliqué dans la prise de décisions complexes (Bechara et al., 2001; Bolla et al., 2003; Rogers et al., 1999; Schoenbaum, Roesch et Stalnaker, 2006), dans l'émotion (Goldstein et al., 2007; Kim et al., 2010; Kringelbach, 2005), ainsi que l'attribution de saillance (Volkow, Fowler et Wang, 2003, 2004), tandis que le DS joue un rôle dans la locomotion (Roland, 1984; Roland, Meyer, Shibasaki, Yamamoto et Thompson, 1982) et l'apprentissage d'habitudes (Cramer et al., 2011; Ersche et al., 2011; Feltenstein et See, 2013; Graybiel, 2008; Sjoerds et al., 2013). Les deux structures sont recrutées lors du développement de la toxicomanie (Everitt et al., 2008; Everitt et Robbins, 2005). De plus, l'OFC envoie des projections glutamatergiques au DS (Berendse, Graaf et Groenewegen, 1992; Schilman, Uylings, Graaf, Joel et Groenewegen, 2008) et beaucoup d'études suggèrent que la neuroplasticité induite par les drogues d'abus découlerait de mécanismes glutamatergiques (Kalivas, 2009; Kalivas et al., 2009; Kalivas et O'Brien, 2008). Ceci dit, la vitesse d'infusion rapide de la cocaïne, ainsi que la consommation par intermittence, sont associées avec des changements comportementaux (augmentation de la motivation pour la drogue) et neurobiologiques (altérations dans la régulation des gènes BDNF, TrkB et c-*fos* dans l'OFC et le DS). Nous avions donc émis l'hypothèse que le circuit OFC-DS

serait impliqué dans la motivation excessive pour la cocaïne observée dans le modèle d'auto administration IntA qui produit des symptômes caractéristiques de la toxicomanie (Article 4). Ainsi, nous avions procédé à la déconnection du circuit OFC-DS via une inactivation pharmacologique et temporaire controlatérale (inactivation d'une structure par hémisphère) avec des microinfusions d'un mélange d'agonistes gabaergiques A et B (muscimol et baclofen, respectivement) infusé directement dans le cerveau immédiatement avant de mesurer la motivation des animaux pour la drogue sous ratio progressif. Selon les données décrites ci-dessus, nous nous attendions à ce que la déconnection du circuit diminue la motivation pour la cocaïne.

6.5 Les objectifs de l'étude

L'objectif principal de cette thèse était de mieux comprendre les changements comportementaux et neurobiologiques provoqués par l'administration d'infusions rapides de la cocaïne, ainsi que la fréquence de la consommation (LgA vs. IntA), dans un modèle d'auto administration de cocaïne chez le rat.

Notre premier objectif était d'étudier les effets induits par l'administration de la cocaïne en i.v. à des vitesses d'infusion entre 5 et 90 secondes. Nous avions mesuré les concentrations de cocaïne et de dopamine en fonction du temps dans le striatum de rats à la suite d'une infusion de cocaïne en 5, 45 ou 90 secondes. À cette fin, nous avions utilisé la microdialyse couplée avec du *high performance liquid chromatography-tandem mass spectrometry* (HPLC-MS/MS) (Article 1). Le deuxième objectif a été de commencer à identifier les changements neurobiologiques provoqués par la vitesse d'infusion et/ou

le patron de consommation chez des rats qui ont chroniquement auto administré la cocaïne et qui montrent une motivation excessive pour la drogue sous ratio progressif. L'hybridation *in situ* a été utilisée pour mesurer l'expression de l'ARN messager de BDNF, TrkB (article 2) et c-*fos* (article 3) dans les régions corticostriatales. Finalement, notre dernier objectif était d'identifier des structures et des circuits potentiellement impliqués dans la motivation excessive pour la cocaïne facilitée par l'auto administration chronique d'infusions rapides et intermittents de la drogue. Basés sur nos études précédentes, nous avions ciblé le circuit OFC-DS, que nous avons déconnecté avec la microinfusion intracérébrale d'un mélange d'agonistes gabaergiques A et B juste avant de mesurer leur motivation pour la cocaïne sous ratio progressif.

Articles

CHAPITRE 7

Varying the rate of intravenous cocaine infusion influences the temporal dynamics of both drug and dopamine concentrations in the striatum

Running Title: Rate of cocaine delivery & striatal drug kinetics

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Abstract

The faster drugs of abuse reach the brain, the greater is the risk of addiction. Even small differences in the rate of drug delivery can influence outcome. Infusing cocaine intravenously over 5 versus 90-100 seconds promotes sensitization to the psychomotor and incentive motivational effects of the drug and preferentially recruits mesocorticolimbic regions. It remains unclear whether these effects are due to differences in how fast and/or how much drug reaches the brain. Here, we predicted that varying the rate of intravenous cocaine infusion between 5-90 seconds produces different rates of rise of brain drug concentrations, while producing similar peak concentrations. Freely-moving male Wistar rats received acute intravenous cocaine infusions (2.0 mg/kg/infusion) over 5, 45 and 90 seconds. We measured cocaine concentrations in the dorsal striatum using rapid-sampling microdialysis (1 sample/minute) and high-performance liquid chromatography-tandem mass spectrometry. We also measured extracellular concentrations of dopamine and other neurochemicals. Regardless of infusion rate, acute cocaine did not change concentrations of non-dopaminergic neurochemicals. Infusion rate did not significantly influence peak concentrations of cocaine or dopamine, but concentrations increased faster following 5-second infusions. We also assessed psychomotor activity as a function of cocaine infusion rate. Infusion rate did not significantly influence total locomotion, but locomotion increased earlier following 5-second infusions. Thus, small differences in the rate of cocaine delivery influence both the rate of rise of drug and dopamine concentrations and psychomotor activity. A faster rate of rise of drug and dopamine concentrations might be an important issue in making rapidly delivered cocaine more addictive.

Keywords: Cocaine addiction, *In vivo* microdialysis, Locomotor activity, Male Wistar rats, Pharmacokinetics

Introduction

The rate of drug delivery to the brain is important in determining the risk of developing addiction. For instance, smoking cocaine or injecting the drug intravenously is associated with a greater vulnerability to addiction than intranasal use (Gossop, Griffiths, Powis et Strang, 1994; Hatsukami et Fischman, 1996). After smoking or intravenous (i.v.) infusion, plasma cocaine concentrations rise more rapidly and reach higher peaks than after intranasal administration (Javaid, Fischman, Schuster, Dekirmenjian et Davis, 1978; Jeffcoat, Perez-Reyes, Hill, Sadler et Cook, 1989; Jones, 1990). Such pharmacokinetic differences are important in determining outcome. For instance, different pharmacokinetic profiles are one reason why the same drug can lead to addiction when taken by one route (nicotine inhaled from tobacco smoke) but can treat addiction when taken by another [nicotine administered orally from a gum or through the skin from a patch (Henningfield et Keenan, 1993)].

Varying the rate of drug delivery to the brain influences the psychological and behavioural effects of drugs. In humans, cocaine (Resnick, Kestenbaum et Schwartz, 1977) and heroin (Comer, Collins, MacArthur et Fischman, 1999) evoke more immediate and stronger pleasurable effects when administered i.v. rather than intranasally. Similarly, increasing the rate of i.v. cocaine (Abreu, Bigelow, Fleisher et Walsh, 2001; Fischman et Schuster, 1984) or morphine (Marsch et al., 2001) infusion produces greater self-reports of euphoria. Early studies in laboratory animals show that relatively large variations in the rate of i.v. drug administration [i.e., injecting a dose between 5-600 seconds (s)] influence cocaine self-administration behaviour (Balster et Schuster, 1973; Woolverton et Wang, 2004). More recent experiments have explored the effects of varying the rate of i.v.

infusion over a smaller range of time (5-100 s). Doing so is important for three main reasons. First, experienced i.v. cocaine users report that they inject the drug rapidly and over a narrow range [3-10 s (Zernig et al., 2003)]. Second, the time difference in the onset of subjective drug effects in i.v. versus intranasal cocaine users is also narrow [2-9 s (Zernig et al., 2003) and 30-120 s (Jones, 1984; Weiss R.D., 1993), respectively]. Finally, injecting cocaine i.v. over 2 versus 60 s influences the magnitude of subjective cocaine effects in humans (Abreu et al., 2001). Such small differences in the rate of drug delivery can have large effects on behaviour in laboratory rats. For instance, injecting cocaine or nicotine i.v. over 5 versus 90-100 s promotes sensitization to the psychomotor (Allain, Roberts, Levesque et Samaha, 2017; Samaha, Li et Robinson, 2002; Samaha, Mallet, Ferguson, Gonon et Robinson, 2004; Samaha et Robinson, 2005) and incentive motivational effects of these drugs (Allain et al., 2017; Bouayad-Gervais, Minogianis, Lévesque et Samaha, 2014; Liu, Roberts et Morgan, 2005; Minogianis, Lévesque et Samaha, 2013). Rats that self-administer i.v. cocaine injections delivered over 5 versus 90 s also take more drug (Bouayad-Gervais et al., 2014; Minogianis et al., 2013; Wakabayashi, Weiss, Pickup et Robinson, 2010), and are more susceptible to relapse following extended abstinence (Wakabayashi et al., 2010).

Variation in the rate of drug delivery influences behaviour presumably because it changes the neurobiological impact of drugs. Studies in rats show that increasing the rate of cocaine delivery to the brain enhances cellular activity in mesocorticolimbic areas [reviewed in (Samaha et Robinson, 2005)]. A first study showed that compared to intraperitoneal cocaine, i.v. cocaine enhances glucose utilization in corticolimbic regions (Porrino, 1993). More recent work shows that injecting cocaine i.v. over 5 versus 90-100

s increases drug-induced immediate early gene expression (Samaha et al., 2004), heat-producing metabolic activity (Brown et Kiyatkin, 2005), and regulation of the growth factor, brain-derived neurotrophic factor and its receptor TrkB (Bouayad-Gervais et al., 2014). Chronic intake of i.v. infusions of cocaine delivered over 5 s but not over 90 s also increases the function of metabotropic group II receptors in the prelimbic cortex and nucleus accumbens (Allain et al., 2017).

Thus, small differences in the rate of i.v. cocaine delivery can predict outcome but is this due to how fast cocaine enters the brain, peak achieved concentration, or both? This question remains unanswered because brain concentrations of cocaine have been *modelled* (Allain et al., 2017; Samaha et al., 2002), but not actually *measured* as a function of the rate of i.v. drug delivery. It is possible that varying the rate of i.v. drug infusion, even over a small range, produces differences in achieved concentration, and this could contribute to the effects seen on brain and behaviour. However, beyond achieved dose, temporal pharmacokinetic variables such as how fast drug levels rise can determine behavioural and neurobiological effects relevant to addiction (Allain, Minogianis, Roberts et Samaha, 2015; Allain et al., 2017). The brain is sensitive to the temporal pattern of cellular stimulation. For instance, in a context outside of drugs of abuse, different temporal patterns of electrical stimulation are differentially effective in producing long-term potentiation (Larson et Lynch, 1986) and dopamine-evoked synaptic plasticity (Wieland et al., 2015).

Here, we hypothesized that varying the rate of i.v. cocaine delivery between 5 and 90 s produces differences in the rate of rise of striatal cocaine and dopamine concentrations, without producing large effects on peak concentrations. We make this

prediction first because it is supported by pharmacokinetic modeling [(Allain et al., 2017; Samaha et al., 2002); where the pharmacokinetic model was based on (Pan, Menacherry et Justice, 1991)]. Second, dopamine and cocaine concentrations are tightly correlated in the brain (Nicolaysen, Pan et Justice Jr, 1988; Shou, Ferrario, Schultz, Robinson et Kennedy, 2006), and injecting cocaine i.v. over 5 versus 100 s produces differences in the rate of rise of extracellular dopamine concentrations in the striatum, without affecting peak concentrations [(Ferrario et al., 2008) but see (Samaha et al., 2004), where brief differences in the half-life of electrically-stimulated extracellular dopamine were observed]. To test our hypothesis, we injected freely-moving rats with i.v. infusions of cocaine delivered over 5, 45 and 90 s, and we used rapid-sampling (1 sample/minute) microdialysis coupled with high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to simultaneously measure cocaine and dopamine concentrations in the dorsal striatum. We measured dopamine concentrations because cocaine-induced increases in dopamine overflow regulate the incentive motivational effects of the drug. The microdialysis samples were also used to determine cocaine effects on 20 other neurochemicals in the striatum. As an additional functional measure, we assessed the psychomotor response to acute cocaine infused over 5, 45 and 90 seconds, in a separate cohort of rats. Based on prior work, we predicted that varying the rate of cocaine delivery over this range would not influence the magnitude of the locomotor response (Samaha et al., 2002), but that rate could influence the time course of cocaine-induced locomotion.

Materials and Methods

Animals and Housing

Twenty male Wistar rats (7 for microdialysis and 13 for cocaine-induced psychomotor activity; Charles River Laboratories, St-Constant, QC) weighing between 225-300 g upon arrival were housed individually in a climate-controlled colony room maintained on a reverse 12 h/12 h light/dark cycle (lights off at 8:30 - 9:00 am). Experiments were conducted during the dark phase of the rats' circadian cycle. Food and water were available *ad libitum*. The animal care committees of the Université de Montréal (CDEA 14-149 and 17-095) and of Concordia University approved all procedures involving animals. These procedures complied with guidelines of the Canadian Council on Animal Care.

Surgery

Following 1-4 weeks of acclimatization to the vivarium, a custom-made, indwelling catheter was implanted into the jugular vein of rats anaesthetized with isoflurane (CDMV; St-Hyacinthe, QC). The other end of the catheter was set to exit between the scapulae. Animals to be used for *in vivo* microdialysis were then placed in a stereotaxic apparatus and a cannula (21-gauge; HRS Scientific, Montreal, QC) was implanted into the dorsal striatum of one hemisphere (counterbalanced across animals; coordinates relative to Bregma; anteroposterior, +1.6 mm, mediolateral, ± 2.5 mm, and dorsoventral, -3.0 mm). Cannulae were anchored to the skull with jeweller's screws, secured in place with dental cement and sealed with obturators (22-gauge; HRS Scientific). We targeted the dorsal

striatum because it is rich in dopamine transporters and it mediates the expression of addiction-related behaviours (Everitt et Robbins, 2005). At the time of surgery, rats received a subcutaneous injection of 5 mg/kg Carprofen (Rimadyl; 50 mg/ml; CDMV, Saint-Hyacinthe, QC) and an intramuscular injection of 0.02 ml of a penicillin G procaine solution (Procillin; 300 000 IU/ml; CDMV, Saint-Hyacinthe, QC). Intravenous catheters were flushed on alternate days with either 0.1 mL physiological saline or a solution containing 0.2 mg/mL Heparin (Sigma-Aldrich, Oakville, ON) and 2 mg/mL of the antibiotic Baytril (CDMV, St- Hyacinthe, QC). Rats recovered in their home cages for 7 days prior to further manipulation.

Determination of microdialysis probe delay time *in vitro*

A first goal was to measure the delay time between placement of our microdialysis probes into a cocaine solution, and detection of the drug in a collected sample. This is important, as it provides a measure of the inevitable delay time that a sample needs to travel from the microdialysis probe, through the tubing set-up and finally into the sample collection vials. Two custom-made microdialysis probes (described below) were perfused at 2 µL/minute with artificial cerebrospinal fluid (aCSF) containing 145 mM NaCl, 2.68 mM KCl, 1.40 mM $CaCl_2*2H_2O$, 1.01 mM $MgSO_4*7H_2O$, 1.55 mM Na_2HPO_4, 0.45 mM $NaH_2PO_4*H_2O$ in HPLC-grade water (pH = 7.4; chemicals from Fisher Scientific, Saint-Laurent, Qc; water from Sigma Aldrich, Oakville, ON). Probes were first placed into a beaker containing a stirred solution of aCSF and ascorbic acid (0.25 mM) at room temperature. After 20 minutes, 10 samples were collected at 1-minute intervals. Probes were then transferred to a second beaker containing aCSF + ascorbic acid solution and

1 μM cocaine, from which 26 samples were taken. Finally, the probes were returned to the aCSF + ascorbic acid solution and 16 additional samples were collected. Samples were collected in 300-μL microcentrifuge vials (VWR, Montreal, QC), and immediately placed in dry ice and stored at -80°C until analysis by HPLC-MS/MS.

In vivo microdialysis probes

In vivo microdialysis was conducted in four custom-made hexagonal chambers (42 x 39 x 33.5 cm) each placed within a larger sound- and light-attenuating cabinet. Microdialysis probes were custom-made. They consisted of a 1 cm length of semi-permeable dialysis membrane (200 μm ID, 216 μm OD, with a molecular weight cut-off of 13 kDa; VWR, Montreal, QC) that extended 4 mm below the tip of the guide cannula. The membrane was inserted into a 20-mm length of 26G stainless steel tubing. The outer end of the membrane was occluded with super glue to create a closed system for dialysate flow. The stainless-steel shaft was inserted into one end of polyethylene (PE) 20 tubing (0.381 mm ID, 1.0922 mm OD; 65 cm long; Scientific Commodities Inc., Lake Havasu City, AZ), while the other end was connected to a dual-channel liquid swivel (Lomir, Notre-Dame-de-l'Ile-Perrot, Qc). The swivel was in turn connected to a syringe pump (Harvard Apparatus, Saint-Laurent, Canada) with PE-20 tubing. Small-diameter fused silica tubing (41 μm ID, 110 μm OD; 2 cm long; HRS Scientific, Montreal, QC) extended into the probe 0.5 mm from the glued tip of the semi-permeable membrane. The other end of the fused silica was glued to PE-1 tubing (0.127 mm ID, 0.254 mm OD; 60 cm long) that extended out of the PE-20 tubing, serving as an outlet for the probe. The microdialysis probe was

secured to the cannula with a stainless-steel collar. The relative recovery rates from the microdialysis probes were 10% for cocaine and 8-15% for dopamine.

In vivo Microdialysis Experiment

Figure 1A illustrates the sequence of experimental events. Following recovery from surgery, microdialysis rats were placed in the test chambers for two, 3.5-hour (h) habituation sessions (one/day). Their catheters were tethered to the i.v. drug infusion line and their cannulae were tethered to the steel spring casing used to protect the microdialysis tubing during sampling. During each habituation session, each rat received 78 µL of saline i.v. over 5, 45 and 90 s, with one infusion given every 90 minutes in counter-balanced order. We injected a volume of 78 µL because for cocaine injections, we would inject 34 µL of saline to account for average catheter volume + 10 µL cocaine solution + 34 µL of saline to ensure that none of the cocaine remains in the catheter. Catheter patency was verified on the second habituation day by i.v. infusion of the short-acting barbiturate, sodium thiopental (20 mg/mL in sterile water, 0.1-0.2 mL/rat; CDMV, St-Hyacinthe, QC). All rats became ataxic within 5 s of the infusion, confirming catheter patency. In rats, sodium thiopental has a $T_{1/2}$ of ~4-5 h (Shideman, Winters, Peterson, Wilner et Gould, 1953), thus we do not expect it to influence microdialysis measurements performed on the next day. On the following day, rats were placed in the test chambers and dialysis probes were lowered into the striatum for a 5-h habituation period. During this period, aCSF was perfused through the microdialysis probe at a rate of 2.0 µL/minute. Rats were awake and freely moving during the experiment. Three hours and fifty minutes into the habituation period, animals were tethered to the cocaine infusion line. It contained

10 µL of cocaine solution (Medisca Pharmaceutique Inc, St-Laurent, QC; 2 mg/kg/infusion in 0.9% physiological saline) separated from additional saline by a small air bubble. The other end of the line was attached to a 3 c.c. BD syringe placed on a syringe pump. A 2 mg/kg dose of cocaine is similar to doses used in prior studies that have measured cocaine or dopamine concentrations in the striatum using *in vivo* microdialysis (Ferrario et al., 2008; Hurd, Kehr et Ungerstedt, 1988; Hurd et Ungerstedt, 1989). This dose also evokes robust immediate early gene expression in the dorsal striatum (Samaha et al., 2004).

Over the last 10 minutes of the 5-h habituation period, 10 baseline dialysate samples were collected, at 1-minute intervals and at a flow rate of 2.0 µL/minute, yielding 2.0 µL/sample. Next, each animal received the first of three i.v. cocaine infusions (2.0 mg/kg/10 µL/infusion), delivered over 5, 45 and 90 s, injected 90 minutes apart, in counter-balanced order. Cocaine infusions were spaced 90 minutes apart because this is 3-4 times longer than cocaine's $T_{1/2}$ in rat brain (Hurd et al., 1988; Nayak, Misra et Mule, 1976). Thus, the 90-minute inter-infusion interval reduces the possibility of carry-over effects between infusions [see also (Hurd et al., 1988)]. Following each cocaine infusion, 15 samples were collected, at 1-minute intervals. Five 1-minute baseline samples were also collected prior to the 2nd and 3rd cocaine infusions. Samples were collected in a 300-µL polypropylene microsampling vial placed on the end of the outlet line. Samples were immediately placed in dry ice and stored at –80°C until processing. At the end of sampling, each microdialysis probe was visually inspected for leaks or breakage. None were detected.

High performance liquid chromatography-tandem mass spectrometry (HP*LC-MS*/*MS*)

Small molecule neurochemical analysis using a triple quadrupole (QQQ) MS. All chemicals were from Sigma Aldrich (St. Louis, MO) unless noted otherwise. Water and acetonitrile for mobile phases were Burdick & Jackson HPLC grade (VWR, Radnor, PA). Artificial CSF consisted of 145 mM NaCl, 2.68 mM KCl, 1.40 mM $CaCl_2$, 1.01 mM $MgSO_4$, 1.55 mM Na_2HPO_4, and 0.45 mM NaH_2PO_4, adjusted pH to 7.4 with NaOH. Cocaine standards (Mallinckrodt Inc., St. Louis, MO) were spiked into a standard mixture for a six-point calibration curve. A modified LC-MS method (Song, Mabrouk, Hershey et Kennedy, 2012; Wong et al., 2016) was used to quantify concentrations of extracellular cocaine and dopamine, as well as acetylcholine, adenosine, aspartate, 3,4-dihydroxyphenylacetic acid, γ-aminobutaric acid, glutamate, glutamine, glucose, glycine, histamine, 5-hydroxyindoleacetic acid, homovanillic acid, 3-methoxytyramine, norepinephrine, normetanephrine, phenylalanine, serine, serotonin, taurine, and tyrosine. Samples were thawed and derivatized with 1.5 µL sodium carbonate, 100 mM; 1.5 µL BzCl, 2% (v/v) BzCl in acetonitrile; 1.5 µL isotopically labeled internal standard mixture diluted in 50% (v/v) acetonitrile containing 1% (v/v) sulfuric acid, and spiked with deuterated acetylcholine and choline (C/D/N isotopes, Pointe-Claire, Canada) to a final concentration of 20 nM. Derivatized samples were analyzed using Thermo Scientific Accela UHPLC system interfaced to a Thermo Scientific TSQ Quantum Ultra triple quadrupole mass spectrometer fitted with a HESI II ESI probe, operating in multiple reaction monitoring. Five-µL samples were injected onto a Phenomenex core-shell biphenyl Kinetex HPLC column (1.7 µm particles, 2.1 mm x 100 mm). Mobile phase A was 10 mM ammonium formate with 0.15% formic acid, and mobile phase B was acetonitrile. Mass transitions for

these derivatized analytes were reported in Wong et al. (2016). Cocaine was detected in its native form with a MS/MS transition of 304 → 182 m/z. The mobile phase was delivered through an elution gradient at 450 µL/minute as follows: initial, 0% B; 0.01 minutes, 19% B; 1 minute, 26% B; 1.5 minutes, 75% B; 2.5 minutes, 100% B; 3 minutes, 100% B; 3.1 minutes, 5% B; and 3.5 minutes, 5% B. Thermo Xcalibur QuanBrowser (Thermo Fisher Scientific) software automatically processed and integrated peaks. Each peak was visually inspected to ensure proper integration. The limits of detection for cocaine and dopamine after derivatization were 0.291 and 0.086 nM, respectively. Limits of detection for the other analytes were 0.045-141 nM.

Histology

Following sampling, animals were anaesthetized with isoflurane and decapitated. Brains were then extracted, frozen and stored at -80°C. The neuroanatomical location of the probes was located on 20-µm thick coronal brain sections, according to the rat brain atlas of Paxinos and Watson (1986).

Psychomotor Activity Experiment

Figure 1B shows the sequence of experimental events. Locomotor activity was assessed in four chambers (31.8 x 25.4 x 26.7 cm; Med Associates, St-Albans, VT), each containing 4 infrared photocells aligned horizontally at the bottom of each cage. The chambers were run by a computer using Med Associates Med-PC version IV software (Med Associates, ST-Albans, VT). Following recovery from surgery, rats were placed in the test chambers

for two, 2-h habituation sessions (one/day). Their catheters were tethered to the i.v. infusion line and a steel spring casing used to protect the line during testing. In the first habituation session, no i.v. infusion was given. During the second habituation session, rats received saline i.v. over 5, 45 or 90 s, thirty minutes into the session. Following the end of this second habituation session, catheter patency was verified by i.v. infusion of Propofol (10 mg/mL; 0.1 mL/rat; CDMV, St-Hyacinthe, QC), a short-acting anaesthetic [$T_{1/2}$ ~27 minutes in Wistar rats; (Dutta, Matsumoto, Gothgen et Ebling, 1997)]. Sodium thiopental was not used in this 2nd experiment because it was no longer available from the manufacturer. All rats became ataxic within 5 s of the Propofol infusion, confirming catheter patency. On the next day, animals were placed in the test chambers and tethered to an infusion line containing the cocaine solution (2.0 mg/kg in 10 µL). The other end of the line was attached to a 1 c.c. BD syringe and placed on a microsyringe pump (HAVARD PHD, 2000; HARVARD Apparatus, Saint-Laurent, Canada). Following a 30-minute habituation period, each animal received the first of three experimenter-administered i.v. cocaine infusions delivered over 5, 45 or 90 s, injected 90 minutes apart and in counter-balanced order. The test session lasted five hours, and locomotor activity was recorded as photocell beam breaks, computed over 10-s intervals. At the end of the study, catheter patency was verified once again with Propofol and animals were immediately euthanized by decapitation while still under anaesthesia. One rat did not become ataxic and was eliminated from the study.

Statistical analysis

Data was analyzed with Prism 7 software (GraphPad Software Inc., La Jolla, CA). Changes in cocaine concentration as a function of time during the *in vitro* assay were analyzed using one-way analysis of variance (ANOVA). Repeated measures two-way ANOVA was used to analyze the effects of cocaine infusion rate on average extracellular concentrations of cocaine and dopamine over time, and on locomotor activity over time ('Infusion Rate' and 'Time' as within-subjects variables). Repeated measures one-way ANOVA, followed by Tukey's multiple comparison tests or two-tailed paired *t*-tests, were used to analyse the effects of cocaine infusion rate on cocaine and dopamine Cmax (the highest value for each analyte obtained from each rat after cocaine, averaged by infusion rate), Tmax (time to reach Cmax in each rat, averaged by infusion rate), and time to first significant increase (the first value > 2 standard deviations above baseline in each rat, averaged by infusion rate). One-way ANOVA was also used to assess the effects of cocaine infusion rate on both total locomotor activity (from minute '0' to minute '30') and locomotor activity within the first minute after injection. Linear regression was used for the correlations and Fisher's r-to-z transformation was then used to compare correlation coefficients (r). Effects were considered statistically significant when $P < 0.05$.

Results

Determination of microdialysis probe delay time *in vitro*

Figure 2 shows cocaine concentrations at 1-minute intervals when microdialysis probes were placed in an aCSF/ascorbic acid solution, then in an aCSF/ascorbic acid/1 μM cocaine solution, and back again. Of note, apparent cocaine concentrations do not start at or return to '0' when the probes are placed in the aCSF/ascorbic acid solution, before and after probe immersion in the cocaine solution. This background signal is likely produced by a contaminant with a similar mass transition to cocaine. We overcame this issue by taking the background contaminant signal into account when assigning the lower limit of quantification (LLOQ) of the LC-MS assay. This LLOQ was estimated as being 3X greater than the signal generated in aCSF/ascorbic acid alone, or 23 nM. The baseline values in Figure 2 are all below 23 nM and thus these values can be considered to be a background signal. It is only when the dialysis probe is placed in the cocaine-containing solution that cocaine concentrations rise above the LLOQ. Once the microdialysis probes were placed into the cocaine solution, cocaine concentrations increased significantly from baseline levels (One-way ANOVA on minutes -10 to 26; $F(35,36) = 26.08$; $P < 0.0001$). Cocaine concentrations began to increase 5-6 minutes after probe immersion into the cocaine solution, and reached near-maximum concentrations 1-2 minutes later. Thus, the delay time between the microdialysis probe and the collection vial is 5-6 minutes, and after this delay, near peak concentrations of cocaine were detected within 1-2 minutes. When the microdialysis probes were placed back into the solution that did not contain cocaine, drug concentrations significantly decreased (One-way ANOVA on minutes 0 to

42; F(42,43) = 17.51; $P < 0.0001$). Cocaine concentrations began to decrease 5-6 minutes after removal of the probes from the cocaine solution, and they returned to baseline levels 1-2 minutes later. Thus, there was a 5-6-minute delay time to detect the presence and then absence of cocaine in a solution. This delay is the time it takes for aCSF to be transported across the microdialysis probe membrane, through the probe outlet tubing and into the collection vial. Residual cocaine is also removed from the microdialysis probes within 2 minutes after this delay time. Based on these findings, the curves illustrating brain cocaine and dopamine concentrations *in vivo* (Figure 3) were corrected for a 5-minute delay time.

Varying the rate of i.v. cocaine delivery between 5 and 90 seconds significantly influences striatal cocaine and dopamine Tmax, but not Cmax

In all 7 rats, the unilateral microdialysis probe lay in the dorsal striatum between 2.2 and 1.2 mm anterior to Bregma (Figure 3a). Some probe tips extended slightly into the nucleus accumbens core. However, there would be little to no sampling from the probe tips as tips were occluded with super glue to create a closed system for dialysate flow. All animals received all three intravenous cocaine infusions administered over 5, 45 and 90 s, in counter-balanced order. Baseline levels of cocaine and dopamine during the 5 minutes prior to each infusion were comparable (Cocaine: F(2, 18) = 1.42, $P = 0.27$; Dopamine: F(2,18) = 0.15, $P = 0.86$; data not shown). Thus, cocaine and dopamine concentrations returned to pre-cocaine baseline levels before each infusion, and there were no significant carry-over effects from one infusion to the next. At all rates, i.v. cocaine administration

increased extracellular concentrations of both cocaine and dopamine above baseline (Figures 3b and 3c, respectively). The rate of i.v. cocaine infusion significantly influenced cocaine and dopamine concentrations over time (Infusion rate x Time interaction effect; Figure 3b, $F_{36, 216}$ = 4.38; Figure 3c, $F_{36, 216}$ = 3.56; all *P*'s < 0.0001). There was no significant effect of the rate of i.v. cocaine infusion on peak concentrations (Cmax values) of cocaine (Figure 4a; $F_{1.61, 9.67}$ = 1.71, *P* = 0.23; 5 s: 267.82 ± 52.11; 45 s: 223.69 ± 41.19; 90 s: 185.09 ± 27.18 nM) or dopamine (Figure 4b $F_{1.41, 8.44}$ = 0.87, *P* = 0.42; 5 s: 3.50 ± 0.49, 45 s: 3.79 ± 0.82; 90 s: 3.06 ± 0.53 nM). However, as hypothesized, peak concentrations of cocaine (Figure 4c; $\underline{F}_{1.5, 9}$ = 16.23, *P* = 0.0016; 5-45 s < 90 s; 5 s: 4.33 ± 0.86; 45 s: 6.86 ± 1.28; 90 s: 11.71 ± 0.47 minutes) and dopamine (Figure 4d; $F_{1.53, 9.17}$ = 7.34, *P* = 0.02; 5 < 90 s; 5 s: 4.33 ± 0.42; 45 s: 6.71 ± 0.89; 90 s: 9.29 ± 1.49 minutes) were reached earlier following faster i.v. infusions. To further examine this effect, we analyzed the effects of infusion rate on the time interval before cocaine and dopamine concentrations were greater than baseline concentrations by two standard deviations. This further confirmed that a 5-s infusion led to the fastest increases in extracellular concentrations of cocaine and dopamine (Figure 4e; $F_{2, 12}$ = 6.42, *P* = 0.01; 5 versus 90 s: t_6 = 2.74, *P* = 0.03; 45 versus 90 s: t_6 = 2.59, *P* = 0.04; Figure 4f; $F_{2, 12}$ = 4.31, *P* = 0.04; 5 versus 90 s: t_6 = 2.54, *P* = 0.04). In parallel, we observed the animals during testing and we noted that locomotor activity increased earlier following rapid (5 s) versus more sustained (45-90 s) i.v. cocaine infusions.

In summary, at all cocaine i.v. infusion rates, we measured a significant increase in drug and dopamine concentrations in the dorsal striatum. Increasing the rate of i.v. cocaine delivery between 90 and 5 s did not significantly influence peak concentrations

of the drug or of dopamine. However, cocaine and dopamine concentrations reached peak levels earlier following more rapid infusions.

Cocaine and dopamine concentrations are tightly coupled across time

Data from representative rats show that, at all infusion rates, cocaine and dopamine concentrations were very closely linked across time (Figures 5a-c). Across all experimental rats and infusion rates, there was a significant positive correlation between extracellular cocaine and dopamine concentrations at each sampling time after drug infusion (Figure 5d; $r^2 = 0.77$, $P < 0.0001$). There was also a significant positive correlation between extracellular cocaine and dopamine concentrations at each infusion rate (5 s, $r^2 = 0.61$, $P = 0.0006$; 45 s, $r^2 = 0.88$, $P < 0.0001$; 90 s, $r^2 = 0.91$, $P < 0.0001$, Data not shown). This correlation was stronger following a 90-s infusion compared to a 5-s infusion (Z=-2.02, $P = 0.04$; no other comparisons were statistically significant). The linear relationship between cocaine and dopamine was observed up to 187-220 nM cocaine, the highest concentrations measured following i.v. infusion of the drug over 5-90 s, respectively.

I.v. cocaine infusion does not significantly influence striatal levels of other neurochemicals

In addition to dopamine, we also measured extracellular concentrations of 20 other neurochemicals in the striatum after cocaine infusion. These were acetylcholine, adenosine, aspartate, γ-aminobutyric acid, glucose, glutamate, glutamine, glycine, histamine, norepinephrine, phenylalanine, serine, serotonin, taurine, tyrosine and the

metabolites 3,4-dihydroxyphenylacetic acid, homovanillic acid, 3-methoxytyramine, 5-hydroxyindoleacetic acid and normetanephrine in the dorsal striatum. There were no significant effects of cocaine or of the rate of infusion on the concentrations of any of these (see supplementary figures S1-S3).

Locomotor activity increases earlier when cocaine is infused over 5 seconds

I.v. infusion rate did not influence the locomotor response to saline (Saline; $F_{2,9} = 0.21$, $P = 0.81$). Thus, saline-induced locomotion was pooled across rates and this served as the control condition. At all infusion rates, cocaine increased locomotor activity relative to saline (Figure 6; $F_{3,33} = 6.80$, $P = 0.001$; 5 s vs. Saline: $F_{1,11} = 11.43$, $P = 0.006$; 45 s vs. Saline: $F_{1,11} = 16.24$, $P = 0.002$; 90 s vs. Saline: $F_{1,11} = 27.74$, $P = 0.0003$), and all rates evoked a comparable increase in locomotion (One-way ANOVA on total locomotor counts over the 30 min after cocaine injection; $F_{1.10,12.13} = 2.17$, $P = 0.17$). However, infusion rate significantly influenced cocaine-induced locomotion across time (Infusion rate x Time interaction effect; $F_{68,\ 748} = 1.47$, $P = 0.01$). To analyse this further, we compared locomotor activity in the first minute following cocaine injection. This showed that injecting cocaine i.v. over 5 versus 90 s evokes greater locomotor activity in the first minute post-injection ($F_{1.57,17.24} = 10.87$, $P = 0.002$; 5 vs. 90 s, $P = 0.004$).

Discussion

To our knowledge, the present experiment is the first to simultaneously measure brain concentrations of extracellular cocaine and dopamine in awake, freely-moving rats, and to also assess the influence of the rate of i.v. cocaine infusion. We found that injecting cocaine i.v. between 5 and 90 s robustly increases cocaine and dopamine concentrations in the dorsal striatum, without significantly altering peak concentrations of either analyte [see also (Ferrario et al., 2008)]. However, drug and dopamine concentrations rose faster when cocaine was administered over 5 s, such that peak concentrations were reached earlier. Previous work shows that compared to slower i.v. cocaine infusions (25-100 s), rapid infusions (5-16 s) promote the development of sensitization to both the psychomotor activating and incentive motivational effects of cocaine (Allain et al., 2017; Bouayad-Gervais et al., 2014; Liu et al., 2005; Minogianis et al., 2013; Samaha et al., 2002; Samaha et al., 2004), lead to greater drug intake (Bouayad-Gervais et al., 2014; Minogianis et al., 2013; Wakabayashi et al., 2010), and increase the risk of cocaine-primed relapse following extended abstinence (Wakabayashi et al., 2010). Rapid cocaine delivery to the brain also preferentially engages mesocorticolimbic cells (Brown et Kiyatkin, 2005; Ferrario et al., 2008; Porrino, 1993; Samaha et al., 2004). If rapid i.v. cocaine administration promotes these addiction-relevant effects by changing dopamine neurotransmission, our findings suggest that the critical factor is likely the time it takes to transition from low/baseline to high extracellular dopamine concentrations rather than any large differences in peak concentrations (Ferrario et al., 2008).

Our results confirm predictions derived from pharmacokinetic modeling. Samaha et al. (2002) used a pharmacokinetic model validated by Pan et al. (1991) and estimated that injecting cocaine i.v. over 5, 50 or 100 s would not significantly influence peak brain concentrations of the drug, but would change the rate of rise of drug concentrations. Our findings confirm this to be the case. The temporal profile of brain cocaine concentrations we report also matches that in Samaha *et al*. (2002). However, Samaha et al. (2002) estimated higher peak cocaine values in the brain than measured here (~4 μM after 1 mg/kg i.v. cocaine compared to 0.2-0.26 μM after 2 mg/kg i.v. cocaine here). Samaha et al. (2002) used a pharmacokinetic model that estimates whole brain concentrations corrected for probe recovery rate, while here we report uncorrected concentrations from dialysate samples. Uncorrected concentration values in dialysate samples would be lower. In accordance with this, in the present study, an *in vitro* test showed that peak concentrations of cocaine detected by the probes were ten-fold lower than the concentration in the prepared solution (0.1-0.14 μM were detected in a 1-μM cocaine solution). This represents a 10% probe recovery at the 2 μL/minute flow rate used here. As such, keeping in mind the 10% recovery rate from the microdialysis probe, the striatal cocaine and dopamine values we report can be used to select behaviourally-relevant cocaine and dopamine concentrations in *in vitro* experiments (Nicolaysen et al., 1988). Indeed, the cocaine dose we used (2 mg/kg) also produced a robust increase in psychomotor activity (detailed below).

Variation in the rate of i.v. cocaine infusion significantly influenced the temporal dynamics of cocaine and dopamine levels in the dorsal striatum. By sampling at 1-minute intervals, we found that peak brain concentrations of cocaine were reached 4 minutes

after a 5-s infusion, 7 minutes after a 45-s infusion and 12 minutes after a 90-s infusion. Similarly, Hurd et al. (1988), who sampled every 10 minutes, found that following an 85-s i.v. cocaine infusion, peak drug concentrations in the striatum are seen within the first 10-minute sample. The cocaine Tmax values we observed here after a 5- versus 90-s i.v. infusion are also generally similar to those seen in rats following i.v. (1-2 minutes) and intranasal (15 minutes) cocaine administration, respectively (Chow, Chen et Matsuura, 1999). This suggests that injecting cocaine i.v. over 5 versus 90 s in rats can to some extent model the temporal kinetics of i.v. versus intranasal cocaine. Our findings also agree with those of Ferrario et al. (2008) showing that infusing cocaine i.v. between 5 and 90 s does not produce large effects on peak dopamine concentrations in the striatum (or on area under the curve values for dopamine), but it produces significant differences in dopamine Tmax. Ferrario et al. (2008) also reported that dopamine concentrations start to rise 1-2 minutes after a 5-s i.v. cocaine infusion, and 3-3.5 minutes after a 100-s infusion. Similarly, here we found that dopamine concentrations begin to increase 1 minute after a 5-s cocaine infusion, and 5 minutes after a 90-s infusion. We have previously used *in vivo* voltammetry techniques in urethane-anesthetized rats and found that peak levels of dopamine reuptake inhibition occur within 1 minute after a 5-s i.v. cocaine infusion (Samaha et al., 2004). This is much sooner than the time to reach peak brain concentrations of dopamine we observed here using awake, freely-moving animals and *in vivo* microdialysis (4 minutes after a 5-s infusion). However, our findings agree with those of others using *in vivo* voltammetry techniques in awake, freely-moving rats, and showing that peak dopamine inhibition does not occur until 6 minutes after a 12-15 s i.v. cocaine infusion (Kiyatkin, Kiyatkin et Rebec, 2000). The reasons for the discrepancy

between these findings could involve the use of anesthetized versus awake animals. It is also possible that there is a non-linear relationship between the ability of an i.v. infusion of cocaine to occupy dopamine transporters and its ability to block the reuptake of dopamine, and thus increase dopamine overflow (Brodnik, Ferris, Jones et España, 2017).

Brain cocaine and dopamine concentrations were tightly coupled across time, at all infusion rates. This agrees with previous studies where cocaine was given via the intraperitoneal or i.v. routes (Nicolaysen et al., 1988; Shou et al., 2006). Thus, once in the brain, cocaine quickly occupies dopamine transporters, producing rapid dopamine reuptake blockade and overflow into the extracellular space. The cocaine-induced increases in dopamine concentrations we measured here are likely largely due to blockade of the dopamine transporter, but cocaine could also be altering dopamine release (Mejias-Aponte, Ye, Bonci, Kiyatkin et Morales, 2015; Venton et al., 2006). As this matter is resolved, the very close temporal relationship between brain cocaine and dopamine concentrations we observed suggests that the pharmacokinetic profile of one compound can be used as a proxy for the other.

In contrast to what we observed with dopamine, acute i.v. cocaine injections did not change the concentrations of other neurochemicals in the striatum. We tested only one (albeit relatively high) dose of cocaine. In addition, our rats received a total of 3 cocaine infusions. This might not be sufficient to reliably alter extracellular concentrations of some of the compounds we measured. For instance, several exposures to cocaine are generally needed to significantly increase striatal glutamate concentrations (Zhang, Loonam, NOAILLES et Angulo, 2001). Interestingly, here and in prior work (Hurd et

Ungerstedt, 1989), cocaine did not significantly change dopamine metabolite concentrations (DOPAC, HVA and 3-MT), even though the drug robustly increased extracellular dopamine concentrations. This suggests that the relationship between dopamine release and metabolism is not simple. Cocaine might not significantly change dopamine metabolite concentrations in the dorsal striatum because the drug potently blocks dopamine reuptake, thus preventing dopamine metabolism in the cell terminal (Hurd et Ungerstedt, 1989). Although acute cocaine can significantly increase norepinephrine overflow in the ventral striatum (Reith et al. 1997), we found no effects on norepinephrine overflow in the dorsal striatum. To our knowledge, this is the first report on how systemic cocaine administration influences norepinephrine overflow in the dorsal striatum. The dorsal striatum contains a limited density of scattered noradrenergic axons and very little norepinephrine (Fornai et al., 1996). We also saw no effect of i.v. cocaine on serotonin concentrations in the dorsal striatum. This was surprising since cocaine has similar affinities for brain serotonin and dopamine transporters (Miller, Yatin, De La Garza Ii, Goulet et Madras, 2001; Rothman et al., 2001). Others have reported that acute cocaine increases extracellular concentrations of serotonin in the dorsal (Bradberry et al., 1993) and ventral (Essman, Singh et Lucki, 1994) striatum. The discrepancy between our findings and these reports could be due to the use of anaesthetized rats (Bradberry et al., 1993), the route of cocaine administration (Bradberry et al., 1993), and the striatal subregion sampled (Essman et al., 1994).

The rate of cocaine infusion also influenced the temporal dynamics of psychomotor activity. While i.v. infusion rate did not significantly influence total levels of cocaine-induced locomotion, faster i.v. infusions evoked a greater locomotor response in the first

minute after infusion. Thus, the rate of cocaine infusion influenced the temporal dynamics of both dopamine concentrations and drug-induced psychomotor activity. However, the relationship between these two measures is not straightforward. First, at all infusion rates, cocaine-induced locomotion both peaked earlier and returned to baseline levels sooner than drug or dopamine concentrations (compare Figures 6 and 3). We measured cocaine/dopamine concentrations and cocaine-induced locomotor activity in different animals. However, others have taken the two measures in the same animals and also found that in previously drug-naive rats, cocaine-induced locomotion peaks earlier than cocaine-induced dopamine concentrations [(Kalivas et Duffy, 1990) but see (Hemby, Jones, Hubert, Neill et Justice, 1994)]. Other studies with methylphenidate (Gerasimov et al., 2000) or nicotine (Benwell et Balfour, 1992) also show a similar effect. Combined, the present study and these prior reports have measured drug-induced dopamine overflow in both the ventral and dorsal striatum. Thus, it is possible that drug-induced dopamine accumulation outside of these brain regions also significantly contributes to the psychomotor response (Kalivas et Duffy, 1990). It is also possible that cocaine increases dopamine concentrations in the synapse to induce psychomotor activity, without proportionate or immediate diffusion of dopamine outside of the synapse, to the microdialysis probe (Gonon, 1988; Kalivas et Duffy, 1990). This would explain why cocaine-induced dopamine concentrations detected by the probe peak later in time compared to cocaine-induced locomotion.

There are elements to consider in interpreting our findings. First, we do not know how the rate of i.v. cocaine infusion influences cocaine/dopamine pharmacokinetics in extra-striatal brain regions. We would predict that the cocaine pharmacokinetics we

measured in the dorsal striatum likely reflect those in the rest of the brain. Indeed, cocaine is distributed uniformly in the brain following i.v. or intranasal administration (Reed et Spiehler, 1985). A Positron Emission Tomography study in humans also suggests that drug pharmacokinetics in individual brain regions are very similar to those in whole brain (Berridge et al., 2010). Second, the neurobehavioural effects of cocaine that are relevant to addiction come about following chronic exposure to the drug. We do not know how our measurements would change with more extensive drug exposure. Of note, brain and blood concentrations of cocaine do not significantly change following repeated i.v. administration (Pan et al., 1991). However, the ability of i.v. cocaine to inhibit dopamine reuptake can increase with repeated exposure (Brodnik et al., 2017). This being said, studying brain cocaine and dopamine pharmacokinetics after a single cocaine exposure is important. A single exposure to psychostimulant drugs like cocaine or d-amphetamine can produce effects that are relevant to the addiction process, in both laboratory rats and humans. These effects include psychomotor sensitization (Lin-Chu, Robinson et Becker, 1985; Robinson, Becker et Presty, 1982; Samaha et al., 2002; Strakowski et Sax, 1998) and changes in spine density on medium spiny neurons of the nucleus accumbens (Kolb, Gorny, Li, Samaha et Robinson, 2003). Finally, we only tested a single cocaine dose, but we would predict that, across a range of cocaine doses, varying the rate of i.v. drug infusion changes the rate of rise of drug concentrations in the brain.

In summary, we measured striatal concentrations of cocaine and dopamine, as well as cocaine-induced locomotion across a range of i.v. infusion rates that significantly influences brain and behaviour [Reviewed in (Allain et al., 2015; Samaha et Robinson, 2005)]. We found that increasing the rate of i.v. drug delivery increases the rate of rise

of cocaine and dopamine concentrations in the dorsal striatum, without producing large effects in peak concentrations. This was mirrored by a more immediate increase in locomotor activity following rapid versus slower i.v. cocaine infusions. Thus, our results raise the possibility that differences in the rate of cocaine rise alone can determine outcome, perhaps by influencing the temporal dynamics of dopamine accumulation in the synapse, and the temporal pattern of dopamine receptor occupancy. In support of this, work in humans shows that smoked and intranasal cocaine can produce equivalent levels of dopamine transporter blockade, but smoked cocaine produces a stronger self-reported high (Volkow et al., 2000). Thus, we conclude that differences in the rate of rise of drug and dopamine levels in the brain might be an important issue in thinking about why drugs, formulations, and routes of administration that achieve a rapid drug onset are the most addictive.

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Conflict of Interest

The authors declare no conflicts of interest.

Authors contribution

ANS, EAM, WGB and WMS designed the study. EAM and WMS performed the *in vivo* microdialysis experiments. OSM and JTW analysed microdialysis samples with HPLC-MS/MS. EAM performed the psychomotor activity experiment. EAM, WMS, PS, OSM, JTW, RTK and ANS analyzed the data and interpreted the findings. EAM and ANS drafted the manuscript. PS, RTK, WMS, OSM, JTW and WGB critically revised the manuscript. All authors critically reviewed content and approved final version for publication.

Abbreviations

aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; Cmax, maximal concentration; DS, dorsal striatum; h, hours; i.v., intravenous; μM, micromoles per liter; nM, nanomoles per liter; NacC, nucleus accumbens core; NacSh, nucleus accumbens shell; s, seconds; SEM, standard error of the mean; Tmax, time to reach maximal concentration.

Data accessibility

Raw data generated by these experiments will be stored on Figshare. Data and statistical analyses will be made available upon request.

Figure legends

Figure 1. The sequence of experimental events. Panel a illustrates the timeline of experimental events for the *in vivo* microdialysis study. Rats were implanted with a unilateral cannula into the dorsal striatum and a catheter into the jugular vein. Following recovery, the rats were habituated to the *in vivo* microdialysis apparatus and to the i.v. infusion procedure on 2 daily sessions. On the following test day, microdialysis probes were inserted into the dorsal striatum and i.v. catheters were tethered to the cocaine infusion set up. Each rat received an i.v. infusion of 2 mg/kg cocaine, delivered over 5, 45 or 90 s, in counterbalanced order, with infusions administered 90 minutes apart. Dialysate samples were collected every minute for 5-10 minutes before each infusion and for 15 minutes thereafter. Panel b shows the sequence of experimental events for the psychomotor activity study. Rats were implanted with an intrajugular catheter and allowed to recover for 7 days. Rats were then habituated to the psychomotor activity cages and i.v. infusion lines on 2 daily sessions. On the following test day, rats were tethered to the cocaine infusion lines and locomotor activity was measured. Each rat received i.v. cocaine (2.0 mg/kg/infusion) delivered over 5, 45 and 90 s, in counterbalanced order, with infusions administered 90 minutes apart.

Figure 2. Cocaine concentrations detected when microdialysis probes were placed into a solution containing 1 μM cocaine. Two probes were each transferred from a solution containing aCSF/ascorbic acid to a solution containing aCSF/ascorbic acid/cocaine (1 μM), and back again. All solutions were stirred and tested at room temperature. The data shown are averages (± SEM) from two independent tests carried out with two different probes. Samples were collected at 1-minute intervals, at a flow rate of 2 μL/minute, and analyzed by HPLC-MS/MS. *n* = 2. *μM*, micromoles/liter. *nM*, nanomoles/liter.

Figure 3. Varying the rate of i.v. infusion between 5-90 s influences the temporal kinetics of extracellular cocaine and dopamine concentrations in the dorsal striatum. The location of microdialysis probes in the striatum is shown in panel (a). The distance in millimeters (mm) from Bregma is given for each coronal rat brain section. The white boxes at the tips of each probe indicate the segment where glue was applied, and where no exchange is possible. Panels (b) and (c) show striatal cocaine and dopamine concentrations over time, respectively, as a function of i.v. drug infusion rate. All values are mean ± SEM. *n* = 7 rats/infusion rate. *DS*, Dorsal Striatum, *NacC*, Nucleus Accumbens Core, *NAcSh*, Nucleus Accumbens Shell, *nM*, nanomoles/liter. *s*, seconds.

Figure 4. Varying the rate of i.v. infusion between 5-90 s influences the rate of rise of cocaine and dopamine concentrations (Tmax) in the dorsal striatum without producing large effects on maximum concentrations (Cmax). Panels (a) and (b) show average Cmax values for cocaine and dopamine, respectively, as a function of the rate of i.v. cocaine infusion. The time to reach peak concentrations (Tmax) of cocaine (c) and dopamine (d)

in the dorsal striatum is shown as a function of the rate of i.v. cocaine delivery. Panels (e) and (f) show the time interval before cocaine and dopamine concentrations were significantly greater than baseline levels (> 2 standard deviations above baseline), as a function of the rate of i.v. cocaine delivery. All values are mean ± SEM. *n* = 7 rats/infusion rate. **P* < 0.05 compared to 90 s. *nM*, nanomoles/liter. *s*, seconds.

Figure 5. Extracellular dopamine and cocaine concentrations in the dorsal striatum are linearly correlated. Panels (a-c) show extracellular concentrations of dopamine and cocaine over time from representative rats, for each i.v. infusion rate. Panel (d) shows a significant positive correlation between dopamine and cocaine concentrations in the dorsal striatum, at each sampling time, across all rats and infusion rates. For this analysis, we used linear regression to model the relationship between dopamine concentrations and cocaine concentrations at each of the 15 post-cocaine samples. *n* = 7 rats/infusion rate. *nM*, nanomoles/liter. *s*, seconds.

Figure 6. The influence of the rate of i.v. cocaine infusion on locomotor activity. Locomotor activity (beam breaks per minute) as a function of i.v. drug infusion rate. Saline-induced locomotor activity is also shown for comparison. The shading highlights the first minute after cocaine, where locomotor activity was greater after a 5-s injection than after a 90-s injection. All values are mean ± SEM. n = 12 rats/infusion rate. s, seconds.

Supporting information:

Figure S1. Cocaine had no significant effect on non-dopaminergic neurotransmitters in the dorsal striatum. All values are mean ± SEM. *n* = 7 rats/infusion rate. *nM*, nanomoles/liter. *s*, seconds.

Figure S2. Cocaine had no significant effect on neuromodulators in the dorsal striatum. All values are mean ± SEM. *n* = 7 rats/infusion rate. *nM*, nanomoles/liter. *s*, seconds.

Figure S3. Cocaine had no significant effect on neurotransmitter metabolites in the dorsal striatum. All values are mean ± SEM. *n* = 7 rats/infusion rate. *nM*, nanomoles/liter. *s*, seconds.

Figures

Figure 1

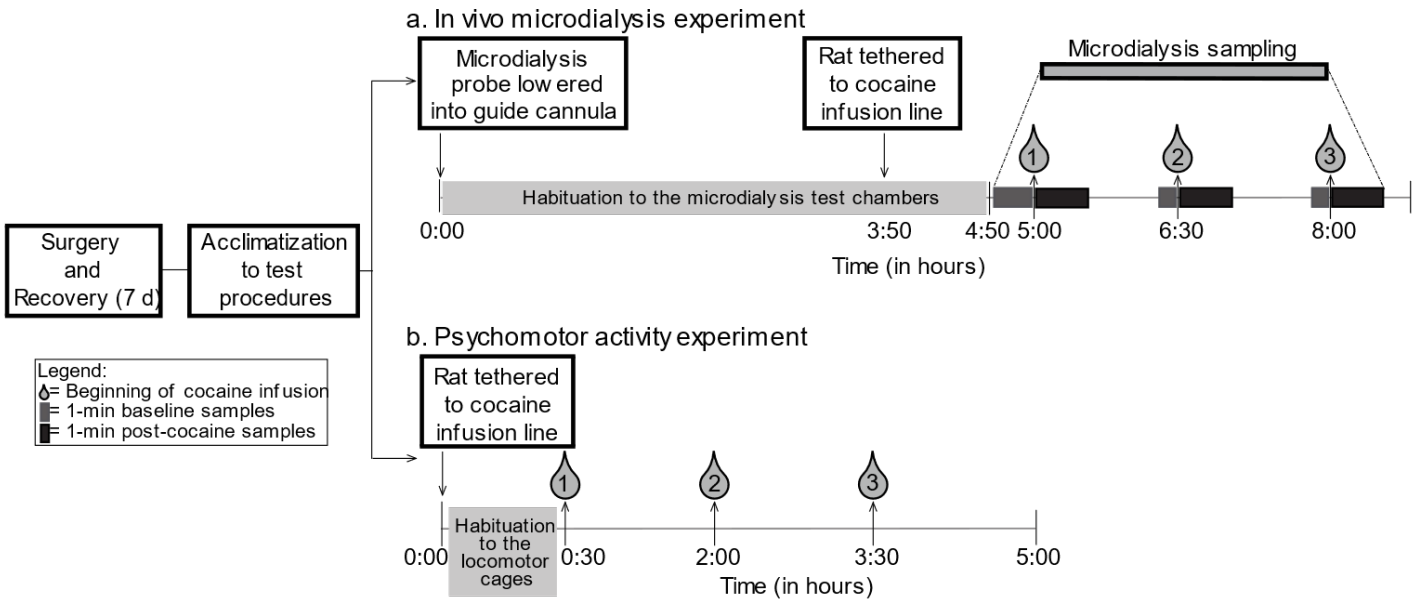


Figure 2

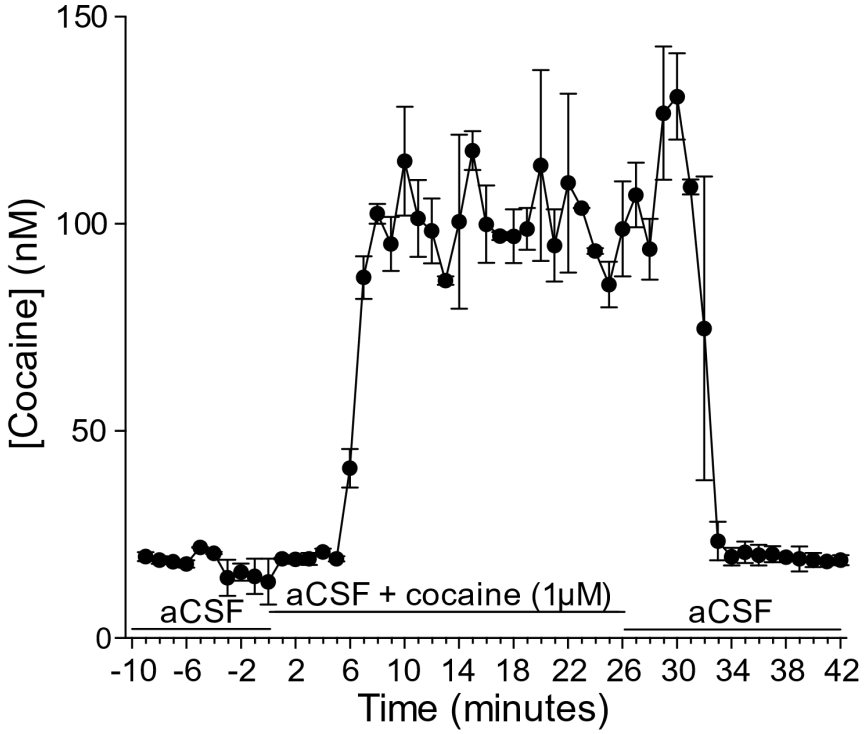

150
100
50
0
[Cocaine] (nM)
aCSF
aCSF + cocaine (1μM)
aCSF
-10 -6 -2 2 6 10 14 18 22 26 30 34 38 42
Time (minutes)

Figure 3

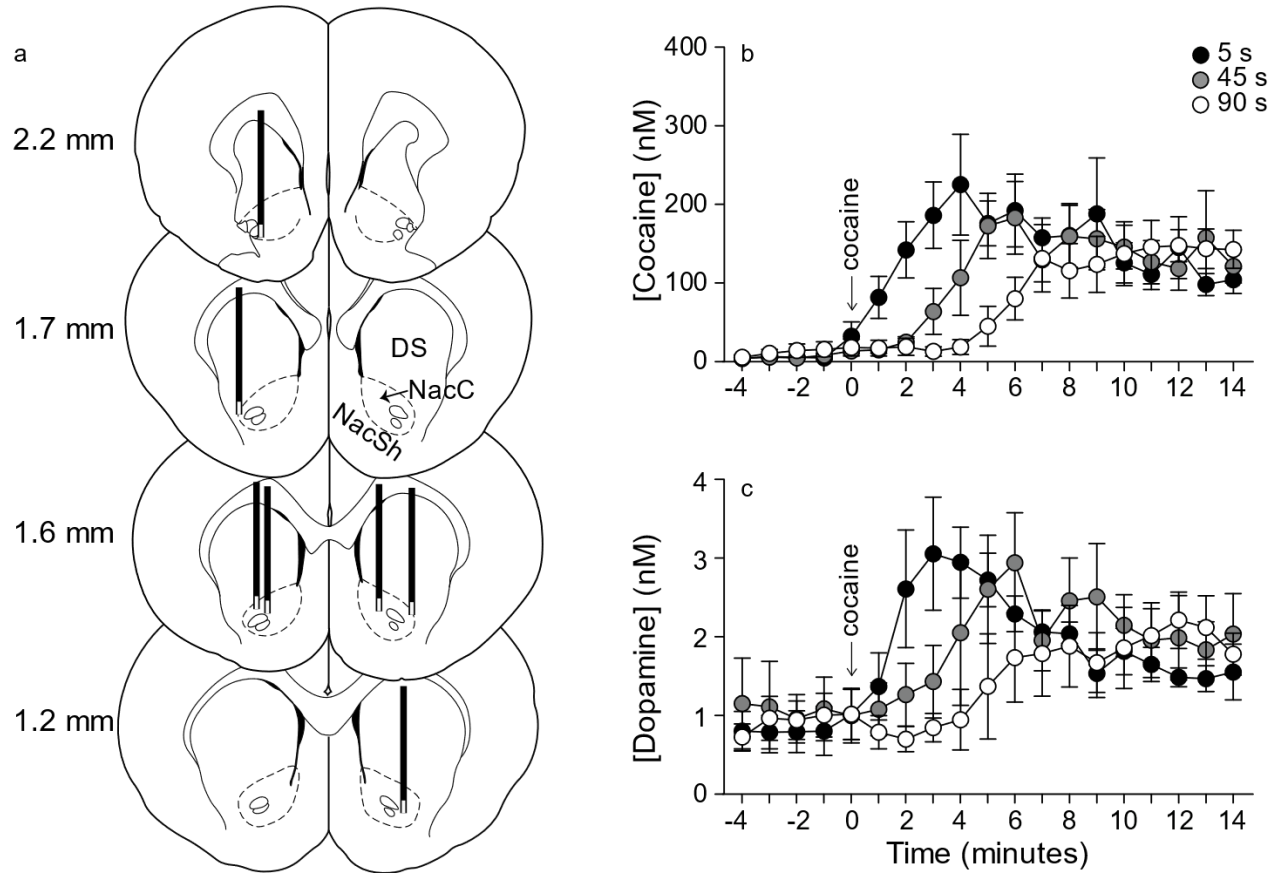

a
2.2 mm
1.7 mm
1.6 mm
1.2 mm
DS
NacC
NacSh
b
5 s
45 s
90 s
[Cocaine] (nM)
cocaine
c
[Dopamine] (nM)
cocaine
Time (minutes)

Figure 4

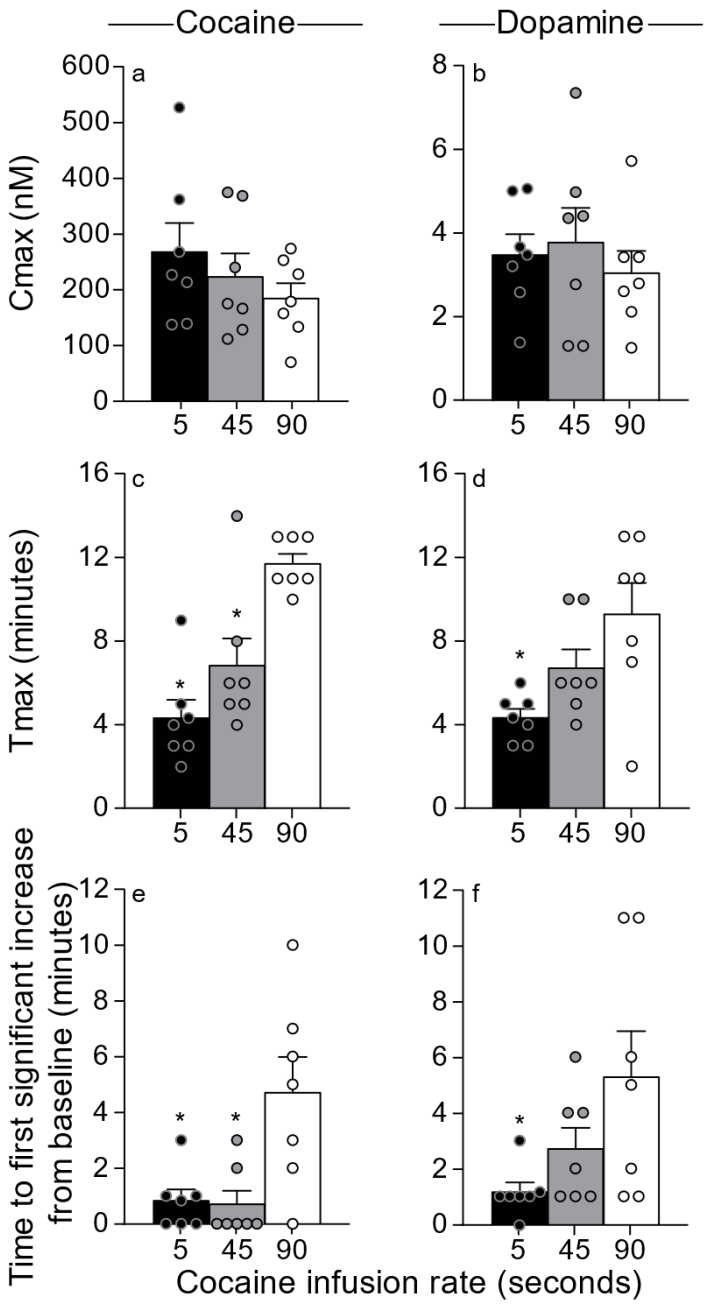

Cocaine
Dopamine
a
b
c
d
e
f
Cmax (nM)
Tmax (minutes)
Time to first significant increase from baseline (minutes)
Cocaine infusion rate (seconds)
5
45
90

Figure 5

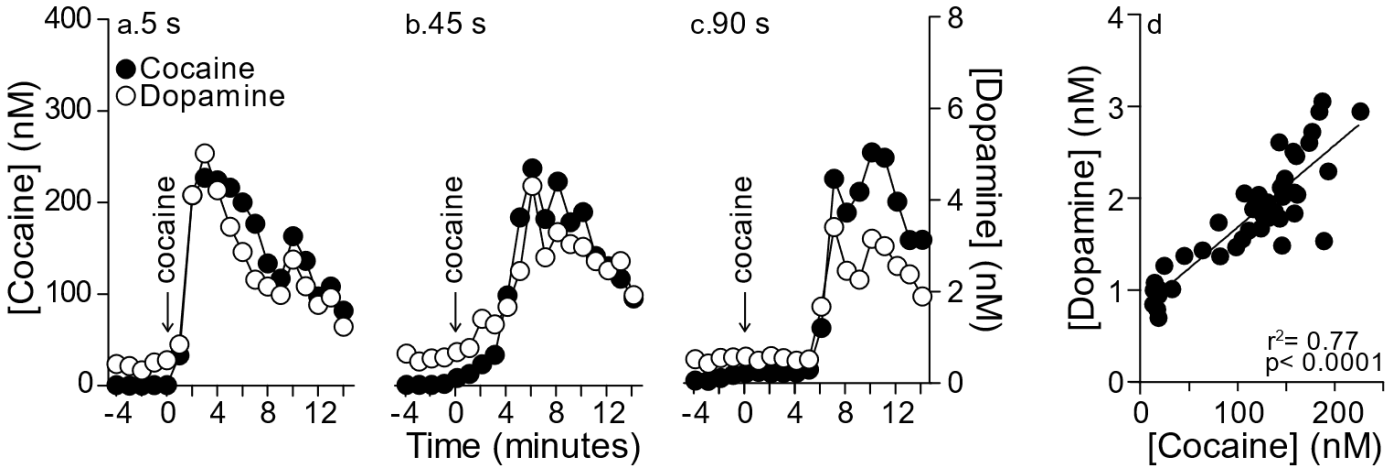
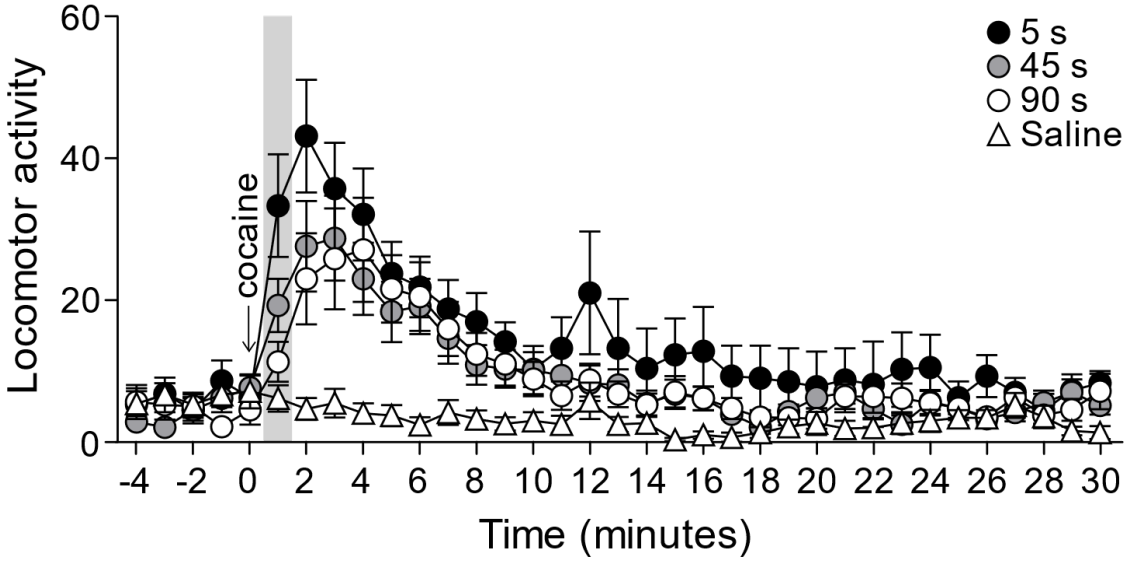

a.5 s
b.45 s
c.90 s
d
Cocaine
Dopamine
[Cocaine] (nM)
[Dopamine] (nM)
cocaine
Time (minutes)
[Dopamine] (nM)
[Cocaine] (nM)
r2= 0.77
p< 0.0001

Figure 6


Locomotor activity
60
40
20
0
cocaine
5 s
45 s
90 s
Saline
-4 -2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Time (minutes)

Supplementary figures

Figure S1

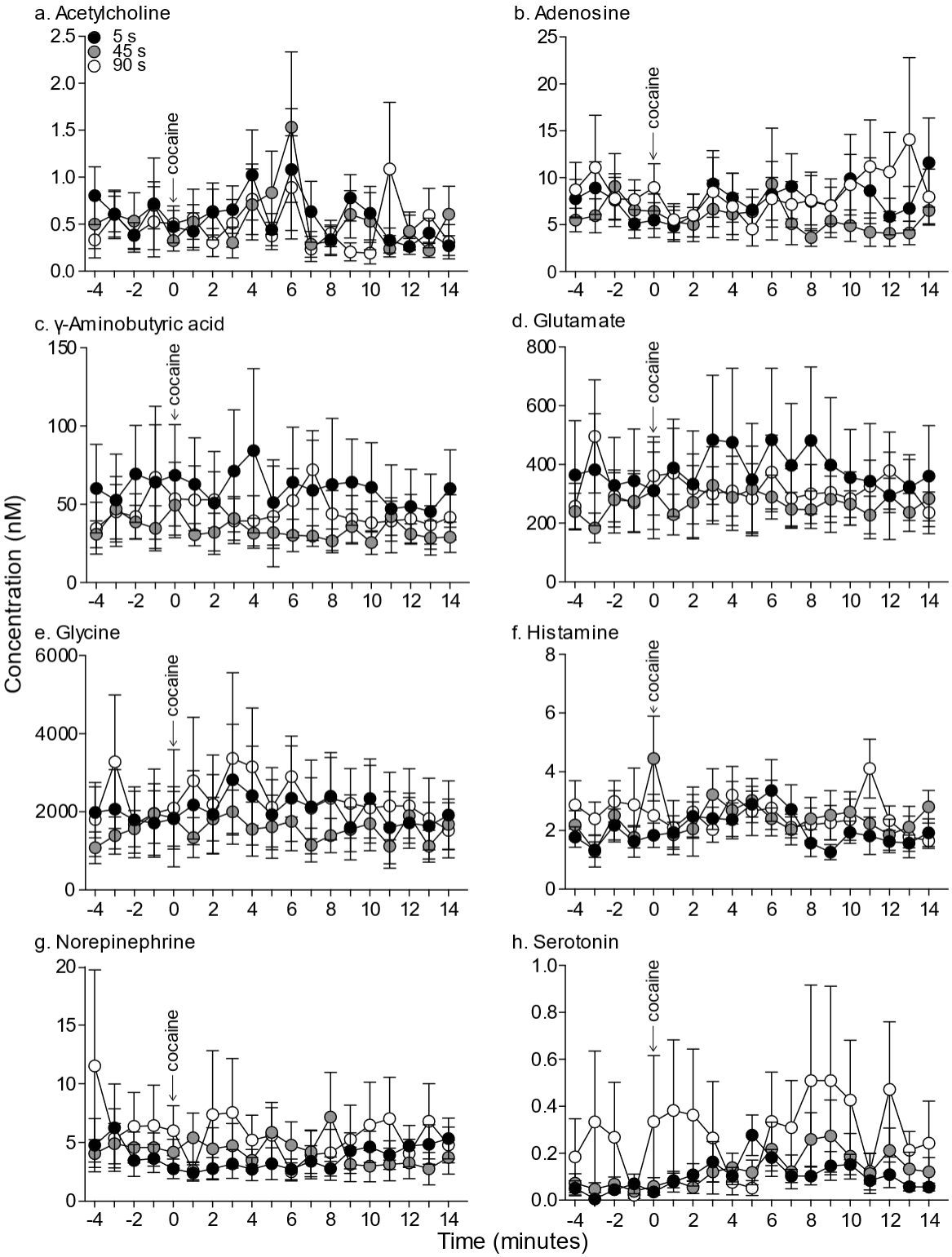


Figure S2

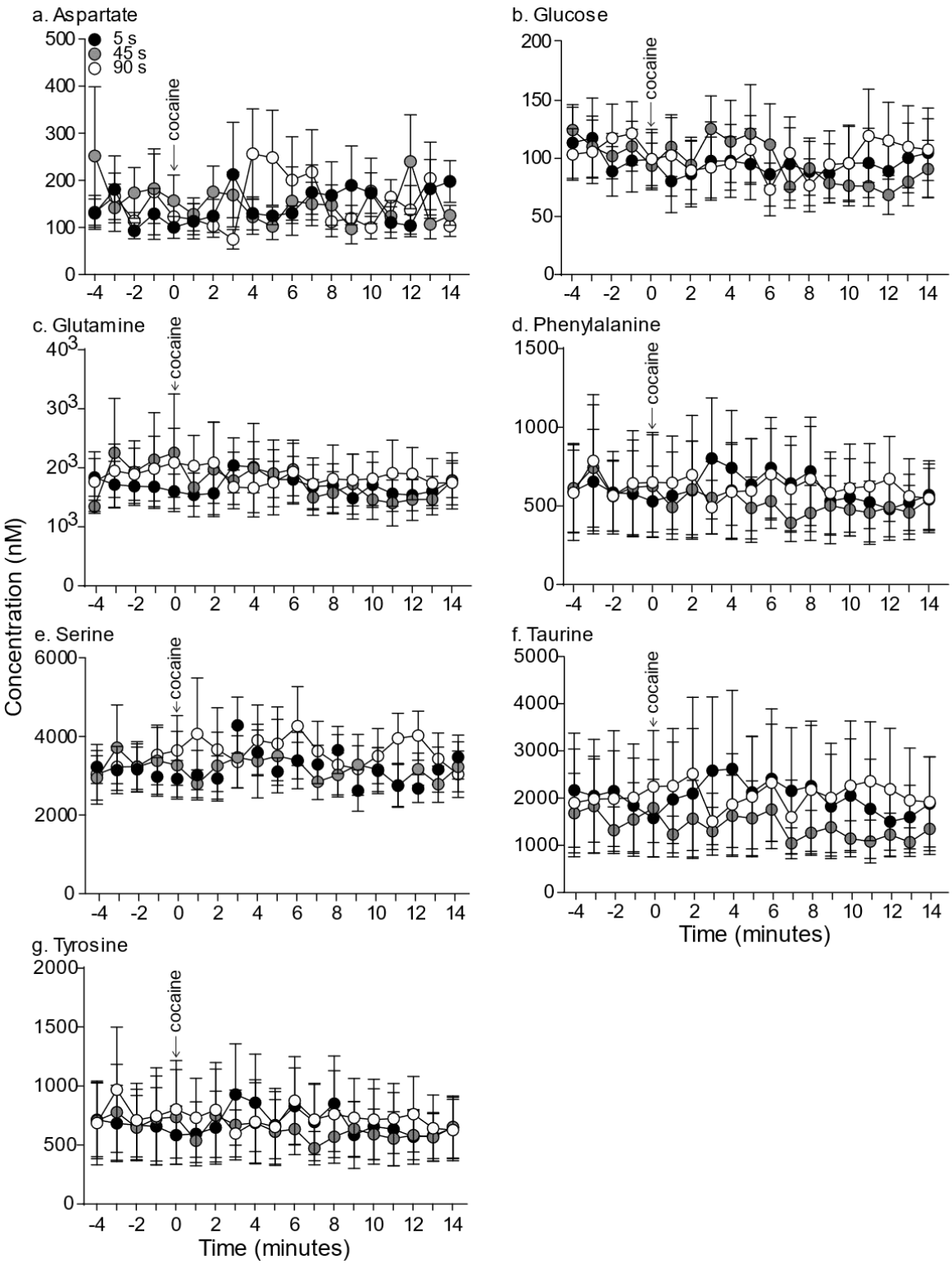
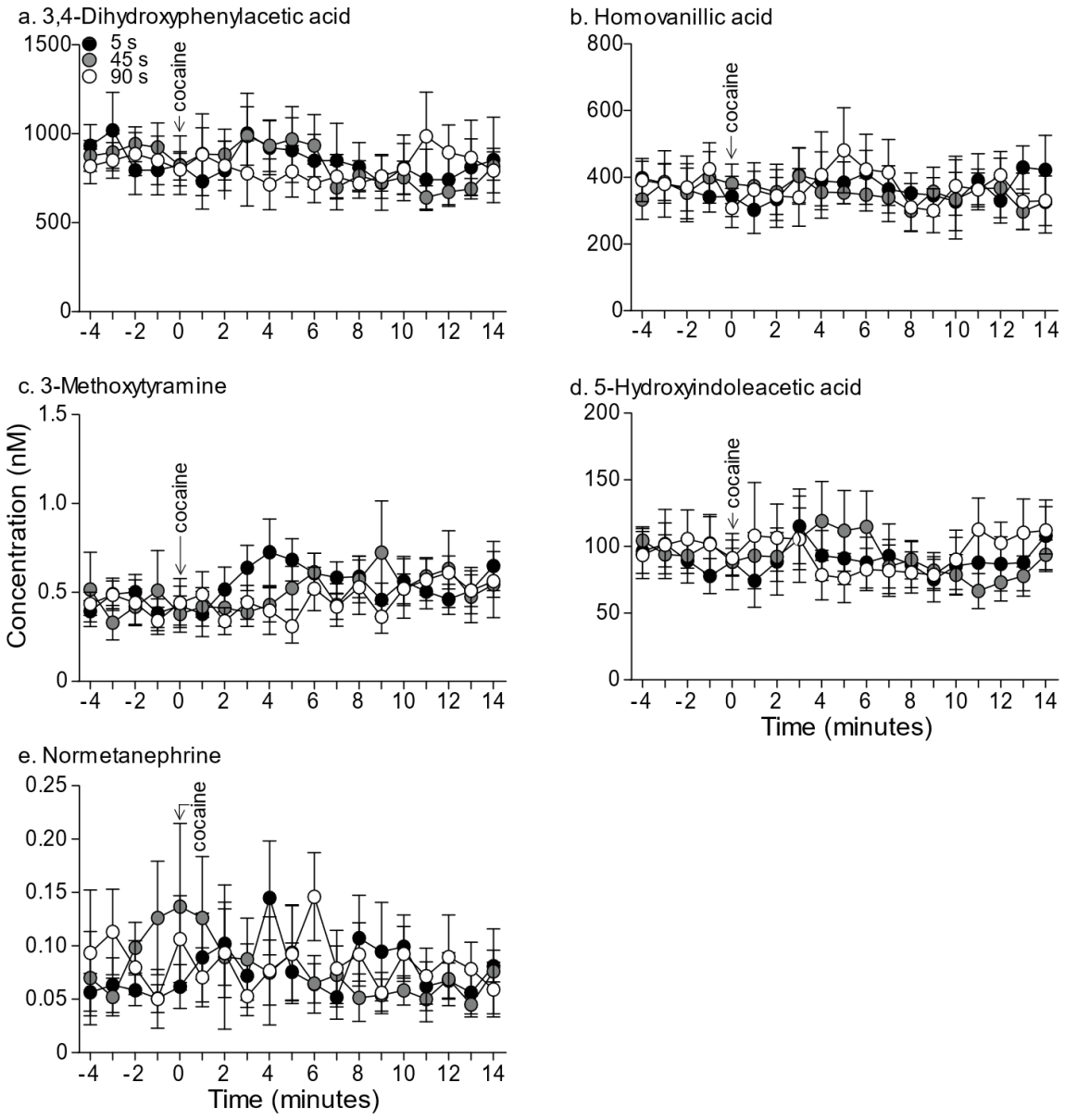

a. Aspartate
5 s
45 s
90 s
cocaine
b. Glucose
cocaine
c. Glutamine
cocaine
d. Phenylalanine
cocaine
e. Serine
cocaine
f. Taurine
cocaine
g. Tyrosine
cocaine
Concentration (nM)
Time (minutes)
Time (minutes)

Figure S3


a. 3,4-Dihydroxyphenylacetic acid
5 s
45 s
90 s
cocaine
b. Homovanillic acid
cocaine
c. 3-Methoxytyramine
Concentration (nM)
cocaine
d. 5-Hydroxyindoleacetic acid
cocaine
Time (minutes)
e. Normetanephrine
cocaine
Time (minutes)

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CHAPITRE 8

The self-administration of rapidly delivered cocaine promotes increased motivation to take the drug: contributions of prior levels of operant responding and cocaine intake

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Please note: KBG provided the work in Experiment 1,

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Abstract

Rationale: Rapid drug delivery to the brain might increase the risk for developing addiction. In rats, increasing the speed of intravenous cocaine delivery (5 versus 90 seconds) increases drug intake and the subsequent motivation to self-administer cocaine. Increased motivation for cocaine could result from more extensive prior drug intake and operant responding for drug, but also from neuroplasticity evoked by rapid drug uptake.

Objective: We determined the contributions of prior drug intake and operant responding to the increased motivation for cocaine evoked by rapid delivery. We also investigated the effects of cocaine delivery speed on corticostriatal expression of brain-derived neurotrophic factor (BDNF) and TrkB mRNA.

Methods: Rats self-administered cocaine (0.25-mg/kg/infusion) delivered over 5 or 90 seconds during short-access (1h/session; ShA) or long-access (6h; LgA) sessions. Motivation for cocaine was then assessed by measuring responding under a progressive ratio schedule of reinforcement. Next, BDNF and TrkB mRNA levels were measured in 5- and 90-s rats.

Results: 5s-ShA and 5s-LgA rats were more motivated for cocaine than their 90-s counterparts. This effect was dissociable from previous levels of drug intake or of operant responding for cocaine. In parallel, only rats self-administering rapid cocaine injections had altered BDNF and TrkB mRNA levels in corticostriatal regions.

Conclusions: Rapid drug delivery augments the motivation for cocaine independently of effects on the levels of drug intake or operant responding for drug. We suggest that rapid delivery might increase the motivation for drug by promoting neuroplasticity within reward pathways. This neuroplasticity could involve increased regulation of BDNF/TrkB.

Keywords: Addiction; Cocaine; Progressive ratio; Intravenous self-administration; Speed of drug delivery; Operant responding, BDNF, TrkB

Introduction

The faster a drug of abuse reaches the brain, the greater is the risk of developing addiction. For example, routes of cocaine administration that result in a rapid onset of drug effects (e.g., smoking or intravenous (i.v.) injection versus the intranasal route) are associated with a greater loss of control over cocaine intake (Gawin et Ellinwood, 1988), greater abuse liability, greater propensity for drug addiction (Chen et Anthony, 2004; Gossop, Griffiths, Powis et Strang, 1994; Hatsukami et Fischman, 1996; O'Brien et Anthony, 2005) and a more rapid progression of addiction (Gorelick, 1992).

Addiction is more likely and more severe when drugs reach the brain rapidly, but it is not known why. Animal studies have investigated the influence of the speed of drug onset on the neurobehavioural impact of drugs by varying the speed of i.v. drug delivery between 5 and 100 seconds (s). These injection speeds produce different magnitudes of subjective drug effects in humans (Abreu, Bigelow, Fleisher et Walsh, 2001), approximate the different rates of rise of plasma cocaine levels when the drug is smoked versus snorted (Jones, 1990), but would hold peak brain levels of cocaine, dopamine and dopamine transporter blockade constant in laboratory animals (while producing different *rates of rise* of all three measures (Ferrario et al., 2008; Samaha, Li et Robinson, 2002; Woolverton et Wang, 2004). Increasing the speed of drug delivery across this range promotes the development of psychomotor sensitization (Samaha et al., 2002; Samaha, Mallet, Ferguson, Gonon et Robinson, 2004; Samaha, Yau, Yang et Robinson, 2005), increases cocaine intake and produces a more persistent vulnerability to relapse (Wakabayashi, Weiss, Pickup et Robinson, 2010). In addition, a key behavioural symptom of addiction is increased motivation for drug that is reflected by the continued

pursuit of drug in the face of rising costs and adverse consequences (Gawin, 1991; Kalivas et Volkow, 2005; Robinson et Berridge, 1993). Our recent work shows that rats self-administering rapid (5 s) versus slower (90 s) cocaine injections take significantly more drug and are later more motivated to self-administer cocaine, as measured by responding for the drug under a progressive ratio (PR) schedule of reinforcement [(Minogianis, Levesque et Samaha, 2013) see also (Liu, Roberts et Morgan, 2005b)]. This is consistent with recent work by Zimmer et al. (Zimmer, Oleson et Roberts, 2012) showing that rapidly spiking brain levels of cocaine (achieved by giving intermittent access to the drug during 6-h self-administration sessions) lead to much greater motivation to take the drug than high, sustained brain levels (achieved by giving continuous drug access during 6-h sessions).

The question now is, how does rapidly delivered cocaine enhance the motivation to self-administer the drug? The available evidence shows that increasing the speed of i.v. cocaine delivery (5-45 vs. 90 s) leads to greater drug intake, followed by increased motivation to self-administer cocaine (Minogianis et al., 2013; Wakabayashi et al., 2010). Thus, one possibility is that the increased motivation for cocaine results from neuroplasticity induced by prior exposure to large amounts of the drug. This neuroplasticity could involve persistent changes to brain reward (Koob et Le Moal, 1997) and decision-making (Jentsch et Taylor, 1999) pathways, and/or an expansion of the neural circuits engaged by cocaine to include networks that mediate obstinate stimulus-response habits (Everitt et Robbins, 2013; Graybiel, 2008; Leyton et Vezina, 2013; Porrino, Daunais, Smith et Nader, 2004). Second, enhanced motivation for cocaine could involve the enduring neuronal effects of prior extensive operant responding for the drug.

Rats that self-administer rapid versus slower cocaine infusions show greater operant responding for cocaine during each self-administration session, thus acquiring more extensive operant training (Minogianis et al., 2013; Wakabayashi et al., 2010). Extensive operant responding for a reward can promote the transition from responding that is goal directed to responding that is habit-based and inflexible, leading to increased reward seeking (Adams, 1982; Adams et Dickinson, 1981; Balleine et Dickinson, 1998; Colwill et Rescorla, 1985; Everitt et Robbins, 2005). In the context of drug self-administration, a high level of operant responding for cocaine has been shown to predict a high level of cocaine-induced reinstatement (Keiflin, Vouillac et Cador, 2008). Third, rapid drug delivery could enhance the motivation for cocaine by promoting changes within brain reward and incentive motivation circuits. For example, the faster drugs like cocaine or nicotine reach the brain, the more readily they activate mesocorticolimbic regions, as measured by indices such as immediate early gene regulation (Samaha et al., 2004; Samaha et al., 2005), heat-producing metabolic activity (Brown et Kiyatkin, 2005) and glucose utilization (Porrino, 1993). In addition, 'spiking' rather than sustained brain levels of cocaine enhance cocaine's potency in inhibiting the dopamine transporter in the nucleus accumbens (Calipari, Ferris, Zimmer, Roberts et Jones, 2013)—an action that is critical to cocaine's reinforcing effects.

Cocaine and related drugs evoke many cellular and molecular changes within brain reward and motivation circuits. Amongst those changes that have been shown to directly modulate drug use are alterations in the expression of the neurotrophin brain-derived neurotrophic factor (BDNF). In response to cell activity, BDNF is secreted (Matsumoto et al., 2008) and activates tropomyosin receptor kinase B (TrkB) receptors (Bibel et Barde,

2000). This in turn modifies intracellular signalling and transcription factor activity (Lu, 2003; Patapoutian et Reichardt, 2001) to produce long-term changes in synaptic plasticity (McGinty, Whitfield et Berglind, 2010). Chronic exposure to cocaine and other psychostimulant drugs alters brain BDNF mRNA and protein levels (Fumagalli, Di Pasquale, Caffino, Racagni et Riva, 2007; Grimm et al., 2003; Im, Hollander, Bali et Kenny, 2010; Meredith, Callen et Scheuer, 2002), and altered BDNF-mediated signalling specifically in midbrain and corticostriatal regions mediates drug-seeking and drug-taking behaviours (Graham et al., 2007; Graham et al., 2009; Im et al., 2010; McGinty et al., 2010). Thus, our objectives were two-fold. First, we sought to determine the contributions of prior level of drug intake and extent of operant responding for drug to the increased motivation to self-administer cocaine evoked by rapid drug delivery. Second, we wished to determine how variations in the speed of cocaine delivery alter the expression of BDNF and TrkB mRNA within corticostriatal regions.

Materials and Methods

Subjects and housing. Male Wistar rats (Charles River Laboratories, St-Constant, QC) weighing between 200-250 g upon arrival were housed individually on a 12h/12 h dark/light cycle (lights off at 8:00 A.M.). Experiments were conducted during the dark phase of the rats' circadian cycle. Water was available *ad libitum*. Except where noted, food was restricted to 25 g of standard rat food chow per day. The animal ethics committee of the Université de Montréal approved all procedures.

Self-administration apparatus. Self-administration training and testing occurred in standard operant conditioning chambers (MED Associates Inc.; St Albans. VT, USA), as described in (Samaha, Minogianis et Nachar, 2011). 3.33 RPM syringe motors were used to deliver cocaine over 5 or 10 seconds and 0.1 RPM motors were used to deliver cocaine over 90 s.

Food training. Figure 1 illustrates the sequence of experimental events. Rats first underwent operant training with food as a reinforcer for 2 sessions (1 session/day). During each session, the house light was turned on and rats could press the left (active) lever to obtain a 45-mg, banana-flavoured food pellet (Grain-based Dustless Precision Pellets; Cedarlane Laboratories LTD, Burlington, ON) under a fixed ratio 1 schedule (FR1) of reinforcement. Pressing on the right (inactive) lever had no programmed consequences. Sessions ended after a maximum of 100 pellets or 30 min. Animals that failed to acquire the task after 2 sessions were given an overnight training session. During food training, animals received 15 g of standard rat food chow in their home cage.

Catheter Implantation and Cocaine self-administration training. Under isoflurane anaesthesia, rats were implanted with custom-made, chronic indwelling catheters into the jugular vein, as described in (Samaha et al., 2011). From then on, catheters were flushed with 0.1 ml of saline or 0.1 ml of saline containing 0.2 mg/ml of heparin (Sigma-Aldrich Inc., Oakville, ON), on alternate days. Following surgery, Baytril (enrofloxacin 10 mg/kg, CDMV, St-Hyacinthe, QC) was added to the rats' drinking water for 7-10 days to prevent

post-surgery infections. Next, self-administration training was initiated in daily 1-h sessions. Rats were trained to press a lever for cocaine hydrochloride infusions (0.25 mg/kg/infusion; Medisca Pharmaceutique, Ville Saint-Laurent, QC) under an FR1 schedule of reinforcement, where each infusion was delivered over 5 s and was followed by a 20-s timeout period. Once rats met minimum training criteria (≥ 5 injections/session, taken at regular intervals) for 2 consecutive days, the response requirement was increased to FR2. Once training criteria were met again, the timeout was extended to 65 s and finally to 85 s. An 85-s timeout period ensured that the projected experimental groups (rats self-administering cocaine infusions over 5 vs. 90 s) had access to the same number of infusions during each session. The average number of injections/session over the last 2 training days was 16.6 ± 0.8 (SEM).

Experiment 1. The contributions of level of drug intake and operant responding for cocaine

Cocaine self-administration under short- and long-access conditions. After cocaine self-administration training, rats were assigned to one of 2 groups such that average drug intake, extent of operant responding (i.e., number of active lever presses), days spent in training and number of inactive lever presses on the last 2 days of training were comparable across groups. For 3 sessions, one group self-administered cocaine infusions delivered over 5 s with an 85-s timeout period and the other group self-administered infusions delivered over 90 s with no timeout. Sessions lasted for 1h (Short access; ShA; referred to as 'Baseline Short Access' in Figure 1). For the next 10 sessions, each group was further divided in two and allowed to self-administer cocaine during either

short (1h)- or long (6h)-access sessions (ShA and LgA, respectively). Thus, four groups were generated; 5s-ShA, 90s-ShA, 5s-LgA and 90s-LgA. Based on prior work (Crombag, Ferrario et Robinson, 2008; Liu et al., 2005b; Minogianis et al., 2013; Wakabayashi, 2010), we predicted that during ShA sessions, drug intake and the level of operant responding for drug would not differ between the 5 and 90 s groups. This would provide two groups that differed only in the speed of the cocaine injections they self-administered. Following 10 ShA or LgA sessions, rats remained in their home cages for 4-5 days. During this period and again at the end of the experiment, catheter patency was assessed by intravenous infusion of a sodium thiopental/sterile water solution (0.2 ml of a 20 mg/ml solution, Vétoquinol, Lavaltrie, QC). Rats that failed to become ataxic within 5 s were removed from the study.

Cocaine self-administration under a progressive ratio schedule of reinforcement. For the next 2 days, the motivation for cocaine was measured using a PR schedule of reinforcement (1 session/day). The number of lever presses required to obtain cocaine increased exponentially with each successive infusion (Response ratio = [$5e^{(\text{injection number} \times 0.2)}$]-5) (Richardson et Roberts, 1996). Sessions ended after a maximum of 5 h or when an hour elapsed since the last infusion. The number of infusions earned prior to this point was used as an index of the motivation for cocaine. Cocaine infusions (0.25 mg/kg/infusion) were delivered over 10 s to all rats. All of the rats in Experiment 1 and 2 attained their breakpoint prior to the 5-h limit.

Experiment 2. The influence of the speed of cocaine delivery on the expression of BDNF and TrkB mRNAs

In a separate set of rats, we examined the influence of the speed of cocaine delivery on BDNF and TrkB mRNA expression within corticostriatal regions. Extended periods of drug taking are thought to be a prerequisite for the emergence of addiction-relevant changes in the brain (Ahmed et Koob, 1998; Belin, Balado, Piazza et Deroche-Gamonet, 2009; Deroche-Gamonet, Belin et Piazza, 2004; Vanderschuren et Everitt, 2004). As such, we gave all animals extended daily access to cocaine (LgA) in this experiment.

Cocaine self-administration. To shorten the length of the total training period, animals did not receive food training. The remainder of the training and testing procedures were as described in Experiment 1, unless noted otherwise. Rats were assigned to one of two groups; a control group allowed to self-administer saline and a group allowed to self-administer cocaine, both delivered over 5 s. Following self-administration training as described above, cocaine-taking rats were assigned to the 5s-LgA or 90s-LgA groups, saline rats were assigned to self-administer saline delivered over 5 or 90s. All animals including the saline controls were then given a total of nine LgA sessions and were also tested under PR as described above, for a total of 4 sessions. Finally, the animals were given one last self-administration session (FR1 for 1h) and sacrificed 15 min later. Following an injection of cocaine, BDNF mRNA levels peak between 1 and 2 h post-injection [though some expression can be seen 0.5 h post-injection (Fumagalli et al., 2007; Graham et al., 2007; Le Foll, Diaz et Sokoloff, 2005)]. The 15-min time point was chosen to ensure that the 5s- and 90s-LgA groups had taken a sufficient number of

cocaine infusions prior to sacrifice. Brains were extracted, plunged into isopentane (-50°C) for 7 seconds and stored at -80°C until processing.

In situ *hybridization*. BDNF or TrkB mRNA expression was labelled on 12-µm-thick coronal brain sections using a [^{35}S]-UTP-labelled riboprobe complementary to BDNF or TrkB, respectively, using procedures described in (Bedard, Maheux, Levesque et Samaha, 2011). The complementary RNA (cRNA) probe for TrkB stems from a 284 bp (nucleotide 2597–2880, NM_008745) EcoRI–BamHI fragment of a full-length mouse TrkB cDNA subcloned into pGEM-4Z and linearized with Kpn I. The cDNA of BDNF was subcloned into pCR 2.1 and linearized with Xho I, and corresponds to a 284 bp (nucleotide 99–448, NM_007540) fragment. Both cRNA probes were synthesized and labelled using a Promega riboprobe kit, [35S]UTP and the RNA polymerase Sp6. Brain sections were then placed against X-ray film (Kodak Biomax-MR; VWR, Town of Mount-Royal, QC) for either 9 (BDNF) or 4 (TrkB) days. An experimenter blind to condition quantified mRNAs on autoradiographs by translating optical grey densities into µCi/g of tissue using a ^{14}C standard curve (ARC-146A, American Radiolabeled Chemicals, St-Louis, MI) and Image J software (NIH, Bethesda, MD). Background values were obtained from the rhinal fissure (+3.4 mm relative to Bregma) or the corpus callosum (+2.6 to 0.0 mm relative to Bregma) of each section and were subtracted from analysis. BDNF and TrkB mRNA values were measured in the ventrolateral (VLO) and lateral (LO) orbitofrontal cortex, the cingulate (Cg1/Cg2; CG), medial prefrontal (prelimbic and infralimbic aspects), frontal (FR1/FR2), and parietal (PAR1) cortices, as well as in the nucleus accumbens core and shell, and the dorsomedial (DM), dorsolateral (DL),

ventromedial (VM) and ventrolateral (VL) quadrants of the caudate-putamen. For each brain region, mRNA levels were averaged over 2-5 sections/rat. Anatomical regions were identified according to (Paxinos et Watson, 1986).

Statistics Repeated measures two-way ANOVA was used to analyse the influence of injection speed (5 or 90 s) and session length (ShA or LgA) on the number of self-administered infusions during ShA or LgA sessions. Mauchly's test is reported in cases where the sphericity assumption is violated. In such cases, degrees of freedom were corrected using the Greenhouse-Geisser estimate of sphericity (ε). Significant Group x Session interaction effects were analysed further using a simple contrast with the first session as reference. Cumulative cocaine intake was calculated as milligrams of cocaine consumed per kilogram across all self-administration sessions, including acquisition sessions. Cumulative active lever presses for cocaine were also summed across all self-administration sessions including acquisition sessions. Each measure was analysed using two-way ANOVA. Significant group effects were analysed further using the Bonferonni post-hoc test. Based on our prior work (Minogianis et al., 2013), an *a priori* prediction was made that the 5-s rats would take more cocaine under progressive ratio than the 90-s rats. Thus, number of infusions taken during PR sessions was analysed using a one-tailed unpaired *t*-test for the ShA and LgA groups. Pearson R correlation was used for all correlations. In Experiment 2, number of infusions prior to sacrifice was analysed using a two-tailed unpaired *t*-test. BDNF and TrkB mRNA levels were analysed using one-way ANOVA followed by Tukey's Multiple Comparison Test. A *p*-value below 0.05 was considered significant.

Results

Experiment 1. The contributions of level of drug intake and operant responding for cocaine

Cocaine self-administration under short- and long-access conditions Figure 2 illustrates the average number of self-administered cocaine infusions as a function of the speed of cocaine delivery and the length of the self-administration sessions. During the first 3 ShA sessions [first 3 sessions in (a) and inset in (b)], the speed of cocaine delivery had no effect on the number of self-administered infusions ($p > 0.05$). However, past these initial 3 sessions, rapid i.v. cocaine injections (delivered over 5 versus 90 s) led to greater drug intake, both in rats given ShA (Figure 2a) and LgA (Figure 2b-c) sessions (a, sessions 4-13, $F(1, 24) = 13.32$; b, sessions 1-10, $F(1, 23) = 24.78$; c, sessions 1-10, $F(1, 23) = 30.60$; all *P*'s < 0.01). In addition, under LgA conditions, the self-administration of rapid, but not slower cocaine injections led to an escalation in drug intake over time, both when considering intake during the first hour and total intake during the 6-h session (Group x Time Interaction; b, Mauchly's test ($X^2(44) = 119.63$; $\varepsilon = 0.46$), $F(4.11, 96.54) = 4.67$; c, $F(9, 207) = 4.91$; all *P*'s < 0.05). Additional *post-hoc* comparisons revealed that in the 5s-LgA rats, escalation of intake in the first hour (Figure 2b) began on the 6th LgA session, (as shown by greater drug intake relative to the first session), and persisted until the last (10th) session, and escalation of total intake during the 6-h session (Figure 2c) began on the 2nd LgA session and persisted until the last (10th) session (All *P*'s < 0.05).

Cumulative cocaine intake and extent of operant responding for cocaine Figure 3 illustrates cumulative cocaine intake (a; the sum of all cocaine injections self-administered

prior to progressive ratio testing multiplied by 0.25 mg/kg) and extent of operant responding for the drug (b; the sum of all active lever presses for cocaine prior to progressive ratio testing) in each experimental group. Increasing the speed of drug delivery increased both cumulative cocaine intake ($F(3, 47)=55.702$, $p < 0.0001$) and extent of operant responding only in rats given LgA sessions ($F(3, 47)=29.165$, $p < 0.0001$). The speed of drug delivery had no effect on either cumulative cocaine intake or extent of operant responding in the ShA groups (all *P*'s > 0.05). It should be noted that as shown in Figure 2a, during some of the ShA sessions, the 5-s group took more cocaine than the 90-s group and thus also engaged in greater active lever pressing behaviour. However, during the preceding acquisition phase, cocaine intake and active lever presses were slightly greater in the 90-s versus 5-s rats (an observation that was not statistically significant). As a consequence, when cocaine intake or active lever presses are summed over all self-administration sessions prior to progressive ratio testing (acquisition + ShA sessions), there are no significant differences between the 5- and 90-s groups given ShA sessions.

Cocaine self-administration under a progressive ratio schedule of reinforcement As illustrated in Figure 4, rats with a history of self-administering rapid cocaine injections during either LgA ($t(23) = 1.81$; $p < 0.05$) or ShA ($t(24) = 2.257$; $p < 0.05$) sessions take more drug under a progressive ratio schedule of reinforcement.

Correlations between prior cumulative cocaine intake and number of infusions taken under progressive ratio. We performed correlations between average number of infusions earned across the 2 progressive ratio sessions on the one hand and prior, cumulative cocaine intake on the other. There was a significant correlation between these two variables only in the 90s-LgA group (data not shown; $r^2 = 0.47$, $p < 0.05$). In this group, the greater the amount of cocaine consumed in the past, the greater the subsequent motivation to self-administer the drug. There were no significant correlations between these two variables in the other experimental groups (5s-LgA, $r^2 = 0.007$; 5s-ShA, $r^2 = 0.019$; 90s-ShA, $r^2 = 0.161$; all *P*'s > 0.05).

Correlations between extent of prior operant responding for cocaine and number of infusions taken under progressive ratio. We also performed correlations between average number of infusions earned across the 2 progressive ratio sessions on the one hand and prior, cumulative active lever presses for cocaine on the other. These two variables were significantly positively correlated in all groups (data not shown, 90s-LgA, $r^2 = 0.406$; 5s-ShA, $r^2 = 0.391$; 90s-ShA, $r^2 = 0.377$; All *P*'s < 0.05), except for the 5s-LgA group ($r^2 = 0.028$, $p > 0.05$).

Experiment 2. The influence of the speed of cocaine delivery on the expression of BDNF and TrkB mRNAs

There were no significant differences in either saline intake or BDNF and TrkB mRNA expression between the two saline groups (data not shown; All *P*'s > 0.05). Thus, these two groups were pooled into one (Sal-LgA). As in Experiment 1, compared to rats in the

90s-LgA group, the 5s-LgA rats took a greater number of cocaine infusions both during the LgA sessions (Figure 5a; $F(1, 9) = 9.90$, $p = 0.01$) and under progressive ratio conditions (Figure 5b; $t(9) = 4.68$; $p = 0.0006$). This cohort of 5-s rats did not escalate their intake during LgA sessions. Doses of cocaine similar to the one used here do not always evoke escalated drug intake (Ferrario et Robinson, 2007; Kippin, Fuchs et See, 2006; Mantsch, Yuferov, Mathieu-Kia, Ho et Kreek, 2004). In the final self-administration session preceding brain collection, cocaine intake was not significantly different between the 5s- and 90s-LgA rats (Figure 5c; $t(9) = 2.06$; $p = 0.07$), although visual inspection of Figure 5c suggests a tendency.

Consistent with reports that the striatum does not synthesize reliably detectable levels of BDNF (Altar et DiStefano, 1998), BDNF mRNA levels in the caudate-putamen and nucleus accumbens core and shell were notably low, with many animals having negative values. Thus, striatal BDNF mRNA levels were not analysed further. In the medial prefrontal cortex there were no group differences in either BDNF or TrkB mRNA expression (data not shown; All *P's* > 0.05). In the nucleus accumbens core and shell, there were no group differences in TrkB mRNA expression (data not shown; All *P's* > 0.05).

Figure 6 illustrates BDNF and TrkB mRNA levels in the orbitofrontal cortex. In this cortical region, BDNF mRNA levels were greater in the 5s-LgA group compared to the 90s-LgA group (Figure 6a and c; All *P's* < 0.05), with no group differences in TrkB mRNA levels (Figure 6b and d). Figure 7 shows BDNF and TrkB mRNA levels in the cingulate, frontal and parietal cortices. In the cingulate cortex, there were no group differences in BDNF mRNA expression (Figure 7a; $p > 0.05$), but TrkB mRNA levels were decreased in the

5s-LgA rats relative to both 90s-LgA and saline rats (Figure 7b; All *P's* < 0.05). In frontal and parietal cortices, the 5s-LgA rats had increased BDNF mRNA levels (Figure 7c and e; All *P's* < 0.01) and decreased TrkB mRNA levels (Figure 7d and f; All *P's* < 0.05), as compared to both 90s-LgA and saline rats. Figure 8 shows TrkB mRNA data in the caudate-putamen. In all quadrants of the caudate-putamen, TrkB mRNA levels were decreased in the 5s-LgA rats (a, 5s-LgA < Sal-LgA; b and c, 5s-LgA < Sal-LgA and 90s-LgA; d, 5s-LgA < Sal-LgA; All *P's* < 0.05). There were no other statistically significant differences.

Thus, in several cortical regions, rats that had self-administered rapid cocaine injections had increased BDNF mRNA levels and decreased TrkB mRNA levels. This was coupled with decreased TrkB mRNA expression within the caudate-putamen. In contrast, in all of the brain regions analysed, rats that had self-administered slow cocaine injections had levels of BDNF and TrkB mRNAs that were no different from control animals (All *P's* > 0.05).

Discussion

Our objectives were to i) determine how the prior levels of cocaine intake and of operant responding for the drug contribute to the ability of rapid drug delivery to enhance the subsequent motivation to take cocaine, and ii) determine how variations in the speed of cocaine delivery alter the expression of BDNF and TrkB mRNA within corticostriatal regions. The self-administration of rapidly delivered cocaine promoted greater drug intake and enhanced the subsequent motivation to self-administer the drug. This is consistent with our recent work showing that rats that have previously taken rapid cocaine infusions are more motivated to self-administer the drug in the future, across a range of doses (Minogianis et al., 2013). In the present study, the increased motivation for cocaine evoked by rapid drug delivery was not accounted for by effects on the prior levels of cocaine intake or of operant responding for the drug (the latter measured as the cumulative number of active lever presses performed to obtain cocaine prior to assessing the motivation for the drug). At the neurobiological level, the self-administration of cocaine altered BDNF and TrkB mRNA expression in corticostriatal regions *only* if the drug was delivered rapidly.

The self-administration of rapidly delivered cocaine promotes increased drug intake

Rapid cocaine injections led to greater drug intake during both LgA and ShA sessions. This is in partial contrast with work showing that increasing the speed of i.v. cocaine delivery (5-100 s) increases drug intake during LgA, but not ShA sessions (Crombag et al., 2008; Minogianis et al., 2013; Wakabayashi et al., 2010). The discordance with the literature might be more apparent than real because prior studies did not test beyond 3-

4 ShA sessions (Crombag et al., 2008; Minogianis et al., 2013; Wakabayashi et al., 2010). We also found that for the first 4 daily ShA sessions, there was no effect of the speed of i.v. delivery on cocaine intake (see Figure 2). However, beyond these initial sessions, faster cocaine injections led to greater drug intake, suggesting an increase in cocaine's reinforcing efficacy with more chronic self-administration experience, even with limited daily access to the drug. This is reminiscent of reports showing that increasing the speed of i.v. cocaine or nicotine delivery does not alter the *acute* psychomotor activating effects of these drugs, but does promote the development of psychomotor sensitization following chronic drug exposure (Samaha et al., 2002; Samaha et al., 2004).

Operant responding

Rapidly delivered cocaine enhanced the motivation for the drug and this was not simply predicted by prior, more extensive operant responding for cocaine (i.e., a greater cumulative number of active lever presses for the drug prior to progressive ratio testing). As an example, the 5- and 90-s rats given ShA sessions had similar histories of operant responding for cocaine, but the 5-s rats showed greater motivation for the drug. Similarly, the 90s-LgA group had access to cocaine 6 h/day and thus acquired a more extensive operant responding history than the 5s-ShA group (1 h/day), yet the two groups showed similar motivation for cocaine. Finally, there was no significant correlation between the extent of prior operant responding for cocaine and the motivation for the drug in 5s-LgA rats.

Drug exposure

Increasing the speed of cocaine delivery increases drug intake [(Minogianis et al., 2013; Wakabayashi et al., 2010) and present findings], but this does not fully account for the ability of rapid drug delivery to enhance the subsequent motivation for cocaine. First, in rats given ShA sessions, total cocaine intake (taking into account both the acquisition and ShA testing phases) was similar in 5- and 90-s rats, yet the 5-s rats were subsequently more motivated to take cocaine. In addition, there was no significant correlation between prior cocaine intake and motivation for the drug in 5s-LgA animals. These observations are consistent with work showing that taking more cocaine in the past does not necessarily predict greater motivation to obtain the drug in the future (Morgan, Liu et Roberts, 2006), and that differences in the motivation for cocaine can be observed between groups of rats with identical prior histories of drug intake (Minogianis et al., 2013). It is also noteworthy that rats given extended daily access to cocaine did not show greater motivation for the drug than rats given limited daily access. This is in accordance with some findings (Liu, Roberts et Morgan, 2005a; Oleson et Roberts, 2009; Quadros et Miczek, 2009) but not others (Hao, Martin-Fardon et Weiss, 2010; Paterson et Markou, 2003; Ramoa, Doyle, Naim et Lynch, 2013; Wee, Mandyam, Lekic et Koob, 2008). As has been discussed elsewhere (Oleson et Roberts, 2009), the discrepancy could involve differences in rat strain, housing, PR ratio requirements and cocaine abstinence periods. As this issue unfolds, the present findings are in agreement with work showing that in predicting the motivation for cocaine, the decisive factor appears to be repeated and rapidly spiking brain levels of the drug, rather than high, stable brain levels of cocaine (Zimmer et al., 2012).

Regulation of BDNF and TrkB mRNA expression

Our findings suggest that effects on the prior levels of cocaine intake or operant responding for cocaine cannot fully explain why the intake of rapidly delivered cocaine promotes increased motivation to self-administer the drug. A reasonable hypothesis is that exposure to rapidly delivered cocaine facilitates the neural plasticity that underlies enhanced motivation to obtain drug. This hypothesis is supported by empirical evidence. For example, increasing the speed of i.v. cocaine delivery (5-6 versus 100-150 s) allows peak levels of striatal dopamine transporter occupancy (Samaha et al., 2004; Woolverton et Wang, 2004) and dopamine overflow (Ferrario et al., 2008) to be reached faster. In addition, the faster drugs like cocaine or nicotine reach the brain, the more readily they evoke cell activity within mesocorticolimbic regions (Brown et Kiyatkin, 2005; Ferrario et al., 2008; Porrino, 1993; Samaha et al., 2004; Samaha et al., 2005). Finally, rapidly spiking brain levels of cocaine promote sensitization to the neurochemical effects of cocaine at the dopamine transporter (Calipari et al., 2013).

The present results add to the previous literature by showing that cocaine alters BDNF and TRKB mRNA expression in corticostriatal structures *only* when it is delivered rapidly. When assessed immediately following a final self-administration session, 5-s rats had increased BDNF mRNA levels in several cortical regions, coupled with decreases in both cortical and caudate-putamen TrkB mRNA levels. This is consistent with evidence that cocaine alters BDNF and TrkB mRNA and protein levels in a time-dependent manner in several mesocorticolimbic structures (Fumagalli et al., 2007; Graham et al., 2007; Graham et al., 2009; Grimm et al., 2003; Im et al., 2010; Le Foll et al., 2005). In contrast,

90-s rats had normal levels of BDNF and TrkB mRNA in all regions analysed. Of note, increased BDNF mRNA was not always accompanied by a decrease in TrkB mRNA (see Figures 6 and 7a-b), suggesting that changes in the transcription of one gene are not simply a compensatory response to changes in the transcription of the other. At this stage, we do not know if the observed changes in mRNA levels translate into protein changes, or how increased regulation of BDNF/TrkB mRNA expression might contribute to the ability of rapid drug delivery to enhance the motivation to self-administer cocaine. With these considerations in mind, a provocative finding here is that variations in the speed of cocaine delivery did not evoke diffuse and nonspecific changes in BDNF/TrkB transcription, but rather the effects were regionally specific. Thus, in specific brain regions (the caudate putamen, orbitofrontal, frontal and parietal cortices, but not the nucleus accumbens or medial prefrontal cortex), BDNF/TrkB transcriptional processes are sensitive to small variations in the temporal dynamics of cocaine delivery. The effects of BDNF/TrkB-mediated signalling on cocaine taking and seeking are regionally specific (McGinty et al., 2010). Increased BDNF-mediated neurotransmission in the ventral midbrain, nucleus accumbens and caudate-putamen enhances drug use (Graham et al., 2007; Graham et al., 2009; Im et al., 2010; Li et al., 2013; Lu, Dempsey, Liu, Bossert et Shaham, 2004), while the same manipulation in the medial prefrontal cortex for example decreases cocaine-seeking behaviour (Berglind et al., 2007).

The largest effect of the speed of cocaine delivery on BDNF mRNA expression was found in the orbitofrontal cortex, where BDNF mRNA levels in the 5-s rats were nearly double those seen in 90-s rats. BDNF is synthesized and secreted in response to cell activity (Matsumoto et al., 2008). The self-administration of rapid cocaine injections

increases the firing rate of orbitofrontal neurons (Guillem, Kravitz, Moorman et Peoples, 2010). Orbitofrontal neurons encode the motivational value of rewards (Tremblay et Schultz, 1999; Wallis et Miller, 2003), and send this information to the striatum to modulate goal-directed behaviour (Pennartz, McNaughton et Mulder, 2000; Schultz, Tremblay et Hollerman, 2000). In this regard, it is of interest that BDNF synthesized in the orbitofrontal cortex (and other cortical areas) is transported to the caudate-putamen (Altar et DiStefano, 1998; Gourley et al., 2013), where increased BDNF activity triggers increased drug taking behaviour (Im et al., 2010). Thus, one can speculate that the rapid delivery of cocaine increases BDNF synthesis within the orbitofrontal cortex and other cortical areas, leading to increased BDNF-mediated signalling in the downstream caudate-putamen, and this in turn promotes increased drug intake and motivation to obtain cocaine. This hypothesis can be evaluated in the future by measuring and manipulating BDNF protein levels. In the meantime, we observed a decrease in TrkB mRNA levels within the caudate-putamen of rats that had self-administered rapid cocaine injections. Such a decrease would be expected in the presence of excess BDNF-mediated signalling.

In summary, independent of any effects on the extent of drug intake or operant responding for cocaine, increasing the speed of drug delivery augments the motivation to obtain cocaine. At the neurobiological level, this is potentially linked to increased regulation of BDNF and TrkB mRNA in corticostriatal nuclei. Our findings add to an emerging literature suggesting that the intake of cocaine under conditions that lead to a rapid rise in drug levels facilitates the brain changes that promote excessive motivation to take the drug (Minogianis et al., 2013; Wakabayashi et al., 2010; Zimmer et al., 2012).

Thus, we suggest that drugs, routes of administration and formulations that allow drugs to reach the brain rapidly might increase addiction liability by evoking neuroadaptations in the brain circuits that modulate motivation.

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Author's contributions

KBG, EAM, DL and ANS designed the studies. EAM and KBG performed the surgical procedures. KGB performed experiment 1 and EAM performed experiment 2. KGB, EAM and ANS analyzed the data and interpreted the findings. KGB, EAM and ANS drafted the manuscript. All authors critically reviewed content and approved final version for publication.

Figure legends

Fig. 1 Timeline of experimental events. h, hour. s, second

Fig. 2 Rats self-administering intravenous cocaine infusions delivered over 5 versus 90 s take more drug during both ShA (a) and LgA (b-c) sessions. LgA rats received 3 ShA sessions prior to the 10 LgA sessions shown. The inset in (b) shows the average numbers of self-administered infusions during these 3 sessions. All values are mean ± SEM. n's = 12-13/condition. s, second. ShA, Short Access sessions (1h/day). LgA, Long Access sessions (6h/day). #$p < 0.05$ compared to the 90-s group. *$p < 0.05$ compared to the first LgA session in the 5s group

Fig. 3 Increasing the speed of intravenous cocaine delivery increases cumulative cocaine intake (a) and extent of operant responding for cocaine (b) only in rats given LgA sessions. n's = 12-13/condition. s, second. ShA, Short Access sessions (1h/day). LgA, Long Access sessions (6h/day). *$p < 0.05$ compared to the 90s-LgA group

Fig. 4 When tested under a progressive ratio schedule of reinforcement, rats that have previously self-administered intravenous cocaine infusions delivered over 5 versus 90 s take more of the drug. Corresponding ratios are shown for comparison. During progressive ratio testing, cocaine was injected over 10 s to all groups. All values are mean

± SEM. n's = 12-13/condition. s, second. ShA, Short Access sessions (1h/day). LgA, Long Access sessions (6h/day). * $p < 0.05$ compared to the corresponding 90-s group

Fig. 5 Self-administration data for the rats used to measure BDNF and TrkB mRNA levels. During long access sessions, rats self-administering intravenous cocaine infusions delivered over 5 versus 90 s take more drug (a) and are subsequently more motivated to take cocaine as measured under progressive ratio (b). In the final self-administration session prior to brain collection (c), there was no statistically significant group difference in cocaine intake between the 5s- and 90s-LgA groups. All values are mean ± SEM. n's = 5-8/condition. s, second. LgA, Long Access sessions (6h/day). *$p < 0.05$ compared to the 90-s group.

Fig. 6 The self-administration of rapid cocaine injections increases BDNF mRNA levels in the orbitofrontal cortex (a and c) with no changes in TrkB mRNA levels (b and d). The figure shows brain-derived neurotrophic factor (BDNF) and tropomyosin-related kinase B (TrkB) mRNA levels in the ventrolateral (VLO; a-b) and lateral (LO; c-d) aspects of the orbitofrontal cortex. All values are mean ± SEM. n's = 5-8/condition. s, second. LgA, Long Access sessions (6h/day). *$p < 0.05$ compared to the 90-s group.

Fig. 7 The effects of the speed of cocaine delivery on BDNF and TrkB mRNA levels in the cingulate (a-b), frontal (c-d) and parietal (e-f) cortices. All values are mean ± SEM. n's = 5-8/condition. s, second. LgA, Long Access sessions (6h/day). CG, cingulate cortex. FR,

frontal cortex. PAR1, parietal cortex. *p < 0.05 compared to the 90-s group. #p < 0.05 compared to the saline (Sal-LgA) group.

Fig. 8 The self-administration of rapid cocaine injections decreases TrkB mRNA expression in the caudate-putamen. The figure shows TrkB mRNA levels in the dorsomedial (DM; a), dorsolateral (DL; b), ventromedial (VM; c) and ventrolateral (VL; d) aspects of the caudate-putamen. All values are mean ± SEM. n's = 5-8/condition. s, second. LgA, Long Access sessions (6h/day). *p < 0.05 compared to the 90-s group. #p < 0.05 compared to the saline (Sal-LgA) group.

Figures

Figure 1

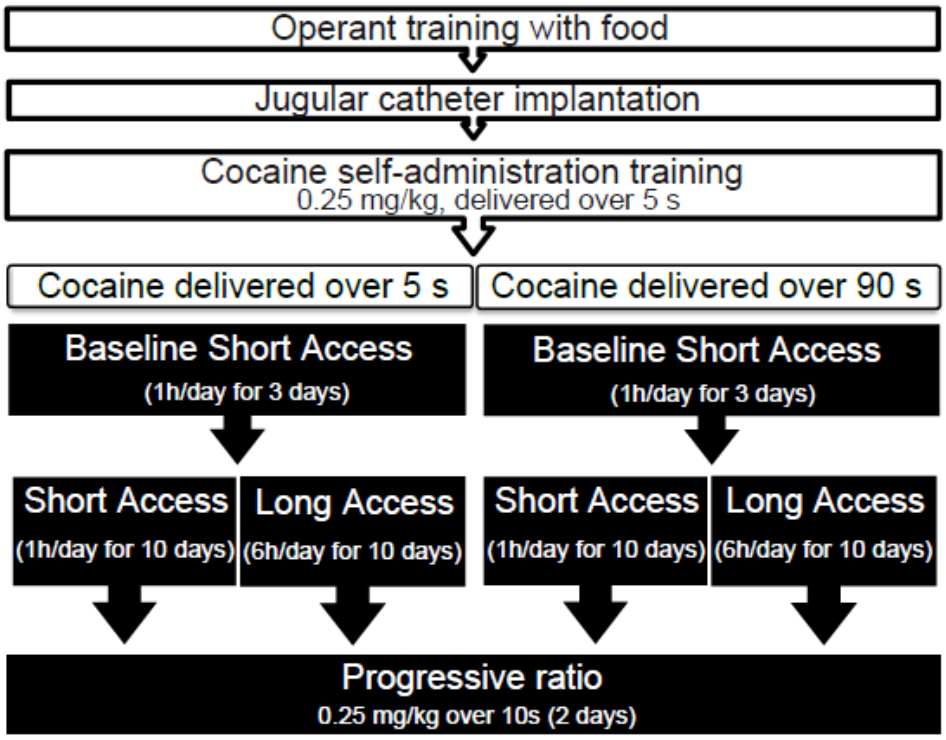


Figure 2

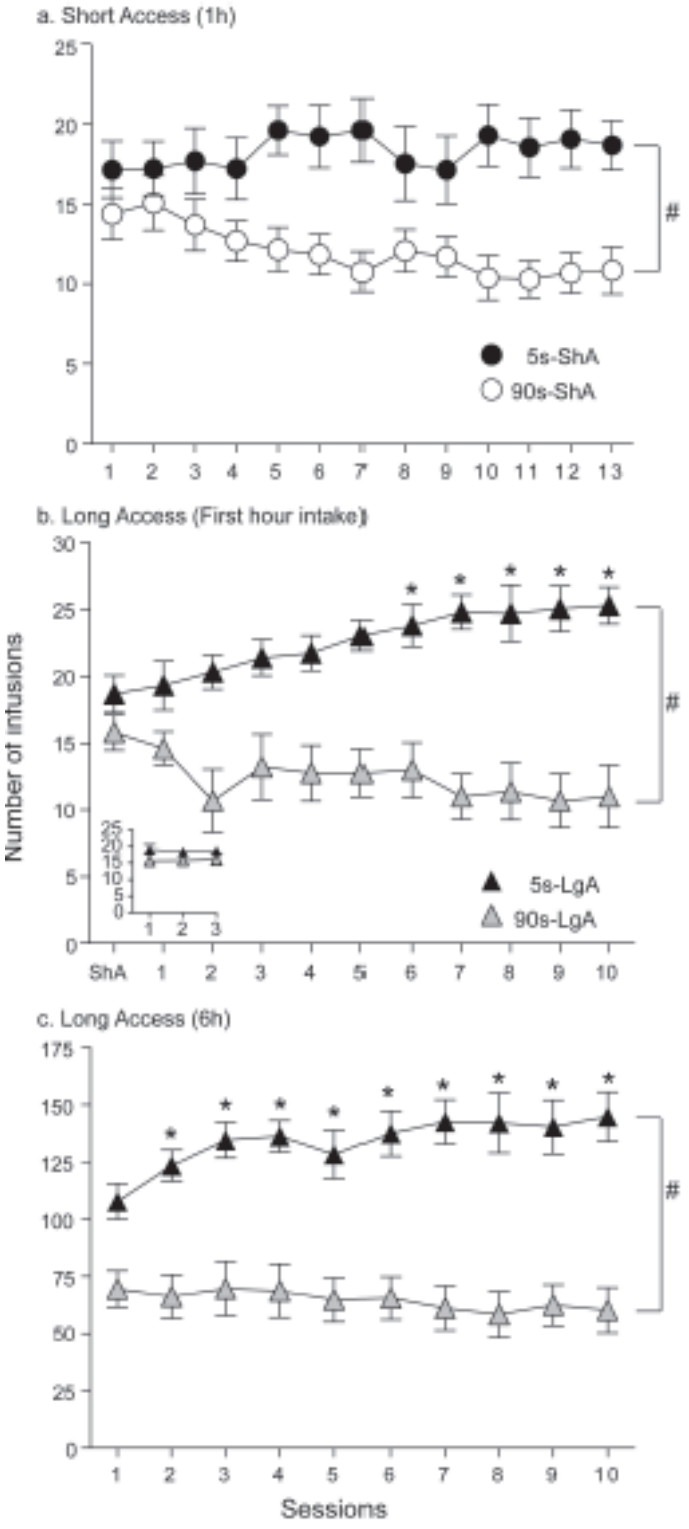

a. Short Access (1h)
5s-ShA
90s-ShA
#
b. Long Access (First hour intake)
Number of infusions
5s-LgA
90s-LgA
ShA
#
c. Long Access (6h)
#
Sessions

Figure 3

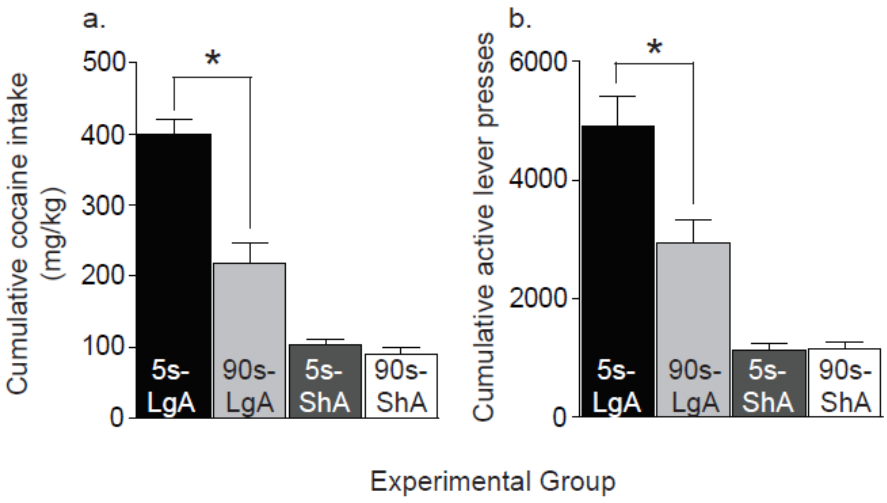

a.
b.
Cumulative cocaine intake (mg/kg)
Cumulative active lever presses
500
400
300
200
100
0
6000
4000
2000
0
*
*
5s-LgA
90s-LgA
5s-ShA
90s-ShA
Experimental Group

Figure 4

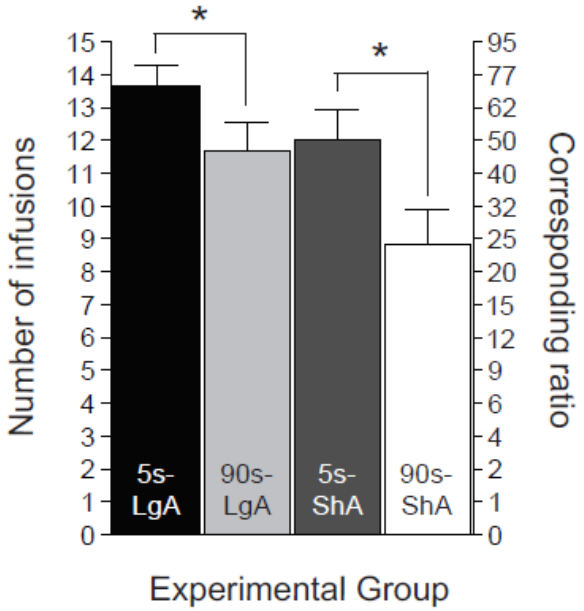

*
*
Number of infusions
Corresponding ratio
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
95
77
62
50
40
32
25
20
15
12
9
6
4
2
1
0
5s-LgA
90s-LgA
5s-ShA
90s-ShA
Experimental Group

Figure 5

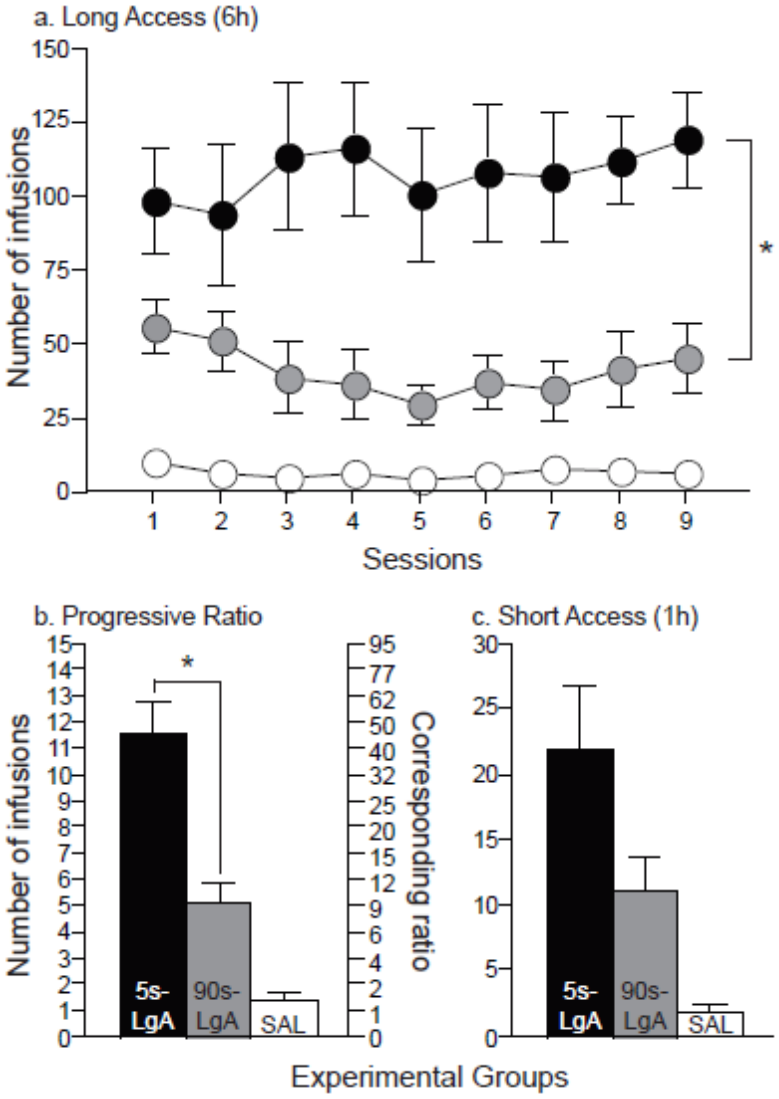

a. Long Access (6h)
Number of infusions
150
125
100
75
50
25
0
1
2
3
4
5
6
7
8
9
Sessions
*
b. Progressive Ratio
Number of infusions
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
*
95
77
62
50
40
32
25
20
15
12
9
6
4
2
1
0
Corresponding ratio
5s-LgA
90s-LgA
SAL
c. Short Access (1h)
30
25
20
15
10
5
0
5s-LgA
90s-LgA
SAL
Experimental Groups

Figure 6

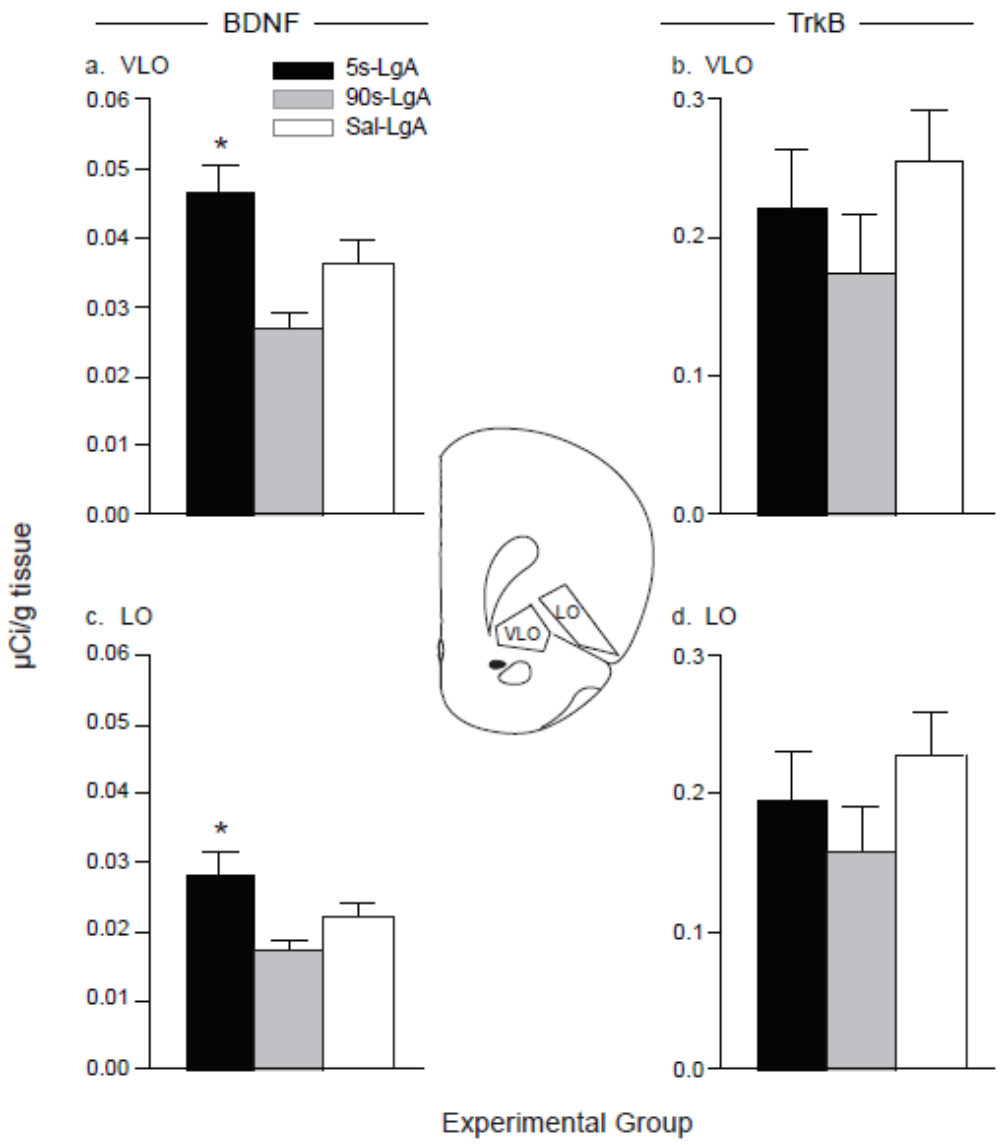

BDNF
TrkB
a. VLO
b. VLO
c. LO
d. LO
5s-LgA
90s-LgA
Sal-LgA
µCi/g tissue
Experimental Group
VLO
LO
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.3
0.2
0.1
0.0
*

Figure 7

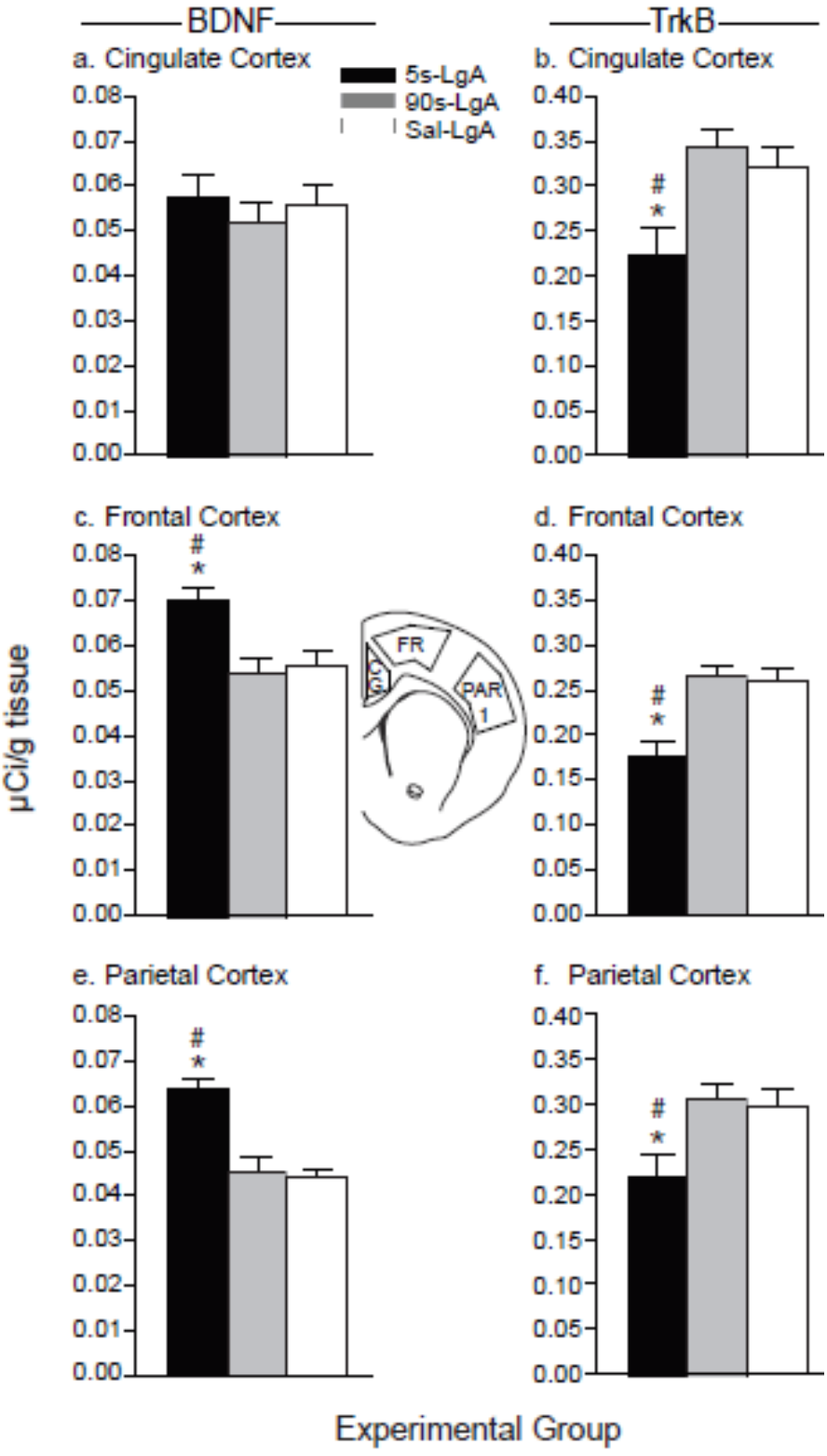
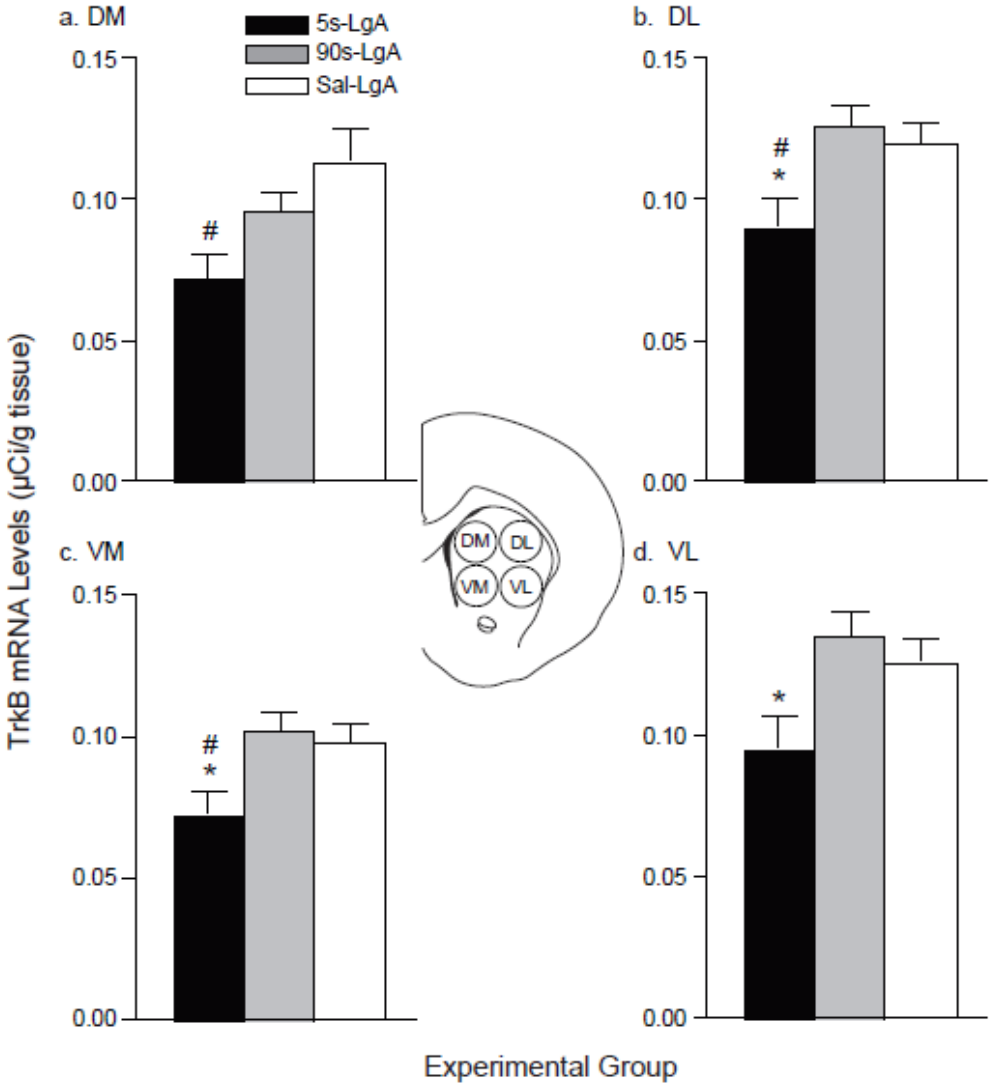

BDNF
TrkB
a. Cingulate Cortex
b. Cingulate Cortex
5s-LgA
90s-LgA
Sal-LgA
c. Frontal Cortex
d. Frontal Cortex
e. Parietal Cortex
f. Parietal Cortex
FR
PAR 1
µCi/g tissue
Experimental Group

Figure 8


a. DM
5s-LgA
90s-LgA
Sal-LgA
b. DL
c. VM
d. VL
TrkB mRNA Levels (µCi/g tissue)
0.15
0.10
0.05
0.00
#
*
DM
DL
VM
VL
Experimental Group

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CHAPITRE 9

Taking rapid and intermittent cocaine infusions increases incentive motivation for the drug and promotes gene regulation in corticostriatal regions

Running Title: Cocaine self-administration kinetics and c-*fos* expression

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Manuscrit en préparation

Abstract

A goal in addiction research is distinguishing neuroplasticity involved in the transition to addiction from that involved in mere drug taking. Animal models of drug self-administration are essential in this context. Some models produce robust addiction-like symptoms. Others do not. Here, c-*fos* mRNA was used to compare cocaine-induced recruitment of corticostriatal regions in two animal models of intravenous cocaine self-administration that differ in the extent to which they produce addiction-like symptoms. Male Wistar rats self-administered intravenous cocaine infusions (0.25 mg/kg/infusion) for seven daily 6-hour sessions. One group had intermittent access to rapid infusions (delivered over 5 seconds). This models the temporal pharmacokinetics of human cocaine use and also produces robust addiction-like behaviour. The other group had continuous access (Long Access or LgA) to slower infusions (90 seconds). This supports high levels of intake without promoting robust addiction-like behaviour. LgA-90s rats took twice as much cocaine as IntA-5s rats, but IntA-5s rats showed greater motivation for the drug, as assessed using a progressive ratio schedule of reinforcement. Immediately following a final self-administration session, brains were processed for *in situ* hybridization of c-*fos* mRNA. Compared to LgA-90s rats, IntA-5s rats expressed more c-*fos* mRNA in the orbitofrontal and prelimbic cortices and the caudate-putamen. Thus, compared to continuous intake of sustained cocaine injections, intermittent intake of rapid injections promotes both incentive motivation for the drug and cocaine-induced recruitment of corticostriatal regions. Increased drug-induced gene regulation in these regions could contribute to the neural and behavioural plasticity underlying the transition to addiction.

Keywords: C-*fos*, Frontal Cortex, Striatum, Speed of Drug Onset, Intermittent Access, Long Access, Progressive Ratio

1. Introduction

Drug addiction is a chronic and relapsing disorder that is characterised by recurring drug-seeking and drug-taking in spite of adverse consequences (APA 2013). Addiction is not an inevitable consequence of drug use. Many people experiment with drugs, but only a subset become addicted (Anthony et al. 1994; Gawin 1991; Shaffer and Eber 2002). A challenge is to distinguish the changes in the brain that are linked specifically to the development of drug addiction from those that result from mere drug taking. To this end, preclinical models have been developed, often using cocaine as a prototypical drug, and whereby animals not only voluntarily take drug, but also develop behavioural symptoms of addiction. To promote patterns of drug use relevant to addiction, these models have manipulated how much drug gets to the brain, how fast, and how often [see (Allain et al. 2015) for review].

A first approach to promote behavioural symptoms of addiction in animal models is to produce a rapid rate of drug onset by increasing the speed of intravenous (i.v.) drug administration (Bouayad-Gervais et al. 2014; Liu et al. 2005; Minogianis et al. 2013; Wakabayashi et al. 2010). This is based on the clinical observation that addiction is less likely to occur in people who take cocaine via slower routes of administration [snorting or oral use versus i.v. injection or smoking; (Ferri and Gossop 1999; Gossop et al. 1992; Gossop et al. 1994; Hatsukami and Fischman 1996)]. Similarly, rats taking slower i.v. infusions of cocaine (injected over 90 versus 5 seconds (s)) consume less drug (Allain et al. 2018; Bouayad-Gervais et al. 2014; Minogianis et al. 2013; Wakabayashi et al. 2010), are less likely to develop psychomotor sensitization (Allain et al. 2017), respond less for cocaine under progressive ratio [PR; (Allain et al. 2017; Bouayad-Gervais et al. 2014; Liu

et al. 2005; Minogianis et al. 2013)], and are less vulnerable to drug-primed relapse following extended abstinence (Wakabayashi et al. 2010). The effects are likely due to differences in the speed of cocaine delivery to the brain rather than to differences in the amount of cocaine reaching the brain (Minogianis et al. 2018). Thus, chronic intake of rapid i.v. cocaine infusions could be a model of addiction-relevant drug use, while chronic intake of slower i.v. infusions could model a less risky, non-disordered drug use.

A second approach to promote addiction-like behaviours in animals involves providing intermittent (IntA) rather than continuous drug access during each self-administration session (Zimmer et al. 2011). During an IntA session (4-6 hours), drug-available periods (5-6 minutes (min)) are separated by no drug-available periods [25-26 min; (Zimmer et al. 2011)]. This produces the peaks and troughs in brain cocaine concentrations that are thought to model how experienced cocaine users take the drug (Beveridge et al. 2012; Zimmer et al. 2011). Compared to continuous-access procedures such as Long Access [LgA; (Ahmed and Koob 1998)], IntA is uniquely effective in producing addiction-like symptoms. Both LgA and IntA experience can produce escalation of cocaine intake over time (Allain et al. 2018; Kawa et al. 2016; Pitchers et al. 2017). However, although LgA rats take much more drug, IntA rats show more incentive motivation for cocaine, as measured either by behavioural economic indicators or responding for the drug under PR (Allain et al. 2018; Kawa et al. 2016; Zimmer et al. 2012). IntA rats also take cocaine in spite of adverse consequences, they persist in responding for cocaine when it is not available, and they show stronger cue-induced relapse behaviour than generally seen in LgA-rats (Kawa et al. 2016; Singer et al. 2018).

Thus, an intermittent pattern of drug intake is more effective in changing drug use over time than continuously high levels of intake.

Thus, addiction-like symptoms are more likely in animals taking rapid versus slower cocaine injections and in animals taking drug intermittently versus continuously during each self-administration session [see also (Allain et al. 2015)]. Here we exploited these pharmacokinetic principles to compare cocaine-induced gene regulation in corticolimbic regions following a pattern of cocaine use that readily produces an addiction phenotype, versus a pattern of cocaine use that does not. We gave one group of rats IntA to rapid (5 s) i.v. cocaine injections and we gave a second group of rats LgA to slow (90 s) i.v. cocaine injections. We then measured cocaine-induced changes in c-*fos* mRNA in corticostriatal regions. Based on the literature above, we expected that the IntA-5s rats would show increased incentive motivation for cocaine and would therefore be the addiction-relevant condition, while the LgA-90s rats would model the effects of non-disordered cocaine use that may potentially be less severe than IntA-5s. Thus, comparing these two groups would distinguish the changes in cocaine-induced gene regulation that could be relevant to the transition to addiction from those that are a consequence of simple chronic cocaine use.

2. Materials & methods

2.1 Animals & housing

Forty male Wistar rats (Charles River Laboratories, St-Constant, QC) weighing between 225-250 g upon arrival were housed individually in a climate-controlled colony room

maintained on a reverse 12 h/12 h light/dark cycle (lights off at 8:00 am). Only male rats were studied because the IntA model of cocaine self-administration is recent (Zimmer et al. 2011). However, we are characterizing IntA self-administration behaviour in female animals in ongoing studies. Experiments were conducted during the dark phase of the rats' circadian cycle. Food and water were available *ad libitum*, until the day after surgery, from which point on, animals received 5 food pellets at the end of the day (25 g). The animal care committee of the Université de Montréal approved all procedures (Protocol #14-149).

2.2 Drugs

Cocaine hydrochloride (Medisca Pharmaceutique Inc, St-Laurent, QC) was dissolved in 0.9% physiological saline (Medical Mart, Mississauga, ON) and filtered with Corning bottle-top filters (0.22 µm PES membrane; Fisher Scientific, Whitby, ON).

2.3 Surgery and operant cages

Figure 1 depicts the timeline of experimental events. After 1 week of habituation to the vivarium, rats were implanted with a catheter into the jugular vein (Samaha et al. 2011; Weeks 1962). To avoid blood clots in the catheters, they were flushed on alternate days with either 0.1 ml physiological saline or saline containing 0.2 mg/ml Heparin (Sigma-Aldrich, Oakville, ON) and 2mg/ml Baytril (CDMV, St-Hyacinthe, QC). On the day before progressive ratio testing, and on the day that brains were extracted (see below), catheter patency was verified by i.v. infusion of a sodium thiopental/sterile water solution (0.2 ml

of a 20 mg/ml solution; CDMV, St-Hyacinthe, QC). All animals became ataxic within 5 s of the infusion and were included in data analyses. At least one week after surgery, the rats were trained to press a lever to obtain i.v. cocaine (0.25 mg/kg/infusion) in standard operant chambers (Med Associates, St-Albans, VT). The chambers were in a room separate from the rats' housing room. Each chamber was placed within a light and sound attenuating cabinet equipped with a ventilation fan that also masked external noise. Infusion pumps with 3.33-RPM motors were used to deliver cocaine over 5 or 10 s at a rate of 29.68 µl/s, while 0.1-RPM motors delivered cocaine over 90 s at a rate of 0.803 µl/s. Each chamber contained two retractable levers and 4 photobeam sensors (Med Associates, St-Albans, VT) to measure locomotor activity. A computer running Med Associates Med-PC version IV software (Med Associates, St-Albans, VT) controlled parameters in the chambers and collected data.

2.4 Self-administration training

After 7 days of recovery from surgery, rats were trained to self-administer cocaine (0.25 mg/kg/infusion) delivered over 5 s, during daily 1-h sessions. Sessions began with the illumination of the house light and insertion of both levers. Projected IntA-5s rats were trained to lever-press for cocaine under a fixed ratio (FR) 3 schedule of reinforcement. During each 5-s infusion and for 20 s thereafter, both levers were retracted and the light located above the active lever was turned on, as in (Allain et al. 2018). Projected LgA-90s rats were trained to lever-press for cocaine under FR2, with the timeout period gradually increasing from 20 s to 85 s, as in (Minogianis et al. 2013). We increased the timeout period in the projected LgA-90s rats to 85 s, such that they would get used to

waiting 90 s between injections (5-s infusion + 85-s timeout). We used FR3 instead of FR2 in the projected IntA-5s rats because in our experience, this increases discrimination between the active and inactive levers in these rats. Pressing on the inactive lever had no programmed consequences. Rats were considered to have acquired reliable cocaine self-administration behaviour if — for 2 consecutive sessions— they took ≥ 5 infusions/session, at regular time intervals during the session (visualized by SoftCr, Med Associates, St-Albans, VT), and if they pressed twice as much on the active versus inactive lever. In parallel to the cocaine-taking rats, a third group of animals from the same cohort could self-administer i.v. saline injections. Following acquisition of cocaine self-administration behaviour, all rats were given 3 additional 1-h sessions (1 session/day). During these sessions, the projected IntA-5s rats self-administered cocaine (0.25 mg/kg/infusion, delivered over 5 s) under FR3, with no timeout period. The projected LgA-90s rats self-administered cocaine under FR2, but each infusion was now delivered over 90 s, with no timeout period. The control rats continued to self-administer saline. During each infusion, the light above the active lever was turned on. After these 3 sessions, all rats were given 7 daily, 6-h sessions. Projected IntA-5s rats now self-administered cocaine under IntA. Projected LgA-90s rats now self-administered cocaine under LgA. One half of the control rats self-administered saline under IntA, and the other half self-administered saline under LgA.

2.5 Self-administration under intermittent access (IntA) conditions

Following the training phase above, IntA-5s rats transitioned to 6-hour IntA self-administration sessions. Each IntA session had twelve, 6-min drug periods separated by

26-min no-drug periods during which levers were retracted and cocaine was not available. During each 6-min drug period, the animals could self-administer a maximum of 2 infusions, as described in (Allain et al. 2018; Allain et al. 2017). Once the two infusions were self-administered or the 6-min drug period had elapsed, a 26-min no-drug period was initiated. Thus, the animals could take a maximum of 24 infusions per IntA-session. The last 6-min drug period was followed by a 2-min no-drug period such that the session lasted no more than 6 h. One half of the control rats self-administered saline under IntA conditions, with saline delivered over 5, 45 or 90 s.

2.6 Self-administration under long access (LgA) conditions

After self-administration training, the LgA-90s rats transitioned to 6-hour LgA sessions. During each self-administration session, cocaine was available continuously, except during each 90-s infusion, where further lever presses did not produce additional infusions. The other half of the control rats self-administered saline (delivered over 5, 45 or 90 s) under LgA conditions.

2.7 Estimating brain cocaine concentrations

We estimated brain cocaine concentrations (C; in μM) as a function of time during the seventh IntA-5s and LgA-90s session of a representative rat from each cocaine-taking group, using a mathematical model developed by (Pan et al. 1991) and described in (Allain et al. 2017). This model has recently been used to estimate cocaine brain levels following IntA self-administration studies (Allain et al. 2018; Allain et al. 2017; Allain and

Samaha 2018; Zimmer et al. 2011; Zimmer et al. 2012). The model uses the following formula:

$$C = dA \cdot e^{-\beta t} - e^{-\alpha t} \text{ with } A = \frac{k}{v \cdot (\alpha - \beta)}$$

where *d* represents the cocaine dose self-administered (0.25 mg/kg/infusion) and *t* signifies the time (in minutes) after each subsequent cocaine infusion. Further details on this formula can be found in (Allain et al. 2018; Allain et al. 2017). The Python script used to model brain cocaine concentrations was kindly provided by Dr. David C. S. Roberts.

2.8 Cocaine self-administration under a progressive ratio schedule of reinforcement (PR)

Five days after the last IntA or LgA session, we assessed incentive motivation for cocaine by allowing the rats to self-administer the drug (0.063-0.25, in descending order, 1-2 sessions/dose) under PR. Control rats self-administered saline under PR. During each PR session, the number of lever presses required to obtain each successive infusion increased exponentially, according to the formula (5 $e^{(\text{injection number x } 0.2)}$ – 5), as described by (Richardson and Roberts 1996). Cocaine (or saline) infusions were delivered over 10 s. Thus, dose and injection speed were held constant across groups, such that any group differences in responding for cocaine would be due to drug-taking history. We used a standard PR procedure, where both levers are present in the cage throughout the session, except during each 10-s injection (Richardson and Roberts 1996). Each PR session ended when an hour had elapsed since the last infusion or after 5 hours. All

animals in this study completed their PR sessions prior to 5 hours. The total number of active lever-presses was used as a measure of motivation for cocaine. The day after the last PR session, the rats received 2 final IntA-5s or LgA-90s sessions. Control rats also underwent 2 more sessions under IntA or LgA at their initial respective speed (5, 45 or 90 s).

2.9 Final cocaine self-administration session and brain extraction

One day after the last IntA-5s or LgA-90s session, all rats were given a final self-administration session before brains were extracted for *in situ* hybridization of c-*fos* mRNA. During this session, cocaine (or saline) was available under a FR2 schedule of reinforcement, and each infusion was administered over 10 s. To avoid the potential confound of differences in drug intake on this last session before brain extraction, the cocaine rats were allowed to take a maximum of 10 infusions. The session ended once the 10 infusions were taken or after 1 hour. Control rats were also given 1 h to self-administer a maximum of 10 saline infusions. c-*fos* mRNA levels peak 0.5-1 h after i.v. cocaine administration (Ennulat et al. 1994). As such, all rats were sacrificed 45 minutes after the end of the session. The animals were decapitated, and brains were extracted, plunged into cold isopentane (-50°C) and stored at -80°C.

2.10 *In situ* hybridization

A subset of representative animals from each group was used for *in situ* hybridization. C-*fo*s mRNA expression was labelled on 12-µm-thick coronal brain sections using a [^{35}S]-

UTP-labelled riboprobe complementary to c-*fos*, as in (Bédard et al. 2011). The complementary RNA probe for c-*fos* mRNA was derived from 1.8 kb *EcoRI* fragment of a full-length rat c-*fos* cDNA. It was subcloned into pBluescript SK-1 plasmid and linearized with SmaI (Tremblay et al. 1999). The probe was synthesized using a Promega riboprobe kit (Fisher Scientific, St-Laurent, QC), [^{35}S]-UTP (Perkin Elmer, Woodbridge, ON) and polymerase T7 (Promega, Fisher Scientific, St-Laurent, QC). Labelled brain slices were placed against Kodak Biomax MR X-ray film (VWR, Town of Mount-Royal, QC) for 4 days.

2.11 Quantification of c-*fos mRNA*

An experimenter blind to experimental condition translated optical gray densities from autoradiographs into microcuries (µCi) per gram of tissue using a ^{14}C standard curve (ARC-146A, American Radiolabeled Chemicals, St-Louis, MI). Image J software was used for the quantification (NIH, Bethesda, MD). Background values were obtained from the rhinal fissure (+ 4.7 to 3.0 mm relative to Bregma) or the corpus callosum (+ 2.6 to 0.0 mm relative to Bregma) of each section. Background values were then subtracted from analysis. C-*fos* mRNA expression was measured in the orbitofrontal cortex [ventrolateral (VLO) and lateral (LO)], medial prefrontal cortex [prelimbic (PrL) and infralimbic (IL) cortex], cingulate cortex area 1 (Cg1), frontal cortex area 2 (FR2), caudate-putamen [(CPu; dorsomedial (DM), dorsolateral (DL), ventromedial (VM) and ventrolateral (VL) quadrants] and nucleus accumbens core (NacC) and shell (NacSh). Brain regions were identified according to (Paxinos and Watson 1986). For each brain region, mRNA levels were averaged over 2-5 sections/rat.

2.12 Statistical analysis

Two-way repeated-measures ANOVA was used to assess group differences in cocaine (or saline) intake, inter-infusion interval, lever presses and cumulative cocaine intake (Group x Session; the latter as a within-subjects variable). Two-way repeated-measures ANOVA was also used to assess group differences in number of lever presses and session length during PR testing (Group x Dose; the latter as a within-subjects variable). One-way ANOVA followed by Tukey's multiple comparisons' test or Kruskal-Wallis H test followed by Dunn's multiple comparisons' test were used to assess group differences in number of infusions, session length and locomotor activity on the last self-administration session before brain extraction. One-way ANOVA followed by Tukey's multiple comparison test was used to analyse group differences in c-*fos* mRNA levels. Pearson's r coefficients (two-tailed) were computed to assess relationships in c-*fos* mRNA expression between different brain regions.

3. Results

3.1 LgA-90s rats take more cocaine than IntA-5s rats

IntA-saline (IntA-Sal) and LgA-saline (LgA-Sal) rats took similar numbers of injections and pressed an equivalent number of times on the active lever during their 6-h sessions (Figures 2b and 2d, respectively; all *P*'s > 0.05); such that they were pooled into a single control group (Sal-Ctrl). Figure 2a shows patterns of cocaine intake and estimated brain cocaine concentrations from a representative rat from each condition

during the 7th IntA or LgA self-administration session. IntA would evoke peaks and troughs in brain cocaine concentrations, while LgA would produce continuously high concentrations [also see (Allain et al. 2018; Zimmer et al. 2012)]. Figure 2b shows the number of self-administered cocaine (or saline) infusions in the IntA-5s, LgA-90s and control groups. Note that there is a break between sessions 7 and 8 in Figures 2b-e because rats were given four PR sessions between these sessions. LgA-90s rats had continuous access to cocaine during each session, and each infusion was delivered over 90 s. IntA-5s rats had intermittent access to cocaine during each session, where drug was available for twelve 6-min periods, separated by 26-min no-drug periods. IntA-5s rats were limited to 2 infusions/6-min cocaine period, providing a maximum of 24 cocaine infusions per 6-h session, as indicated by the dotted line in Figure 2b. Under these conditions, LgA-90s rats took significantly more cocaine than IntA-5s rats (Main effect of Group; $F(1,17) = 13.99$, $p = 0.0016$). Both cocaine-taking groups also self-administered more infusions than both saline groups (Main effect of Group; IntA-5s vs. IntA-Sal, $F(1,22) = 323.4$; vs. LgA-Sal, $F(1,19) = 254.3$; LgA-90s vs. IntA-Sal, $F(1,17) = 43.15$; vs. LgA-Sal, $F(1,14) = 29.99$; all P's < 0.0001). Figure 2c shows the inter-infusion interval for the cocaine groups, calculated as the time elapsed between the end of one injection and the beginning of the next. For IntA-5s rats, the inter-infusion interval was computed during the 6-min cocaine periods. During these periods, IntA-5s rats took one injection every 26 s on average (± 5 s SEM). This was significantly shorter than the LgA-90s rats (11 min ± 4 min SEM; Main effect of Group; $F(1,17) = 11.22$, $p = 0.004$). The self-imposed inter-infusion interval in the LgA-90s rats was also significantly longer than the 90-s infusion length. This suggests that the 90-s infusion length did not artificially constrain cocaine

self-administration behaviour in these animals. Figure 2d shows active lever presses. Both cocaine groups pressed more on the active lever than control rats (Main effect of group; IntA-5s vs. IntA-Sal, $F(1,22) = 286.5$; vs. LgA-Sal, $F(1,19) = 211.8$; LgA-90s vs. IntA-Sal, $F(1,17) = 43.91$; vs. LgA-Sal, $F(1,14) = 31.61$; all *P*'s < 0.0001). LgA-90s rats also pressed more on the active lever than IntA-5s rats (Main effect of Group; $F(1,17) = 18.46$, $p = 0.0005$). LgA-90s rats also pressed more on the inactive lever than the other two groups (LgA-90s vs. IntA-Sal, $F(1,17) = 10.76$; vs. LgA-Sal, $F(1,14) = 7.27$; vs. IntA-5s, $F(1,17) = 10.36$, all *P*'s < 0.02; data not shown). Figure 2e shows cumulative intake over the 9 self-administration sessions (number of injections taken x 0.25 mg/kg). LgA-90s animals self-administered more cocaine than IntA-5s animals (Main effect of Group; $F(1,17) = 14.83$, $p = 0.001$). Overall, LgA-90s rats took twice as much cocaine as IntA-5s rats. No other comparisons were statistically significant. Thus, compared to IntA-5s rats, LgA-90s rats took cocaine at a slower pace but they took twice as much drug.

3.2 IntA-5s rats respond more for cocaine under a progressive ratio schedule of reinforcement than LgA-90s rats

Figure 3 shows operant responding for cocaine (0.063-0.25 mg/kg/infusion; or saline in the Sal-Ctrl group) under PR. During PR testing, all infusions were administered over 10 s. As such, any group differences in responding for cocaine would be due to differences in drug-taking history. IntA-5s and LgA-90s rats pressed more on the active lever than Sal-Ctrl rats (Figure 3a; Main effect of Group; $F(2,37) = 22.49$; IntA-5s vs. Sal-Ctrl, $F(1,31) = 42.55$; LgA-90s vs. Sal-Ctrl, $F(1,26) = 42.17$, all *P*'s < 0.0001), and lever-pressing increased with cocaine dose (Main effect of Dose; $(2,74) = 11.03$, $p < 0.0001$;

IntA-5s vs. Sal-Ctrl, $F(2,62) = 16.11$, $p < 0.0001$; LgA-90s vs Sal-Ctrl, $F(2,52) = 5.26$, $p = 0.008$). IntA-5s lever pressed more for cocaine than LgA-90s rats (Dose x Group interaction effect; IntA-5s vs. LgA-90s: $F(2,34) = 3.87$, $p = 0.03$), in particular at the highest cocaine dose ($p = 0.01$). IntA-5s and LgA-90s animals did not differ in the number of inactive lever presses during PR sessions, but both groups pressed more on the inactive lever than Sal-Ctrl rats (Figure 3b; Main effect of Group: $F(2,37) = 14.15$, IntA-5s vs. Sal-Ctrl, $F(1,31) = 30.37$; ContA-90 s vs. Sal-Ctrl, $F(1,26) = 24.56$; all *P*'s < 0.0001) and pressing on the inactive lever also increased with cocaine dose (Main effect of Dose; $F(2,74) = 35.78$; $p < 0.05$). PR sessions lasted longer in the IntA-5s and LgA-90s rats than in Sal-Ctrl rats (Figure 3c; $F(2,37) = 42.72$; all P's < 0.002). PR sessions also lasted longer at higher cocaine doses (Main effect of dose; $F(2,34) = 3.87$, $p = 0.03$). No other comparisons were statistically significant. Figures 3d-f show cumulative number of drug infusions taken during the 5-h progressive-ratio sessions, as a function of time, at each cocaine dose. Visual inspection of Figures 3d-f suggests that, in particular at the highest dose tested, the IntA-5s rats earned more cocaine than the LgA-90s rats, at all time points during the PR session. Thus, though IntA-5s rats had taken significantly less cocaine in the past than LgA-90s rats, they showed greater incentive motivation for the drug when tested under PR.

3.3 Self-administration behaviour and locomotor activity on the last session before brain extraction

On the final self-administration session before brain extraction (Figure 4), animals could self-administer a maximum of 10 infusions, each delivered over 10 s. The Sal-Ctrl

rats self-administered saline. The session ended once 10 infusions were taken or after a maximum of 1 h. Figure 4a shows that IntA-5s and LgA-90s rats took a similar number of cocaine infusions ($\chi^2_{(2)}$ = 23.93, p > 0.9999), and both groups self-administered more infusions than Sal-Ctrl rats ($\chi^2_{(2)}$ = 23.93, p < 0.0001; IntA-5s vs. Sal-Ctrl, p < 0.0001; LgA-90s vs. Sal-Ctrl, p = 0.004). The self-administration session was shorter in IntA-5s rats compared to Sal-Ctrl rats (Figure 4b; ($\chi^2_{(2)}$ = 22.31, p < 0.0001; IntA-5s vs. Sal-Ctrl: p <0.0001; LgA-90s vs. Sal-Ctrl p = 0.12). Session length did not differ between cocaine-taking groups (p = 0.29); yet, there was a tendency for IntA-5s rats to take their cocaine infusions at a more rapid rate than LgA-90s rats (data not shown; Unpaired *t*-test on the inter-infusion interval; $t_{(7,68)}$ = 1.98, p = 0.08). This observation is further supported by visual inspection of the cocaine intake pattern from a representative rat from each group found in figure 4d-e. Figure 4c shows that the IntA-5s and LgA-90s rats showed similar levels of cocaine-induced locomotion during this final self-administration session, and both groups showed greater locomotion than the Sal-Ctrl rats ($\chi^2_{(2)}$ = 27.21, p < 0.0001; IntA-5s vs. LgA-90s, p > 0.9999, IntA-5s vs. Sal-Ctrl, p < 0.0001 and LgA-90s vs. Sal-Ctrl, p = 0.0008). In summary, during the last self-administration session before brain extraction, IntA-5s rats and LgA-90s rats took similar amounts of cocaine and showed similar levels of drug-induced locomotion. A subset of the animals shown in Figure 4 were used for *in situ* hybridization (IntA-5s and LgA-90s, n = 6/group; Sal-Ctrl, n = 10). Supplementary Figure 1 shows the behaviour of this subset of rats during the last self-administration session before brain extraction. This subset of IntA-5s rats and LgA-90s rats also took similar amounts of cocaine (Figure S1a; $\chi^2_{(2)}$ = 16.03, p > 0.9999), with

similar session length (b; $F(2,19) = 13.15$, $p = 0.1808$), and similar levels of drug-induced locomotion (c; $\chi^2_{(2)} = 15.65$, $p > 0.9999$).

3.4 Compared to LgA-90s rats, IntA-5s rats show greater cocaine-induced c-*fos* mRNA expression in the frontal cortex and the striatum

In all brain regions sampled, c-*fos* mRNA levels were similar in rats that had self-administered saline under either IntA or LgA conditions (data not shown, all *P*'s > 0.05). Thus, the two control groups were pooled into one (Sal-Ctrl). Figure 5 shows c-*fos* mRNA levels in the orbitofrontal, medial prefrontal, cingulate and frontal cortices. In all of these cortical regions, cocaine self-administration increased c-*fos* mRNA expression compared to saline self-administration (all *P*'s < 0.05). The mode of cocaine intake in the past also significantly influenced cocaine-induced c-*fos* mRNA levels. IntA-5s rats had more c-*fos* mRNA than LgA-90s rats in the ventrolateral and lateral orbitofrontal cortices and in the prelimbic cortex (Figures 5b-d; VLO: $F(2,107) = 57.5$; LO: $F(2,63) = 32.57$; PrL: $F(2,85) = 37.88$; all *P*'s < 0.0001). There were no differences between these two groups in the infralimbic, cingulate or frontal area 2 cortices (Figures 5e-g; all *P*'s > 0.05).

Figure 6 shows c-*fos* mRNA levels in the caudate putamen (b-e) and in the nucleus accumbens (f-g). Cocaine self-administration increased c-*fos* mRNA expression in both regions compared to saline self-administration (all *P*'s < 0.05). The history of cocaine intake also altered cocaine-evoked c-*fos* mRNA expression. Compared to LgA-90s rats, IntA-5s animals had increased c-*fos* mRNA levels in all quadrants of the caudate-putamen (Figures 6b-e; DM: $F(2.85) = 35.14$; DL: $F(2.85) = 27.66$; VM: $F(2.85) = 41.38$;

VL: 31.78; all *P*'s < 0.0001). There were no differences between these two groups in the nucleus accumbens core or shell (Figures 6f-g; all *P*'s > 0.05).

3.5 C-*fos* mRNA levels in the frontal cortex and the dorsal striatum are positively correlated only in IntA-5s rats

Corticostriatal afferents are a massive source of glutamate in the striatum (Berendse et al. 1992; Carter 1982; Gerfen 1992; McGeorge and Faull 1989) and psychostimulant-induced immediate early gene expression in the striatum depends on glutamatergic inputs from the cortex (Cenci and Björklund 1993; Ferguson and Robinson 2004; Hess et al. 2003). These are the cortical regions where IntA-5s and LgA-90s rats showed significant differences in c-*fos* mRNA density. The prelimbic cortex sends excitatory inputs to the dorsomedial caudate-putamen (Berendse et al. 1992; Mailly et al. 2013). Also, the ventrolateral and lateral areas of the orbitofrontal cortex send glutamatergic afferents to the centrolateral caudate-putamen (Berendse et al. 1992; Mailly et al. 2013; Schilman et al. 2008). For these reasons, we performed correlations between c-*fos* mRNA levels in the caudate-putamen and c-*fos* mRNA levels in each of the orbitofrontal and prelimbic cortices. Figure 7 shows that there was a significant positive correlation between c-*fos* mRNA levels in these cortical regions and the caudate-putamen *only* in IntA-5s rats. In this group only, higher levels of c-*fos* mRNA in the ventrolateral and lateral orbitofrontal cortex significantly predicted higher levels of the immediate early gene in the dorsal caudate-putamen (Figure 7a, $r^2 = 0.85$, $p = 0.009$). Similarly, in the IntA-5s group only, a higher density of c-*fos* mRNA in the prelimbic cortex significantly predicted a higher density of the immediate early gene in the dorsomedial

caudate-putamen (Figure 7d, r^2 = 0.94; p = 0.001). Thus, cocaine-induced gene regulation in frontal cortical areas and in the caudate-putamen was significantly correlated only in rats with a history of taking rapid and intermittent cocaine injections.

4. Discussion

Compared to slow cocaine delivery and continuous within-session intake, rapid cocaine delivery and intermittent drug intake more effectively promote the development of the excessive patterns of drug use that characterize addiction (Allain et al. 2018; Allain et al. 2017; Bouayad-Gervais et al. 2014; James et al. 2018; Kawa et al. 2016; Liu et al. 2005; Minogianis et al. 2013; Singer et al. 2018; Wakabayashi et al. 2010; Zimmer et al. 2012). The effects of these different temporal patterns of cocaine use on drug-seeking and drug-taking behaviours have been well characterized, but much less is known about effects on neurobiology. Here we used *in situ* hybridization histochemistry to quantify cocaine-induced c-*fos* mRNA expression in rats with a history of intermittent intake of rapid cocaine injections (IntA-5s) versus a history of continuous intake of slower injections (LgA-90s). LgA-90s rats took twice as much cocaine as IntA-5s rats, but IntA-5s animals showed greater incentive motivation for the drug, as measured by responding under a PR schedule of reinforcement. We also found that IntA-5s rats had greater cocaine-induced increases in c-*fos* mRNA levels in the orbitofrontal and prefrontal cortices and in the caudate-putamen when compared to the LgA-90s group. These findings demonstrate that the temporal pattern of cocaine intake influences both incentive motivation for the drug and cocaine-induced gene regulation in the brain. The findings also suggest that greater

incentive motivation for cocaine is potentially linked to increased recruitment of corticostriatal regions.

Our results show that high levels of cocaine exposure in the past do not necessarily produce greater cocaine wanting in the future, and that intermittent intake of rapid drug injections is particularly effective in producing this change [see also (Allain et al. 2018; Zimmer et al. 2012)]. This is consistent with a growing literature showing that how fast and how often cocaine is consumed is more important than how much in promoting addiction-like symptoms in preclinical models (Allain et al. 2018; Allain et al. 2015; Allain et al. 2017; Bouayad-Gervais et al. 2014; James et al. 2018; Kawa et al. 2016; Liu et al. 2005; Minogianis et al. 2013; Singer et al. 2018; Wakabayashi et al. 2010; Zimmer et al. 2012). LgA cocaine self-administration results in high levels of drug exposure, but IntA experience produces greater incentive motivation for the drug, as measured either by behavioural economic indicators (James et al. 2018; Zimmer et al. 2012) or responding for cocaine under PR [(Allain et al. 2018) and present findings]. It appears therefore, that exposing the brain to high amounts of cocaine is not necessary to produce change in drug use over time, and that intermittent cocaine intake, which achieves peaks and troughs in brain drug concentrations could be more effective (Allain et al. 2018; Kawa et al. 2016; Zimmer et al. 2012). In further support of this idea, very brief exposure to IntA cocaine self-administration can evoke changes in brain and behaviour that are relevant to addiction. For instance, as little as three 6-h IntA sessions can produce sensitization to both the incentive motivational effects of cocaine as measured using behavioral economics, and the drug's effects at the dopamine transporter (Calipari et al. 2015). Short daily bouts of IntA cocaine self-administration (2 h/session) are also sufficient to produce

levels of incentive motivation for cocaine, cocaine-induced reinstatement, burst-like patterns of drug-taking and psychomotor sensitization that are similar to those seen with 6-h IntA sessions (Allain and Samaha 2018). Importantly, IntA procedures might also be more clinically representative. It is unlikely that cocaine users maintain continuously high brain concentrations of drug when consuming cocaine, as achieved by LgA procedures. Instead, drug use in human cocaine addicts is defined by intermittency, both between and within bouts of consumption (Beveridge et al. 2012; Cohen and Sas 1994; Simon et al. 2001). Together, this literature suggests that compared to continuous access procedures (e.g., LgA), IntA procedures might be the most useful to study the neurobiology of cocaine addiction.

In almost every brain region studied, self-administered cocaine evoked greater c-*fos* mRNA levels in IntA-5s rats than in LgA-90s rats or cocaine-naïve animals that had chronically self-administered i.v. saline. Group differences in behaviour or testing conditions immediately before brain extraction can confound interpretation of c-*fos* results. To minimize this risk, we limited the IntA-5s and LgA-90s animals to 10 cocaine injections during the last test session, delivered at a common speed (10 s). The two groups took similar levels of cocaine and also showed similar levels of cocaine-induced psychomotor activity on this last self-administration session. As such, group differences in brain c-*fos* mRNA expression cannot be due to any differences in achieved cocaine dose or motor activity. Being placed in a testing context can also evoke c-*fos* expression in the brain (Badiani et al. 1998; Uslaner et al. 2001). However, this likely did not contribute to group differences in c-*fos* expression because all three groups (IntA-5s, LgA-90s and Sal-Ctrl animals) received similar instrumental training experience and exposure

to the operant test cages. In all brain regions examined, both cocaine-taking groups had greater c-*fos* mRNA levels than cocaine-naïve animals, and in most brain regions examined (orbitofrontal cortex, medial prefrontal cortex and caudate-putamen), c-*fos* mRNA density was also highest in the IntA-5s rats. The principal difference between IntA-5s and LgA-90s rats concerns the temporal pattern of chronic cocaine use in the past, which resulted in greater cumulative drug exposure in LgA-90s rats but more incentive motivation for cocaine in IntA-5s rats. Thus, two non-mutually exclusive conclusions can be drawn. First, the intake of large quantities of cocaine (i.e., in the LgA-90s condition) blunts the c-*fos* mRNA response to subsequent cocaine. Previous work has found that acute intraperitoneal or intravenous cocaine administration leads to the rapid and transient expression of c-*fos* mRNA (Daunais and McGinty 1994; Ennulat et al. 1994; Graybiel et al. 1990; Hope et al. 1992; Steiner and Gerfen 1993), while chronic and/or repeated intravenous cocaine self-administration (Daunais et al. 1993; Daunais et al. 1995) or intraperitoneal administration (Daunais and McGinty 1994; Hope et al. 1992; Steiner and Gerfen 1993) seem to suppress this effect. While the data from these studies suggest that c-*fos* mRNA may not play a role in the long-term effects of cocaine, our results disagree and report that both IntA and LgA cocaine self-administration increases c-*fos* mRNA levels in all corticostriatal regions analyzed when compared to saline controls. One potential explanation for these differences is that repeated cocaine administration is provided by the experimenter (Daunais and McGinty 1994; Hope et al. 1992; Steiner and Gerfen 1993). Also, both the intraperitoneal [4-14 sessions; (Daunais and McGinty 1994; 1995; Hope et al. 1992)] and intravenous self-administration [3 sessions; (Daunais et al. 1995)] studies reported above tend to be executed over much

shorter period of time than we report here (3-4 weeks) which led to a lesser cumulative cocaine intake. Daunais et al. (1993) were the exception, since animals had previous undisclosed intravenous self-administration experience under fixed and progressive ratio schedules of reinforcement for 2-6 weeks prior to a week of 5-h sessions under FR1, which was then followed by brain extraction. Even though the mechanism behind the differences observed in c-*fos* mRNA levels between IntA and LgA access is unknown, the literature and our data suggest that LgA to cocaine may act like chronic cocaine administration and thus promotes tolerance to the ability of the drug to induce c-*fos* mRNA expression [(Hope et al. 1992) and reviewed in (Hammer et al. 1997)], while IntA may resemble acute use and may not lead to this desensitization. This agrees with the findings of Ennulat et al. (1994) who compare acute (a single i.v. cocaine infusion), repeated (1 day of 3 i.v. cocaine infusions followed by a single cocaine infusion on day 2) and extended (4 days of 3 i.v. infusions followed by a single challenge infusion on day 5) cocaine treatment on the induction of c-*fos* mRNA. They report that repeated cocaine administration leads to sensitization of cocaine-induced c-*fos* mRNA following the cocaine challenge on the final test day, while extended treatment does not, and instead seems to produce desensitization versus the acute treatment. A second conclusion which could be drawn is that a sensitized c-*fos* mRNA response to cocaine in corticostriatal regions contributes to increased incentive motivation for the drug. These two possibilities are consistent with findings showing that IntA cocaine self-administration promotes sensitization-related neuroadaptations, while LgA promotes tolerance-related changes, at least in terms of dopamine system function. IntA sensitizes to cocaine's effect at the dopamine transporter in the nucleus accumbens core (Calipari et al. 2013) and also

produces cross-sensitization to both methylphenidate' and amphetamine's effect at the transporter (Calipari et al. 2014b). In contrast, LgA blunts dopamine-mediated neurotransmission—at least early after the last self-administration session (Calipari et al. 2014a; Ferris et al. 2011; Willuhn et al. 2014).

It is also important to note that variations in the speed of i.v. cocaine delivery alone can also change cocaine-induced immediate early gene expression. When cocaine is injected acutely, a rapid i.v. infusion (5 s) produces greater c-*fos* mRNA expression in the medial prefrontal and orbitofrontal cortices (Samaha et al. 2004) and in the striatum (Ferrario et al. 2008; Samaha et al. 2004) than a more sustained infusion (25-100 s). Unfortunately, in none of these studies (including this one) was drug-induced neuroplasticity measured in rat brains extracted immediately after the PR session. Therefore, while we cannot make a direct link between the detected neuroplasticity and excessive motivation for cocaine, potential effects of these drug-induced changes may cause this increased willingness to obtain the drug and need to be explored.

The temporal pattern of cocaine intake in the past produced not only quantitative differences in c-*fos* mRNA activity, but qualitative differences as well. The OFC is thought to be involved in the motivational control of goal-directed behaviours (Tremblay and Schultz 1999; Wallis and Miller 2003), while it is believed that the CPu is involved in the development of drug-motivated habits that can contribute to addiction (Everitt et al. 2008; Robbins and Everitt 1999; Tiffany 1990). Human brain imaging studies report alterations in these brain structures following long-term cocaine experience. For instance, 25-day-abstinent cocaine abusers show greater OFC activation during the Iowa Gambling Task, a decision-making task, compared to the control group (Bolla et al. 2003), suggesting

differences in the anticipation of reward between the two groups. Also, cocaine-associated cues induce changes in both the OFC (Wang et al. 1999) and the CPu (Garavan et al. 2000; Volkow et al. 2006), providing a potential role for this structure in drug craving. Not only are these two brain structures individually involved in the addiction process, but they are also anatomically interconnected via considerable glutamatergic projections from the OFC to the CPu (Berendse et al. 1992; Carter 1982; Gerfen 1992; McGeorge and Faull 1989; Schilman et al. 2008). Glutamate from corticostriatal afferents is thought to mediate c-*fos* response to drugs in the CPu, particularly when drugs are experienced under conditions that promote sensitization-related changes in both the brain and behaviour (Ferguson et al. 2003; Ferguson and Robinson 2004; Snyder-Keller 1991). Given this, we found evidence of apparent differences in interregional connectivity between our experimental groups, such that a strong positive correlation between c-*fos* mRNA expression in the orbitofrontal/prelimbic cortices and the caudate-putamen was only found in the IntA-5s rats. The unique pattern of 'connectivity' between corticostriatal regions in IntA-5s rats could reflect the extent to which specific, functionally-linked brain regions are recruited when cocaine has increased incentive motivational properties. This will require studies to determine how activity in these interconnected brain areas might be involved in the pursuit of cocaine when incentive motivation for the drug is increased versus when it is not. Indeed, comparing IntA-5s and LgA-90s rats in this context could be useful. The fact that we only observe a significant and strong positive correlation in the IntA-5s rats, our addiction-relevant model, suggests that the striatal c-*fos* response observed here might preferentially involve cocaine-induced activation of corticostriatal glutamate inputs, and although hypothetical, may even facilitate the development of

excessive motivation seen here, and other addiction-like behaviours. However, a recent report from Singer et al. (2018) using the Puzzle Self-Administration Procedure (a method that avoids the development of habitual drug-seeking behaviour by forcing test rats to solve a new puzzle each day to access the drug) suggests that addiction-like symptoms can develop in the absence of strong stimulus-response seeking habits, and that in this context, inhibiting neuronal activity in the CPu may not necessarily influence cocaine-taking behaviour.

Laboratory animals are more likely to show addiction-like patterns of drug use if they are given access to cocaine intermittently versus continuously, and if they self-administer rapid versus more sustained cocaine infusions. Here we leveraged these pharmacokinetic principles to identify brain regions that are engaged by cocaine in animals that show high versus lower levels of incentive motivation for the drug. We report that self-administered cocaine engages frontal cortical areas and the caudate-putamen, as indicated by c-*fos* mRNA induction, preferentially in animals that show increased sensitivity to the incentive motivational effects of the drug. Thus, we conclude that cocaine-induced recruitment of these brain systems is potentially involved in the expression of the increased incentive motivation for cocaine that defines the transition to addiction, rather than in the expression of non-disordered cocaine use. Ongoing studies are characterizing the link between neuronal activity in these corticostriatal regions and the response to cocaine. Together, these studies could identify the brain circuits that mediate the changes in behaviour and psychological function involved in the transition to cocaine addiction.

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Conflicts of interest

The authors declare no conflicts of interest.

Author's contributions

EAM and ANS designed the study. EAM performed the surgical procedures. EAM performed the experiments. EAM and ANS analyzed the data and interpreted the findings. EAM and ANS drafted the manuscript. All authors critically reviewed content and approved final version for publication.

Figure Legends

Figure 1. Timeline of experimental events. Rats were implanted with an intrajugular catheter and trained to self-administer cocaine (0.25 mg/kg/infusion) or saline delivered over 5,45 (saline group only) or 90 s seconds during 1-hour sessions under a fixed ratio schedule of reinforcement. Following three more 1-hour sessions at their respective infusion speed, animals were further divided into groups as a function of access condition, such that cocaine-taking animals received either intermittent access to rapid cocaine infusions (IntA-5s) or continuous access to slower infusions (LgA-90s; ≤ 6 hours/session over 7 sessions). Similarly, saline-taking rats were given access to saline under either IntA or LgA conditions. Following 5 days of withdrawal, motivation for cocaine (0.063-0.25 mg/kg/infusion) or saline delivered over 10 s was tested under a progressive ratio schedule of reinforcement. After PR, rats were given 2 more IntA/LgA sessions. On the following day, rats underwent a final test session where an infusion criterion of 10 was imposed or the session ended after 1 hour, and brains were extracted. *h*, hour. *IntA*, intermittent access. *LgA*, long access. *s*, seconds.

Figure 2. Rats consumed greater amounts of cocaine when given continuous, rather than intermittent, access to the drug. IntA-5s rats were limited to 2 infusions (infused over 5 s) per 6-minute drug access phase, each followed by a 26-minute inactive phase during which no drug was available (12 cycles/session), while LgA-90s rats had unlimited access to slow infusions to the drug (delivered over 90 s) for 6 hours. Here, patterns of cocaine intake (top) and estimated cocaine brain levels (bottom) provided by a representative rat from each group on the 7th day of IntA or LgA (a), cocaine intake (b), inter-infusion interval

(c), active (d) lever presses, and cumulative cocaine intake (e) are shown. All values are mean ± SEM. n = 7-21 rats/group. *h*, hours. *IntA*, intermittent access. *LgA*, long access. *min*, minutes. *s*, seconds. *Sal-Ctrl*, saline control animals. **p* < 0.05 vs. Sal-Ctrl or IntA-5s groups.

Figure 3. IntA-5s rats showed greater motivation for cocaine than LgA-90s rats under a progressive ratio schedule of reinforcement, even though they had consumed less cocaine in the past. Motivation for cocaine was assessed as the number of active lever presses made under PR. All infusions were administered over 10 s. Active (a) and inactive lever presses (b), session length (c) and cumulative number of infusions taken over 15-minute bins during each PR test session (d) are shown as a function of dose. All values are mean ± SEM. n = 7-21 rats/group. *h*, hours. *IntA*, intermittent access. *LgA*, long access. *s*, seconds. *Sal-Ctrl*, saline control animals. **p* < 0.05 vs. Sal-Ctrl group. $^{\#}p$ < 0.05 vs. IntA-5s group.

Figure 4. On their final test session, both cocaine groups consumed the same amount of drug and exhibited similar locomotor activity. During the final self-administration session prior to brain extraction, all rats were limited to a maximum of 10 cocaine infusions (0.25 mg/kg/infusion) delivered over the common speed of 10 s. Sessions ended once the infusion criterion was attained or after 1 hour. No statistically significant difference was detected in either cocaine intake (a), session length (b) or locomotor activity (represented as total beam breaks per minute; c) between the two cocaine-taking groups. The pattern

of cocaine intake as a function of time from a representative rat from each group is shown in d (IntA-5s) and e (LgA-90s). All values are mean ± SEM. n = 7-21 rats/ group. *IntA*, intermittent access. *LgA*, long access. *min*, minutes. *s*, seconds. *Sal-Ctrl*, saline control animals. *$p < 0.05$ vs. Sal-Ctrl group.

Figure 5. Access to rapid and intermittent cocaine induces greater c-*fos* mRNA expression in the orbitofrontal and prelimbic cortices. The effects cocaine on rats following a history of either rapid and intermittent (IntA-5s) or slower and continuous (LgA-90s) cocaine infusions on c-*fos* mRNA levels in the orbitofrontal (ventrolateral (b) and lateral (c)), prelimbic (d), infralimbic (e), cingulate (f) and frontal (g) cortices. While cocaine self-administration increases *c-fos* mRNA expression in all structures examined, c-*fos* mRNA levels are greater in IntA-5s rather than LgA-90s rats in the ventrolateral and lateral orbitofrontal cortices (b,c) and the prelimbic cortex (d). Localization of the regions quantified and in situ autoradiographs for c-*fos* mRNA expression from a sample rat from each group can be found in panel (a). All values are mean ± SEM. *n* = 6-10 rats/group. *Sal-Ctrl*, saline control animals. *IntA*, intermittent access. *LgA*, long access. *s*, seconds. *VLO*, ventrolateral orbitofrontal cortex. *LO*, lateral orbitofrontal cortex. *PrL*, prelimbic cortex. *IL*, infralimbic cortex. *Cg1*, cingulate cortex area 1. *Fr2*, frontal cortex area 2. *$p < 0.05$ vs. Sal-Ctrl group. $^{\#}p < 0.05$ vs. LgA-90s group.

Figure 6. Intermittent access to rapid cocaine infusions induces greater c-*fos* mRNA expression in the dorsal striatum. The effects of the chronic self-administration of rapid

and intermittent (IntA-5s) or slow and continuous (LgA-90s) cocaine infusions on c-*fos* mRNA levels in the four quadrants of the caudate putamen (dorsomedial (b), dorsolateral (c), ventromedial (d) and ventrolateral (e)) and the nucleus accumbens (core (f) and shell (g)). The self-administration of cocaine given rapidly and intermittently leads to greater c-*fos* mRNA expression than continuous access to more gradual infusions in the CPu (b-e). Localization of the regions quantified and in situ autoradiographs for c-*fos* mRNA expression from a sample rat from each group can be found in panel (a). All values are mean ± SEM. *n* = 6-10 rats/group. *Sal-Ctrl*, saline control animals. *IntA*, intermittent access. *LgA*, long access. *s*, seconds. *CPu*, caudate putamen. *DM*, dorsomedial quadrant. *DL*, dorsolateral quadrant. *VM*, ventromedial quadrant. *VL*, ventrolateral quadrant. *NacC*, nucleus accumbens core. *NacSh*, nucleus accumbens shell. **p*<0.05 vs. Sal-Ctrl group. $^{\#}p$<0.05 vs. LgA-90s group.

Figure 7. The self-administration of rapid and intermittent, but not slow and continuous, cocaine infusions leads to a significant positive correlation between the expression of c-*fos* mRNA in the orbitofrontal and prelimbic cortices, and the caudate-putamen. The OFC projects to the ventrolateral CPu (Berendse et al. 1992; Schilman et al. 2008). A significant positive correlation was found in the c-*fos* mRNA expression of these two regions only when the animals were given access to cocaine to rapid and intermittent cocaine infusions (a; IntA-5s, r^2 = 0.85, p = 0.009). Correlations for LgA-90s and Sal-Ctrl groups are shown in b and c, respectively. The PrL sends projections to the dorsomedial CPu (Berendse et al. 1992; Schilman et al. 2008). Similarly, a significant positive correlation was found in the c-*fos* mRNA expression of these two regions in IntA-5s rats

(d; r^2 = 0.94, p = 0.001), but not LgA-90s (e) and Sal-Ctrl (f) rats. *Sal-Ctrl*, saline control animals. *IntA*, intermittent access. *LgA*, long access. *s*, seconds. CPu, caudate-putamen. *VLO*, ventrolateral orbitofrontal cortex. *LO*, lateral orbitofrontal cortex. *PrL*, prelimbic cortex.

Supplementary Figures

Supplementary figure 1. Animals whose brains were used in the *in situ* hybridization procedure for c-*fos* mRNA expression did not differ in cocaine intake or locomotor activity on their final test session. Rats were limited to a maximum of 10 cocaine infusions (0.25 mg/kg/infusion) delivered over the common speed of 10 s. Sessions ended once the infusion criterion was reached or after 1 hour. No statistically significant difference was detected in either cocaine intake (a), session length (b) or locomotor activity (represented as total beam breaks per minute; c) between the two cocaine-taking groups. The pattern of cocaine intake as a function of time from each rat is shown in d (IntA-5s) and e (LgA-90s). All values are mean ± SEM. n = 6-10 rats/group. *IntA*, intermittent access. *LgA*, long access. *min*, minutes. *s*, seconds. *Sal-Ctrl*, saline control animals. *p < 0.05 vs. Sal-Ctrl group.

Figures

Figure 1

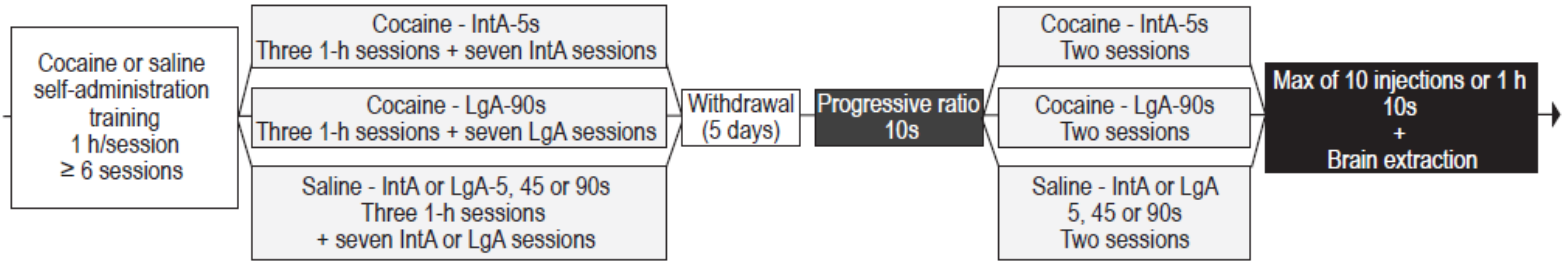


Figure 2

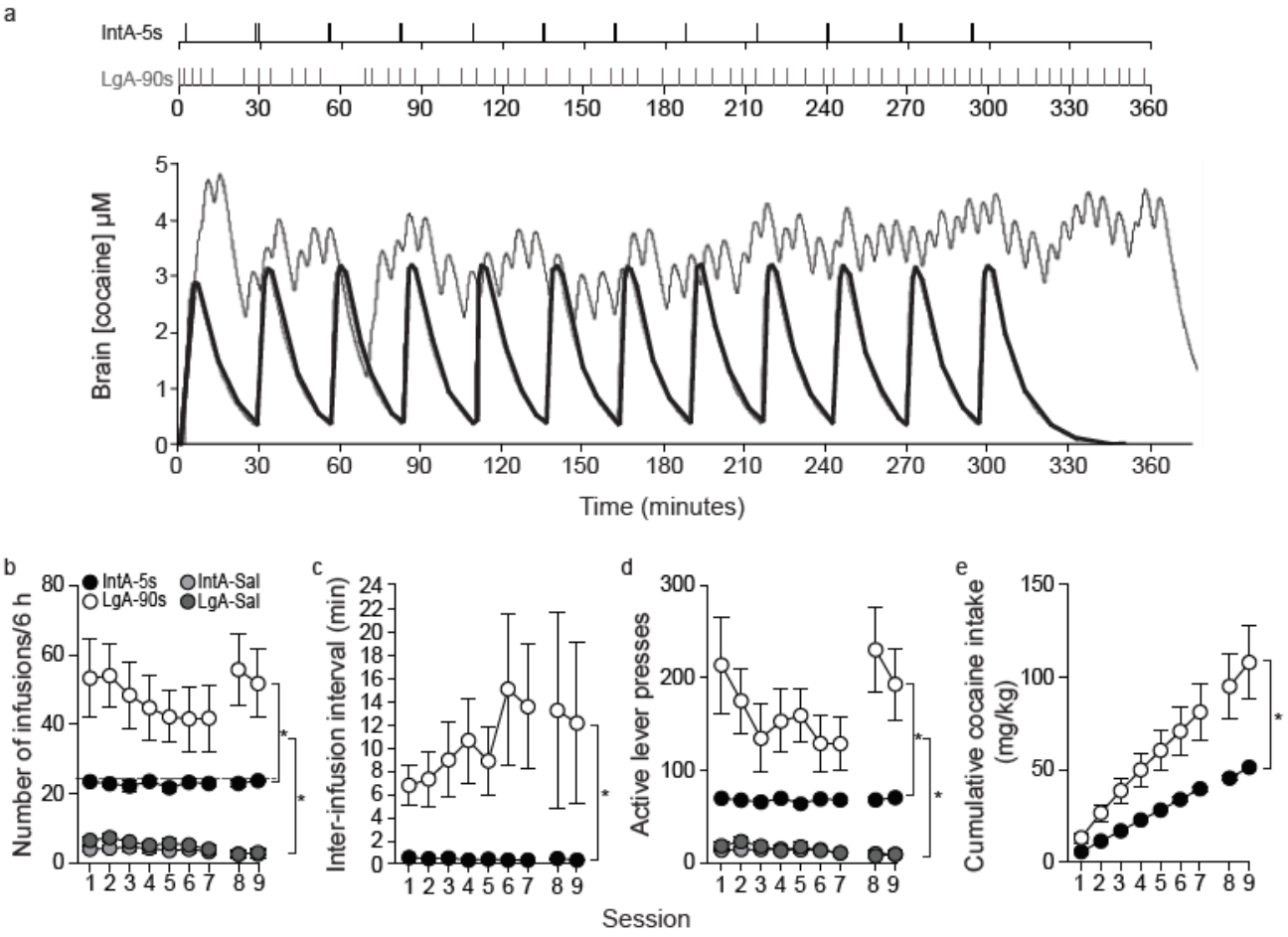

a
IntA-5s
LgA-90s
0 30 60 90 120 150 180 210 240 270 300 330 360
Brain [cocaine] µM
0 1 2 3 4 5
0 30 60 90 120 150 180 210 240 270 300 330 360
Time (minutes)
b
IntA-5s
LgA-90s
IntA-Sal
LgA-Sal
Number of infusions/6 h
0 20 40 60 80
1 2 3 4 5 6 7 8 9
c
Inter-infusion interval (min)
0 2 4 6 8 10 12 14 16 18 20 22 24
1 2 3 4 5 6 7 8 9
d
Active lever presses
0 100 200 300
1 2 3 4 5 6 7 8 9
e
Cumulative cocaine intake (mg/kg)
0 50 100 150
1 2 3 4 5 6 7 8 9
Session

Figure 3

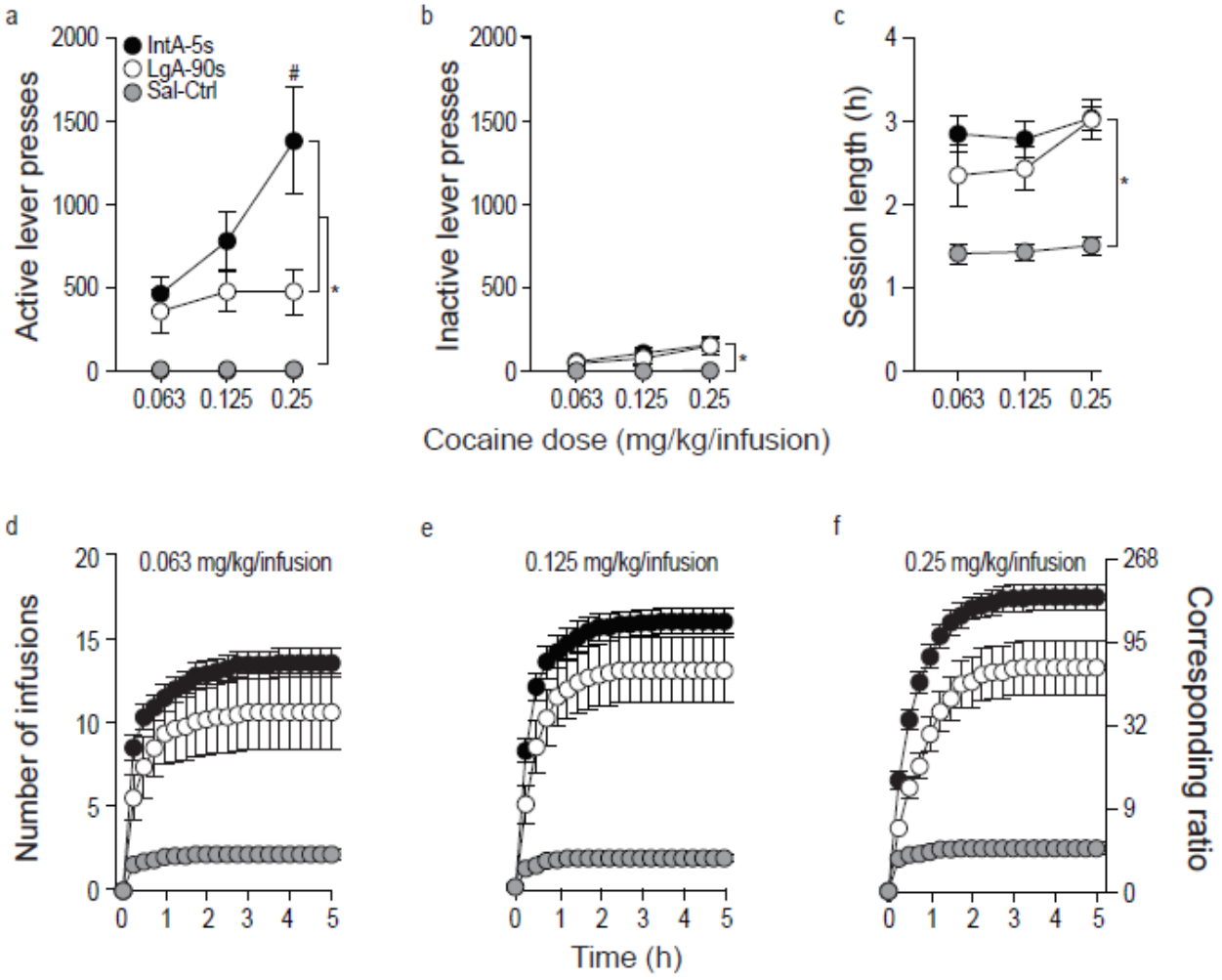

a
b
c
IntA-5s
LgA-90s
Sal-Ctrl
Active lever presses
Inactive lever presses
Session length (h)
Cocaine dose (mg/kg/infusion)
0.063
0.125
0.25
d
e
f
0.063 mg/kg/infusion
0.125 mg/kg/infusion
0.25 mg/kg/infusion
Number of infusions
Corresponding ratio
Time (h)

Figure 4

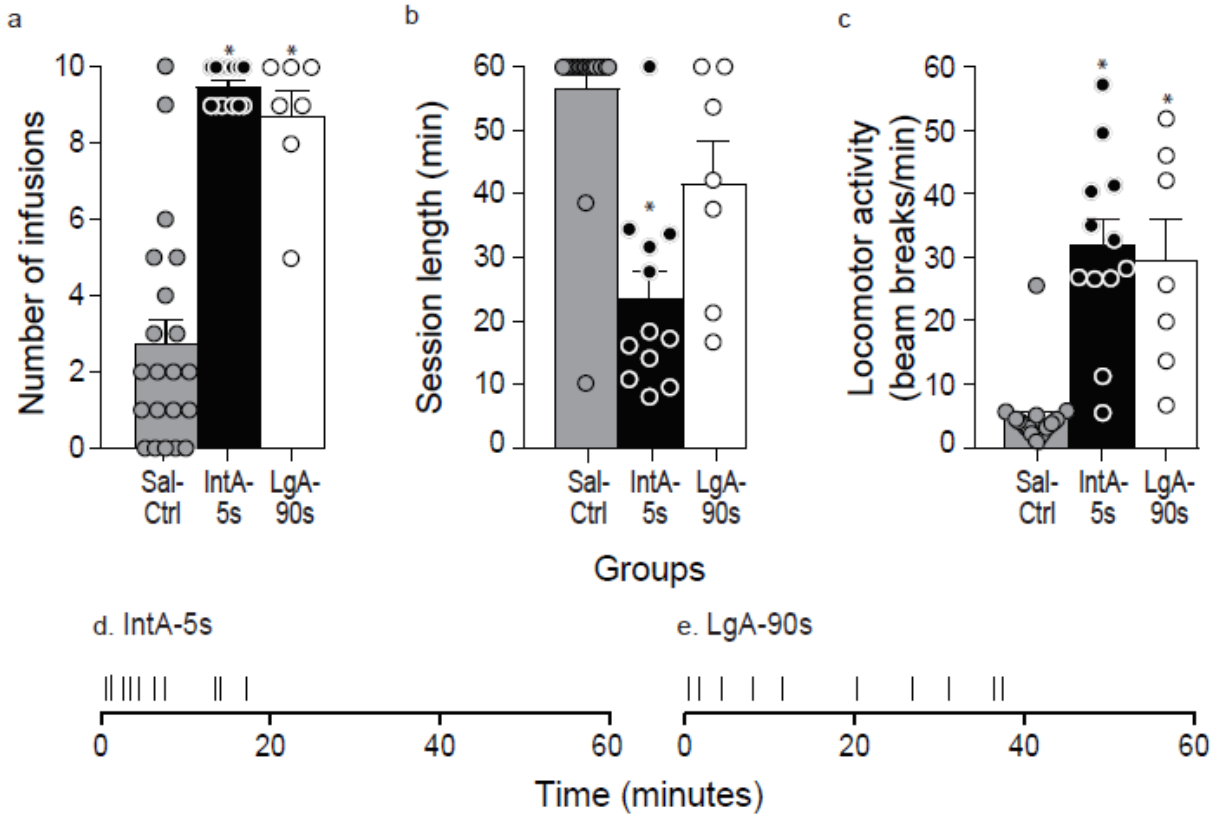

a
b
c
Number of infusions
Session length (min)
Locomotor activity (beam breaks/min)
Sal-Ctrl
IntA-5s
LgA-90s
Groups
d. IntA-5s
e. LgA-90s
Time (minutes)

Figure 5

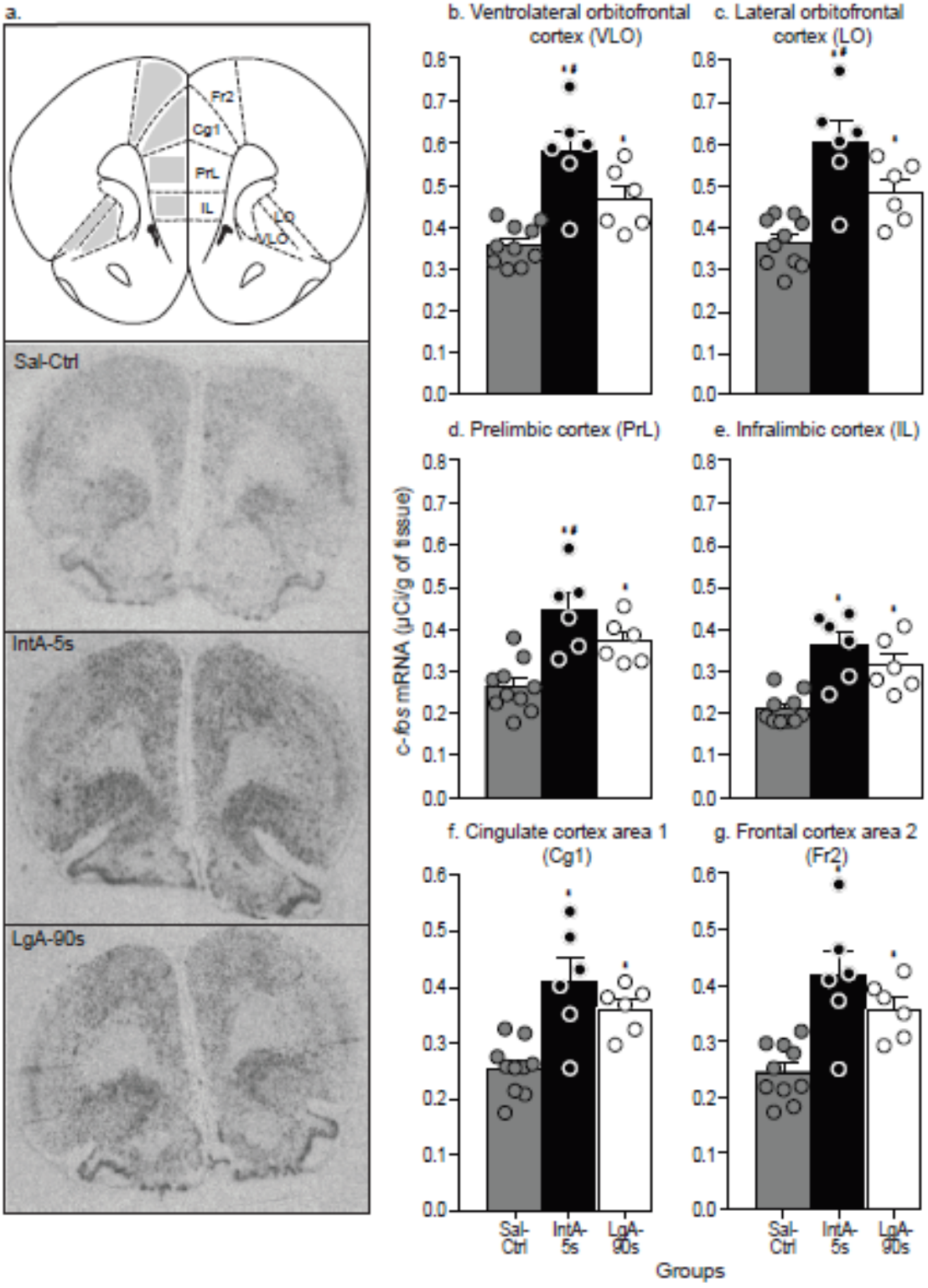

a.
Fr2
Cg1
PrL
IL
LO
VLO
Sal-Ctrl
IntA-5s
LgA-90s
b. Ventrolateral orbitofrontal cortex (VLO)
c. Lateral orbitofrontal cortex (LO)
d. Prelimbic cortex (PrL)
e. Infralimbic cortex (IL)
f. Cingulate cortex area 1 (Cg1)
g. Frontal cortex area 2 (Fr2)
c-fos mRNA (µCi/g of tissue)
Sal-Ctrl
IntA-5s
LgA-90s
Groups

Figure 6

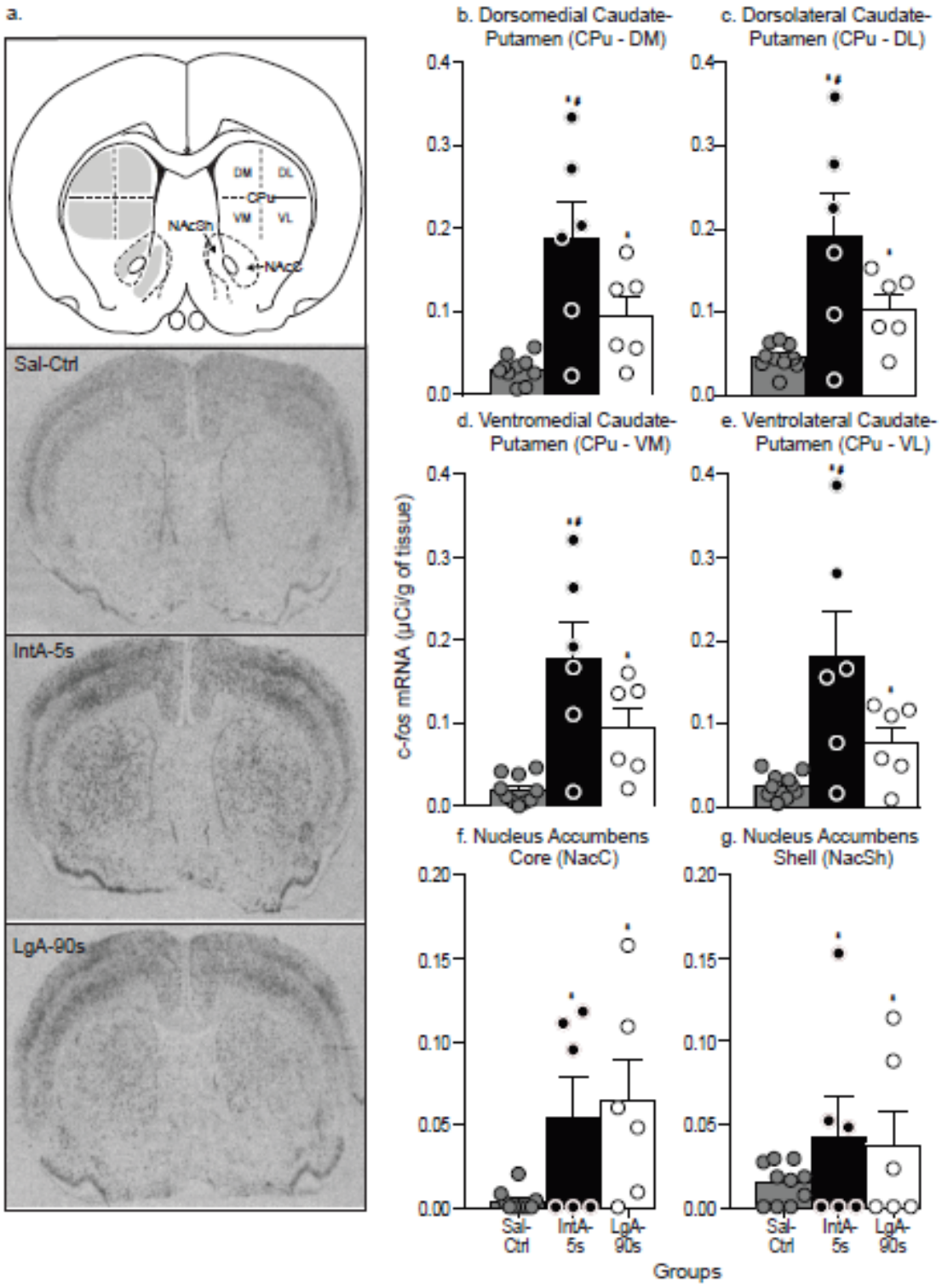

a.
DM
DL
CPu
VM
VL
NAcSh
NAcC
Sal-Ctrl
IntA-5s
LgA-90s
b. Dorsomedial Caudate-Putamen (CPu - DM)
c. Dorsolateral Caudate-Putamen (CPu - DL)
d. Ventromedial Caudate-Putamen (CPu - VM)
e. Ventrolateral Caudate-Putamen (CPu - VL)
f. Nucleus Accumbens Core (NacC)
g. Nucleus Accumbens Shell (NacSh)
c-fos mRNA (µCi/g of tissue)
0.0
0.1
0.2
0.3
0.4
0.00
0.05
0.10
0.15
0.20
Sal-Ctrl
IntA-5s
LgA-90s
Groups

Figure 7

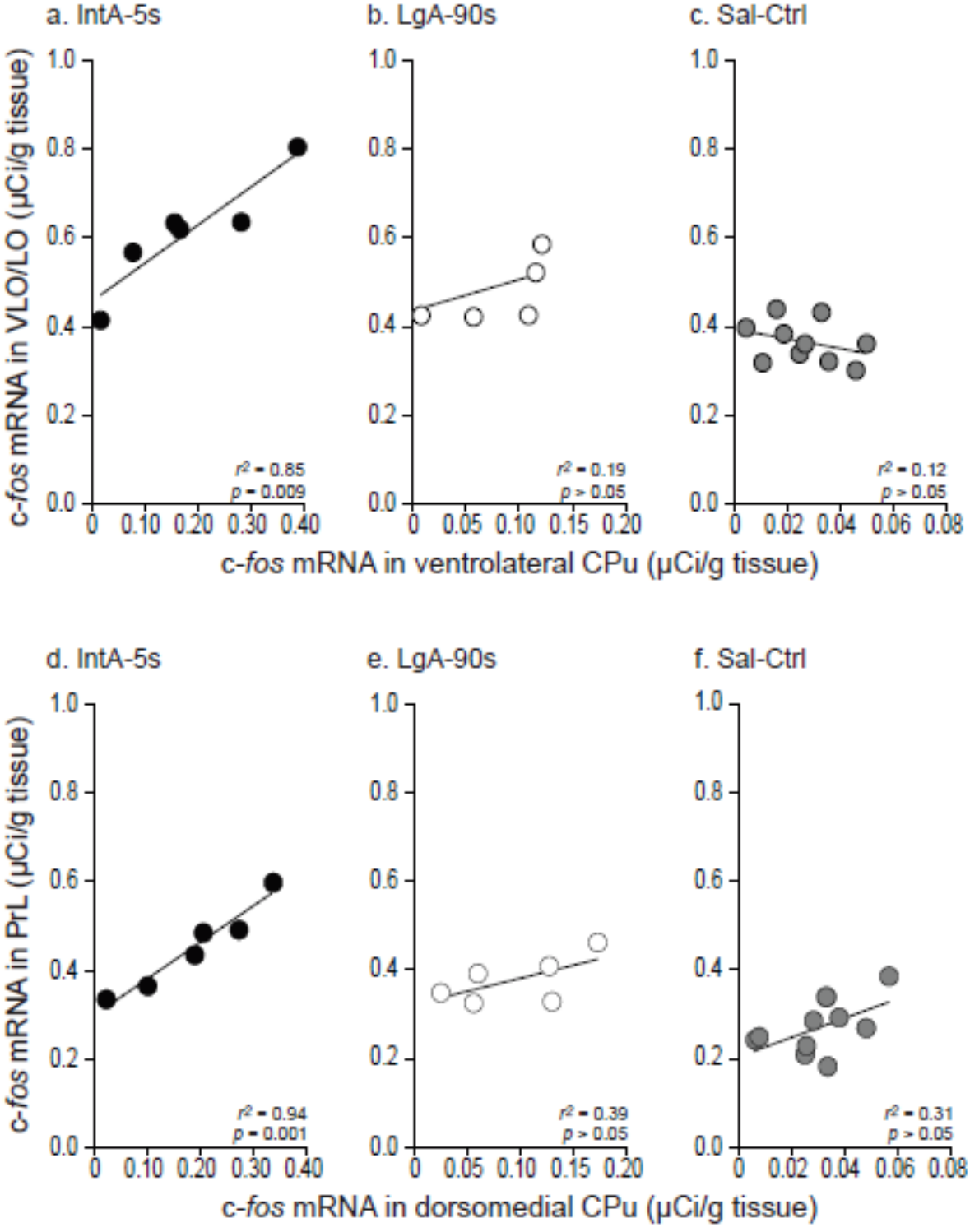
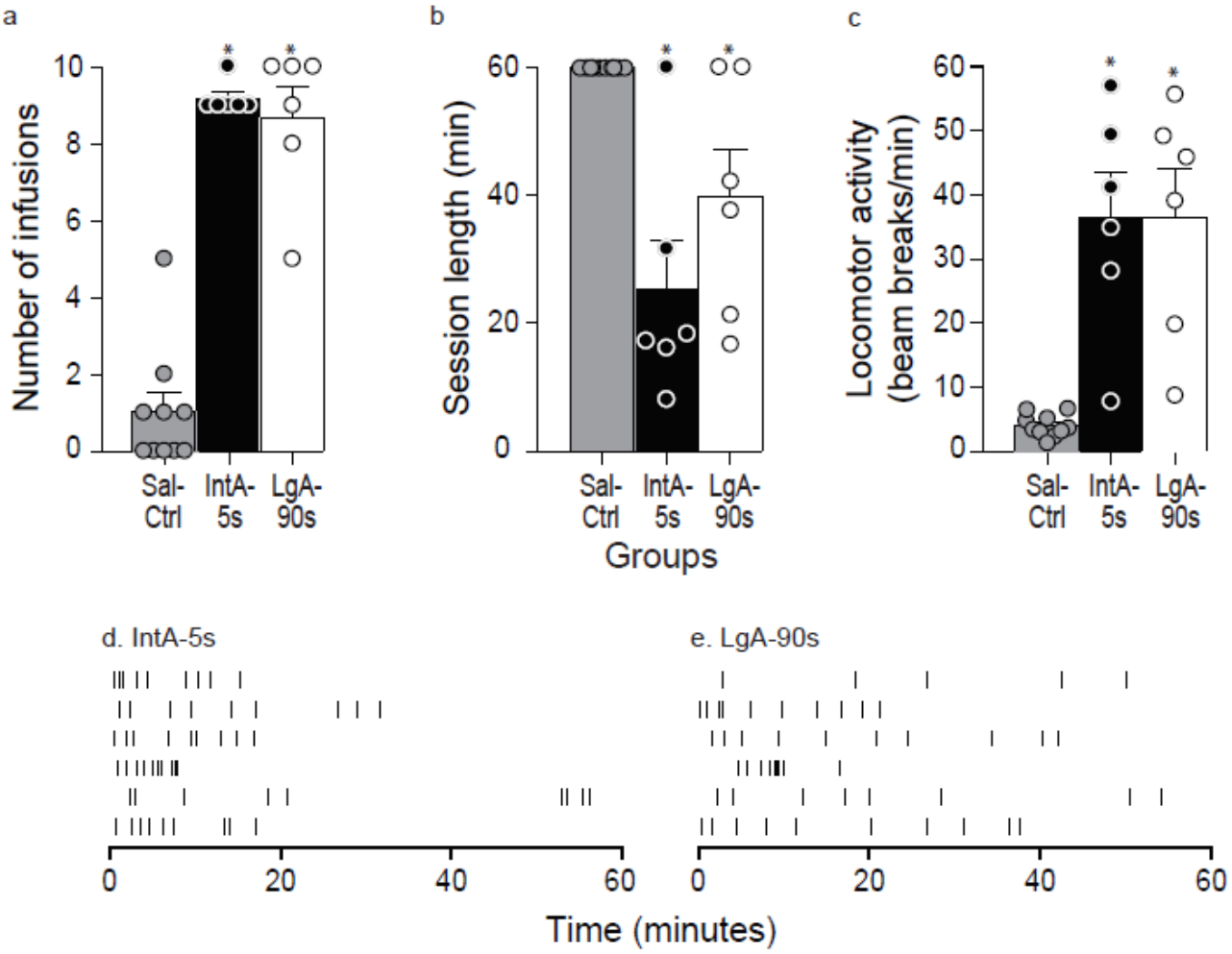

a. IntA-5s
b. LgA-90s
c. Sal-Ctrl
c-fos mRNA in VLO/LO (µCi/g tissue)
r² = 0.85
p = 0.009
r² = 0.19
p > 0.05
r² = 0.12
p > 0.05
c-fos mRNA in ventrolateral CPu (µCi/g tissue)
d. IntA-5s
e. LgA-90s
f. Sal-Ctrl
c-fos mRNA in PrL (µCi/g tissue)
r² = 0.94
p = 0.001
r² = 0.39
p > 0.05
r² = 0.31
p > 0.05
c-fos mRNA in dorsomedial CPu (µCi/g tissue)

Figure S1


a
b
c
Number of infusions
Session length (min)
Locomotor activity (beam breaks/min)
Sal-Ctrl
IntA-5s
LgA-90s
Groups
d. IntA-5s
e. LgA-90s
Time (minutes)

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CHAPITRE 10

Role of the orbitofrontal cortex and the dorsolateral striatum in the motivation for cocaine

Running title: Corticostriatal involvement in motivation for cocaine

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Manuscrit soumis

Abstract

Drug addiction involves increased incentive motivation for drug. Intermittent access to cocaine (IntA; where drug-available periods alternate with drug-unavailable periods within each session) enhances incentive motivation for the drug. The orbitofrontal cortex (OFC) and the dorsal striatum (DS) are part of a corticolimbic circuit that encodes incentive value and regulates goal-directed behaviour. We tested the hypothesis that pharmacological inactivation of the OFC, DS or both suppresses incentive motivation for cocaine after intermittent access (IntA) experience. Male Wistar rats had IntA to intravenous cocaine (0.25 mg/kg/infusion) for 10 sessions. The rats developed a 'loading' pattern of intake, taking most of their cocaine in the first minute of each drug-available period. The rats also developed psychomotor sensitization to self-administered cocaine. We then used a progressive ratio schedule of reinforcement (PR) to assess incentive motivation for cocaine. Before some PR tests, rats received microinfusions of a baclofen/muscimol cocktail (0.3 and 0.03 nmol/hemisphere, respectively, or saline) to temporarily inactivate the OFC or DS, or to bilaterally disconnect the two regions. None of these treatments changed spontaneous locomotor behaviour in cocaine-naïve rats. However, baclofen/muscimol and saline infusions both influenced cocaine self-administration behaviour. Infusing baclofen/muscimol or saline into the OFC or into the OFC and contralateral DS decreased responding for cocaine under PR, with baclofen/muscimol and saline having similar effects. Infusing baclofen/muscimol or saline into the DS also reduced responding for cocaine under PR, but baclofen/muscimol was more effective. We conclude that neuronal activity in the OFC and DS might mediate incentive motivation for cocaine.

Keywords: Cocaine, Intermittent access, Progressive ratio, Baclofen/Muscimol, Dorsal striatum, Orbitofrontal cortex.

Abbreviations**:** d, days; DS, dorsal striatum; FR, fixed ratio; h, hours; IntA, intermittent access; i.m., intramuscular; min, minutes; OFC, orbitofrontal cortex; PR, progressive ratio; s, seconds; s.c., subcutaneous.

1. Introduction

Addiction is defined by excessive incentive motivation for drug. This is linked to persistent desire for drug, craving and the investment of much time and energy to obtain the next dose, at the expense of psychosocial and professional activities [1]. In most people who take drugs, drug use remains under control with minimal clinical distress [2, 3]. In others, chronic drug use induces forms of neural plasticity that sensitize to the incentive motivational properties of drugs, and this promotes the patterns of drug use that define addiction [4, 5]. A challenge for addiction research is to identify the neurobiological mechanisms involved in excessive incentive motivation for drug.

In rats, intermittent access (IntA) to cocaine during each self-administration session produces the spiking pattern of brain cocaine concentrations thought to model the temporal dynamics of human cocaine use during a bout of consumption [6-8]. IntA to cocaine is also particularly effective in producing addiction-like symptoms in rats. This includes recurring episodes of burst-like drug use (taking many injections within a few minutes (min); [9, 10]), escalation of cocaine intake [9, 11-14], psychomotor sensitization [9, 10, 15], enhanced incentive motivation for cocaine [6, 9, 11, 14], increased willingness to take cocaine despite adverse consequences [11, 13, 14], and very robust drug- or cue-induced reinstatement of drug seeking behaviour [11, 13, 14, 16].

The influence of IntA cocaine experience on drug seeking and taking behavior has been examined, but little is known about underlying neurobiological mechanisms. The available evidence shows that IntA cocaine experience produces sensitization to cocaine-, methylphenidate- and amphetamine-induced inhibition of the dopamine transporter in

the nucleus accumbens [17-19]. This is associated with increased incentive motivation for cocaine, as measured by behavioural economics measures [20]. IntA to rapid (injected over 5 seconds) but not slower (90 seconds) intravenous injections of cocaine also increases mGlu2/3 receptor function in the prelimbic cortex, nucleus accumbens and amygdala, and this has been linked to increased incentive motivation for cocaine, as measured by responding for the drug under PR [15]. Extended abstinence from IntA to rapid, but not slower cocaine injections also increases brain-derived neurotrophic factor protein levels in the prelimbic cortex, nucleus accumbens and ventral tegmental area [16], and this could be associated with incubation of drug craving. Finally, IntA cocaine experience increases orexin cell function in the lateral hypothalamus and either administration of an orexin-1 receptor antagonist or knockdown of these orexin neurons attenuates addiction-like behaviors in IntA rats [14].

The orbitofrontal cortex (OFC) and the dorsal striatum (DS) are reciprocally connected and they modulate goal-directed behaviour. The OFC encodes the motivational value of rewards [21, 22]. Via substantial monosynaptic glutamatergic inputs, the OFC sends this information to the striatum to influence reward-directed behaviour [23, 24]. The DS is a prominent nucleus within a circuitry that mediates deeply entrenched, tenacious stimulus-response habits [25-27]. The DS becomes increasingly recruited as drug intake progresses and becomes chronic [28], and the DS mediates the acquisition and expression of difficult-to-break habits [27, 29]. Drug use can induce plasticity within this corticostriatal network, and this is thought to evoke a state where incentive motivation for drug is potentiated [5, 30-32]. The role of certain corticostriatal projections has been characterized extensively in some addiction-relevant behaviours (e.g., the role of

prefrontal cortex to nucleus accumbens projections in relapse behaviour [5, 33]). Less is known about the role of the OFC-DS pathway specifically in incentive motivation for cocaine. Furthermore, because the IntA procedure is uniquely effective in producing patterns of cocaine use relevant to addiction, there is great interest in studying the underlying brain mechanisms [34]. Here we hypothesized that neuronal activity in the OFC or DS alone, or in the OFC-DS circuit is necessary for the full expression of incentive motivation for cocaine following IntA experience. To this end, rats chronically self-administered cocaine under IntA. We then used intracerebral infusions of the GABA receptor agonists baclofen and muscimol to temporarily inactivate the OFC or DS alone, or to pharmacologically disconnect the two regions before assessing responding for cocaine under PR.

2. Materials and Methods

2.1 Animals and Housing

Nineteen male Wistar rats (Charles River Laboratories, St-Constant, QC) weighing between 400-425 g (Experiment 1; n = 4) or 225-250 g (Experiment 2; n = 15) upon arrival were individually housed in a climate-controlled colony room maintained on a reverse 12 h/12 h light/dark cycle (lights off at 8:30 am). Experiments were conducted during the dark phase of the rats' circadian cycle. Food and water were available ad libitum, except where noted otherwise. The animal care committee of the Université de Montréal approved all experiments (CDEA #16-108). Only male rats were studied here. Among human cocaine users, women can be more susceptible than men to some addiction-related effects of the

drug [35, 36]. New work also shows that IntA experience evokes greater incentive motivation for cocaine in female versus male rats, as determined using behavioural economics measures [37]. In ongoing work, we are also examining sex differences in cocaine self-administration behaviour under IntA conditions.

2.2 Drugs

Cocaine hydrochloride (Medisca Pharmaceutique Inc, St-Laurent, QC) was dissolved in 0.9% sodium chloride solution [saline; (Medical Mart, Mississauga, ON)] and filtered with a Corning bottle-top filter (0.22 μm PES membrane; Fisher Scientific, Whitby, ON). To temporarily inactivate the OFC and/or DS, we microinfused a baclofen/muscimol cocktail (GABA-A and GABA-B receptor agonists, respectively; 0.03 nmol/side for muscimol and 0.3 nmol/side for baclofen; Sigma-Aldrich, Oakville, ON. Drugs were dissolved in 0.9% sodium chloride solution). Infusing baclofen/muscimol into the brain produces rapid and reversible effects. Via the inhibitory effects of GABA receptor activity, baclofen/muscimol inactivate neuronal cell bodies while sparing fibers of passage [38-40]. The muscimol and baclofen concentrations are based on previous reports using similar behavioural paradigms in rats [41-43].

2.3 Apparatus

We measured spontaneous locomotion in Plexiglas chambers (27 x 48 x 20 cm) located in a testing room separate from the rats' housing room. Each chamber was equipped with a row of 6 infrared photocells aligned horizontally at the bottom of each

cage. Beam lengths (number of times a rat crossed the length of the chamber) were used as an index of locomotor activity and were computed over 5-min intervals.

Rats self-administered cocaine in standard operant chambers (31.8 x 25.4 x 26.7 cm; Med Associates, St-Albans, VT) located in a testing room separate from the rats' housing room, as described in Samaha, Minogianis and Nachar [44]. Each chamber was placed within a light and sound attenuating cabinet equipped with a ventilation fan that also masked external noise. Cocaine was delivered over 5-10 s, at a rate of 30.16 µl/s using 3.33-RPM syringe pumps. Each chamber contained two retractable levers and 4 horizontally-aligned infrared photocells (Med Associates, St-Albans, VT) to measure locomotor activity during cocaine self-administration sessions. A computer running Med Associates Med-PC version IV software (Med Associates, St-Albans, VT) controlled testing parameters in the operant chambers and collected data.

2.4 Surgeries

Implantation of cannulae into the OFC and the DS. We targeted the lateral and ventrolateral subareas of the OFC because in unpublished work, we found that IntA experience enhances cocaine-induced c-fos mRNA expression in these regions, but not in the medial or dorsolateral OFC [45]. We targeted the centrolateral DS because it is most strongly innervated by the lateral and ventrolateral OFC [46-48]. Finally,OFC projections to the DS are mainly ipsilateral [46, 48]. Therefore, to temporarily disconnect the OFC-DS circuit in both hemispheres, we injected baclofen/muscimol into the OFC of one hemisphere and into the DS of the other hemisphere. Rats in experiment 1 were used

to determine the effects of baclofen/muscimol microinfusions on spontaneous locomotor activity in otherwise naïve rats. Seven days after arrival, rats were anesthetised with isoflurane (5% for induction, 2-3% for maintenance) and placed in a stereotaxic apparatus. Animals were implanted with bilateral guide cannulae (22 GA, model C313G, Plastics One, HRS Scientific, Montreal, Canada) 2 mm dorsal to the OFC (relative to Bregma; A/P + 3.7, M/L +/- 2.7 and D/V - 3.2 mm, with a 10º M/L angle) and 2 mm dorsal to the centrolateral DS (relative to Bregma; A/P - 1.2, M/L +/- 3.7 and D/V - 5.6 mm, with a 10º A/P angle). Four stainless steel screws were anchored into the skull and cannulae were fixed in place with dental cement. Cannulae were sealed with obturators (model C313DC, Plastics One). At the time of surgery, rats were injected with 5 mg/kg Carprofen (s.c.; Rimadyl; 50 mg/ml; CDMV, Saint-Hyacinthe, QC) and 0.02 ml of a penicillin G procaine solution (i.m.; Procillin; 300 000 IU/ml; CDMV, Saint-Hyacinthe, QC). Animals were given 7 days to recover from surgery before behavioural testing. Rats in Experiment 2 were used to study the effects of OFC or DS inactivation, and bilateral OFC-DS disruption on incentive motivation for cocaine. The rats were smaller than those used in Experiment 1 and the stereotaxic coordinates were adjusted in consequence (OFC; A/P + 3.8, ML +/- 3.0, and D/V - 3.2 mm, with a 10º M/L angle; DS (A/P - 1.3, ML +/- 3.9 and D/V - 5.7 mm, with a 10º A/P angle; all coordinates are relative to Bregma).

Implantation of intrajugular catheters. Rats were implanted with a homemade catheter in the right jugular vein, as described in Samaha, Minogianis and Nachar [44]. During surgery, animals received a subcutaneous (s.c.) injection of 5 mg/kg Carprofen (Rimadyl; 50 mg/ml; CDMV, Saint-Hyacinthe, QC) and an intramuscular (i.m.) injection of

0.02 ml of a penicillin G procaine solution (Procillin; 300 000 IU/ml; CDMV, Saint-Hyacinthe, QC). From this point on, catheters were flushed on alternate days with either 0.1 ml physiological saline or a solution containing 0.2 mg/ml Heparin (Sigma-Aldrich, Oakville, ON) and 2 mg/ml Baytril (CDMV, St-Hyacinthe, QC). Rats were given seven days to recover before cocaine self-administration began. Following surgery, animals received 25 g of food/day for the remainder of the experiment.

2.5 Intracerebral infusions

Injectors (28 GA, model C313I; HRS Scientific, Anjou, QC) were inserted to lie 2 mm below the tips of the guide cannulae. Using a microsyringe pump (Harvard PhD, Harvard Apparatus, Saint-Laurent, Canada), rats were injected with either baclofen/muscimol or saline (0.5 µL/hemisphere for the OFC and 2 µL/hemisphere for the DS, 0.5 µL/min with the injectors left in place for an additional minute for diffusion). Saline is routinely used as the control condition in in vivo intracerebral inactivation studies using baclofen/muscimol [41, 42, 49-51].

2.6 Experiment 1: Effects of bilateral OFC-DS disruption, and OFC or DS inactivation on locomotor activity

Figure 1a illustrates the sequence of experimental events. Following cannulae implantation and recovery, we determined whether microinfusions influenced spontaneous locomotor behaviour. If so, this could confound interpretation of results in Experiment 2, where we assessed the effects of brain microinfusions on operant

responding for cocaine under PR. Locomotor activity sessions [2 hours (h)] were given 2 days apart, in a within-subjects design. To limit potential brain tissue damage, each rat received a maximum of 4 microinfusions in each structure. The rats received 5 locomotor activity test sessions (Figure 1a). In the 1st session, we measured baseline levels of locomotor activity (i.e., no microinfusion). In the 2nd session, we measured locomotion after bilateral saline microinfusions into both the OFC and the DS. In the 3rd session, we measured locomotion after pharmacologically disconnecting the OFC-DS circuit in both hemispheres. This was done by microinfusing baclofen/muscimol into the OFC of one hemisphere, and the DS of the other hemisphere. In the 4th session, we assessed locomotion after bilateral baclofen/muscimol infusions into the OFC or the DS. In the 5th and final session, we measured locomotion following bilateral saline microinfusions into both the OFC and the DS.

2.7 Experiment 2: Effects of bilateral OFC-DS disruption, and OFC or DS inactivation on responding for cocaine under a progressive ratio schedule of reinforcement

Figure 1b illustrates the sequence of experimental events. Following 7 days of acclimatization to the colony room, animals were trained to lever-press for banana-flavoured food pellets (45-mg, grain-based; VWR, Town of Mount-Royal, QC) on a fixed ratio 1 schedule of reinforcement (FR1) over daily 1-h sessions. This was to facilitate acquisition of lever-pressing behaviour. Sessions began with the illumination of the house light and the insertion of two levers into the operant cage. Pressing the inactive lever was computed but had no programmed consequences. Pressing the active lever produced one food pellet under FR1, followed by a 45-s timeout period during which levers were

retracted and the light above the active lever was turned on. Once animals learned this task (self-administration of > 20 food pellets/session, and pressing twice more on the active vs. inactive lever, over two consecutive sessions), the timeout period was increased to 65 s. Next, the schedule of reinforcement was increased to 3 (FR3) to enhance active versus inactive lever discrimination. During the food training phase, the rats received 15 g of standard laboratory chow (Purina Chow, Charles River, St-Constant, QC) in their home cages, at least 1 h after each food self-administration session. Once the rats learned the food self-administration task, they received 35 g of food per day until catheter implantation.

2.7.1 Cocaine self-administration training. Rats were trained to self-administer cocaine (0.25 mg/kg/infusion delivered over 5 s) during daily 1-h sessions under FR3, first with a 65-s timeout period, then with an 85-s timeout period. During the timeout, levers were retracted and the light above the active lever was turned on. Inactive lever-presses had no programmed consequences. Taking ≥ 5 infusions/session, at a regular pace over the session and for 2 consecutive sessions indicated that cocaine self-administration behaviour was acquired. Finally, for 3 additional 1-h sessions, the timeout period was removed.

2.7.2 Cocaine self-administration under intermittent access (IntA). Following the training phase above, the rats were given 10 IntA sessions, 1 session/day. Each IntA session had twelve, 6-min drug available periods separated by 26-min no-drug available

periods during which levers were retracted [6, 7]. During each 6-min drug period, the rats could take up to 2 infusions, with no timeout between infusions, as described in [9, 15]. Under IntA conditions, limiting or not limiting cocaine intake during each drug available period produces the same levels of responding for cocaine under PR, across a range of cocaine doses [9]. Thus, the animals could take a maximum of 24 infusions per IntA-session. Once the two infusions were self-administered or the 6-min drug period had elapsed, a 26-min no-drug period was initiated. Session duration was determined by how quickly the rats took their allotted injections or was a maximum of 6 h. After 10 IntA sessions, the rats remained in their home cages for 5 days before PR testing.

2.7.3 Cocaine self-administration under a progressive ratio schedule of reinforcement (PR). Incentive motivation for cocaine was assessed by determining the number of infusions self-administered under PR (0.063, 0.125 and 0.25 mg/kg/infusion, delivered over 10 s, in counterbalanced order, 1-2 sessions per dose; the 0.063 mg/kg/infusion PR session is also referred to as '**Pre-surgery session**' below). Cocaine was injected over 10 s during PR test sessions to allow comparison with previous IntA studies [15, 45]. The number of lever presses necessary to obtain each consecutive drug infusion increased exponentially, according to the formula $(5\ e^{(\text{injection number} \times 0.2)} - 5)$, as described by [52]. The PR session ended once 1 h had elapsed since the last cocaine infusion or after 5 h. Thus, shorter PR sessions indicate that responding for cocaine ceased sooner, and this suggests reduced incentive motivation for the drug. As the primary index of incentive motivation for the drug, we used the number of cocaine infusions taken prior to the end of the session. Following the final PR session, catheter

patency was verified by intravenous injection of Propofol (10 mg/mL; 0.1 mL/rat; CDMV, St-Hyacinthe, QC), a short-acting anaesthetic. One rat failed to become ataxic within 5 s of the injection and was excluded from final data analysis.

2.7.4 Brain microinfusions and PR testing. After establishing baseline levels of PR responding for cocaine (0.063, 0.125 and 0.25 mg/kg/infusion), the rats were implanted with cannulae targeting the OFC and the DS. Seven days later, rats were habituated to the microinfusion procedure by receiving bilateral saline infusions into the OFC and then into the DS. On the next day, we again assessed responding for cocaine (0.063 mg/kg/infusion) under PR, without microinfusions (referred to as '**Post-surgery session**' below). We used 0.063 mg/kg/infusion cocaine for all baseline and experimental PR sessions. This is because cocaine-experienced animals reach breakpoints for this dose in ~ 3 h [9, 45], and the behavioural effects of baclofen/muscimol microinfusions last at least 2 h [38-40, 53]. This ensures that in the present study, baclofen/muscimol would be pharmacologically active during the PR test sessions. To assess the effects of bilateral OFC-DS disruption on incentive motivation for cocaine, rats were injected with baclofen/muscimol or saline into the OFC of one hemisphere and the DS of the other hemisphere immediately before cocaine self-administration under PR. To assess the effects of OFC or DS inactivation on incentive motivation for cocaine, rats received bilateral baclofen/muscimol or saline microinfusions into either the OFC or the DS immediately before PR testing. Each rat was tested with saline and baclofen/muscimol, 1 session/day every 2 days, in counter-balanced order. Each rat received a total of 4 microinfusions/region. Finally, we determined whether repeated microinfusions had long-

lasting effects on responding for cocaine. To this end, after all test sessions, we gave rats a final PR session with no microinfusions (referred to as '**Final session**' below).

2.8 Histology

At the end of Experiment 1, rats were decapitated, brains were extracted, quickly frozen in cold isopentane (- 50°C) and stored at - 80°C. Following the final test session in Experiment 2, catheter patency was verified by i.v. infusion of Propofol (10 mg/mL; 0.1 mL/rat; CDMV, St-Hyacinthe, QC) and then we proceeded with the brain extraction. Injector tip placement was estimated on 30 µm-coronal slices using the Paxinos and Watson [54] rat brain atlas. All animals in Experiment 1 had their cannulae in the targeted brain regions and were included in the results. In Experiment 2, one rat had injector tips outside of the targeted brain regions and was excluded from final data analysis.

2.9 Statistics

Data was analyzed with Prism 7 (GraphPad Software Inc., La Jolla, CA) and IBM SPSS Statistics version 25 software. The effects of baclofen/muscimol or saline microinfusions on locomotor activity were analyzed using two-way repeated-measures analysis of variance [ANOVA; Treatment x Session Time (min), both as within-subjects variables]. Self-administration behaviour (infusions and inter-infusion interval) were analyzed with a Friedman's test followed by Dunn's multiple comparisons tests. Lever presses, self-administered infusions and locomotor activity during IntA sessions were analyzed with two-way repeated measures ANOVA (with 'Session' or 'Time (min) as

within-subjects variables). During PR sessions, locomotor activity was computed as average photocell beam breaks over 60 min. This is because PR session length can vary between rats. During PR sessions, the effects of cocaine Dose on Self-administered Infusions or Session Duration were analyzed with one-way repeated-measures ANOVA, and Dose x Lever type effects (both as within-subjects variables) were analyzed with two-way repeated measures ANOVA. Self-administration behaviour during PR sessions given pre- versus post-surgery versus during the Final PR session was analyzed with one-way repeated measures ANOVA, and effects on lever presses and locomotor activity were analysed with a Friedman's test (all variables were within-subjects). The effects of baclofen/muscimol or saline microinfusions on self-administration and locomotor behaviour during PR sessions were analyzed with one-way repeated measures ANOVA followed by Tukey's multiple comparisons tests or with a Friedman test, followed by Dunn's multiple comparisons tests when data did not follow a normal distribution. All data in the figures are mean ± standard error of the mean.

3. Results

3.1 Estimated cannulae placements

Figure 2 shows estimated location of injector tips in the OFC (a) and DS (b). For Experiment 1, injector tips terminated in the ventrolateral and lateral aspects of the OFC between + 3.7 and + 3.2 mm relative to Bregma and in the centrolateral DS between + 0.48 and – 0.26 mm relative to Bregma. For Experiment 2, injector tips terminated in the

ventrolateral and lateral aspects of the OFC between + 4.7 and + 3.2 mm relative to Bregma and in the centrolateral DS between + 0.7 and – 0.4 mm relative to Bregma.

3.2 Experiment 1

3.2.1 Microinfusions of saline or baclofen/muscimol do not change spontaneous locomotor activity

Figure 3 shows locomotor activity under baseline conditions and after baclofen/muscimol or saline microinfusions. Saline was microinfused on 2 test sessions, given before and after baclofen/muscimol test sessions. There were no differences between the two saline sessions (main effect of Saline Treatment Session, $F(1,3) = 2.14$, $p = 0.24$) and they were pooled together in Figure 3. Locomotor activity decreased as a function of time under all conditions (main effect of Time; Figure 3a, $F(23,207) = 15.9$; Figure 3b, $F(23,69) = 11.82$; Figure 3c, $F(23,69) = 12.91$, all P's < 0.0001). This suggests that exploratory behaviour progressively decreased as the rats habituated to the test cages. Microinfusing saline or baclofen/muscimol into the OFC and/or DS did not change locomotor behaviour compared to baseline levels, and locomotor counts were also similar following saline or baclofen/muscimol microinfusions (main effect of Treatment; Figure 3a, $F(2,9) = 2.69$, $p = 0.12$; Figure 3b, $F(2,3) = 0.20$, $p = 0.83$; Figure 3c, $F(2,3) = 1.78$, $p = 0.31$). In summary, although a small number of rats was evaluated ($n = 4$), at the concentrations tested (0.3 nmol baclofen and 0.03 nmol muscimol), baclofen/muscimol microinfusions into the OFC and/or the DS did not change spontaneous locomotion. This suggests that the treatments had no significant motor side effects in otherwise naïve rats.

3.3 Experiment 2

3.3.1 IntA cocaine self-administration promotes a drug 'loading' pattern of intake, psychomotor sensitization and dose-dependent responding for the drug under PR

Figure 4 shows cocaine self-administration and locomotor behaviour during IntA sessions. The rats were limited to 24 infusions/session, as indicated by the dotted line in Figure 4a. The rats took an average of 20 injections/session (± 1.5 SEM), and this did not change over sessions (Figure 4a; $\chi^2 = 4.54$, $p = 0.87$). Figure 4b shows average inter-infusion interval during the 10 sessions, defined as the time elapsed between the end of one infusion and the beginning of the next. Over the 10 sessions, the rats took one injection on average every 46 s (± 12 SEM), and this did not significantly change over sessions ($\chi^2 = 15.66$, $p = 0.07$). The animals pressed significantly more on the active versus inactive lever (Figure 4c; $F(1,26) = 130.1$, $p < 0.0001$) and lever-pressing behaviour was stable over sessions ($F(9,234) = 0.47$, $p = 0.89$). Prior work shows that IntA rats take most of their injections in the first 60-90 s of each drug-available period, and that this 'loading' effect sensitizes over sessions [9, 10, 37]. To assess this here, Figure 4d shows the number of infusions taken during each minute of the 6-min cocaine-available period, for sessions 1, 5 and 10. The rats took most of their cocaine infusions in the first minute of each drug-available period (main effect of 60-s Bin; $F(5,65) = 60.46$, $p < 0.0001$). There was a tendency for this 'loading' effect to sensitize over sessions ($p = 0.12$). We also measured locomotor activity during each IntA session. Figure 4e represents the time course of locomotor activity averaged over the twelve, 26-min no cocaine-available periods during the 1st and 10th IntA session. We analysed locomotion

during these periods because they consistently last 26 minutes across sessions and across rats and because lever-pressing behaviour does not confound locomotor activity during these periods. In contrast, the drug-available periods can be of different lengths across rats, as these periods end when each rat takes its 2 allotted infusions. Cocaine-induced locomotor activity decreased over the 26-min no cocaine-available periods. This supports the idea that IntA achieves peaks and troughs in brain cocaine concentrations during each self-administration session [6, 7, 10, 15]. Even though rats consumed similar amounts of cocaine across IntA sessions, cocaine-induced locomotor activity was greater on the 10^{th} than on the 1^{st} session (Figure 4e, Time x Session interaction effect; $F(25,300) = 10.68$, $p < 0.0001$), specifically during the first 7 minutes of the no drug-available periods ($p < 0.05$). This suggests that the rats developed psychomotor sensitization to cocaine (see also [15]).

Figure 5 shows responding for cocaine (0.063-0.25 mg/kg/infusion) under PR. The rats worked harder for higher cocaine doses, taking more infusions at 0.25 than at 0.063 mg/kg/infusion (Figure 5a; $F(1.37, 17.75) = 4.83$, $p < 0.05$). Total active lever presses/PR session were also highest at 0.25 mg/kg/infusion cocaine (Figure 5b; main effect of Lever Type; $F(1,13) = 29.94$, $p = 0.0002$; main effect of Dose; $F(2,26) = 6.27$; 0.063 vs. 0.25 mg/kg/infusion, $p = 0.01$; 0.125 vs. 0.25 mg/kg/infusion, $p = 0.005$). There was no significant effect of cocaine dose on inactive lever presses (Figure 5b; $p > 0.05$). PR sessions also lasted longest at the highest cocaine dose (Figure 5c; $F(1.74,22.66) = 9.07$; 0.063 vs. 0.25 mg/kg/infusion: $p = 0.006$; 0.125 vs. 0.25 mg/kg/infusion: $p = 0.004$). Thus, incentive motivation for cocaine increased with dose.

3.3.2 Intracranial cannulae implantation and repeated microinfusions have no long-term effects on responding for cocaine under PR

Following the baseline PR sessions above, we assessed the effects of microinfusing saline or baclofen/muscimol into the OFC and DS on responding for cocaine (0.063 mg/kg/infusion) under PR. Figure 6 shows cocaine (0.063 mg/kg/infusion) self-administration behaviour and locomotor activity during PR sessions given 1-5 days before (pre-surgery) and 8 days after (post-surgery) intracranial cannulae implantation, as well as 2 days after the last brain microinfusion (Final session). The number of self-administered infusions (Figure 6a; $F(2,24) = 1.71$, $p = 0.20$) and lever pressing behaviour (Figure 6b; active lever, $p = 0.79$; inactive lever, $p = 0.58$) did not significantly change across PR sessions. Figure 6b shows that during each PR session, the rats pressed more on the active versus inactive lever (Pre-surgery, $t_{12} = 4.37$, $p = 0.001$; Post-surgery, $t_{12} = 3.21$, $p = 0.008$; Final session, $t_{12} = 4.87$, $p = 0.0004$). This indicates that lever discrimination remained stable after intracranial manipulations. Figure 6c shows that locomotor activity during PR sessions also did not significantly change across sessions ($p = 0.29$). In summary, neither the passage of time nor intracranial surgery and microinfusions significantly changed baseline levels of responding for cocaine or cocaine-induced locomotor activity under PR. The three PR sessions shown in Figure 6 were therefore averaged as an index of baseline levels of incentive motivation for cocaine in the analyses below (‘Basal’; Figures 7-9).

3.3.3 Infusing baclofen/muscimol or saline into the OFC and the contralateral DS decreases responding for cocaine under PR

We assessed the effects of bilaterally disconnecting the OFC-DS circuit on responding for cocaine (0.063 mg/kg/infusion) under PR. This was achieved by microinfusing baclofen/muscimol (or saline) into one OFC and into the contralateral DS immediately before PR testing. We were surprised to observe that intracerebral infusions of saline disrupted cocaine self-administration behaviour under PR test conditions. Figure 7 shows that compared to baseline (i.e., no microinfusion), microinfusing baclofen/muscimol or saline reduced both the number of self-administered cocaine infusions and pressing on the active lever (Figure 7a; $F(2,22) = 12.93$; Figure 7b; $\chi^2 = 13.17$; All P's ≤ 0.02). There were also effects that were specific to baclofen/muscimol. Figure 7 shows that compared to baseline, baclofen/muscimol microinfusions decreased inactive lever presses, increased the inter-infusion interval, and reduced locomotor activity during PR testing (Figure 7c; $\chi^2 = 11.02$; Figure 7d; $\chi^2 = 6.0$; Figure 7f; $\chi^2 = 12.17$, All P's ≤ 0.05). No other comparisons were statistically significant.

Thus, following IntA cocaine experience, microinfusing baclofen/muscimol or saline into one OFC and into the contralateral DS suppressed responding for cocaine under PR, and baclofen/muscimol also attenuated both inactive lever presses and cocaine-induced locomotion (Figure 7).

3.3.4 Infusing baclofen/muscimol or saline into the OFC decreases responding for cocaine under PR

We also assessed the effects of bilateral microinfusions of saline or baclofen/muscimol into the OFC on responding for cocaine (0.063 mg/kg/infusion) under PR. Figure 8 shows that compared to baseline, microinfusing saline or baclofen/muscimol into the OFC reduced the number of self-administered cocaine infusions, active lever presses and locomotor activity during PR testing (8a; $F(2,22) = 12.93$; Figure 8b; $F(1.24, 13.67) = 22.26$; Figure 8f; $\chi^2 = 13.17$; All P's ≤ 0.02). Compared to baseline or saline microinfusions, baclofen/muscimol microinfusions also significantly suppressed inactive lever presses (Figure 8c; $\chi^2 = 8.0$, baclofen/muscimol $<$ Basal and Saline; All P's ≤ 0.04). Saline also significantly increased the inter-infusion interval compared to baseline levels (Figure 8d; $F(2,22) = 4.34$; Saline vs. Basal; All P's ≤ 0.03). No other comparisons were statistically significant.

In summary, following IntA cocaine experience, bilateral microinfusions of baclofen/muscimol or saline into the OFC suppressed both responding for cocaine and cocaine-induced locomotion during PR testing (Figure 8).

3.3.5 Infusing baclofen/muscimol or saline into the DS decreases responding for cocaine under PR, but baclofen/muscimol infusions are more effective

Finally, we assessed the effects of bilateral saline or baclofen/muscimol microinfusions into the DS on responding for cocaine (0.063 mg/kg/infusion) under PR. Figure 9 shows that compared to baseline, intra-DS microinfusions of saline or baclofen/muscimol

reduced the number of self-administered cocaine infusions, active lever presses and locomotion during PR testing (Figure 9a; $F(1.34, 14.78) = 23.46$; Figure 9b; $\chi^2 = 18.50$; Figure 9f; $\chi^2 = 17.17$; All P's ≤ 0.04). However, baclofen/muscimol microinfusions were more effective than saline in reducing cocaine intake (Figure 9a; $p = 0.04$). Consequently, PR sessions were shortest following baclofen/muscimol microinfusions (Figure 9e; $\chi^2 = 10.50$; All P's ≤ 0.04). Baclofen/muscimol microinfusions also decreased inactive lever presses compared to baseline (Figure 9c; $\chi^2 = 13.06$; vs Basal; All P's ≤ 0.002). No other comparisons were statistically significant.

Thus, following IntA cocaine experience, bilateral microinfusions of baclofen/muscimol or saline into the DS suppressed both cocaine-induced locomotion and responding for cocaine under PR (Figure 9). Notably, DS inactivation with baclofen/muscimol was more effective than intra-DS saline in reducing both cocaine intake under PR and PR session duration compared to baseline levels (Figure 9). This suggests that temporary pharmacological inactivation of the DS attenuates incentive motivation for cocaine.

4. Discussion

IntA to cocaine is thought to model the temporal kinetics with which cocaine addicts take the drug [8, 55, 56]. IntA is also especially effective in increasing incentive motivation for cocaine in rats [6, 9-11, 13-15]. The OFC and the DS are interconnected brain regions that modulate goal-directed behaviour [21-24]. Here we determined whether neuronal activity in the OFC-DS projection is necessary for the expression of incentive motivation for cocaine following IntA experience. We temporarily inactivated the OFC or DS with

bilateral microinfusions of baclofen/muscimol. We also temporarily functionally disconnected the OFC and DS in both hemispheres by microinfusing baclofen/muscimol into one OFC and into the contralateral DS. Immediately after the microinfusions, we assessed incentive motivation for cocaine by measuring responding for the drug under PR. In a first experiment we found that these treatments had no effect on spontaneous locomotion in cocaine-naïve rats. This was tested in 4 rats and more work is needed to fully assess the effects of intra-OFC and intra-DS baclofen/muscimol infusions on spontaneous motor behaviour. However, our findings concord with other studies similar to ours showing that compared to either saline microinfusions or testing with no microinfusions, baclofen/muscimol microinfusions do not significantly change spontaneous locomotion or pressing on an inactive lever, in either cocaine-experienced [41, 57, 58] or cocaine-naïve rats [41]. In a second experiment, we found that compared to baseline conditions (i.e. no microinfusions), baclofen/muscimol microinfusions into the OFC, the DS, or into one OFC and the contralateral DS reduce both responding for cocaine and locomotor activity during PR test sessions. To our surprise, saline microinfusions had similar effects. The only region where baclofen/muscimol and saline microinfusions had significantly different effects on behaviour was the DS. These findings suggest two things. First, neuronal activity in the OFC and DS might not play a major role in mediating spontaneous locomotion, but it might influence both cocaine-induced locomotion and incentive motivation for the drug following IntA experience. Second, in cocaine-experienced rats, saline microinfusions into the OFC and/or DS can attenuate both cocaine-induced locomotion and operant responding for the drug.

In the present study, IntA rats had relatively low levels of cumulative cocaine intake but they showed high levels of incentive motivation for the drug. This is consistent with prior work [6, 9-11, 13, 15]. We only had an IntA group and comparison with other studies is done with caution. However, cumulative cocaine exposure in our IntA rats was comparable to or lesser than that in Short-Access rats at similar unit doses, but our IntA rats reach much higher breakpoints for cocaine than generally seen in Short-Access rats under similar PR test conditions [59-61]. The IntA rats in the present study also achieved much higher breakpoints for cocaine than generally seen in Long-Access rats [60, 62-64] - even though Long-Access rats reach high levels of cumulative drug intake (~250-350 mg/kg, compared to 51 mg/kg ± 3.05 SEM here). In addition, IntA experience produced significant psychomotor sensitization here, in accord with previous IntA studies [10, 15]. Psychomotor sensitization is thought to reflect brain changes that also underlie sensitized drug wanting [4, 65, 66]. Thus, our findings agree with a growing literature showing that IntA is uniquely effective in producing the patterns of drug seeking and taking that contribute to the transition to addiction [6, 9-15, 45].

4.1 Effects of microinfusions into the OFC-DS circuit or into the OFC alone

Functionally disconnecting the OFC-DS circuit with baclofen/muscimol or microinfusing saline into the circuit similarly decreased responding for cocaine under PR. Injecting baclofen/muscimol or saline into the OFC alone also had similar effects. However, saline and baclofen/muscimol microinfusions also had distinguishable effects. Baclofen/muscimol infusions into the OFC-DS decreased intake, active and inactive lever presses and locomotor activity during PR testing. In contrast, saline microinfusions only

reduced the number of active lever presses and thus the number of infusions earned under PR, suggesting a milder effect on behaviour. One possibility is that the effects of the microinfusions on behaviour reflect motor impairments. At present, this cannot be ruled out. However, it is likely that simple motor impairments do not fully explain the results, since the microinfusions did not change locomotor behaviour in cocaine-naïve rats (Experiment 1). Another possibility is that saline and baclofen/muscimol microinfusions each disrupt neuronal activity, and that intact neuronal activity in the OFC-DS circuit and/or the OFC alone is required for the full expression of incentive motivation for cocaine. Evaluating the effects of microinfusions into neighbouring brain regions would provide additional information on neuroanatomical specificity. Still, in support of this hypothesis, baclofen/muscimol infusions into the OFC suppress reinstatement of cocaine- [58], alcohol- [51] and heroin-seeking behaviour [42], without affecting operant responding for food or saccharin [42, 51]. Thus, the OFC mediates the response to several drugs of abuse. Our findings extend this work using a clinically-pertinent IntA cocaine self-administration procedure. Our findings further suggest that the influence of the OFC on incentive motivation for cocaine could involve OFC-DS projections.

4.2 Effects of microinfusions into the DS

Both baclofen/muscimol and saline infusions into the DS reduced responding for cocaine under PR compared to baseline, but the effect was more pronounced with baclofen/muscimol. This suggests that intra-DS baclofen/muscimol (and to a lesser degree, intra-DS saline) could be decreasing neuronal activity in a brain region that is part of a circuit involved in incentive motivation for drug. Consistent with this, disrupting cellular

activity in the DS (pharmacologically or surgically) suppresses responding for cocaine or morphine under PR [67, 68], decreases cocaine-, cue- and context-induced reinstatement of cocaine-seeking [57, 69], and attenuates the operant pursuit of cocaine-paired cues [70]. In contrast, baclofen microinfusions into the DS do not influence lever-responding for food in drug-naïve rats [67]. Similarly, in the present study, intra-DS baclofen/muscimol infusions did not influence locomotor behaviour in cocaine-naïve rats. This suggests that intra-DS baclofen/muscimol likely does not decrease responding for cocaine under PR through simple suppression of motor behaviour. Instead, the expression of incentive motivation for cocaine might require intact neurotransmission in the DS.

4.3 Saline microinfusions into the brain influence behaviour

We were surprised to find that saline microinfusions into the OFC and/or the DS decreased responding for cocaine during PR testing. This suggests that saline could have modified neuronal activity in these brain regions. In support of this, when awake, behaving rats receive saline microinfusions into the basolateral amygdala, this reduces excitation of nucleus accumbens core neurons in response to a food-paired cue [71]. Saline is routinely used as the control condition in intracerebral inactivation studies using baclofen/muscimol [41, 42, 49-51]. Here, we also had a no-microinfusion condition, and this allowed us to detect significant effects of saline microinfusions on behaviour. As cautioned by Wakim [72], “normal" 0.9% salt solution might be neither "normal" nor “physiological”. Saline solution is acidic (pH is generally 5.5) and its constituents differ chemically, qualitatively and quantitatively from those found in extracellular fluids. This and our findings suggest that brain microinfusion studies should include a no-

microinfusion control condition. Many brain microinfusion studies do not [41, 42, 49-51]. Our results might also suggest that in brain microinfusion studies, vehicle solutions other than saline might be better to use. Other vehicles include phosphate-buffered saline (PBS; [58, 69, 73-75]) and artificial cerebrospinal fluid (aCSF; [76, 77]). Saline, PBS and aCSF are all “isotonic”, but they have very different salt compositions. Because the [Na+]:[Cl-] ratio is lower in saline than in PBS or aCSF, saline might disturb ion channel function and homeostasis ([78] see also [79]). It is also possible that microinfusing any fluid into the brain can change cellular activity through mere mechanical stimulation. This further highlights the importance of a no-microinfusion control condition to correctly interpret results.

4.4 Concluding remarks

Using a preclinical model relevant to cocaine addiction, we provide new information on the potential roles of the OFC and DS in incentive motivation for cocaine. Our findings suggest that intact neuronal activity in the OFC and DS alone, or in the OFC-DS projection is not necessary for the expression of spontaneous locomotor behaviour in otherwise naïve rats, but it is necessary for the full expression of incentive motivation for cocaine and cocaine-induced psychomotor activity following a history of chronic drug use. Saline microinfusions into the OFC and DS had similar effects. We interpret these data to suggest that both saline and baclofen/muscimol microinfusions influence neuronal activity in the OFC and DS, and that these regions are part of a network that could be critical for cocaine wanting after chronic drug use. More work is needed to further investigate the roles of the OFC, DS and their connections in incentive motivation for

cocaine. This work could involve additional cocaine doses and behavioural assays (e.g., behavioural economics measures of incentive motivation), and other brain regions for comparison. Future studies using projection-specific chemogenetic and optogenetic methods will also be informative in this context. Finally, our findings also suggest that studies examining behaviour after baclofen/muscimol microinfusions into the brain should include a no-microinfusion control condition and should also consider vehicles other than saline. These issues notwithstanding, the present findings could guide the design of future studies aimed at characterizing the cellular and molecular mechanisms in the OFC-DS projection that could mediate the expression of incentive motivation for cocaine.

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Conflicts of Interest

ANS is a scientific consultant for H. Lundbeck A/S. This had no influence on the manuscript’s content. The other authors declare no conflicts of interest.

Author contributions

EAM and ANS designed the study. EAM and AS performed the surgical procedures. EAM and MPF performed the experiments. EAM and ANS analyzed the data and interpreted the findings. EAM and ANS wrote the manuscript with input from AS. All authors critically reviewed content and approved final version for publication.

Figure Legends

Figure 1. Timeline of experimental events. (a) Experiment 1. Rats were implanted with bilateral cannulae targeting the orbitofrontal cortex (OFC) and the dorsal striatum (DS). First, baseline levels of locomotor activity were measured. Two days later, locomotor activity was assessed following bilateral saline microinfusions in both the OFC and the DS. Next, locomotion was measured after bilateral disruption of the OFC-DS circuit (baclofen/muscimol into the OFC of one hemisphere, and the DS of the contralateral hemisphere), followed by the bilateral inactivation of the OFC or the DS two days later. The effects of bilateral saline microinfusions into both the OFC and the DS were assessed during a final test session. All sessions lasted 2 h. (b) Experiment 2. One week after implantation of an intrajugular catheter, rats were trained to self-administer 0.25 mg/kg/infusion cocaine during daily 1-h sessions. Next, rats self-administered cocaine under intermittent access (IntA) conditions for 10 sessions, where they were limited to 2 infusions/6-min cocaine-available period (max. 24 infusions/session). Following IntA, we assessed baseline levels of responding for cocaine (0.063-0.25 mg/kg/infusion) under a progressive ratio schedule of reinforcement (PR). Rats were then implanted with bilateral cannula into the OFC and the DS. We determined the effects of bilateral disruption of the OFC/DS circuit or bilateral inactivation of each individual structure on responding for cocaine (0.063 mg/kg/infusion) under PR. *B+M*, Baclofen + Muscimol. *d*, days. *DS*, dorsal striatum. *h*, hour. *IC*, intracerebral. *IntA*, intermittent access. *OFC*, orbitofrontal cortex. *PR*, progressive ratio.

Figure 2. Estimated injector tip placements in the OFC (a) and DS (b) from experiment 1 (*) and 2 (●). n = 4 for Experiment 1 and n = 12 for Experiment 2. *DS*, dorsal striatum. *LO*, lateral orbitofrontal cortex. *VLO*, ventrolateral orbitofrontal cortex. Numbers indicate millimetres (mm) from Bregma.

Figure 3. Baclofen/muscimol or saline microinfusions into the orbitofrontal cortex (OFC) and dorsal striatum (DS) do not influence spontaneous locomotor activity. Locomotor activity was assessed under baseline conditions or after injecting a baclofen/muscimol solution (0.3 and 0.03 nmol, respectively, or saline) into the OFC of one hemisphere and the DS of the contralateral hemisphere (a), into the OFC of each hemisphere (b) and into the DS of each hemisphere (c). All values are mean ± SEM. n = 2-4 rats. *DS*, dorsal striatum. *OFC*, orbitofrontal cortex.

Figure 4. Under our intermittent access (IntA) conditions, cocaine self-administration behaviour remains stable over sessions, but rats show significant psychomotor sensitization. Rats self-administered cocaine under IntA over 10 sessions. Rats could take a maximum of 2 infusions/6-min cocaine-available period, for a maximum of 24 infusions, as indicated by the dotted line in (a). The figure shows the number of self-administered infusions (a), the inter-infusion interval (b). lever-pressing behaviour (c) and the breakdown of drug infusions taken during each minute of the 6-min cocaine-available periods (d), across self-administration sessions. The figure also shows the time course of locomotor activity averaged over the twelve 26-min no-drug available periods of the 1st

and 10th IntA sessions (e). *$p < 0.05$ vs. inactive lever presses. #p < 0.05 vs. session 1. All values are mean ± SEM. n = 14 rats. *s*, seconds.

Figure 5. When tested under a progressive ratio schedule of drug reinforcement, rats respond more for increasing doses of cocaine. As cocaine dose is increased, the rats self-administer more cocaine infusions (a), respond more on the active lever (b) and have longer test sessions (c), suggesting increased incentive motivation for cocaine at higher cocaine doses. *$p < 0.05$. n = 14 rats. *h*, hours.

Figure 6. Responding for cocaine (0.063 mg/kg/infusion) under a progressive ratio schedule of drug reinforcement is comparable before and after surgery, and after repeated intracerebral injections (Final session). The figure compares the number of self-administered infusions (with corresponding ratio; a), lever-pressing behaviour (b) and locomotor activity (c) under baseline conditions (Pre-surgery), following intracerebral cannula implantation (Post-surgery) and following all intracerebral treatments (Final session). *$p < 0.05$. n = 12 rats. *A*, active lever. *I*, inactive lever.

Figure 7. Injecting baclofen/muscimol or saline into the orbitofrontal cortex (OFC) of one hemisphere and the dorsal striatum (DS) of the other hemisphere decreases responding for cocaine (0.063 mg/kg/infusion) under a progressive ratio schedule of reinforcement. Both baclofen/muscimol and saline treatments decreased the number of self-administered infusions (a) and active lever presses (b) compared to baseline.

Baclofen/muscimol treatment also significantly reduced inactive lever presses (c) and increased the inter-infusion interval (d). Session duration remained constant across conditions (e). Finally, baclofen/muscimol treatment reduced locomotor activity (f) during progressive ratio testing. *p< 0.05. n = 12 rats. *B+M*, Baclofen + Muscimol. *h*, hours. *min*, minutes. *Sal,* saline.

Figure 8. Bilateral baclofen/muscimol or saline infusions into the orbitofrontal cortex (OFC) decrease responding for cocaine (0.063 mg/kg/infusion) under a progressive ratio schedule of reinforcement. Baclofen/muscimol or saline treatment reduced the number of self-administered infusions (a), active lever presses (b), and locomotor activity (f) compared to baseline. Baclofen/muscimol treatment also reduced inactive lever presses (c), while saline treatment increased the inter-infusion interval (d). Session duration remained constant across conditions (e). *p < 0.05 vs. basal. $^{\#}p$ < 0.05 vs. saline. n = 12 rats. *B+M*, Baclofen + Muscimol. *h*, hours. *min*, minutes. *Sal*, saline.

Figure 9. Bilateral baclofen/muscimol infusions into the dorsal striatum (DS) decrease responding for cocaine (0.063 mg/kg/infusion) under a progressive ratio schedule of reinforcement compared to either saline or baseline. Baclofen/muscimol decreased the number of self-administered infusions (a) and both active (b) and inactive (c) lever presses. Saline treatment also reduced the number of self-administered infusions (a) but less so than baclofen/muscimol. Saline reduced active lever presses (b). Inter-infusion interval times did not change as a function of treatment (d). Baclofen/muscimol treatment

decreased the length of PR test sessions (e). Finally, both baclofen/muscimol and saline diminished locomotor activity compared to baseline (f). *p< 0.05 vs. basal. $^{\#}p$ < 0.05 vs. saline. n = 12 rats. *B+M*, Baclofen + Muscimol. *h*, hours. *min*, minutes. *Sal*, saline.

Figures

Figure 1

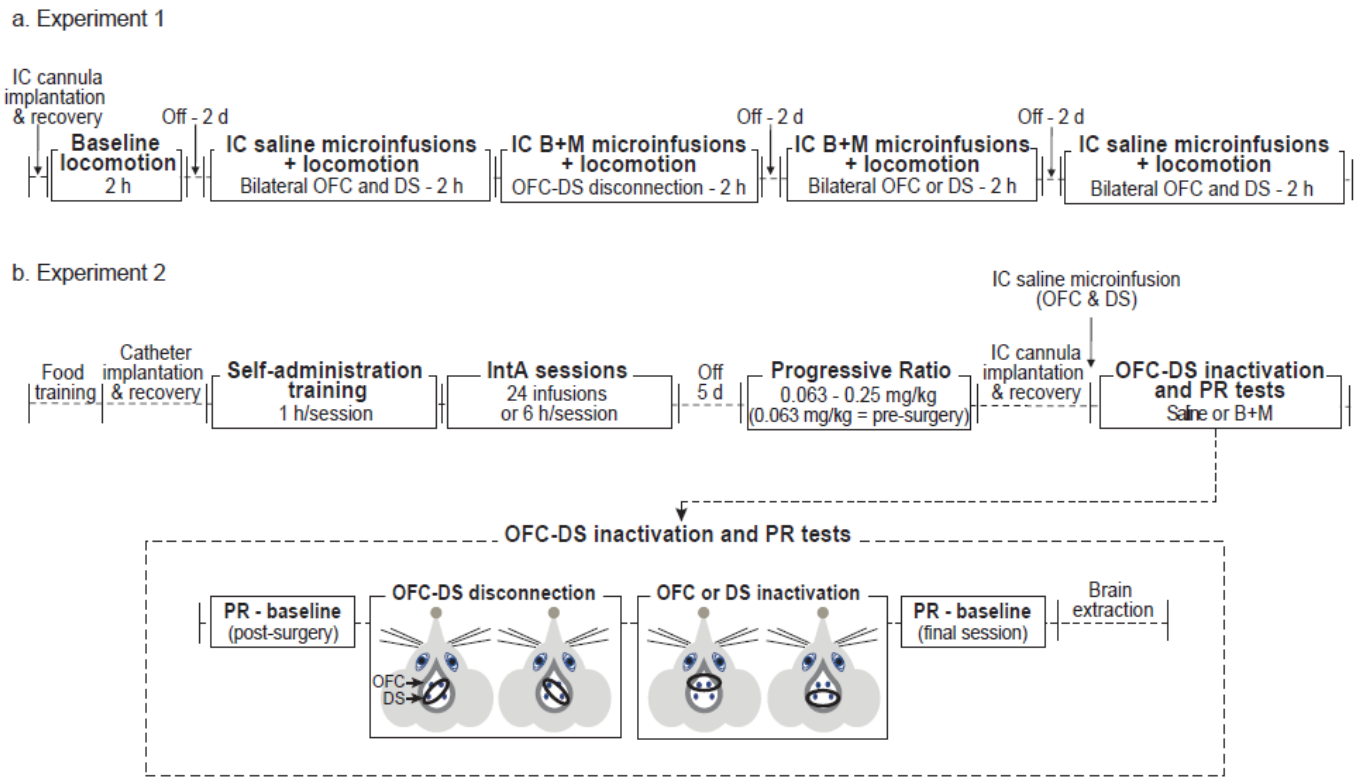


Figure 2

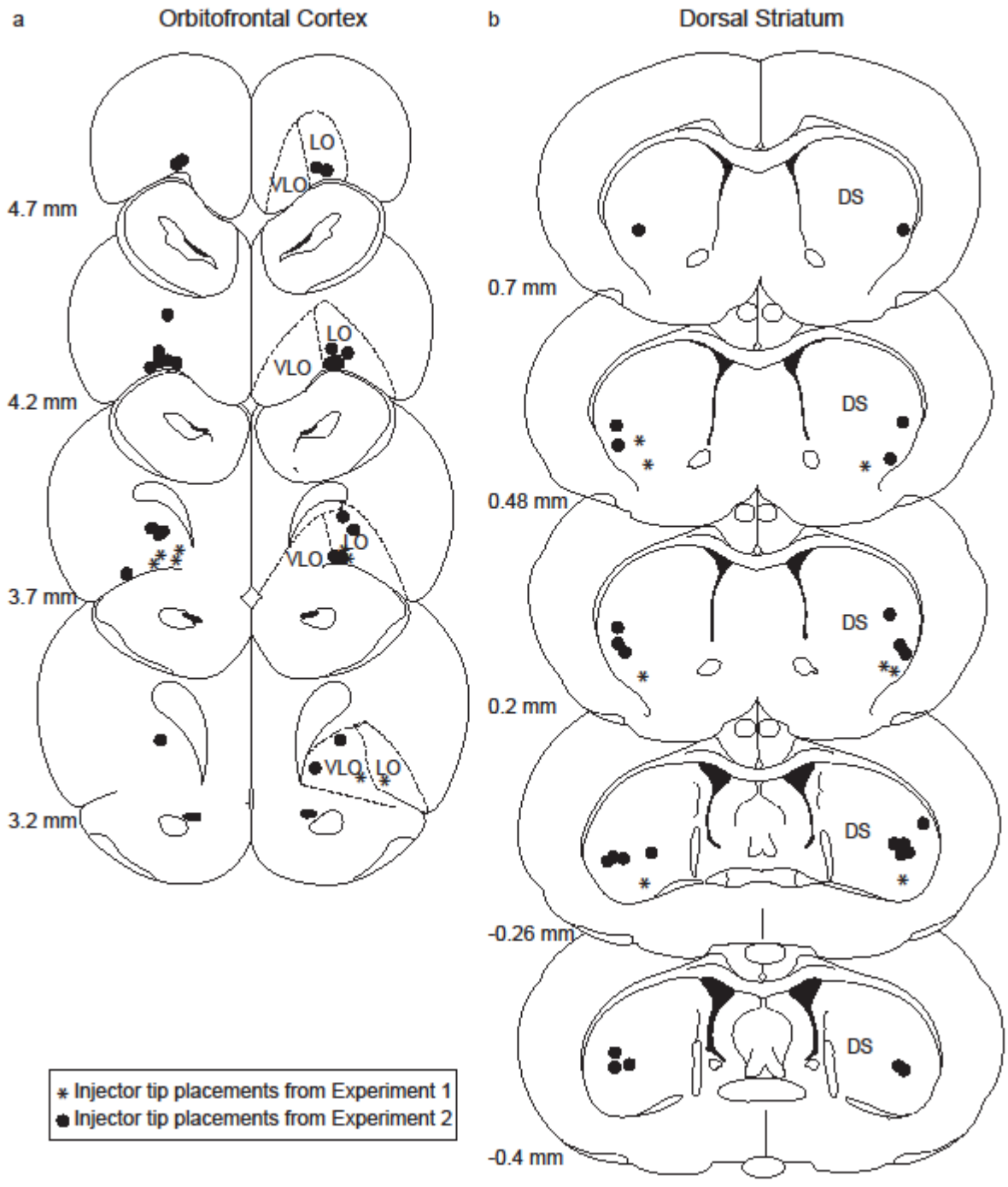

a
Orbitofrontal Cortex
b
Dorsal Striatum
4.7 mm
4.2 mm
3.7 mm
3.2 mm
LO
VLO
DS
0.7 mm
0.48 mm
0.2 mm
-0.26 mm
-0.4 mm
Injector tip placements from Experiment 1
Injector tip placements from Experiment 2

Figure 3

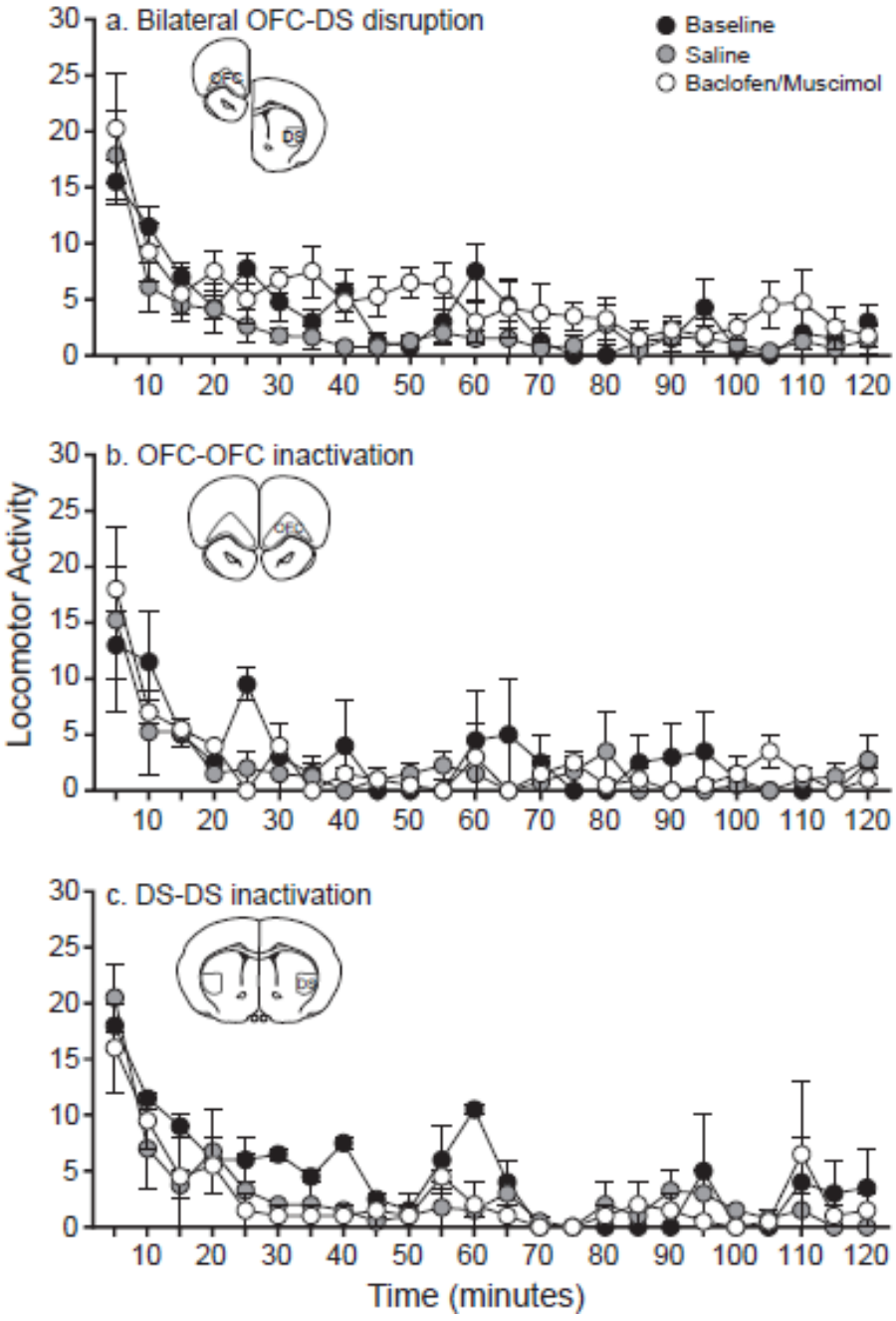

a. Bilateral OFC-DS disruption
Baseline
Saline
Baclofen/Muscimol
OFC
DS
b. OFC-OFC inactivation
c. DS-DS inactivation
Locomotor Activity
Time (minutes)
0
5
10
15
20
25
30
10
20
30
40
50
60
70
80
90
100
110
120

Figure 4

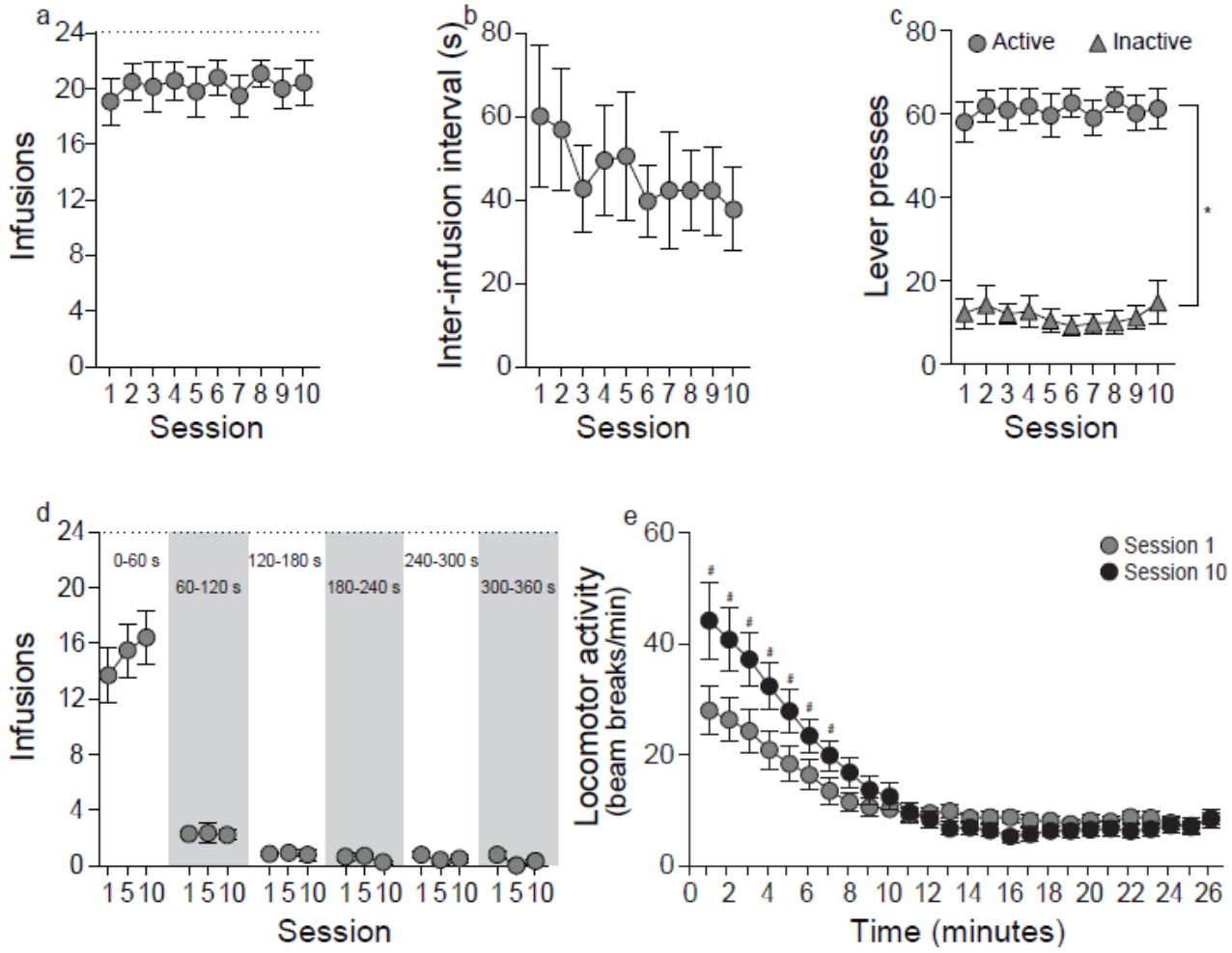

a
Infusions
Session
b
Inter-infusion interval (s)
Session
c
Active
Inactive
Lever presses
*
Session
d
0-60 s
60-120 s
120-180 s
180-240 s
240-300 s
300-360 s
Infusions
Session
e
Session 1
Session 10
Locomotor activity (beam breaks/min)
Time (minutes)

Figure 5

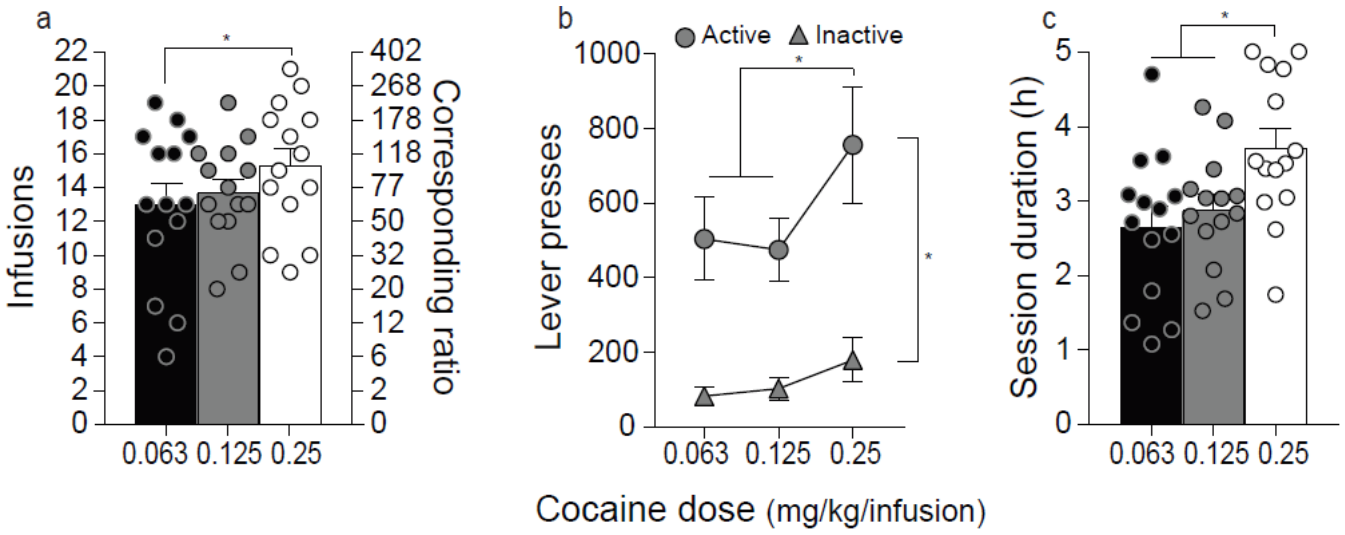

a
b
c
Infusions
Corresponding ratio
Lever presses
Active
Inactive
Session duration (h)
0.063 0.125 0.25
Cocaine dose (mg/kg/infusion)

Figure 6

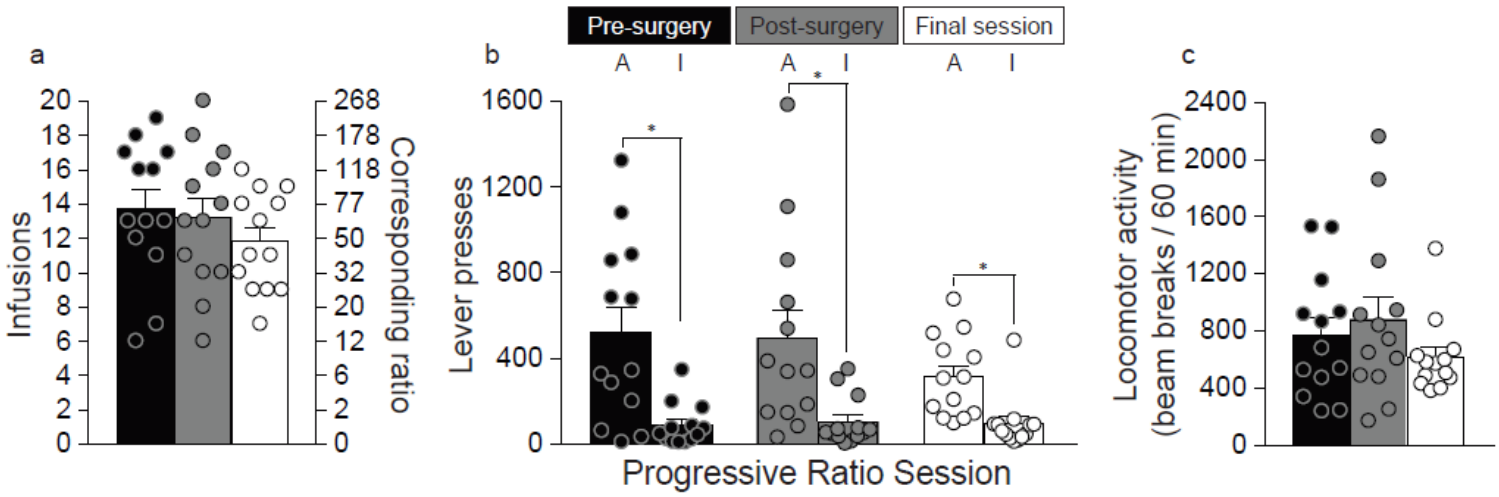

a
Infusions
Corresponding ratio
b
Pre-surgery
Post-surgery
Final session
A
I
Lever presses
Progressive Ratio Session
c
Locomotor activity
(beam breaks / 60 min)

Figure 7

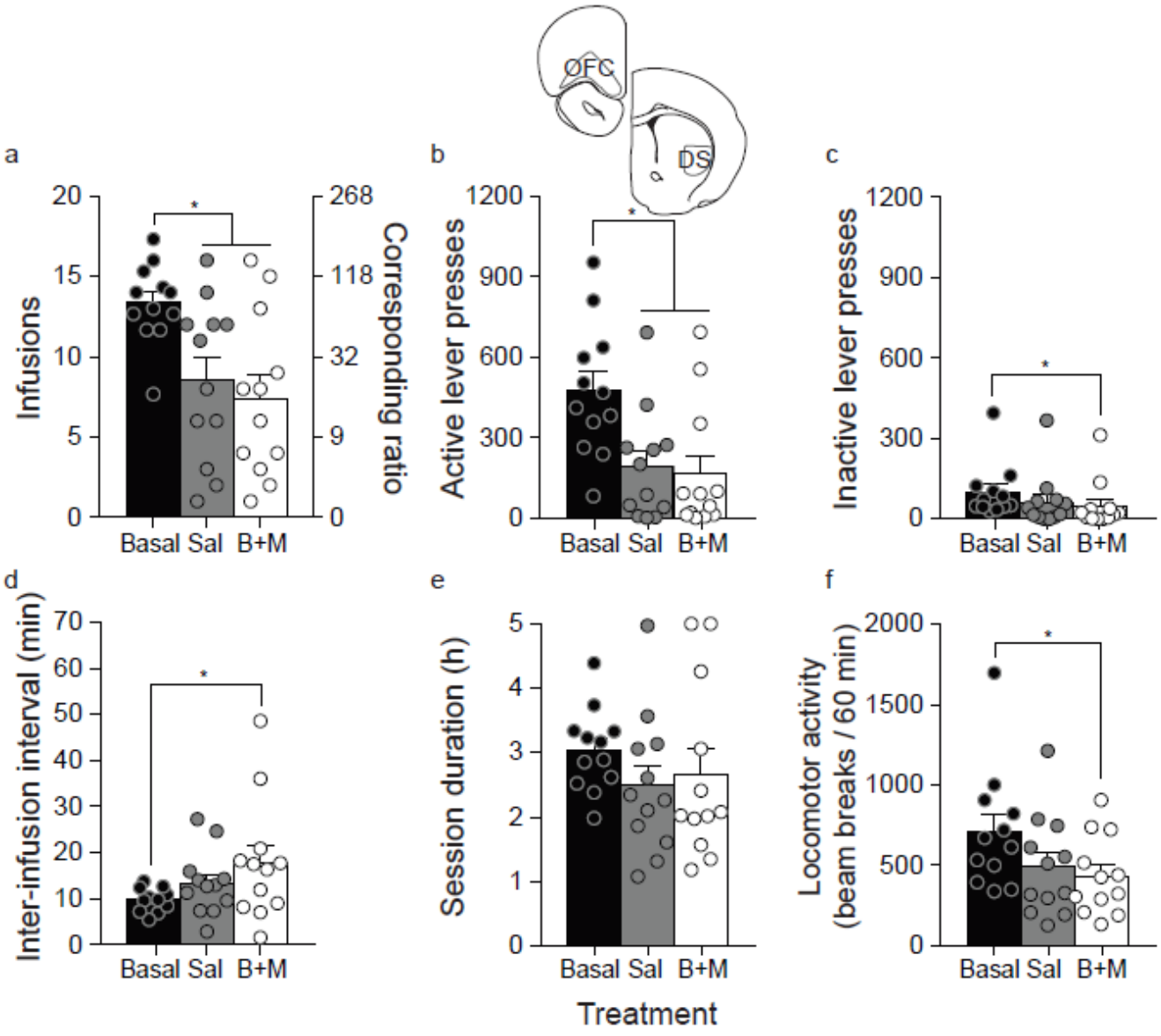

a
b
c
d
e
f
OFC
DS
Infusions
Corresponding ratio
Active lever presses
Inactive lever presses
Inter-infusion interval (min)
Session duration (h)
Locomotor activity (beam breaks / 60 min)
Basal Sal B+M
Treatment
*

Figure 8

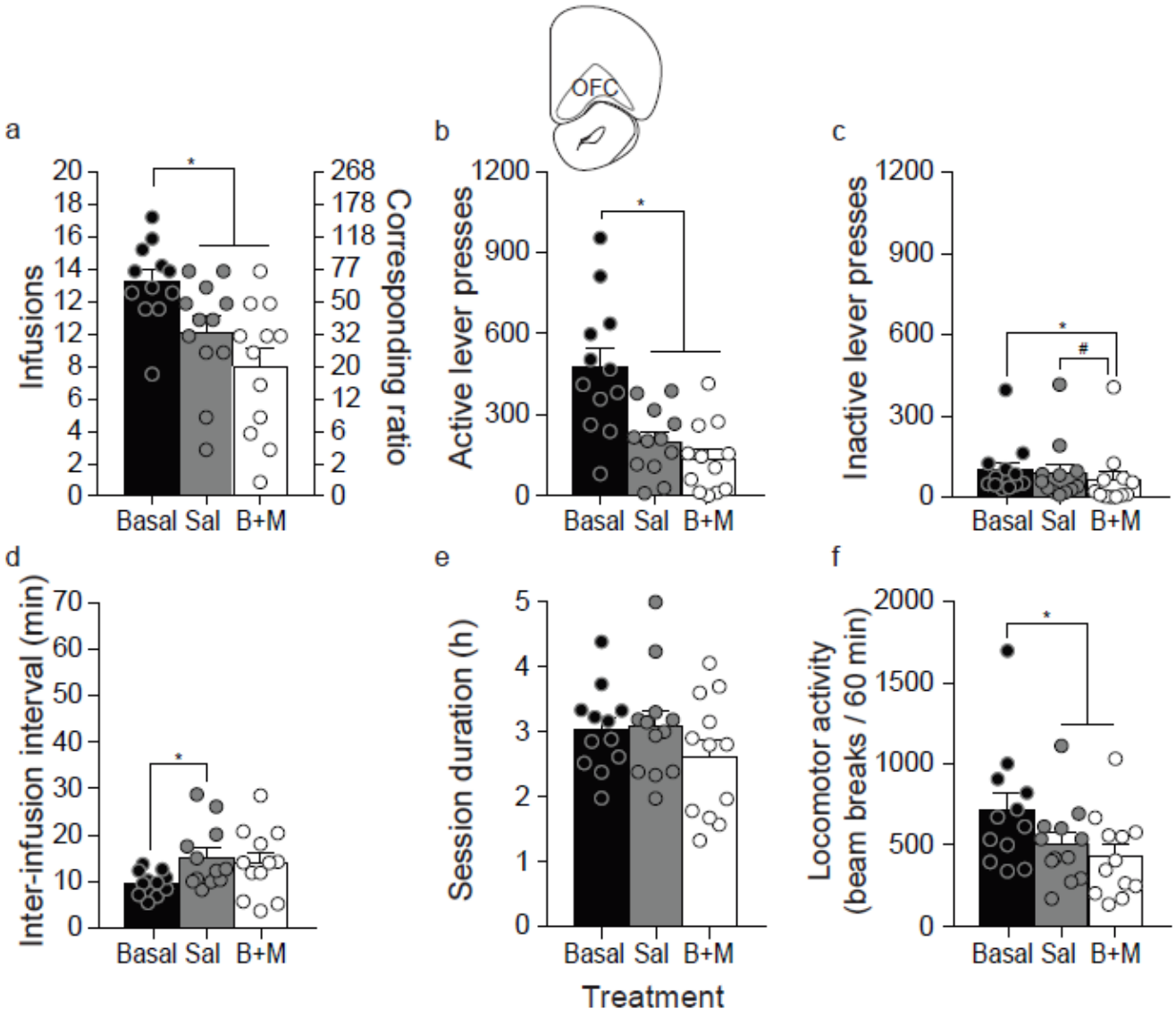
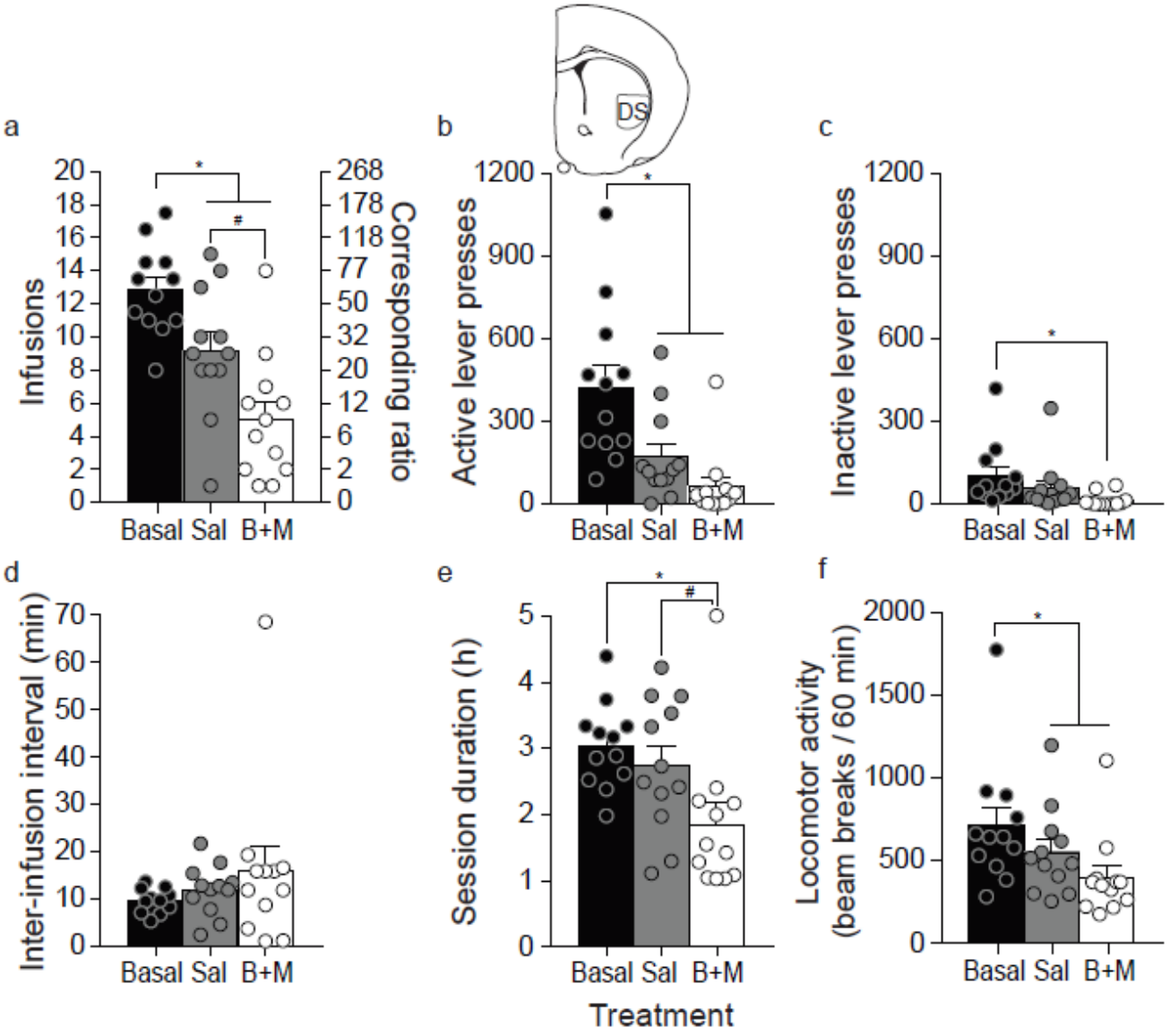

a
b
c
d
e
f
OFC
Infusions
Corresponding ratio
Active lever presses
Inactive lever presses
Inter-infusion interval (min)
Session duration (h)
Locomotor activity
(beam breaks / 60 min)
Basal Sal B+M
Treatment
*
#

Figure 9


a
b
c
d
e
f
DS
Infusions
Corresponding ratio
Active lever presses
Inactive lever presses
Inter-infusion interval (min)
Session duration (h)
Locomotor activity (beam breaks / 60 min)
Basal Sal B+M
Treatment
*
#

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Discussion

CHAPITRE 11 – Résumé des résultats

La toxicomanie est un trouble complexe, ce qui explique pourquoi l'identification des mécanismes qui la sous-tendent n'est pas une tâche facile. Bien que le nombre d'individus qui consomment de la drogue est élevé, peu parmi ces derniers passeront d'un usage récréatif à la toxicomanie (O'Brien et Anthony, 2005; Wagner et Anthony, 2002). Il est donc nécessaire de comprendre quels changements prennent lieu dans le cerveau de ces individus, ainsi facilitant le développement de la toxicomanie. Pour commencer à répondre à cette question, la recherche en toxicomanie doit commencer par développer un bon modèle animal dans lequel étudier ces changements. Le travail décrit dans cette thèse souligne l'importance des facteurs pharmacocinétiques dans le développement de modèles animaux pour étudier les comportements et la neuroplasticité qui sont caractéristiques à la toxicomanie.

Premièrement, les propriétés de la drogue consommée peuvent influencer l'évolution de la toxicomanie (Farré et Camí, 1991; Hatsukami et Fischman, 1996). Une de ces caractéristiques est la vitesse à laquelle la drogue arrive au cerveau. En effet, plus la cocaïne arrive rapidement à ses sites d'actions dans le cerveau, plus le risque de toxicomanie est élevé (Hatsukami et Fischman, 1996; Jones, 1990). Ceci met les individus qui consomment la cocaïne par des voies rapides (la voie intraveineuse ou en fumant du crack) plus à risque de développer des troubles liés à l'usage des drogues que les individus qui utilsent les voies plus lentes [voie intranasale; (Ferri et Gossop, 1999; Gossop et al., 1992; Gossop et al., 1994; Hatsukami et Fischman, 1996)]. De plus, malgré que la majorité des individus commencent avec la voie intranasale [87%; (Dunn et Laranjeira, 1999; Guindalini, Vallada, Breen et Laranjeira, 2006)], un grand nombre parmi

ces derniers passeront aux voies plus rapides (68% fumeront du crack et 20% utiliseront la voie intraveineuse), ce qui rend nécessaire la meilleure compréhension de ce qui arrive dans le cerveau lorsque la drogue y parvient rapidement. Nombreuses études chez le rat démontrent que les infusions intraveineuses rapides de cocaïne (en 4-5 versus 25-100 secondes) favorisent le développement de comportements caractéristiques de la toxicomanie (Allain et al., 2017; Bouayad-Gervais et al., 2014; Minogianis et al., 2013; Samaha et al., 2002; Samaha et al., 2004; Wakabayashi et al., 2010) et induisent de la neuroplasticité dans le cerveau qui est différente de celle qui est provoquée par des infusions plus lentes (Allain et al., 2017; Bouayad-Gervais et al., 2014; Brown et Kiyatkin, 2005; Ferrario et al., 2008; Minogianis et al., 2013; Samaha et al., 2004). Malgré cette grande littérature, une question restait sans réponse: Est-ce que ces effets sont dus au fait que 1) la cocaïne arrive plus rapidement au cerveau, 2) une infusion rapide de cocaïne mène à un taux plus élevé de drogue dans le cerveau ou 3) pour les deux raisons? L'étude dans l'article 1 (chapitre 7) permet de répondre à cette question. En effet, les infusions rapides de cocaïne (5 vs. 45-90 secondes) ne changent pas significativement les concentrations maximales de la cocaïne et de la dopamine dans le cerveau, mais fassent en sorte que les pics de ces deux molécules soient atteints plus rapidement. Ces résultats renforcent les prédictions du modèle pharmacocinétique de Pan, Menacherry et Justice (1991) utilisé pour prédire les courbes de concentration de cocaïne dans le cerveau suite à l'infusion de la drogue en 5, 25, 50 et 100 secondes (Samaha et al., 2002). De plus, nos résultats sont en accord avec Ferrario et al. (2008) qui montrent qu'une infusion aiguë de cocaïne en 5 versus 100 secondes augmente les niveaux de dopamine dans le cerveau plus rapidement, sans provoquer de changements dans la

concentration maximale de la drogue. Finalement, nous montrons que la vitesse d'administration de la cocaïne change aussi la vitesse d'induction de l'activité psychomotrice, sans modifier la locomotion totale, et ceci pourrait être lié à l'augmentation des niveaux de dopamine dans le cerveau. En prenant en considération le fait que les niveaux de la cocaïne et de la dopamine sont fortement corrélés dans le cerveau (Nayak et al., 1976; Shou et al., 2006), ces données suggèrent que le profil pharmacocinétique de l'une peut être utilisée pour estimer les niveaux de l'autre dans le cerveau. Cependant, le résultat le plus intéressant est le fait que les infusions rapides de la cocaïne faciliteraient le développement de symptômes caractéristiques de la toxicomanie en provoquant l'arrivée plus précoce de la drogue dans le cerveau, et non en changeant la concentration. Ceci aiderait à expliquer pourquoi fumer du crack ou s'injecter la drogue augmenterait le risque de développer la toxicomanie comparativement à l'insufflation (Hatsukami et Fischman, 1996; Jones, 1990).

La deuxième partie de ce travail s'est concentrée sur un aspect important de la recherche en toxicomanie : la caractérisation de la neuroplasticité induite par la consommation de drogue. Il est nécessaire de différencier la neuroplasticité impliquée dans la toxicomanie de celle qui est causée par le simple fait de consommer la drogue. Dans Minogianis et al. (2013), nous avions démontré que la consommation répétée d'infusions rapides de cocaïne (en 5 versus 90 s) par i.v. lors de longues sessions d'accès à la drogue (6 h/session) augmente la motivation pour la cocaïne sous ratio progressif. Une augmentation dans le temps et l'énergie dépensée à chercher et à consommer la drogue est un symptôme caractéristique de la toxicomanie (APA, 2013) qu'on peut modéliser chez l'animal grâce au programme à ratio progressif. Dans une première étude

nous avons examiné la neuroplasticité induite par des infusions rapides de cocaïne en LgA sur l'expression de l'ARN messager de BDNF et son récepteur TrkB dans les régions corticostriatales impliquées dans la toxicomanie (article 2 – chapitre 8). Seulement les infusions rapides de cocaïne, et non les infusions lentes de la drogue ni les infusions de salin, ont provoqué des changements dans l'expression de BDNF et de TrkB dans le cerveau. Nous avons observé une augmentation de l'expression de l'ARN messager de BDNF dans les cortex orbitofrontal, frontal et pariétal, tandis qu'il y a eu une baisse dans les niveaux de TrkB dans les cortex cingulé, frontal et pariétal et dans le striatum dorsal. Par la suite, la littérature rapporte que les individus ayant un trouble lié à l'usage de la cocaïne ne consomment pas de grandes quantités de drogue de façon continue, mais plutôt attendent entre chaque session de consommation, ainsi créant des fluctuations de drogue dans le cerveau (Beveridge et al., 2012). Ainsi, nous avons utilisé le modèle animal d'auto administration avec accès intermittent à la drogue de Zimmer et al. (2012) qui permet des fluctuations de drogue dans le cerveau et qui favorise l'apparition d'une motivation excessive pour la drogue, malgré la faible quantité de drogue consommée (Allain et al., 2018; Allain et al., 2017; Allain et Samaha, 2018; Kawa et al., 2016; Singer et al., 2018; Zimmer et al., 2012) pour comparer l'expression de l'ARN messager du c-*fos* chez deux groupes d'animaux (article 3 – chapitre 9). Le premier groupe était un modèle animal pertinent à la toxicomanie (IntA à des infusions rapides de cocaïne; IntA-5s). Le deuxième était un modèle de consommation plus atténué (LgA à des infusions lentes de cocaïne; LgA-90s), équivalent à des animaux qui consomment une quantité significative de drogue, sans montrer des comportements caractéristiques de la toxicomanie (Minogianis et al., 2013; Wakabayashi et al., 2010). La cocaïne augmente

l'expression de c-*fos* dans toutes les structures corticostriatales quantifiées comparativement à l'auto administration du salin. Par contre, les rats IntA-5s ont exprimé des taux d'ARN messager c-*fos* encore plus élevés que les rats LgA-90s dans les cortex orbitofrontal et prélimbique, ainsi que le striatum dorsal. Ces données montrent que la vitesse d'infusion et la fréquence de consommation prédisent le développement de neuroplasticité associée à la toxicomanie.

Ces deux études soulignent des changements dans l'activité de l'OFC et du DS chez des animaux qui ont également démontré une motivation excessive pour la drogue. L'OFC et le DS sont recrutés avec la progression de la toxicomanie (Everitt et al., 2008; Everitt et Robbins, 2005). De plus, l'OFC envoie des projections glutamatergiques vers le DS (Berendse et al., 1992; Schilman et al., 2008). Ces résultats nous ont donc donné une piste pour un circuit qui serait possiblement impliqué dans le développement d'une motivation excessive pour la cocaïne dans un modèle animal pertinent à la toxicomanie (article 4 – chapitre 10). Ainsi, la déconnection temporaire et pharmacologique de ce circuit et l'inactivation individuelle du OFC ou du DS a baissé la motivation pour la cocaïne sous ratio progressif. Ces résultats suggèrent l'implication possible, malgré qu'ils ne soient pas concluants, de l'OFC et du DS dans la motivation incitative pour la cocaïne et la toxicomanie. Des études supplémentaires seraient nécessaires pour mieux étudier le rôle de ce circuit dans la motivation excessive pour la drogue.

Enfin, ces résultats montrent que la vitesse d'infusion et la fréquence de consommation sont importantes dans l'évolution de la toxicomanie. Ces facteurs pharmacocinétiques doivent être inclus dans le développement de modèles animaux adéquats, car ils favorisent des comportements et de la neuroplasticité qui sont

caractéristiques à ce trouble. Ces résultats pourraient donc servir à mieux comprendre pourquoi certaines personnes sont plus à risque de développer des troubles liés à l'usage de la drogue que d'autres. Finalement, l'OFC et le DS pourraient potentiellement être des cibles dans le développement d'une motivation excessive pour la drogue.

CHAPITRE 12 – Comment l'arrivée plus rapide d'une drogue au cerveau pourrait faciliter le développement de la toxicomanie?

Dans notre première étude, nous avions voulu déterminer comment la vitesse d'infusion change la dynamique temporelle de la cocaïne et de la dopamine dans le cerveau à la suite d'une injection intraveineuse de drogue infusée en 5, 45 ou 90 secondes. De plus, nous avions examiné les effets de la vitesse de cocaïne sur une vingtaine de neurotransmetteurs, neuromodulateurs et métabolites, pour voir par quels autres mécanismes une infusion rapide de cocaïne en aiguë pourrait influencer le développement de la toxicomanie.

12.1 Effets de la vitesse d'administration d'une infusion de cocaïne en aiguë sur les niveaux de cocaïne et de dopamine dans le striatum

Nos données montrent que des petites variations dans la vitesse d'infusion de la cocaïne en aiguë (entre 5-90 secondes) produisent de grandes différences dans le temps nécessaire pour atteindre la concentration maximale de la drogue dans le cerveau (Tmax), sans significativement modifier la concentration maximale (Cmax). Ces résultats sont en accord et renforcent les estimations générées par le modèle pharmacocinétique de Pan et al. (1991) comparant des vitesses d'infusion entre 5 et 100 secondes (Samaha et al., 2002). Nous avions aussi trouvé que la vitesse d'infusion de la cocaïne produit des effets similaires sur les niveaux de dopamine dans le cerveau. Ces données coïncident avec le travail de Ferrario et al. (2008), qui montrent qu'une infusion de cocaïne en 5 versus 100 secondes ne modifie pas significativement la concentration maximale et la

quantité totale de dopamine dans le striatum dorsal, mais le Tmax de la dopamine est atteint plus tôt. Similairement, Samaha et al. (2004) montrent que les infusions rapides de la cocaïne font varier la cinétique de l'inhibition du transporteur de la dopamine. Cependant, ils rapportent que la vitesse d'infusion modifie aussi l'amplitude de la recapture de la dopamine déterminée par la capacité de la drogue à prolonger la demi-vie de la dopamine dans le noyau accumbens (Samaha et al., 2004), une différence qui pourrait être expliquée par les méthodologies différentes appliquées.

12.2 Aucun effet de la vitesse d'administration d'une infusion de cocaïne en aiguë sur les niveaux des autres neurotransmetteurs, neuromodulateurs et métabolites étudiés.

La cocaïne influence non seulement la recapture de la dopamine, mais aussi celle d'autres neurotransmetteurs, soit la noradrénaline et la sérotonine (Heikkila et al., 1975; Koe, 1976). Nous avions donc voulu mesurer les effets de la vitesse d'infusion de la cocaïne sur ces neurotransmetteurs, ainsi que d'autres molécules qui seraient changées par la vitesse d'administration de la drogue. Cependant, aucune autre différence dans la concentration de neurotransmetteurs ou de leurs métabolites a été détectée dans le striatum dorsal à la suite d'une infusion intraveineuse aiguë de cocaïne. Par exemple, aucune augmentation des concentrations de la norépinephrine a été détectée, malgré que la cocaïne soit aussi un inhibiteur de la recapture de ce neurotransmetteur en agissant sur le transporteur de la norépinephrine (Ritz, Cone et Kuhar, 1990). L'absence de changements des niveaux de norépinephrine pourrait être attribuable à la densité réduite d'axones noradrénergiques et aux faibles niveaux du neurotransmetteur dans cette structure (Fornai et al., 1996). De plus, nous n'avions pas trouvé de changements

provoqués par la drogue sur les niveaux de glutamate, similairement aux résultats des travaux de Ferrario et al. (2008) dans le striatum dorsal et Miguéns et al. (2008) dans le noyau accumbens. Ceci est potentiellement dû au fait que de hautes doses de cocaïne en aiguë [15-30 mg/kg, i.p.; (Reid, Hsu et Berger, 1997; Smith, Qiu, Guo, Kunko et Robinson, 1995)] ou des infusions répétées sont nécessaires pour induire des changements dans le niveau de glutamate (Zhang, Loonam, Noailles et Angulo, 2001). Malgré qu'une augmentation des niveaux de la sérotonine dans le noyau accumbens et l'aire tegmentale ventral après une infusion systémique a été rapportée dans la littérature (Essman, Singh et Lucki, 1994; Reith, Li et Yan, 1997), aucun changement dans les concentrations extracellulaires de la sérotonine a été trouvé dans le striatum dorsal après une infusion i.v. [cette étude et (Bradberry et al., 1993)] ou i.p. (Bradberry et al., 1993). Ces résultats étaient aussi surprenants, car comme pour la norépinephrine, la cocaïne est un inhibiteur du transporteur de la sérotonine (Ritz et al., 1990). Les divergences entre ces études peuvent aussi être causées par des disparités dans la méthodologie, telles que la voie d'administration utilisée (Bradberry et al., 1993), l'anesthésie (Bradberry et al., 1993) et la région échantillonnée (Essman et al., 1994). Finalement, une infusion aiguë de cocaïne n'a pas provoqué des changements dans les concentrations extracellulaires des métabolites de la dopamine [DOPAC, HVA et 3MT; voir aussi (Hurd et Ungerstedt, 1989)], de la sérotonine [5-HIAA; voir aussi (Kalivas et Duffy, 1990)] et de la norépinéphrine (Normétanéphrine). Le manque d'effets sur les concentrations de ces métabolites est important, car ça suggère la forte capacité de la cocaïne à bloquer les transporteurs de la dopamine, de la norépinéphrine et de la sérotonine, prévenant ainsi leur métabolisme dans les terminaisons axonales (Hernandez et Hoebel, 1988; Hurd et

Ungerstedt, 1989) et ainsi permettant leur action continue dans la synapse. Cependant, il faut noter que la littérature n'est pas constante sur les effets d'une infusion aiguë de cocaïne sur ces métabolites. Par exemple, l'infusion de cocaïne en aigu diminue les niveaux de DOPAC dans le noyau accumbens (Hernandez et Hoebel, 1988; Kalivas et Duffy, 1990) et le cortex frontal (Karoum, Chrapusta, Brinjak, Hitri et Wyatt, 1994). Similairement, Kalivas et Duffy (1990) soulignent la diminution des niveaux de HVA dans le noyau accumbens, tandis que Hernandez et Hoebel (1988) ne montrent aucun changement.

12.3 Comment des petites variations dans l'arrivée de la drogue au cerveau peuvent-elles faciliter le développement de la toxicomanie?

Les données de l'article 1 suggèrent que des variations dans la vitesse d'arrivée de la cocaïne au cerveau peuvent prédire le résultat subséquent. Ainsi, nos données suggèrent que l'arrivée rapide de la cocaïne pourrait changer l'impact neurobiologique de la drogue au cerveau en modulant la dynamique temporelle de la dopamine, en influençant soit les niveaux de dopamine dans la synapse via l'inhibition de la recapture de la dopamine au niveau du transporteur, soit l'occupation des récepteurs dopaminergiques. Cette hypothèse est soutenue par deux études. Dans un premier cas, une étude de neuroimagerie utilisant la tomographie par émission de positrons et [^{11}C]-cocaïne rapporte que la cocaïne fumée ou prise par voie intranasale produit des niveaux équivalents de cocaïne plasmatique et de blocage du transporteur de la dopamine. Par contre, la cocaïne fumée produit des effets subjectifs plus intenses et plus rapides que la voie intranasale (Volkow et al., 2000). Similairement, lorsque deux formulations du

psychostimulant méthylphénidate (MPH) sont comparées (MPH via libération immédiate (MPH-IR) et MPH par libération osmotique (MPH-OR; produit une libération graduelle de MPH dans le plasma)), les deux formulations produisent des Cmax semblables. Cependant, le Tmax et l'occupation maximale du DAT, la cible principale de MPH (Volkow et al., 1998), sont atteints plus lentement avec le MPH-OR (Spencer et al., 2006). Ceci suggère que les différences entre les effets de renforcement de la cocaïne en fonction de la route d'administration ne sont pas dues à l'efficacité de la drogue à ses sites d'actions, mais plutôt à la vitesse à laquelle la drogue et la dopamine augmentent dans le cerveau. Ceci pourrait expliquer pourquoi les voies d'administration et les formulations de la cocaïne qui mènent la drogue rapidement au cerveau mettraient les individus qui la consomment plus à risque pour le développement de la toxicomanie.

CHAPITRE 13 – La vitesse d'infusion et la fréquence de consommation induisent de la neuroplasticité dans les régions corticostriatales du cerveau : implications pour le développement de la toxicomanie

La neuroplasticité induite par la cocaïne facilite le développement de comportements et de symptômes caractéristiques de la toxicomanie. Un des objectifs de cette thèse était de séparer la neuroplasticité impliquée dans la toxicomanie, de celle qui est générée par la simple consommation de la drogue. Afin de commencer à faire cette distinction, nous avons utilisé des modèles animaux qui sont pertinents à la toxicomanie. Pendant longtemps, on croyait que le développement de la toxicomanie nécessitait la consommation de grandes quantités de drogue (Ahmed, 2012; Ahmed et Koob, 1998). Bien que la quantité de drogue consommée soit un facteur pharmacocinétique important dans le développement de la toxicomanie, les études présentées dans cette thèse montrent que la vitesse d'infusion et la fréquence de consommation seraient des facteurs aussi importants, et même plus. La neuroplasticité induite par ces trois facteurs sera discutée ci-dessous.

13.1 La quantité de cocaïne consommée induit des changements neurobiologiques dans le cerveau

13.1.1 Effets de l'administration répétée de la cocaïne par la voie intraveineuse

La consommation chronique de la cocaïne est associée à de nombreux changements neuronaux persistants au niveau moléculaire, cellulaire et morphologique

[voir (Kalivas et O'Brien, 2008; Nestler, 2001, 2004b)]. Ces changements neuronaux incluent l'altération de l'expression de gènes à la suite de la consommation répétée de drogue (Graham et al., 2007; Pich et al., 1997; Zahm et al., 2009), ainsi qu'après de longues périodes de retrait (Grimm et al., 2003). Au niveau structurel, il a été démontré que les neurones épineux moyens situées dans les régions de la récompense du cerveau sont modifiés à la suite de la prise répétée de drogues d'abus. Les neurones épineux moyens sont important dans le développement de la toxicomanie car ils sont impliqués dans la signalisation excitatrice du cerveau (Shepherd, 1996). La densité et l'organisation des dendrites des neurones épineux moyens serait modifiée à la suite de l'utilisation de drogues d'abus, dont la morphine et les psychostimulants amphétamine et cocaïne (Li, Kolb et Robinson, 2003; Robinson et al., 2001; Robinson et Kolb, 1997, 1999a; Robinson et Kolb, 1999b). Plus spécifiquement, l'administration persistante de la cocaïne mène à l'augmentation de l'arborisation dendritique et de la densité épineuse des dendrites sur les neurones épineux moyens dans le noyau accumbens et les cellules pyramidales du cortex préfrontal et du cortex pariétal (Robinson et al., 2001; Robinson et Kolb, 1999a). Cette réorganisation de la connectivité synaptique dans des structures impliquées dans la toxicomanie pourrait faciliter le développement de la toxicomanie.

13.1.2 Effets de l'administration de grandes quantités de cocaïne

De nombreuses études suggèrent que l'exposition continue à la cocaïne pendant de longues sessions (LgA) est associée avec le développement de neuroplasticité. Premièrement, chez des rats s'auto administrant de la cocaïne de façon répétée et continue sous accès prolongé (LgA; 6 h/session), et non pas lors d'accès limité à la drogue (ShA; 1-2 h/session), il y des modifications au niveau des récepteurs

dopaminergiques et glutamatergiques. Par exemple, les rats-LgA, mais non les rats-ShA, ont une diminution de l'expression de l'ARN messager du récepteur dopaminergique D2 dans les cortex préfrontal médian et orbital, et de la protéine dans le cortex préfrontal médian. Une baisse significative des niveaux de la protéine des sous-unités GluA1 et GluA2 du récepteur glutamatergique AMPA dans le cortex préfrontal dorsomédian a aussi été signalée suite à l'administration de drogue en LgA, mais non en ShA (Sun et al., 2013). De plus, l'auto administration de la cocaïne chez les rats-LgA, mais non chez les rats-ShA, induit l'augmentation des niveaux de protéine des sous-unités Homer1b/c, NR2a et NR2b du récepteur glutamatergique NMDA dans le cortex préfrontal médian, des changements qui pour certaines sous-unités persistent jusqu'à 60 jours après le retrait de la drogue (Ben-Shahar et al., 2009). Des neuroadaptations structurelles ont aussi été reportées après un mois d'abstinence, où une augmentation dans la densité dendritique des épines des neurones épineux moyens a été observée dans le *core* du noyau accumbens seulement chez les rats-LgA (Ferrario et al., 2005). Finalement, l'auto administration de la cocaïne peut causer des changements ou des débalancements dans la plasticité synaptique normale du cerveau, qui a comme but de contrôler le bon fonctionnement des circuits neuronaux et leur communication adéquate, afin de permettre l'adaptation de nos comportements aux changements continuels de l'environnement [voir (Malenka et Bear, 2004)]. Ainsi, la consommation de grandes quantités de cocaïne de manière chronique et répétée (pendant 40-50 sessions) engendre des déficiences au niveau de la dépression à long terme (long-term depression; LTD) dans le noyau accumbens (Kasanetz et al., 2010), ainsi que la suppression de la LTD médiée par le récepteur glutamatergique 2/3 et l'augmentation des ratios des

récepteurs AMPA/NMDA dans le cortex prélimbique (Kasanetz et al., 2013). Ainsi, des changements persistants dans des structures qui sont impliquées dans la motivation et la récompense pourraient faciliter la transition vers la toxicomanie.

13.2 L'administration d'infusions rapides de cocaïne induit de la neuroplasticité dans les régions corticostriatales du cerveau

L'arrivée rapide de la cocaïne à ses sites d'action dans le cerveau favorise aussi le développement de neuroplasticité dans plusieurs régions corticostriatales impliquées dans la toxicomanie. Premièrement, l'infusion rapide de la cocaïne par i.v. (infusée en 5 versus 100 s), augmente l'expression des gènes précoces c-*fos* (Ferrario et al., 2008; Samaha et al., 2004) et *arc* (Samaha et al., 2004), qui sont des marqueurs d'activité neuronale, dans plusieurs régions corticostriatales. De plus, des infusions rapides de cocaïne (infusées en 4 versus 64 s) produisent des changements dans l'activité métabolique productrice de chaleur au niveau du noyau accumbens et de l'aire tegmentale ventrale (Brown et Kiyatkin, 2005). Similairement, l'administration aiguë de cocaïne par voie intraveineuse (voie rapide) induit plus d'utilisation de glucose, un signe d'activation neuronale, dans le système méso-corticolimbique comparativement à la voie intrapéritonéale [voie lente; (Porrino, 1993)]. Aussi, l'accès intermittent à la cocaïne infusée rapidement (en 5 versus 90 secondes) augmente le fonctionnement des récepteurs métabotropes glutamatergiques de la famille 2/3 dans le cortex prélimbique et le noyau accumbens (Allain et al., 2017). D'autre part, l'activation de ces récepteurs avec l'agoniste LY379268 diminue la motivation pour la cocaïne mesurée sous ratio progressif spécifiquement chez les rats qui avaient consommé des infusions rapides et

qui avaient montré une motivation pour la cocaïne supérieure à celle des rats consommant des infusions plus lentes (Allain et al., 2017). Les neuroadaptations énumérées ci-dessus, se manifestent dans des animaux qui montrent des comportements qui sont caractéristiques de la toxicomanie, ce qui suggère que la vitesse d'infusion est un facteur important dans la vulnérabilité à la toxicomanie.

13.3 L'auto administration d'infusions intermittentes de cocaïne induit de la neuroplasticité dans différentes régions du cerveau.

Le modèle animal de consommation de drogue par IntA est relativement nouveau, donc il n'y a qu'une quantité limitée d'études qui reportent des altérations dans le cerveau à la suite de la consommation de cocaïne en IntA. Par contre, une étude importante par Calipari et al. (2013) suggère que la fréquence de consommation (IntA ou LgA) modifie la régulation du transporteur de la dopamine. Leur étude propose que la consommation continue de drogue (LgA) produit une tolérance aux effets inhibiteurs de la cocaïne sur le transporteur, tandis que l'accès intermittent induit une sensibilisation aux effets de la cocaïne, ce qui augmenterait la capacité de la drogue à être plus renforçatrice. Une étude plus récente implique la consommation IntA dans l'induction de neuroplasticité dans le système orexine hypothalamique, une région impliquée dans la récompense et la motivation. Les rats-IntA de cette étude, qui ont aussi éprouvé plusieurs symptômes caractéristiques de la toxicomanie, ont montré une augmentation dans la densité et l'activité des neurones qui expriment l'orexine dans l'hypothalamus latéral lorsqu'ils sont comparés aux rats LgA et ShA. De plus, cette neuroplasticité a persisté pendant au moins 6 mois (James et al., 2018).

13.4 Qu'est-ce que nos résultats ajoutent à cette littérature?

Malgré la grande diversité de neuroadaptations induites par la consommation de quantités importantes de cocaïne, de nombreuses études proposent que la consommation excessive de drogue ne soit pas nécessaire [(Allain et al., 2018; Allain et al., 2017; Allain et Samaha, in press; Calipari et al., 2013; Kawa et al., 2016; Singer et al., 2018; Zimmer et al., 2012) et révisé dans (Allain et al., 2015)]. Ces études montrent qu'une quantité limitée de drogue consommée de façon intermittente peut faciliter le développement de comportements associés à la toxicomanie, incluant l'augmentation de la consommation, de la motivation et de la vulnérabilité à la rechute. Nos données sont en accord avec cette littérature. Dans l'article 3, les animaux du groupe plus à risque pour la toxicomanie (accès intermittent à des infusions rapides de drogue; IntA-5s) ont consommé moins que la moitié de la quantité totale prise par le groupe qui a eu des infusions lentes en continu (LgA-90s). Néanmoins, le groupe IntA-5s a montré une motivation plus grande pour la drogue sous ratio progressif. De plus, ils ont exprimé de manière plus importante l'ARN messager de c-*fos* dans les cortex orbitofrontal et prélimbique, et le striatum dorsal. Au contraire, les rats LgA-90s n'ont montré aucune différence dans la consommation ou de motivation excessive pour la drogue, malgré les grandes quantités de cocaïne consommées dans le passé. Également, nos données montrent que la vitesse d'infusion induit aussi de la neuroplasticité qui favorise potentiellement des comportements caractéristiques de la toxicomanie. Dans l'article 2, nous montrons que chez un modèle animal qui montre une motivation excessive pour la drogue, les infusions rapides de cocaïne (en 5 versus 90 secondes) modulent

l'expression du facteur de croissance BDNF et de son récepteur TrkB dans plusieurs structures corticostriatales, dont le cortex orbitofrontal et le striatum dorsal. Ainsi, chez des rats qui montrent une motivation excessive pour la drogue, nous pouvons proposer que la neuroplasticité induite par la vitesse d'infusion pourrait en être la cause [voir aussi (Allain et al., 2017; Bouayad-Gervais et al., 2014; Minogianis et al., 2013)]. Similairement, la vitesse d'infusion induirait potentiellement des neuroadaptations qui favoriseraient le développement d'autres comportements, dont l'augmentation de la consommation (Bouayad-Gervais et al., 2014; Minogianis et al., 2013; Wakabayashi et al., 2010) et le risque de rétablissement de la recherche et la prise de drogue après une période de sevrage (Wakabayashi et al., 2010).

13.5 Quel est le mécanisme?

Les changements associés à la sensibilisation envers les effets de la drogue sont nécessaires au développement de la toxicomanie (Ferrario et al., 2005; Robinson et Berridge, 1993). Ces changements peuvent être au niveau neurobiologique, mais peuvent aussi affecter le comportement. En effet, les circuits qui contrôlent les effets psychomoteurs de cocaïne, modulent aussi les propriétés motivationnelles incitatives (Wise et Bozarth, 1987). De nombreuses études portant sur les effets de la vitesse d'infusion de la cocaïne montrent le développement de la sensibilisation psychomotrice (Allain et al., 2017; Samaha et al., 2002; Samaha et al., 2004). L'accès intermittent à la drogue la promeut aussi (Allain et al., 2018; Allain et al., 2017; Allain et Samaha, 2018; Kawa et al., 2016). Au contraire, les études où la cocaïne est disponible en continue, le phénomène de sensibilisation psychomotrice est généralement absent [(Ben-Shahar et

al., 2004b; Knackstedt et Kalivas, 2007), mais pas dans (Ferrario et al., 2005)]. Ces données sont en accord avec Calipari et al. (2013), qui proposent que l'accès intermittent induise la sensibilisation du transporteur de la dopamine aux effets inhibiteurs de la cocaïne, tandis que l'accès continu (LgA) induit la tolérance.

En effet, la consommation chronique de drogues d'abus favorise souvent le développement de la tolérance, qui consiste en une réponse réduite à la drogue suite à une exposition répétée (Meyer et Quenzer, 2005). Ainsi, l'auto administration répétée de cocaïne caractéristique de l'accès en continu (LgA) peut potentiellement engendrer le développement de tolérance aux effets renforçateurs de la cocaïne. Ceci expliquerait que les animaux augmentent leur consommation dans le but d'atteindre et de maintenir des niveaux élevés de drogue (Ahmed, 2012; Ahmed et Koob, 1998; Ahmed, Kenny, Koob et Markou, 2002). Au contraire, l'administration d'infusions rapides et/ou intermittentes peut induire la sensibilisation aux propriétés incitatives et motivationnelles de la drogue. L'exposition aux substances d'abus peut rendre les circuits neuronaux impliqués dans l'attribution de pouvoir d'attraction hypersensibles aux drogues et aux stimuli qui y sont associés. Ainsi, la sensibilisation de la saillance incitative favorise la transition vers la recherche et la prise de drogue compulsives (Robinson et Berridge, 1993). Ceci indique que les infusions rapides de cocaïne et les fluctuations de la cocaïne dans le cerveau peuvent engendrer une plus grande sensibilisation aux propriétés incitatives et motivationnelles de la cocaïne [révisé dans (Allain et al., 2015)]. Un indice de cette sensibilisation est l'augmentation de la motivation incitative pour la drogue lorsque mesurée sous ratio progressif (Allain et al., 2018; Allain et al., 2017; Bouayad-Gervais et al., 2014; Minogianis et al., 2013) ou par des indicateurs économiques du comportement

(Kawa et al., 2016; Zimmer et al., 2012) chez des animaux qui s'auto administrent de la cocaïne infusée rapidement et/ou en accès intermittent.

En résumé, les infusions rapides de drogue ainsi que l'intermittence de la consommation induisent de la neuroplasticité qui facilite le développement de comportements caractéristiques de la toxicomanie dans nos modèles animaux. De plus, la consommation de grandes quantités de drogue, malgré un facteur pharmacocinétique important, n'est pas nécessaire à la production de changements neurobiologiques pertinents à la toxicomanie.

CHAPITRE 14 – Rôles du cortex orbitofrontal et du striatum dorsal dans la motivation pour la cocaïne

Nos résultats montrent que les infusions rapides de cocaïne, ainsi que les fluctuations de cocaïne dans le cerveau provoquées par un accès intermittent à la drogue, produisent de la neuroplasticité dans de nombreuses structures corticostriatales. Ces données sont en accord avec la littérature qui montre que la neuroplasticité induite par la vitesse d'infusion (Allain et al., 2017; Ferrario et al., 2008; Samaha et al., 2004) et le patron de consommation (Calipari et al., 2013) altère les circuits corticostriataux. De plus, ces changements sont observés chez des rats qui ont préalablement montré une motivation excessive pour la cocaïne (Allain et al., 2017; Bouayad-Gervais et al., 2014) ou une sensibilisation psychomotrice aux effets de la drogue (Allain et al., 2017). De plus, les effets de la cocaïne sur l'expression des gènes précoces comme le c-*fos* dans les structures corticostriatales du cerveau impliquent des mécanismes dopaminergiques (Berretta, Robertson et Graybiel, 1992; Young et al., 1991) et glutamatergiques (Berretta et al., 1992; Torres et Rivier, 1993). Ensemble, ces données suggèrent un rôle potentiel du circuit corticostriatal dans le développement de la toxicomanie.

Les données des articles 2 et 3 de cette thèse (chapitres 8-9) ajoutent à cette littérature, en montrant que l'administration d'infusions rapides de cocaïne et/ou de la consommation intermittente de drogue induisent des neuroadaptations dans le cortex orbitofrontal (OFC) et le striatum dorsal (DS), identifiées par l'expression du facteur de croissance BDNF ou le facteur de transcription c-*fos*. De plus, les changements dans l'expression de l'ARN messager de c-*fos* dans le cortex orbitofrontal sont fortement

corrélés avec les changements produits dans le striatum dorsal (Figure 7a – article 3). Les données des articles 3 et 4 nous ont permis de proposer que le circuit OFC-DS serait potentiellement impliqué dans la motivation excessive pour la cocaïne et la toxicomanie et explique la raison pour laquelle nous avons déconnecté ce circuit dans l'article 4. La littérature chez l'humain soutient cette hypothèse, car les études d'imagerie cérébrale rapportent des altérations dans ces structures suite à la consommation répétée de cocaïne (Bolla et al., 2003; Franklin et al., 2002; Garavan et al., 2000; Goldstein et Volkow, 2002; Volkow et Fowler, 2000; Volkow, Mullani, Gould, Adler et Krajewski, 1988; Volkow et al., 2006; Wang et al., 1999).

Notre étude sur la déconnection du circuit OFC-DS suggère que le circuit entre ces deux structures est important dans la motivation pour la cocaïne, et ceci bien que nos études portant sur l'expression de l'ARNm de BDNF et de c-*fos* n'ont pas directement examinées les changements dans l'expression de ces gènes immédiatement après les sessions à ratio progressif, afin de corréler ces changements avec la motivation pour la drogue. La microinfusion de baclofen/muscimol ou de salin dans l'OFC, le DS ou l'OFC et le DS controlatéral diminue la motivation pour la cocaïne versus la motivation basale déterminée en absence de traitement. Toutefois, infuser du baclofen/muscimol dans le DS diminue la motivation pour la drogue encore plus et cette fois-ci comparativement à la motivation basale et au traitement de salin. De plus, ces données laissent croire que la microinfusion de salin dans ce circuit ou dans l'OFC et le DS individuellement peut influencer le comportement.

Malgré les effets prononcés sur la motivation pour la cocaïne à la suite des microinfusions OFC et/ou DS, il faut mentionner que cette étude compte certaines limites.

Premièrement, le traitement au baclofen/muscimol et au salin a diminué aussi la locomotion, ce qui peut potentiellement expliquer la baisse dans le nombre d'infusions auto administrées sous ratio progressif. Cependant, nous ne croyons pas que c'est le cas, car le traitement baclofen/muscimol administré en concentrations et volumes similaires à ceux utilisés dans cette étude n'influence pas la locomotion ni chez des animaux naïfs à la cocaïne [étude 1 de l'article 4 (malgré un nombre limité d'animaux) et (McFarland et Kalivas, 2001)], ni chez des animaux qui ont été chroniquement exposés à la drogue (Fuchs et al., 2006; Fuchs et al., 2004; McFarland et Kalivas, 2001). De plus, la microinfusion de baclofen/muscimol dans l'OFC ou le DS diminue la vulnérabilité au rétablissement de recherche et prise de cocaïne induite par la drogue elle-même ou des indices associés à celle-ci (Fuchs et al., 2006; Fuchs et al., 2004; Gabriele et See, 2011). Aussi, ce traitement atténue la poursuite opérante d'indices associés à la cocaïne (Di Ciano, Robbins et Everitt, 2008) sans affecter la réponse opérante pour la nourriture (Bianchi et al., 2018; Brebner, Phelan et Roberts, 2000; Fanous et al., 2012), ce qui suggère que l'inactivation de l'OFC et du DS ne diminue pas la motivation via un effet moteur. Au contraire, la cocaïne est un psychostimulant, donc une baisse de la motivation, induirait une diminution du nombre d'infusions auto administrées et ainsi provoquerait une locomotion atténuée comparativement au niveau basal. Une deuxième limite est le fait que les microinfusions de salin ont aussi diminué la motivation pour la cocaïne ainsi que la locomotion comparativement au niveau basal. Il est donc possible que le salin modifie l'activité neuronale de ces structures aussi, même si la microinfusion de salin chez des animaux naïfs ne modifie pas la locomotion (étude 1 de l'article 4). Les microinfusions de salin sont le principal contrôle des études d'inactivation (Bianchi et al.,

2018; Bossert et al., 2012; Fanous et al., 2012; Forget, Pushparaj et Le Foll, 2010; McFarland et Kalivas, 2001) qui omettent l'utilisation d'un groupe contrôle sans traitement pour vérifier les effets des microinfusions sur le cerveau. Dans notre cas, des solutions plus isotoniques tels que le PBS ou le liquide céphalorachidien artificiel auraient pu être plus avantageux. Finalement, malgré que nous ayons ciblé l'OFC et le DS, nous ne savons pas l'étendue de l'inhibition. En effet, notre étude aurait bénéficié de microinfusions de baclofen/muscimol dans une région anatomique contrôle, tel que le cortex somatosensoriel sus-jacent (Arguello et al., 2017; Fuchs et al., 2006). De plus, l'utilisation d'une technique plus précise, telle qu'une inhibition chimio-génétique de ces structures pourrait potentiellement produire des résultats plus propres [similairement à (Gremel et Costa, 2013)].

Malgré ces limites, nos données sont les premières à montrer que le circuit OFC-DS, ainsi que chacune de ces structures individuellement, seraient possiblement impliqués dans la motivation pour la drogue. Le ciblage pharmacologique de ce circuit aurait donc un potentiel thérapeutique. En effet, le développement de la toxicomanie consiste en la perte du contrôle préfrontal et le recrutement progressif des circuits striataux impliqués dans la formation des habitudes (Graybiel, 2008; Zapata, Minney et Shippenberg, 2010). Ceci dit, des traitements pharmacologiques qui préviennent la neuroplasticité dans ce circuit pourraient potentiellement servir à inhiber cette transition (Kalivas et O'Brien, 2008).

CHAPITRE 15 – IMPLICATIONS DES RÉSULTATS

15.1 Développement de meilleurs modèles animaux pour l'étude de la toxicomanie

Comme tout « modèle », le modèle d'auto administration chez le rat pour l'étude de la toxicomanie n'est pas parfait. Nombreuses différences physiologiques, anatomiques et psychologiques limitent l'interprétation et la traduction de nos résultats chez l'humain. En effet, nous ne pouvons pas observer tous les symptômes retrouvés chez l'humain, De plus, il est impossible d'étudier certains effets subjectifs induits par la cocaïne chez le rongeur, tels que l'euphorie et la stimulation de l'humeur. Cependant, nos résultats suggèrent que la vitesse d'administration de la drogue et la fréquence de la consommation sont deux facteurs pharmacocinétiques qui sont importants dans le développement de symptômes caractéristiques de la toxicomanie, et nier ces facteurs pharmacocinétiques dans la conception de modèles pour l'étude de la toxicomanie serait désavantageux [revisé dans (Allain et al., 2015)].

15.2 Le développement de thérapies potentielles pour prévenir et traiter les troubles liés à la toxicomanie

15.2.1 L'utilisation de facteurs pharmacocinétiques dans le traitement des troubles liés à l'usage de la cocaïne

Les voies d'administration qui mènent la cocaïne rapidement au cerveau (voie intraveineuse ou inhalation) favorisent le développement de la toxicomanie comparativement aux voies plus lentes [voie intranasale; (Hatsukami et Fischman, 1996; Jones, 1990)]. Ceci suggère que les formulations de drogue ou les voies d'administration qui ralentissent l'arrivée de la drogue au cerveau diminuent le risque que la drogue soit

abusée et peuvent servir comme traitement. Nombreux traitements fonctionnent en contrôlant la vitesse à laquelle la drogue arrive au cerveau, tels que le timbre transdermique pour la cessation de fumer des cigarettes (Fiore, Smith, Jorenby et Baker, 1994) et la méthadone pour traiter les personnes ayant un trouble lié à l'usage de l'héroïne (Ling, Rawson et Compton, 1994). Depuis quelques années, modafinil (Provigil®), un faible psychostimulant approuvé par la FDA pour la narcolepsie et le traitement de troubles cognitifs est considéré comme agent prometteur pour la toxicomanie aux psychostimulants (Mereu, Bonci, Newman et Tanda, 2013; Zhang et al., 2017). Cette molécule est aussi un inhibiteur du transporteur de la dopamine (Madras et al., 2006). Des études de microdialyse chez la souris montrent que l'augmentation des concentrations extracellulaires de dopamine est moins importante avec cette molécule, qui possède aussi une durée d'action plus longue que la cocaïne (Loland et al., 2012). Ces caractéristiques pourraient rendre le modafinil promettante comme thérapie de substitution. Chez l'humain, sa consommation par voie orale permet aussi le ralentissement de l'arrivée de la drogue au cerveau, provoquant l'apparition plus lente des effets psychostimulants et ainsi diminuant son potentiel d'abus (Newman, Negus, Lozama, Prisinzano et Mello, 2010). L'utilisation d'un psychostimulant comme modafinil pourrait donc être prometteur.

En accord avec la littérature, nos résultats montrent que la consommation intermittente de la cocaïne crée des hausses et des baisses de cocaïne dans le cerveau des rats, qui favorisent le développement d'une motivation excessive pour la drogue (Allain et al., 2018; Allain et al., 2017; Allain et Samaha, 2018; Zimmer et al., 2012). La sensibilisation du transporteur de la dopamine par ces fluctuations de drogue dans le

cerveau serait potentiellement le mécanisme impliqué (Calipari et al., 2013). Une étude récente de notre laboratoire montre qu'un traitement continue à l'amphétamine via minipompe lors de sessions d'auto administration de cocaïne en IntA diminue la motivation pour la cocaïne et le risque du rétablissement du comportement de recherche et de prise de drogue induite par la cocaïne subséquente [abstract; (Allain et Samaha, 2017)]. Ceci suggère que l'atténuation des fluctuations de cocaïne dans le cerveau lors de l'IntA par l'augmentation du tonus monoaminergique grâce au traitement d'amphétamine pourrait diminuer le risque du développement de comportements caractéristiques à la toxicomanie. En effet, une étude clinique montre que le traitement d'entretien à l'amphétamine produirait des résultats similaires chez l'humain. L'entretien à la D-amphétamine diminue les effets renforçants de la cocaïne prise par la voie intranasale durant une procédure de choix discret (Rush, Stoops, Sevak et Hays, 2010). De plus, l'administration d'agonistes indirects, tels la levadopa (L-DOPA), fonctionneraient aussi à augmenter la libération de dopamine induite par la cocaïne ce qui réduirait la recherche et la prise de drogue chez le rat (Antinori et al., 2018). Les thérapies de remplacement d'agonistes sont déjà utilisées pour les opioides et la nicotine (Henningfield, 1995; Ling et al., 1994), donc elles pourraient aussi servir aux traitements de troubles liés à la cocaïne.

D'autres approches pharmacocinétiques sont aussi en développement pour prévenir et/ou traiter les troubles liés à l'usage de la cocaïne. Ces thérapies ont comme but d'agir directement sur la drogue, de manière à altérer sa distribution, bloquer ses actions ou accélerer son métabolisme et élimination (Zheng et Zhan, 2012). Par exemple, l'administration d'enzymes qui accélèrent le métabolisme de la cocaïne préviendrait

l'entrée de la drogue dans le cerveau et éviterait les effets subséquents de la drogue. Par exemple, la butyrylcholinesterase (BChe) plasmatique humaine, une des principales enzymes impliquées dans le métabolisme de la cocaïne en ecgonine méthyl ester (EME), a déjà été considérée dans le traitement de la toxicité liée à l'usage de la cocaïne (Brimijoin et al., 2008; Gorelick, 1997) et diminue le rétablissement du comportement de recherche et de prise de drogue induit par la cocaïne chez des rats qui avaient chroniquement auto administré la cocaïne (Brimijoin et al., 2008). Un deuxième exemple est le développement d'un vaccin contre la cocaïne qui préviendrait le passage de cette drogue à travers la barrière hémato-encéphalique. Les études précliniques montrent que l'administration passive d'anticorps monoclonaux sélectifs pour la cocaïne via un vaccin réduit la distribution de la drogue dans le cerveau et diminue l'auto administration de la cocaïne (Fox et al., 1996; Kantak et al., 2000). Chez l'humain, les vaccins anti-cocaïne semblent atténuer les effets euphoriques de la drogue et ainsi diminuent la consommation (Haney, Gunderson, Jiang, Collins et Foltin, 2010; Martell, Mitchell, Poling, Gonsai et Kosten, 2005).

15.2.2 Utilisation d'agents qui modulent la transmission glutamatergique pour le traitement de troubles liés à la consommation de cocaïne

La signalisation glutamatergique anormale dans les voies corticostriatales est un exemple de neuroplasticité induite par l'administration chronique de drogues qui peut faciliter le développement de la toxicomanie (Kalivas, Volkow et Seamans, 2005). Dans notre cas, nous avions trouvé des altérations produites par l'administration d'infusions rapides et intermittentes de cocaïne, qui impliqueraient le circuit OFC-DS et qui seraient potentiellement associées avec une motivation excessive pour la drogue. Ainsi, le fait de

contrer cette neuroplasticité glutamatergique pourrait être une approche intéressante pour traiter les troubles liés à l'usage de la cocaïne. En effet, plusieurs études précliniques et cliniques examinent les effets pharmacologiques d'agents qui altèrent la transmission glutamatergique pour le traitement de la toxicomanie. Par exemple, moduler la libération de glutamate synaptique lors de la recherche et la prise de drogue pourrait réduire le risque de rechute. L'anticonvulsivant topiramate, un faible antagoniste des récepteurs AMPA, diminuerait la vulnérabilité à la rechute chez les individus qui souffrent de troubles liés à la cocaïne (Kampman et al., 2004) et à l'alcool (Krupitsky et al., 2007; Rustembegovic, Sofic et Kroyer, 2002).

Un deuxième mécanisme par lequel l'administration répétée de la cocaïne induit de la neuroplasticité au niveau de la transmission glutamatergique est en diminuant l'échange cystine-glutamate par l'échangeur x_c-, ce qui modifie la régulation des voies corticostriatales par le glutamate extrasynaptique (Madayag et al., 2007). Ceci induit la baisse des niveaux basaux de glutamate dans le noyau accumbens et provoque l'augmentation de glutamate induit par la cocaïne, une forme de neuroplasticité qui persiste pendant au moins trois semaines (Baker et al., 2003). Cependant, ces effets sont empêchés par la N-acétylcystéine, une prodrogue de la L-cyctéine, qui bloque le glutamate induit par la cocaïne dans le noyau accumbens (Baker et al., 2003; Madayag et al., 2007) et prévient le rétablissement de la recherche de drogue chez le rat (Baker et al., 2003). Des études cliniques préliminaires rapportent une diminution des symptômes de rechute et de *craving* chez des humains ayant un trouble lié à l'usage de cette drogue, suggérant son application thérapeutique éventuelle (Amen et al., 2010; LaRowe et al., 2006; LaRowe et al., 2007).

CHAPITRE 16 – PERSPECTIVES

Ces travaux suggèrent que la pharmacocinétique de la drogue est importante dans le développement de la toxicomanie. L'auto administration répétée d'infusions rapides et/ou intermittentes augmentent la motivation pour la cocaïne, un symptôme caractéristique de la toxicomanie. De plus, chez ces animaux, nous trouvons des changements dans la régulation des gènes BDNF et c-*fos* induits dans le cortex orbitofrontal et le striatum dorsal. La déconnection de ce circuit diminue la motivation pour la cocaïne, ce qui le rend une cible thérapeutique potentielle. Cependant, nous avons encore plusieurs questions auxquelles il faut répondre.

16.1 Effets potentiels de la vitesse d'infusion sur la Cmax et le Tmax d'animaux chroniquement exposés à la cocaïne

Dans l'article 1, nous avons mesuré les différences dans le Cmax et le Tmax suite à une infusion intraveineuse de cocaïne dans des animaux naïfs. Cependant, nous n'avions pas examiné les effets de la vitesse d'infusion chez des animaux exposés chroniquement à la drogue. La toxicomanie est une maladie chronique, donc les effets neurobiologiques qui se développent nécessitent une exposition répétée à la drogue. Le travail de Pan et al. (1991) suggère que les niveaux de cocaïne dans le cerveau ne varient pas significativement suite à l'administration aigue ou répétée de cocaïne par voie intraveineuse. Pourtant, d'autres études rapportent des disparités. Par exemple, la capacité de la cocaïne à bloquer le transporteur de la dopamine augmente avec des expositions répétées à la drogue (Brodnik, Ferris, Jones et España, 2017). Similairement,

les niveaux de sérotonine sont plus élevés dans le noyau accumbens et l'aire tegmentale ventrale suite à l'administration répétée de la drogue (Parsons et Justice, 1993), tandis que les niveaux extracellulaires des métabolites dopaminergiques (DOPAC et HVA) et sérotoninergiques (5-HIAA) sont réduits dans le noyau accumbens (Hurd et Ungerstedt, 1989; Kalivas et Duffy, 1990). Finalement, l'administration chronique de cocaïne augmente la capacité d'une prochaine dose de cocaïne à augmenter les niveaux de glutamate dans le noyau accumbens et l'aire tegmentale ventral (Kalivas et Duffy, 1998; Miguéns et al., 2008; Pierce et al., 1996). Dans notre cas, le traitement aigu à la cocaïne n'a pas induit des changements dans la sérotonine, le glutamate et les métabolites des différentes neurotransmetteurs. Donc, ces données offrent plusieurs possibilités de mécanismes sur lesquels la cocaïne pourrait potentiellement moduler et favoriser le développement de la toxicomanie. Une deuxième étude de microdialyse *in vivo* où la collecte d'échantillons se fasse chez des animaux ayant été chroniquement exposés à la drogue pourrait identifier ces différences potentielles.

16.2 Identification du rôle potentiel du circuit cortex prélimbique et striatum dorsal (PrL-DS) dans la motivation pour la cocaïne

Les résultats de l'article 3 montrent que l'expression de l'ARN messager de c-*fos* est aussi plus élevé dans le cortex prélimbique (PrL) dans notre modèle de rat pertinent à la toxicomanie, et que ces changements sont corrélés à ceux induits dans le striatum dorsal (Figure 7d – article 3). Ainsi, le circuit PrL-DS pourrait aussi être impliqué dans la motivation pour la drogue. Comme l'OFC, le PrL projette aussi au DS (Berendse et al., 1992; Donoghue et Herkenham, 1986; Gabbott, Warner, Jays, Salway et Busby, 2005)

et ces projections sont glutamatergiques (Berendse et al., 1992; Carter, 1982; Gerfen, 1992; McGeorge et Faull, 1989). Le PrL est impliqué dans les processus de prise de décisions, d'inhibition, de la mémoire de travail et des actions dirigées vers un but (Balleine et Dickinson, 1998; Corbit et Balleine, 2003). La littérature suggère que cette structure est importante dans le développement de la toxicomanie. En effet, les rats s'auto administrent la cocaïne directement dans le cortex préfrontal médian (qui inclut le PrL) (Goeders et Smith, 1983; Goeders et Smith, 1993). De plus, l'inactivation du PrL diminue le risque du rétablissement de recherche et de prise de drogue induite par la cocaïne (Capriles, Rodaros, Sorge et Stewart, 2003; McFarland et Kalivas, 2001), aux indices associés à la drogue (McLaughlin et See, 2003) ou au stress (Capriles et al., 2003; McFarland, Davidge, Lapish et Kalivas, 2004). À l'opposé, l'administration de la cocaïne directement dans le PrL restitue le rétablissement de prise de cocaïne initialement inhibé par l'administration de l'antagoniste dopaminergique flupenthixol (Park et al., 2002). L'inactivation du DS diminue aussi la recherche pour la cocaïne suite à 14 jours d'abstinence. Il serait donc intéressant de déterminer si ce circuit joue un rôle potentiel dans la motivation excessive pour la drogue étudiée dans cette thèse, mais aussi dans le risque de rechute.

16.3 Étudier les effets de microinfusions de baclofen/muscimol et de salin sur la locomotion chez des rats naïfs et expérimentés à la cocaïne

Notre étude portant sur les effets locomoteurs de microinfusions de baclofen/muscimol et de salin dans l'OFC et le DS ne comporte que quatre animaux. Cette étude ne montre aucun effet des microinfusions sur l'activité locomotrice chez des

animaux naïfs comparativement au niveau basal de locomotion, et ceci peu importe le traitement. Cependant, il est possible qu'un nombre plus significatif d'animaux aurait pu mener à une conclusion différente. Il serait donc intéressant de comparer les effets de la microinfusion de baclofen/muscimol et de salin dans l'OFC et/ou le DS chez des animaux naïfs, mais aussi chez des animaux ayant une exposition chronique à la cocaïne. Ceci permettrait : 1) de vérifier les différences entre le baclofen/muscimol vs. le salin vs. la locomotion basale et 2) de voir si les microinfusions en général, potentiellement via les dommages mécaniques causés, diminuent la locomotion. Il serait aussi intéressant de tester d'autres véhicules plus isotoniques (Hombrebueno, Luo, Guo, Chen et Xu, 2014; Robinson, 1969), tels que le PBS ou l'aCSF (Arguello et al., 2017; Chefer, Wang et Shippenberg, 2011; Fuchs et al., 2004; Gabriele et See, 2010, 2011; Gipson et al., 2013; Peters, LaLumiere et Kalivas, 2008). Ceci apporterait des nouvelles informations importantes sur les effets du salin dans le cerveau et sur le comportement, une littérature qui a encore plusieurs lacunes.

CHAPITRE 17 – CONCLUSIONS

Nos résultats indiquent que la vitesse d'infusion rapide de la cocaïne et les fluctuations des niveaux de la cocaïne dans le cerveau produites par une consommation intermittente de la drogue provoquent des changements comportementaux qui sont caractéristiques à la toxicomanie, dont l'augmentation de la motivation pour la drogue. De plus, ces facteurs induisent de la neuroplasticité dans les circuits de la récompense et de la motivation, qui facilitent la transition vers ce trouble. L'identification de neuroadaptations dans l'OFC et le DS, ainsi qu'un rôle potentiel de ce circuit dans la motivation excessive pour la cocaïne, ajoutent à la grande littérature qui cartographie les circuits impliqués dans la toxicomanie. À travers les études de cette thèse, l'importance des facteurs pharmacocinétiques dans l'étude des mécanismes qui sous-tendent la toxicomanie est bien soulignée. Ainsi, la vitesse d'infusion et la fréquence de consommation peuvent prédire le risque de passer d'un usage récréatif vers la toxicomanie, et doivent être considérés lors de la conception de thérapies préventives et de traitements pour les troubles liés à l'usage des drogues.

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ANNEXE

HOW FAST AND HOW OFTEN: THE PHARMACOKINETICS OF DRUG USE ARE DECISIVE IN ADDICTION

Running Title: Pharmacokinetics in addiction

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ABSTRACT

How much, how often and how fast a drug reaches the brain determine the behavioural and neuroplastic changes associated with the addiction process. Despite the critical nature of these variables, the drug addiction field often ignores pharmacokinetic issues, which we argue can lead to false conclusions. First, we review the clinical data demonstrating the importance of the speed of drug onset and of intermittent patterns of drug intake in psychostimulant drug addiction. This is followed by a review of the preclinical literature demonstrating that pharmacokinetic variables play a decisive role in determining behavioural and neurobiological outcomes in animal models of addiction. This literature includes recent data highlighting the importance of intermittent, ‘spiking’ brain levels of drug in producing an increase in the motivation to take drug over time. Rapid drug onset and intermittent drug exposure both appear to push the addiction process forward most effectively. This has significant implications for refining animal models of addiction and for better understanding the neuroadaptations that are critical for the disorder.

Keywords
Drug addiction, Pharmacokinetics, Cocaine, Route of drug intake, Speed of drug delivery, Intermittent drug exposure.

Highlights

- Drug pharmacokinetics determine neurobehavioural changes linked to addiction
- The drug addiction field often ignores pharmacokinetic issues
- Rapid drug onset and intermittent use both facilitate the transition to addiction
- This has implications for refining animal models of the process of addiction

Introduction

Amongst people who use drugs, some keep control over their drug use while others develop addiction. What accounts for this differential vulnerability? Like any complex disease, "*the development of addiction depends on the interaction of agent, host and environment"* (O'Brien, 2008). With this reality, it becomes clear that to identify the brain changes that are critical to addiction, it is important that we understand how agent, host and environment each influence the response to drugs of abuse. The roles of the individual and of the environment are studied extensively. Much less attention has been paid to characteristics of the 'agent', such as drug pharmacokinetics. In the context of drug addiction, important pharmacokinetic parameters include how much drug gets to the brain (achieved dose), how fast drug levels rise in the brain (rate of drug onset) and how often they rise and fall (intermittency).

Thirty-five years ago, Robert Post emphasized the importance of the temporal dynamics of stimulation by drugs and other stimuli in '…*determining the direction and magnitude of adaptive response following repeated presentation*' (Post, 1980). When considering the response to a drug of abuse or any pharmacological agent for that matter, pharmacokinetics determine the pharmacodynamics. That is, pharmacokinetics govern the ability of drugs to interact with transporters and receptors, and to influence intra-cellular signalling cascades. The same drug can have markedly different—sometimes opposite—effects on brain and behaviour depending on whether it is injected intravenously (i.v.), or into the intraperitoneal (i.p.) cavity, administered through the skin, taken orally or given chronically in a subcutaneous (s.c.) osmotic minipump. This is because different routes of drug administration produce markedly different

pharmacokinetic profiles. This is why a drug that can produce addiction when smoked or injected can be used to treat addiction when given orally (methadone) or in a patch (nicotine). Similarly, amphetamine formulated to produce slowly rising and steady-state levels of drug in the brain is currently used as pharmacotherapy for cocaine addiction (Negus and Henningfield, 2015). Methadone, nicotine and amphetamine are striking examples from the clinical literature showing that manipulation of pharmacokinetic variables can fundamentally change the behavioural effects of drugs—rather than having abuse potential they might actually have therapeutic potential. As will be shown below, pharmacokinetics determine all drug effects that are relevant to addiction.

The influence of pharmacokinetics on the response to drugs of abuse remains largely unstudied, save for dose. This is in part because in the addiction field, it is widely assumed that the amount of drug exposure largely determines outcome (Ahmed, 2012; Benowitz and Henningfield, 1994; Jonkman et al., 2012). Some have even suggested the existence of a 'critical level' of drug exposure beyond which "*addiction-causing neuropathological processes could be set in motion*" (Ahmed, 2012). Drug pharmacokinetics are often regarded as secondary, simply a means to vary the amount of drug reaching the brain. This assumption is dangerous. Variation in the pharmacokinetic profile of a drug can produce different effects even when the same amount of drug reaches the brain. In other words, "how fast" and "how often" can be more important than "how much" in determining functional outcome. This principle is at the core of the present review. The general principles that will be discussed apply across drug classes. However, it is not feasible to review all drug classes. Opiates, alcohol and nicotine present their own specific pharmacokinetic issues and these deserve to be reviewed in depth. Here we will focus on

cocaine. We do so for three principle reasons. First, cocaine can be snorted, smoked or injected and these different routes result in very different pharmacokinetic profiles. Second, the clinical literature emphasizes the importance of pharmacokinetics in cocaine addiction (Hatsukami and Fischman, 1996). Third, in animal studies, cocaine is most often used as a prototypical drug of abuse to investigate the contributions of pharmacokinetics to drug response. Thus, here we review the evidence showing that in addition to drug dose, the speed of drug onset and the intermittency of drug exposure both determine brain and behaviour changes that are relevant to addiction, particularly cocaine addiction.

Drug pharmacokinetics influence drug addiction liability

The clinical data are clear; the faster drugs reach the brain, the more likely it is that addiction will develop. Drugs of abuse are often taken by the i.v., smoked, oral or intranasal routes. The route of drug administration determines both how fast (rise time) and how much (area under the curve) drug reaches the general circulation and ultimately, the brain (see Figure 1). However, rise time can be more predictive of addiction liability than other parameters such as area under the curve. Smoking and injecting are the two fastest and most efficient methods of getting drug into the bloodstream (Cone, 1995, 1998; Evans et al., 1996). Peak venous plasma levels of cocaine or radiolabelled cocaine are reached in 2-5 min following i.v. injection or smoking, and 30-60 min after intranasal administration [(Cone, 1998; Javaid et al., 1978; Jeffcoat et al., 1989; Van Dyke et al., 1976). Of note, peak *arterial* plasma levels of drug are reached earlier than peak venous plasma levels, for example time to peak arterial cocaine levels after smoking or intravenous injection is 15 seconds (Evans et al., 1996)]. The oral and intranasal routes

result in slower absorption rates, which in turn result in lower blood concentrations, over a longer period of time (Cone, 1995, 1998). Peak plasma levels of drug correlate well in time with the subjective effects of drugs. For a drug like cocaine, for example, the maximum self-reported 'high' is reached 1-5 min after intravenous injection, and 15-20 min after intranasal administration (Evans et al., 1996; Javaid et al., 1978).

Addiction is more likely and more severe in individuals who take drugs via rapid routes of drug delivery. For instance, addiction to cocaine, amphetamine, methamphetamine, nicotine or heroin is more probable in people who consume these drugs via smoking or i.v. injection than in individuals who use slower routes of drug administration [e.g., the intranasal or transdermal routes (Barrio et al., 2001; Budney et al., 1993; Carpenter et al., 1998; Ferri and Gossop, 1999; Gossop et al., 1992, 1994; Hatsukami and Fischman, 1996; Hughes, 1989; Rawson et al., 2007; Van Dyke and Byck, 1982; Volkow and Swanson, 2003; Winger et al., 1992)]. Compared to intranasal drug users, individuals who smoke or inject drugs i.v. also use drugs more frequently, for a longer time, spend more money on drugs, report a greater loss of control over drug taking and are more likely to overdose (Barrio et al., 2001; Carpenter et al., 1998; Ferri and Gossop, 1999; Gossop et al., 1992, 1994; Hatsukami and Fischman, 1996; Hughes, 1989; Rawson et al., 2007; Van Dyke and Byck, 1982; Volkow and Swanson, 2003; Winger et al., 1992). Finally, users who smoke or inject drugs have more health-threatening patterns of drug use (Carpenter et al., 1998; Ferri and Gossop, 1999; Gossop et al., 1992; Hatsukami and Fischman, 1996; Hughes, 1989; Rawson et al., 2007; Winger et al., 1992), and they suffer more harm, including contracting blood borne viruses such as HIV and hepatitis C, experiencing drug-induced paranoid psychosis, and dying of an overdose (Brady et al., 1991; Gossop et al.,

1992; Hatsukami and Fischman, 1996; Roncero et al., 2012; Winger et al., 1992).

The oral route is much slower and therefore carries less addiction liability. However, the risk exists and drug manufacturers make efforts to reduce the speed of absorption of certain potentially addictive medications. For example, amphetamine (Adderall), methylphenidate (Ritalin) and phenmetrazine (Preludin) are being designed to reduce the speed of brain uptake and the ability of users to smoke or inject the drugs (Connor and Steingard, 2004; Spencer et al., 2006).

The intermittency of drug use is also a recurrent theme in the addiction literature. Systematic research on the temporal pattern of drug use in humans is scarce. There are however anecdotal reports and general agreement that intermittency of intake is a defining feature of psychostimulant drug addiction. There appear to be two, possibly independent intermittency phenomena. The first is the 'abstinence period', which can last for days or weeks. The second is the inter-dosing interval within a bout of intoxication. Drug users adopt intermittent patterns of use, and this is seen both within and between a bout of intoxication. The notion of intermittent drug use in addicts, particularly for drugs like cocaine, is emphasized in classic writings on addiction (Gawin and Kleber, 1986; O'Brien, 2001), and is supported by anecdotal reports and observational studies primarily involving experienced drug users. For example, Ward et al (Ward et al., 1997) state "*anecdotal reports from the majority of cocaine users in our laboratory indicate that binges may last for days, with intervals of heavy cocaine use separated by brief breaks in which the user hustles in order to get enough money to buy more cocaine".* Similarly, Cohen and Sas (Cohen and Sas, 1994) studied patterns of cocaine use in addicts in Amsterdam, and they report that sustained and high levels of use are "*rarely maintained*" and that "*Many users*

lace their cocaine use career with periods of abstention. Such periods may last from a week to several months." This suggests that cocaine addicts are unlikely to maintain continuously high brain levels of drug over extended periods. Addicts do engage in drug binges, where drug is taken at a high frequency during a bout lasting from hours to days. For instance, craving for cocaine is reported to be highest shortly after intake of the drug (Jaffe et al., 1989; O'Brien et al., 1992). This can favour the transition towards a binge pattern of administration and addiction (Gawin, 1991). However, even within a binge, the pattern of use appears to be intermittent. A recent study on this issue suggests that experienced users wait, on average, well over an hour between cocaine uses within a binge (Beveridge et al., 2012). Cocaine has a half-life of ~ 40 min (Javaid et al., 1983). This suggests that during a binge, blood levels of the drug are not maintained at continuously high levels but might rise and fall in a spiking pattern (Zimmer et al., 2012). This intermittent and spiking pattern of drug levels is thought to promote the transition to cocaine addiction (Zimmer et al., 2012). Similarly, smoking cigarettes is also thought to be particularly addictive because the puff-by-puff inhalation of cigarette smoke produces intermittent and fast-rising spikes in brain levels of nicotine (Russell and Feyerabend, 1978) and of other components of tobacco smoke thought to enhance the addictive properties of nicotine (Berlin and Anthenelli, 2001).

Preclinical studies show that drug pharmacokinetics determine brain and behaviour effects relevant to addiction

Clinical studies point to the very important role of pharmacokinetic variables in drug addiction liability—in particular the rapidity of drug onset and the intermittency of drug exposure. The clinical data are correlational, but they make clear predictions that can be

tested empirically in animals. Animal studies provide greater opportunities to study how pharmacokinetic variables influence the response to drugs. Here we review evidence from such studies indicating the powerful influence of pharmacokinetic variables on drug effects relevant to addiction. We begin by presenting simpler models, in which pharmacokinetic variables are limited to dose and route of administration. We then lead through to self-administration studies, where there are challenging interactions to be considered.

Until recently, the great majority of animal studies on the response to drugs used experimenter-administered, i.p. injections, and much has been learned from these experiments. Figure 2 illustrates the pharmacokinetic profiles of an i.p. versus an i.v. injection of cocaine. Comparing Figures 2 and 1 suggests that an i.p. injection produces a pharmacokinetic profile closest to that produced by intranasal or oral drug administration in humans. Though drug absorption is slower from the i.p. cavity, a vast literature shows that i.p. drug injections are nonetheless powerful in producing behavioural and neurobiological effects. This literature also highlights the importance of intermittent drug exposure followed by a withdrawal period in promoting sensitization-like changes in brain and behaviour (Vezina et al., 2007). Thus, studies using i.p. drug injections laid the groundwork for understanding how the temporal pattern of drug exposure influences the response to drug. For instance, intermittent exposure to drug achieved via experimenter-administered i.p. injections of cocaine (Downs and Eddy, 1932; Post, 1980; Post and Rose, 1976; Reith et al., 1987; Stewart and Badiani, 1993) or nicotine (Baker et al., 2013; Di Chiara, 2000) more readily induces sensitization to the psychomotor activating effect of these drugs, while continuous infusion produces tolerance to this effect.

Psychomotor sensitization refers to a gradual and progressive increase in the

locomotor response to the same or lower doses of a drug with repeated treatment (Eikelboom and Stewart, 1982; Kuczenski and Segal, 1988; Robinson and Becker, 1986; Siegel, 1977), and is also characterized by a faster onset of locomotor activation in response to drug administration (Carey and Gui, 1998; Segal et al., 1981). The ability of drugs to produce psychomotor sensitization might reflect their ability to sensitize reward and motivational processes, thus promoting drug use and addiction (Robinson and Berridge, 1993, 2000; Vezina, 2004). For instance, intermittent i.p. injections of amphetamine, morphine, or cocaine produce sensitization to the rewarding effects of these drugs, as measured by the conditioned place preference test (Lett, 1989). Intermittent i.p. injections of amphetamine, cocaine or nicotine also increase the susceptibility to subsequently initiate voluntary self-administration of these drugs (Horger et al., 1990; Neugebauer et al., 2014; Piazza et al., 1990; Pierre and Vezina, 1997, 1998) and increase the work output animals will emit to obtain drug (Mendrek et al., 1998; Neugebauer et al., 2014; Vezina et al., 2002).

The intermittency of drug exposure also determines the nature and direction of cocaine-evoked neuroadaptations within the dopamine system—a system that is fundamental to the reinforcing effects of psychostimulant drugs. Intermittent, once a day i.p. cocaine injections enhance cocaine-induced inhibition of striatal dopamine reuptake (Izenwasser and Cox, 1990), while continuous infusion of the drug over a 24-hour period attenuates the effects of cocaine on reuptake (Izenwasser and Cox, 1992). Intermittent cocaine administration also produces functional subsensitivity of D2 autoreceptors that modulate dopamine release, while continuous cocaine administration produces D2 autoreceptor supersensitivity (Jones et al., 1996). Within an intermittent, i.p. dosing schedule, the time

interval between injections is also important. For instance, even when the total daily dose of cocaine is held constant, injecting the drug at short intervals preferentially evokes dopamine receptor upregulation within cortical and striatal brain regions (Unterwald et al., 2001). This suggests that the frequency of drug-evoked changes in extracellular dopamine levels, and by implication, the frequency of dopamine receptor activation, determines the long-term consequences of cocaine exposure (Unterwald et al., 2001).

Another model used to study drug effects relevant to addiction is the conditioned place preference paradigm. It measures the conditioned reinforcing properties of a physical environment that has been paired with the effects of a drug. In this paradigm, dose is generally the only pharmacokinetic variable studied [and this work shows that the ability of drugs like cocaine to produce a conditioned place preference is dose-dependent (Spyraki et al., 1982)]. A notable exception is work done by Nomikos and Spyraki. They compared the i.v. and i.p. routes and report that across a range of doses, conditioned place preference to i.v. cocaine requires fewer conditioning sessions, is less susceptible to procedural factors, and is of greater magnitude than conditioning with i.p. cocaine (Nomikos and Spyraki, 1988). The same group also reports that the dopamine D2/3 antagonist haloperidol disrupts conditioned place preference evoked by i.v. but not i.p. cocaine (Spyraki et al., 1987). This was one of the first findings to suggest that the neurobiology mediating the reinforcing effects of cocaine varies as a function of the route of drug administration. The speed of drug onset differs markedly when cocaine is injected i.p. versus i.v. (Figure 2). Thus, the work of Spyraki and colleagues recalls what the clinical literature has taught; the speed of drug onset is critical in determining outcome.

The i.p. route cannot be used to manipulate the speed of drug onset; however, the i.v. route can. In a series of studies in rats we have used experimenter-administered i.v. drug injections to study the effects of variation in the speed of drug onset on the susceptibility to develop psychomotor sensitization. The findings concord with the clinical literature. They show that the speed of drug onset determines behavioural plasticity. These studies using experimenter-administered i.v. injections of cocaine are also important in that they bridge the gap between the literature using i.p. drug injections (the great majority of the work on psychomotor sensitization and conditioned place preference) and the literature using animal models of i.v. drug self-administration. In these studies, we varied the speed of drug onset by varying the speed of i.v. injection between 3 and 100 s. Across this range, there is no effect of injection speed on the acute locomotor response to cocaine or nicotine (Samaha et al., 2002; Samaha et al., 2004; Samaha et al., 2005). However, we found that increasing the speed of cocaine or nicotine delivery by as little as 20 seconds increases the susceptibility to psychomotor sensitization (Samaha et al., 2002; Samaha et al., 2004; Samaha et al., 2005). A first series of experiments showed that across a range of doses (0.5-2 mg/kg/infusion), and using both rotational behaviour in rats with a unilateral 6-hydroxydopamine lesion (a manipulation which destroys nigrostriatal dopamine neurons) and locomotor activity in neurologically intact rats as indices of psychomotor activation, rapid i.v. injections of cocaine (3-16 versus 25-100 s) promote the development of psychomotor sensitization (Samaha et al., 2002). Next, we showed that psychomotor sensitization to a single i.v. injection of cocaine developed when cocaine was injected rapidly (over 5 s) but not when it was injected more slowly [25-100 s; (Samaha et al., 2004)]. Figure 3 illustrates this effect. Finally, we showed that the influence of the speed of drug onset also extends to another drug, nicotine. Rapid (5 versus 25-100 s) i.v.

injection also promoted the development of psychomotor sensitization to nicotine (Samaha et al., 2005). Of course, most studies of psychomotor sensitization use i.p. injections of psychostimulant drugs. Intraperitoneal injection would result in slower drug absorption relative to a 5-s i.v. injection (Figure 2), but it is nonetheless effective in producing psychomotor sensitization. Consistent with this, we found that slower i.v. drug injections can still produce sensitization but this requires the use of higher doses and repeated exposure (Samaha et al., 2002; Samaha et al., 2004; Samaha et al., 2005). Thus, the faster cocaine or nicotine are administered, the more likely they are to induce behavioural sensitization. A clear implication is that even when the route of drug administration is held constant, the speed of drug delivery must influence the effects of drugs on the brain.

Small variations in the speed of cocaine or nicotine onset have large effects on the neurobiological impact of these drugs. Increasing the speed of drug onset promotes changes in cellular activity in mesocorticolimbic structures [(Porrino, 1993; Samaha et al., 2004; Samaha et al., 2005). See Figure 3], greater and more immediate increases in heat-producing, metabolic activity in the ventral tegmental area and nucleus accumbens (Brown and Kiyatkin, 2005), and more immediate increases in dopamine transporter blockade (Samaha et al., 2004) and extracellular dopamine levels (Ferrario et al., 2008) in the striatum. With the exception of Porrino (Porrino, 1993), who compared i.p. and i.v. injections of cocaine, the speed of drug onset was varied by manipulating the speed of i.v. drug injection between 4 and 100 seconds. Pharmacokinetic modelling predicts that across these i.v. injection speeds, peak brain concentrations of cocaine would not vary, but the rate of rise of brain drug levels would (Samaha et al., 2002). Consistent with this

prediction, this range of injection speeds produces differences in the rate of rise of striatal dopamine levels, without affecting peak dopamine overflow (Ferrario et al., 2008; Zernig, 1997). Similarly, Woolverton and Wang (Woolverton and Wang, 2004) manipulated the speed of i.v. cocaine delivery across an even wider range (10-600 seconds) and found that faster i.v. cocaine injections produce a more rapid onset of dopamine transporter occupancy without altering maximum occupancy levels. These findings agree with clinical work suggesting that across a range of cocaine doses that produce similar plasma levels of drug, i.v., smoked or intranasal cocaine produce the same maximum levels of dopamine transporter occupancy, while producing different subjective effects (Volkow et al., 2000).

The effects of pharmacokinetic variables in intravenous drug self-administration models

As a model of drug-taking behaviour, i.v. drug self-administration in laboratory animals provides a strong opportunity to study how dose, speed of drug onset and intermittency of drug exposure interact to determine patterns of drug use. However, the model also offers considerable challenges. The pharmacokinetic issues can become quite complicated. Rather than dealing with a single, isolated injection, self-administration procedures result in multiple infusions, at various time intervals. The pattern of drug taking is influenced by summated brain levels, as well as how fast and how often these levels rise and fall. Understanding how pharmacokinetic variables affect cocaine intake and how cumulative cocaine exposure and the kinetics of this exposure affect cocaine seeking is critical to the design of animal models used in the study of the addiction process.

Drug taking (consummatory) versus drug seeking (appetitive) responses

Drug taking and drug seeking responses represent different categories of behaviour (i.e consummatory and appetitive) that are likely regulated by different neural mechanisms (Roberts et al., 2013). Nonetheless, the sections below highlight that pharmacokinetic variables have parallel effects on the two response categories. The difference between appetitive and consummatory responses is rather obvious in humans; the consummatory response (smoking, drinking/swallowing, injecting, snorting) is an ingestive act that can become ritualistic. Appetitive responses involve more varied behaviours that result in gaining access to the drug (acquiring money, seeking out suppliers, paying for drug). Similarly in a typical operant experiment in which food is used as reinforcement, a lever response or nose poke would be considered an appetitive response that results, according to a defined schedule, in the delivery of a food pellet. The animal then has the option of ingesting the food. Pressing a lever and eating a food pellet are clearly different response types representing the appetitive and consummatory class. This distinction is also important in that it shapes experimental questions. For instance, identifying the processes an individual might use to regulate their intake of free alcohol is quite different from determining how much someone might pay for a particular bottle of wine.

In experiments involving animals self-administering drug via i.v. catheters, consummatory and appetitive responses are not as clearly partitioned. As discussed elsewhere, intravenous drug self-administration studies—particularly under a fixed ratio 1 schedule of reinforcement (one operant response provides one drug infusion; FR1)—are a special case wherein the appetitive and consummatory responses are in fact necessarily conflated (Roberts et al., 2013). Since drug reinforcement is a programmed infusion delivered via a catheter, the consummatory act is entirely circumvented; there is no

external stimulus to direct an ingestive behaviour toward (no smoking, drinking/swallowing, injecting, snorting). The nose poke or lever press becomes not only an appetitive response in the traditional operant sense but also a consummatory response that controls drug ingestion.

Whether the rates and patterns of responding exhibited in an intravenous drug self-administration experiment are controlled by appetitive or consummatory mechanisms will depend, to a large extent, on the schedule of reinforcement used. When using an FR1 schedule, every response leads to the intake of drug, thus every response is a consummatory act (though in light of the discussion above, the response might also reflect an appetitive process). An FR1 schedule therefore provides an opportunity to study the mechanisms that control drug intake. Such studies can presumably model specific human drug taking patterns and therefore allow the opportunity to study phenomena associated with limited versus extended drug access and “binges” for example. Below we will discuss how dose, speed of injection and intermittency of access affect the rates and patterns of cocaine consumption. Finally, we will discuss how these same pharmacokinetic variables influence appetitive responding, as measured using schedules that require an exponential increase in work demand in order to obtain each successive reinforcer, thus producing higher rates of operant behaviour prior to the delivery of the drug, in particular the progressive ratio schedule of drug reinforcement (PR). It could well be argued that FR schedules above FR1 also measure appetitive responses because they require that several operant responses be emitted before delivery of the reinforcer. However, this raises the difficult question of how many operant responses under an FR schedule are required to convincingly tap into appetitive processes. For example, it is not clear whether an FR2 schedule measures a psychological process different from that measured by FR1.

In contrast, responses under a PR schedule of reinforcement are unequivocally appetitive. Another important difference between a PR schedule and an FR1 schedule is that a PR schedule produces very high work demand. Under a PR schedule, obtaining each successive drug injection requires an increasing number of operant responses, until the subject ceases drug self-administration. The number of operant responses performed to obtain the last drug infusion is termed the breakpoint and it is used to infer the motivation to obtain drug (Hodos, 1961; Richardson and Roberts, 1996). Thus, the PR schedule queries an animal about how much physical work it is willing to emit to obtain a reinforcer, and the answer is used as an index of the motivation to procure that reinforcer. Schedules such as PR lend themselves to standard operant interpretation and can be used to assess motivational issues under conditions of very high work demand (Roberts et al., 2013). Such schedules can be used with operant theory or behavioural economics to infer changes in the motivation to seek drugs or continue a binge.

A substantial body of evidence has accumulated which shows that drug consumption and appetitive responding for drugs have partially distinct neurobiological substrates and are differentially affected by a wide variety of manipulations. For example, drug consumption under conditions of very low work demand (under an FR1 schedule) and appetitive responding for drugs under conditions of high work demand (under a PR schedule) are differentially affected by neurotoxic lesions (Loh and Roberts, 1990; Roberts, 1989; Roberts et al., 1994), pharmacological pretreatments (Brebner et al., 2000; Espana et al., 2010), the estrous cycle (Roberts et al., 1989) and diurnal rhythms (Bass et al., 2010; Fitch and Roberts, 1993). Note that while human addicts do not self-administer drug on PR schedules per se, they are willing to suffer significant escalations

in both price and work output in order to obtain drug. In addition, PR tests in animal models are essential because they provide tools to assess how various patterns of drug taking influence the motivation for further use. In the following sections, we will use the rough distinction between FR1 schedules of reinforcement versus other schedules with higher response requirements such as the PR procedure to discuss the effect of pharmacokinetic variables on drug taking versus drug seeking, respectively.

Pharmacokinetic variables influence drug-taking (consummatory) behaviour

Drug-taking behaviour during limited daily access to drug

Pharmacokinetic variables such as dose and speed of drug onset have minor effects on rate of drug intake in the acute stage – but in situations that would be expected to promote a change in the pattern of intake, these variables are critical. First, it is important to recognize that cocaine self-administration can be remarkably stable. Although drug self-administration during daily 1-3 hour, FR1 sessions can produce psychomotor and neurochemical sensitization, and sensitization-related neuroadaptations can promote increased drug intake (Hooks et al., 1994; Lorrain et al., 2000; Phillips and Di Ciano, 1996), cocaine intake during short daily sessions is tightly regulated and changes little over time (Ahmed et al., 2002; Ahmed and Koob, 1998; Deroche-Gamonet et al., 2004; Knackstedt and Kalivas, 2007; Mantsch et al., 2004). Under these stable conditions, manipulations of dose generally have little effect on intake within or between sessions. For instance, if cocaine dose is manipulated during limited FR1 self-administration sessions, rats will self-administer lower doses more frequently than higher doses (Pickens

and Thompson, 1968; Wilson et al., 1971), but hourly intake will typically remain stable both within a session and over days. Figure 4 shows the cocaine dose-response relationship under these conditions, depicted as the responses during a 2-hour session. The figure illustrates that cocaine intake is held constant across a 10-fold range of doses (41 – 421 µg/infusion). Figure 5a shows fluctuations in brain cocaine levels in an animal self-administering 500 µg/infusion under these conditions, estimated using a mathematical model (Ahmed and Koob, 2005; Nicola and Deadwyler, 2000; Pan et al., 1991; Wise et al., 1995). The event record in Figure 5a shows that there is a typical "loading phase" at the beginning of the session – a collection of several injections that produce a rapid rise in brain levels followed by an extremely regular pattern of responding that results in brain cocaine levels being maintained within a narrow range. Under these conditions, both hourly cocaine intake and intake over days remain relatively constant across a wide dose range. It is not clear why intake under these conditions generally does not change over time, or why some conditions that can produce psychomotor and neurochemical sensitization produce unchanging levels of drug intake. Just as the expression of behavioural sensitization is observed more readily following a period of drug withdrawal, it is possible that following limited daily self-administration sessions, an increase in drug intake might be observed after a sufficiently long abstinence period. However, it has been shown that in animals with a history of self-administering cocaine during short daily sessions, drug intake remains stable even following one month of forced abstinence (Ahmed and Koob, 1998; Hollander and Carelli, 2005). Thus, escalation of drug intake is often not observed following short daily self-administration sessions, even though the same conditions can evoke psychomotor and neurochemical sensitization. Based on such evidence, some authors have concluded that psychomotor sensitization

should not be used as a marker for the transition to increased drug use (Ahmed and Cador, 2006), while others maintain that psychomotor sensitization and changes in drug use are more readily linked under conditions of extended access to drug (Ferrario et al., 2005).

During time-limited (1-3 h), FR1 sessions, the speed of drug delivery has minor effects on drug intake acutely, but it can significantly influence change in drug use over time. Some studies show that rapid i.v. injections of cocaine or nicotine lead to greater drug intake within each time-limited session (Kato et al., 1987; Schindler et al., 2011; Schindler et al., 2009), other studies do not show this effect (Crombag et al., 2008; Minogianis et al., 2013; Sorge and Clarke, 2009; Wakabayashi et al., 2010). This suggested that the speed of drug onset might not critically influence drug intake during short, FR1 sessions. However, a more recent study suggests that this lack of effect might be restricted to the initial stages of drug self-administration (the first 3-4 sessions following acquisition of the drug self-administration task). Bouayad-Gervais et al., (2014) showed that, consistent with prior studies (Crombag et al., 2008; Minogianis et al., 2013; Wakabayashi et al., 2010), varying the speed of i.v. cocaine injection (5 or 90 s) has no effect on intake during the first 3-4 self-administration sessions. However, Bouayad-Gervais et al., (2014) bring new data to this literature by showing that beyond these initial sessions, faster i.v. cocaine injections led to greater drug intake than slower cocaine injections. Group differences emerged because the rats given access to slower cocaine injections decreased their intake over days. In contrast, the rats given access to more rapid injections maintained stable drug intake over test days. The latter finding is consistent with the great majority of studies using short daily self-administration sessions (1-3 h), where drug is delivered at

speeds comparable to 5 seconds, and intake remains stable over time. It remains to be determined why the self-administration of sustained cocaine infusions decreases over repeated test days. As research on this issue unfolds, the findings of Bouayad-Gervais et al., (2014) support the idea that variation in the speed of drug delivery evokes neuroadaptations over time, leading to differences in drug intake with repeated drug exposure. The next section will show that the effect of pharmacokinetic variables including the speed of drug onset on drug intake over days is even more dramatic in animals given longer daily sessions (long-access sessions; LgA) – a procedure designed to promote addiction-related neuroplasticity.

Drug-taking behaviour during extended daily access to drug

"Escalation of drug intake" has become a major focus in the cocaine self-administration literature and this phenomenon also serves to illustrate the importance of pharmacokinetic variables in behavioural plasticity. Here we will show that the capacity for one day of cocaine intake to influence the rate of intake on the next day is facilitated by the use of large doses, intermittent drug access, and doses injected quickly. Lengthening the daily session length, a procedure termed long access (LgA), has been shown to increase the rate of within-session intake by about 40-70% over 2-3 weeks (Ahmed and Koob, 1998, 1999; Ben-Shahar et al., 2004; Knackstedt and Kalivas, 2007; Wee et al., 2008). The LgA procedure is a powerful animal model because if one wishes to model addiction in laboratory animals, one expects these animals to change their drug-taking behaviour over time. In the LgA model, escalation of drug intake is modulated by several variables. For

instance, rats can show significant inter-individual variability in the propensity to escalate their cocaine intake (Deroche-Gamonet et al., 2004; Wakabayashi et al., 2010). Pharmacokinetic variables also play a role. For instance, using nicotine, it has been shown that when periods of drug self-administration are interspersed with periods of forced abstinence, this promotes an escalation in nicotine intake (Cohen et al., 2012). Furthermore, escalation might be more likely at high doses of cocaine. Dose-response curves are not widely studied in the literature on the escalation of cocaine intake. A notable exception is Mantsch et al. (Mantsch et al., 2004), who showed that escalation occurs earlier at higher doses (2 versus 0.5 mg/kg/injection). This is in line with a number of studies that have failed to see escalation of cocaine intake at lower drug doses (0.25 – 0.6 mg/kg/injection) (Ferrario and Robinson, 2007; Kippin et al., 2006; Mantsch et al., 2004; Minogianis et al., 2013). Escalation is also facilitated by a rapid speed of cocaine onset. Studies showing that prolonged daily access to drug can promote the escalation of drug intake use rapid i.v. injections of drug (Ahmed and Koob, 1998, 1999; Ben-Shahar et al., 2004; Knackstedt and Kalivas, 2007; Wee et al., 2008). This prompted us and others to ask whether the speed of drug delivery plays a role in the propensity to escalate drug consumption. Work on this issue showed that when daily access to cocaine is increased from 1 to 6 h, rats given access to rapid (5-45 seconds) versus slower (90 seconds) injections of cocaine take more drug and are also more likely to escalate their consumption over days (Bouayad-Gervais et al., 2014; Minogianis et al., 2013; Wakabayashi et al., 2010). Figure 6 illustrates this effect. Such effects are observed in spite of the fact that in these latter studies, all rats had equal opportunity to take cocaine (one injection was available every 90 seconds). Thus, extended access to drug by itself is not sufficient to promote escalated drug use, and the speed of drug onset is decisive in producing this

behavioural change. In summary, in agreement with clinical observations and the literature on psychomotor sensitization, dose, the intermittency of drug use, and the speed of drug onset are key determinants of behavioural change in the LgA model of drug self-administration. This serves to highlight the role of pharmacokinetic factors in the plastic changes associated with the addiction process.

Pharmacokinetic variables influence drug-seeking (appetitive) behaviour

A key question in addiction is how specific patterns of drug taking predispose an individual to further drug use. This is a critical question to address if one wishes to understand why some drug users maintain control over their drug use, while others develop excessive and pathological patterns of drug use, and ultimately addiction. Accordingly, there is now a trend in the literature to assess how cocaine consumption affects the appetitive response – or put another way – how patterns of consumption subsequently affect an animal's motivation to seek and use drugs. In the next sections, we will review some procedures that affect appetitive responding for drug. These include the long access (LgA), discrete trials (DT), and progressive ratio training (PR) procedures. Data from these models hint at the importance of the intermittency of drug use and speed of drug onset in determining the subsequent motivation to seek and take drug. Finally, we will review findings from a new model of drug self-administration behaviour that powerfully illustrates how intermittent 'spikes' in brain levels of cocaine can be more effective than high and sustained levels in determining the subsequent motivation to seek and take the drug.

The long-access model

While some inconsistencies exist, there are numerous reports of drug self-administration under LgA conditions changing the susceptibility to relapse, drug seeking in spite of punishment, and, responding for drug under a PR schedule of reinforcement. For instance, compared to rats given limited daily access to cocaine, rats given LgA sessions are more vulnerable to drug-primed reinstatement of previously extinguished responding for cocaine (Ahmed and Cador, 2006; Ahmed and Koob, 1998; Knackstedt and Kalivas, 2007; Mantsch et al., 2004). LgA rats also show greater responding for cocaine in spite of punishment (footshock) (Vanderschuren and Everitt, 2004), and also reach higher breakpoints for cocaine under progressive ratio conditions [(Hao et al., 2010; Paterson and Markou, 2003; Wee et al., 2008); note however that this latter effect is not always observed (Bouayad-Gervais et al., 2014; Liu et al., 2005a; Oleson and Roberts, 2009)].

While testing of pharmacokinetic variables with the LgA model has been limited, the data again support the idea that intermittent drug access, high doses and a rapid speed of drug onset are critical to the ability of extended self-administration sessions to influence appetitive responding. The data further show that there are strong parallels in the effects of pharmacokinetic variables on consummatory and appetitive behaviours. For instance, when access to cocaine is given intermittently by interspersing LgA sessions with periods of forced abstinence, this promotes the development of increased motivation for cocaine, as assessed using a progressive ratio schedule of cocaine reinforcement (Morgan and Roberts, 2004). The ability of LgA sessions to promote cocaine-primed reinstatement of drug-seeking behaviour is also facilitated by the use of high doses during the LgA phase

(Mantsch et al., 2004). Finally, when the speed of i.v. drug injection is varied during LgA sessions, rats allowed to self-administer rapid cocaine injections (5 versus 90 seconds) subsequently show increased motivation to obtain the drug (Bouayad-Gervais et al., 2014; Minogianis et al., 2013). This effect is illustrated in Figure 7. Rats with a history of taking rapid cocaine injections are also more vulnerable to drug-primed reinstatement of cocaine-seeking behaviour after a long period of forced abstinence [45 days; (Wakabayashi et al., 2010)]. The ability of rapidly administered cocaine to increase motivation to obtain drug and the susceptibility to reinstatement is not just a consequence of the amount of drug previously consumed. Even when the rats self-administering rapid cocaine injections take the same amount as rats self-administering slower injections, the former show greater motivation for drug and a more persistent vulnerability to reinstatement (Bouayad-Gervais et al., 2014; Minogianis et al., 2013; Wakabayashi et al., 2010). Thus, how much, how often and how fast a drug reaches the brain all determine the neuroplasticity that underlies changes in appetitive behaviours characteristic of addiction.

What accounts for the ability of LgA self-administration sessions to influence subsequent appetitive responding for drug? The early literature suggested that increased appetitive responding for drug results from elevated amounts of drug consumed (i.e., area under the curve). Some suggest the existence of "…*a threshold level that can readily establish and sustain addiction*" (Benowitz and Henningfield, 1994), and that "…*below this critical level of exposure, there would be no drug-induced neuropathological changes, and drug use would remain under control, at least in the majority of drug-exposed animals*" (Ahmed, 2012). Said differently, to induce addiction, "more is better" and sustained levels

are important. This is a reasonable assumption given that compared to ShA sessions, LgA sessions are much more effective in promoting future appetitive responding for drug, and an obvious difference between the two procedures is the level of prior drug exposure (modelled in Figure 5). If this is correct, then elevated brain levels in the past should be sufficient to enhance the motivation to obtain drug in the future. Another possibility is that the *number* of injections is important. That is, that the frequency at which brain levels of drug rise and fall might influence future patterns of drug use. The fact that dose and speed of injection are critical factors suggests that the size and speed of the "spike" in cocaine levels may be important. However, it is difficult to test the relative contributions of area under the curve versus spiking brain levels in conventional LgA/ShA models. The length of the session and the number of injections will always be confounded. Whether 'spikes' are necessary and/or sufficient to drive the important behavioural and neurochemical changes associated with the addiction process needs to be tested using different experimental procedures. As will be detailed further below, recent work tests this directly.

The discrete trials model

Discrete trials procedures (DT) were a first attempt in determining whether an increase in the motivation to obtain drug could be seen in animals with a history of intermittent rather than continuous drug access during each self-administration session. The DT procedure allows animals to self-administer 24 hours a day, but manipulates hourly drug intake. Animals have the opportunity to self-administer one injection and must then wait until the next trial within the hour for another opportunity. For example, DT4 involves giving 4 opportunities to take a single drug injection per hour. Under this schedule, rats tend to

take an injection during every trial for the first 24 hours and then settle into a regular daily pattern of intake, with drug taking restricted to the dark phase of the activity cycle (Roberts et al., 2002). Interestingly, self-administration on a DT4 protocol for 10 days increases breakpoints under a PR schedule of reinforcement, and this increase depends on an abstinence period. If animals are tested immediately after 10 days of DT4 there are no changes in breakpoints (Morgan et al., 2002; Morgan et al., 2005). But if a seven-day abstinence period is introduced, then sensitization of breakpoints is observed (Morgan and Roberts, 2004). The DT procedure thus illustrates that high and sustained brain levels of drug (as achieved with a conventional LgA session) are not a necessary condition to enhance the subsequent motivation for drug. The DT4 model also demonstrates that intermittent drug exposure followed by an abstinence period facilitates the development of sensitization to the motivational effects of cocaine. This concords with the literature on psychomotor sensitization, where intermittent drug exposure and a withdrawal period can be decisive (Downs and Eddy, 1932; Post, 1980; Post and Rose, 1976; Reith et al., 1987; Robinson and Becker, 1986; Stewart and Badiani, 1993). The DT model does have its limits. It can be argued that the parameters are arbitrary and restrictive, and that the daily pattern does not resemble the way human addicts take cocaine. Still, it is an important demonstration that an intermittent pattern of cocaine intake evokes sensitization of appetitive responding for cocaine.

Daily testing under a progressive ratio schedule of cocaine reinforcement

Daily testing on a PR schedule of drug reinforcement is another procedure illustrating that sustained brain levels of drug are not a necessary condition to augment subsequent

appetitive responding for drug. With daily testing, breakpoints maintained by cocaine escalate to very high levels over time (Liu et al., 2005b). Intriguingly, very low levels of prior cocaine intake result in rapid sensitization of breakpoints, whereas high levels of prior intake suppress the development of this sensitization (Morgan et al., 2006). Daily testing under a PR schedule of drug reinforcement was not intended as a model of human drug taking. However, the observation that breakpoints escalate over time – with about the same drug exposure as ShA – suggests that something interesting is going on. One possibility is that the schedule imposes a constraint on intake. It takes time to complete the high response ratios and brain levels of drug fall in between each injection. As such, the PR schedule would promote greater 'spiking' from lower brain levels of drug than the LgA procedure. Are these 'spikes' in brain drug levels important? Indeed they are. The susceptibility to escalate breakpoints for cocaine is determined by dose and the speed of drug onset (Liu et al., 2005b). When rats were given access to cocaine injections delivered i.v. over 5, 25 or 50 sec, only rats taking 5-sec injections showed an escalation in breakpoints. Similarly, only larger unit injection doses produced escalation of breakpoints (Liu et al., 2005b). These findings suggest that a rapid rise from low to high brain levels of drug is an important determinant of the future motivation to seek and take cocaine.

A new model: Intermittent access to drug during a long-access session

The protocols reviewed above suggest that rapidly rising and intermittent spikes in brain levels of cocaine might be decisive in predicting subsequent appetitive responding for drug, but this awaited formal investigation. Recently, one of the authors of the present review (D.C.S.R.) set about testing whether fast, "spiking" levels might drive the change

in appetitive responding. To this end, a new model of cocaine self-administration was developed involving intermittent, within-session access to drug (Zimmer et al., 2012). The recent survey data in experienced cocaine users that we described above provided additional impetus to develop this model. These survey data suggested that experienced cocaine users likely do not maintain high brain levels of the drug, but might instead voluntarily achieve marked reductions in brain levels, which then rapidly rise with each drug self-administration (Beveridge et al., 2012). The intermittent access (IntA) procedure models this by limiting cocaine availability during a 6-hour session to twelve 5-min discrete trials separated with 25-min timeout periods. Brain cocaine levels cannot be maintained; instead the animals experience twelve rapidly rising spikes in cocaine levels during each session. Thus the IntA protocol tests the effect of a history of cocaine 'spikes' on the motivation to self-administer the drug later on, and it also allows for a direct test of the importance of 'spiking' versus sustained cocaine levels (Zimmer et al., 2012). To this end, IntA rats were compared to LgA rats. Predictably, the LgA group took much more cocaine than the IntA group. In addition, pharmacokinetic modelling suggested that LgA sessions would produce continuously high brain cocaine levels while IntA sessions would produce repeated, fast-rising spikes in brain cocaine levels (modelled in Figure 5). Remarkably, in spite of being exposed to significantly less cocaine, the IntA rats were more motivated to self-administer the drug in the future (Zimmer et al., 2012). This is consistent with other findings showing that even extremely high levels of cocaine intake are not sufficient on their own to increase the subsequent motivation to take the drug (Roberts et al., 2002). Such findings are a challenge to the belief that simply maintaining high levels of drug intake is sufficient to develop an addicted phenotype. Instead, it appears that when it comes to producing an increase in the motivation to obtain drug, 'how often' drug is taken

can be more important than ‘how much’. In support of this idea, recent work in rats shows that the early occurrence of a burst-like pattern of cocaine intake is a behavioural marker of vulnerability to compulsive drug use (Belin et al., 2009). Amongst a group of rats allowed to self-administer cocaine, the subset that spontaneously and rapidly developed a high-frequency pattern of intake (spontaneous intake of 5 infusions in less than 5 minutes) was particularly vulnerable to develop addiction-like symptoms. These symptoms included increased motivation to obtain drug, persistence of drug-seeking behaviour in spite of signalled unavailability of cocaine and self-administration in spite of physical punishment. Importantly, the rats that developed a burst-like pattern of cocaine use did not differ from the other rats with respect to cumulative cocaine intake. Similarly, by dividing rats into two groups based on their spontaneous, self-imposed interval between cocaine infusions, Martin-Garcia et al. (Martin-Garcia et al., 2014) found that rats showing high-frequency cocaine self-administration are more vulnerable to cocaine-primed reinstatement of drug-seeking behaviour relative to rats with low-frequency cocaine intake—in spite of equivalent levels of prior drug intake. Together, these findings suggest that an intermittent pattern of use, more than the amount of drug used, governs the susceptibility to increased drug use.

A key question is how the IntA procedure maps onto what we know about human cocaine intake. As discussed, new work suggests that experienced cocaine users take cocaine in a pattern different than previously thought, achieving intermittent and rapidly rising brain levels of the drug, rather than maintaining high and sustained levels (Beveridge et al., 2012). Even in between bouts of drug self-administration, human addicts likely do not have relatively continuous access to drug, several hours a day, for

weeks/months on end (as modelled by the LgA procedure). Due to the interaction of several factors, some willed by the user, others not (jail time, lack of money, etc) drug intake, particularly cocaine intake, is intermittent, both within and between bouts of intoxication. Given this, understanding what happens to the brain when cocaine intake is intermittent is important. By modelling this in laboratory animals, the IntA procedure clearly shows that in producing change in the motivation to take drug over time, sustained brain levels of drug are not necessary, and spiking levels look to be the prime determinant. This is consistent with clinical observations, and also corroborated by evidence from studies using the more traditional LgA, DT and daily PR testing procedures, where a rapid speed of drug onset and high doses are critical for the development of behavioural change.

Pharmacokinetics determine the neuroplasticity evoked by drugs

Pharmacokinetic variables determine the impact drugs have on the brain. Everything that we have described for behaviour above has important parallels with neurochemical and neurobiological findings. A first series of studies showed that the speed of drug onset determines drug-induced effects on gene regulation. Rats with a history of taking rapid cocaine injections during LgA sessions show desensitization of cocaine-evoked Fos expression in the nucleus accumbens, while rats exposed to slower injections do not (Wakabayashi et al., 2010). This is reminiscent of findings using experimenter-administered nicotine, showing that rats that were previously treated with rapid i.v. injections of the drug (5 versus 90 seconds) show greater desensitization of nicotine-evoked c-*fos* and *arc* mRNA expression in the nucleus accumbens shell and caudate-putamen (Samaha et al., 2005). The functional significance of these changes is unknown.

However, an interesting hypothesis is that the time-course of drug-induced plasticity in gene regulation might vary as a function of the speed of drug onset (Samaha et al., 2005). Variation in the speed of i.v. cocaine delivery also alters the brain expression of the neurotrophin, brain-derived neurotrophic factor (BDNF) and of its receptor, tropomyosin receptor kinase B (TrkB) (Bouayad-Gervais et al., 2014). Chronic exposure to cocaine and other psychostimulant drugs regulates brain BDNF mRNA and protein levels (Asan, 1997; Fumagalli et al., 2007; Grimm et al., 2003; Im et al., 2010), and BDNF-mediated signalling specifically in midbrain and corticostriatal regions mediates drug-seeking and drug-taking behaviours (Graham et al., 2007; Graham et al., 2009; Im et al., 2010; Unterwald et al., 2001). In rats given LgA session, only those taking rapid cocaine injections (5 versus 90 seconds) show altered BDNF and TrkB mRNA levels in corticostriatal structures (caudate-putamen, orbitofrontal, frontal and parietal cortices, but not the nucleus accumbens or medial prefrontal cortex) (Bouayad-Gervais et al., 2014). At present, it remains unknown how the mRNA changes might translate to protein changes and whether increased regulation of BDNF and TrkB in corticostriatal nuclei plays a causal role in the behavioural effects of rapid cocaine delivery. Dopamine D2 receptor changes have also been reported in rats with a history of self-administering rapid versus more sustained cocaine injections during LgA sessions. Following 2 weeks of withdrawal from cocaine, rats with a history of taking slow cocaine injections have decreased D2 receptor levels in the caudate-putamen, and D2 receptors with greater agonist affinity (Minogianis et al., 2013). As with the BDNF-TrkB mRNA findings, additional work is needed to determine how the observed D2 receptor changes might contribute to the behavioural effects of the speed of cocaine delivery. Future studies can determine

whether the D2-receptor related changes modify striatal signalling in ways that protect from excessive motivation to take cocaine (Minogianis et al., 2013).

How often drug levels rise and fall within a bout of drug self-administration is also a prime determinant of drug effects on the brain. The intake of cocaine at short (2 min) rather than longer (6 min) intervals within each self-administration session preferentially increases c-Fos protein levels in the prelimbic and infralimbic cortices, the nucleus accumbens shell and core and the basolateral nucleus of the amygdala (Martin-Garcia et al., 2014). Optogenetic manipulations also show that neuronal activity within the prelimbic cortex regulates cocaine-seeking and -taking behaviours only in animals allowed to self-administer cocaine at short inter-infusion intervals (Martin-Garcia et al., 2014). This suggests that cocaine intake at short inter-infusion intervals promotes the ability of the prelimbic cortex to control drug use. Finally, Calipari et al. (Calipari et al., 2014; Calipari et al., 2013) have assessed the influence of intermittent spikes in brain cocaine levels on the dopamine transporter (DAT). They compared LgA rats to IntA rats. IntA rats developed sensitization to the ability of cocaine, methylphenidate and methamphetamine to inhibit the DAT within the nucleus accumbens, while LgA rats developed tolerance to cocaine's effects at the transporter (Calipari et al., 2014; Calipari et al., 2013). This is in agreement with studies using experimenter-administered drug and showing that intermittent access promotes sensitization of cocaine's effects on dopamine reuptake, while continuous access promotes tolerance (Izenwasser and Cox, 1990, 1992). The findings of Calipari et al (Calipari et al., 2014; Calipari et al., 2013) also concord with recent work showing that LgA rats show decreased phasic dopamine levels in the nucleus accumbens core (Willuhn et al., 2014).

Why is it useful to study the temporal dynamics of drug delivery as an active principle in addiction?

In attempting to reduce the enormous impact of drug addiction on health and society, the first step is to understand the factors that can modulate the development of the disorder. As the literature reviewed above demonstrates, in determining the effects of drugs on brain and behaviour, the dynamics of drug delivery are not just secondary to achieved dose or to the crossing of certain threshold levels of drug exposure. Instead, they determine brain and behaviour changes that are relevant to the addiction process. A growing literature suggests that, in keeping with clinical observations, animals that take drugs like cocaine in a pattern that produces rapidly rising and intermittent 'spikes' in brain levels of drug more readily develop an addiction phenotype. Thus, evidence is emerging to help us better understand what routes and patterns of drug administration are the most addictive. This information has tremendous implications. First, it can be used to identify vulnerable drug users most susceptible to progress towards addiction, allowing early intervention strategies to be implemented. Second, this work has implications for new treatment avenues. Pharmacokinetic principles are already exploited to treat addiction to some drugs (nicotine patch/gum versus smoking a cigarette; methadone versus heroin). Pharmacokinetic principles might also be used in the development of agents to treat addiction to drugs like cocaine. If spiking brain levels of drug do indeed contribute to addiction, pharmacological ways of flattening these spikes can be envisioned. Finally, upstream of this, data from the basic science literature can be used to educate the public and influence choices not only about whether to take drugs, but how. Scientists have long known that pharmacokinetics matter, the layperson might not. The public would benefit

from education about the consequences of using certain routes, patterns of drug use and drug formulations.

Where do we go from here?

Great strides have been made in understanding the importance of pharmacokinetic factors in drug addiction, but important data are still missing. It is known that pharmacokinetics influence the behavioural response to drugs and some neurobiological correlates have been reported. However, the neurobiological findings remain largely correlational. There is little evidence on how specific brain changes might play a causal role in the ability of the speed of drug onset or the intermittency of drug use to influence behaviour. In parallel, we know little about what brain levels of drug look like when experienced drug users are given control over drug pharmacokinetics (e.g., dose, intermittency of dosing, speed of drug onset). Such information would be critical for the design of both representative brain imaging studies in humans and drug self-administration experiments in laboratory animals.

Concluding remarks

If we as drug addiction researchers ignore pharmacokinetics, we do so at our peril. There is strong evidence that chronic exposure to intermittent and rapidly rising brain levels of drug (cocaine in particular) promote an escalation in consummatory and appetitive responding for drug. This has notable parallels with extensive literature on psychomotor sensitization, where there is agreement that intermittency of drug exposure

and abstinence periods are key (Post, 1980; Robinson and Becker, 1986). The ability of rapidly rising brain levels of drug to facilitate excessive patterns of drug use is associated with changes in gene regulation, dopamine neurochemistry and cell function that are thought to be important in the addiction process. In several instances, such rapidly spiking brain levels of drug were directly compared with high and sustained brain levels (e.g., IntA versus LgA). This work reveals that the two pharmacokinetic profiles produce different outcomes and that out of the two, drug 'spikes' appears to push the addiction process forward more effectively. This has profound implications for better understanding the neuroadaptations that are critical for addiction. There is a great amount of data on the ability of LgA procedures to promote changes in brain and behaviour. This model is currently dominating the literature on drug self-administration in animals [even in our own past work (Bouayad-Gervais et al., 2014; Minogianis et al., 2013)], and it has shed important new light on how extensive exposure to sustained and high levels of drug change brain and behaviour. LgA is widely used because many would say that it has face validity since it produces escalation from more modest drug intake to binge use. However, as argued above, it is unlikely that human cocaine addicts have relatively free access to cocaine several hours a day, for days on end, enabling them to maintain high and sustained brain levels of drug. Rather, they might voluntarily achieve intermittently rising brain levels of drug during a bout of intoxication (Beveridge et al., 2012), and also use intermittently over time (Cohen and Sas, 1994; O'Brien, 2001). Moreover, work in animals shows that continuously high versus intermittently high brain levels of cocaine can produce opposite effects on DA system function for example (Calipari et al., 2014; Calipari et al., 2013). This deserves research attention, as we want to be sure which model more closely captures the way humans take drugs. The temporal pattern of drug use is of

tremendous importance in addiction, particularly cocaine addiction, and this should be reflected in the animal models we use to study addiction in the laboratory.

Figure legends

Figure 1. The pharmacokinetic profiles of plasma cocaine levels in humans as a function of the route of drug administration. Plasma cocaine levels rise sharply and decline rapidly when cocaine is injected intravenously or smoked. In contrast, plasma cocaine levels rise and decline more slowly following intranasal or oral administration of the drug. Data represent the mean for ten human subjects in each condition. Adapted from (Jones, 1990).

Figure 2. The pharmacokinetic profiles of plasma cocaine levels in rats following intravenous (i.v) and intraperitoneal (i.p) administration. Plasma cocaine levels rise sharply and decline rapidly following an intravenous injection. In contrast, plasma cocaine levels rise and decline more slowly following an intraperitoneal injection of the drug. Note that following intravenous injection, cocaine concentrations in plasma rise rapidly and reach Cmax within the first 5 min, which was the earliest sampling interval in this experiment. Adapted from (Pan et al., 1991)

Figure 3. Increasing the speed of intravenous cocaine delivery facilitates the development of psychomotor sensitization and promotes c-*fos* mRNA expression in corticolimbic regions. The panel on the left illustrates psychomotor activity averaged over the first 12 min following an intravenous injection of 2.0 mg/kg cocaine, delivered by an experimenter over 5, 25 or 100 seconds, on two consecutive days. Data are mean ± SEM. Panels on the right show representative densitograms illustrating c-*fos* mRNA levels in the brains of

rats injected with 2.0 mg/kg cocaine at different speeds. Coc, cocaine. s, second. Adapted from (Samaha et al., 2004)

Figure 4. The effect of manipulating dose on rate of responding (A) and total intake (B) for a group of rats self-administering intravenous injections of various unit doses of cocaine during daily two-hour sessions. Panel A illustrates an inverted 'U' shaped curve, and at the high end of this curve, responding decreases as dose increases. Panel B illustrates that over the dose range characterized by a decrease in the number of injections (41 – 421 µg/infusion, framed within dotted lines), intake is relatively constant. Data are mean ± SEM. Adapted from (Oleson and Roberts, 2009).

Figure 5. The pattern of intake and modelled cocaine levels in the brain for representative animals tested during three distinct self-administration procedures. Each panel shows the modelled brain levels of cocaine corresponding to the pattern of intake indicated by the event record. Curves were generated using a model developed by Pan et al., (1991). Pan et al., (1991) used microdialysis to measure brain concentrations of cocaine injected intravenously. The relevant pharmacokinetic parameters were then estimated by fitting a two-compartment open model to the data using nonlinear regression. The equations used by the model and the parameter estimates are provided in Pan et al., (1991). ShA, 2-hour, Short Access session (0.75 mg/kg/infusion, with a 20-second time out period). LgA, 6-hour, Long Access session (0.75 mg/kg/infusion, with a 20-second time out period). IntA, 6-hour, Intermittent Access session (12 discrete 5-minute trials separated by a 25-minute

inter-trial-interval; 0.375 mg/kg/infusion). Note that in the intermittent access sessions, no time out was imposed, allowing animals to self-administer multiple injections within a few seconds. Adapted from (Zimmer et al., 2012).

Figure 6. In rats given extended daily access to cocaine, only those self-administering rapid cocaine injections escalate their drug intake over time. Panel A shows cocaine intake in the first hour of the 6-hour session, in rats self-administering rapid intravenous cocaine injections (delivered over 5 seconds) and in rats self-administering slower injections (90 seconds). Panel B shows total cocaine intake during each 6-hour session in the same animals. Rats received three short-access sessions (ShA; 1 h/session) prior to the 10 long-access sessions (LgA; 6 h/session). In (A), 'ShA' shows the average number of infusions taken over these three sessions. Data represent mean ± SEM. N = 12-13/group. s, second. #$p<0.05$ compared to the 90-s rats. *$p<0.05$ compared to the 1st session of LgA in 5-s rats. Adapted from (Bouayad-Gervais et al., 2014).

Figure 7. The self-administration of rapid cocaine injections in the past leads to increased motivation to take the drug in the future. The figure illustrates responding for intravenous cocaine injections under a progressive ratio schedule of reinforcement in animals that have previously self-administered rapid (delivered over 5 seconds) or more sustained (90 seconds) cocaine infusions. Corresponding ratios are included. Panel A illustrates that rats in the 5-second group show an increased motivation to take cocaine, across a range of doses during progressive ratio testing. In (A), cocaine was delivered over 5 seconds for

the 5-second group, and over 90 seconds for the 90-second group. Panel B shows that rats in the 5-second group also show an increased motivation to take cocaine regardless of drug injection speed during progressive ratio testing. Data represent mean ± SEM. N = 11-16/group. s, second. PR 5 s, cocaine was delivered over 5 seconds during progressive ratio testing. PR 90 s, cocaine was delivered over 90 seconds during progressive ratio testing. #$p<0.0001$ and *$p<0.05$ compared to the 90-s rats. Adapted from (Minogianis et al., 2013).

Figures

Figure 1

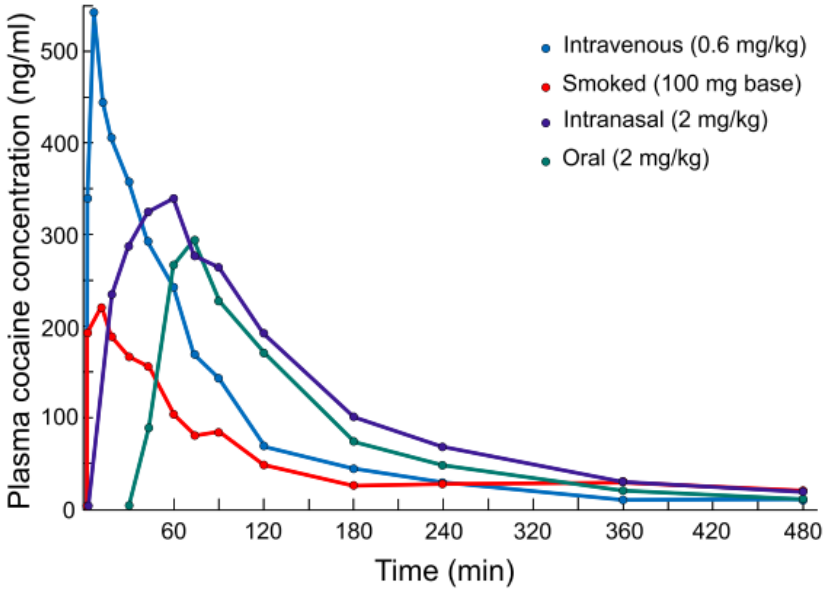


Figure 2

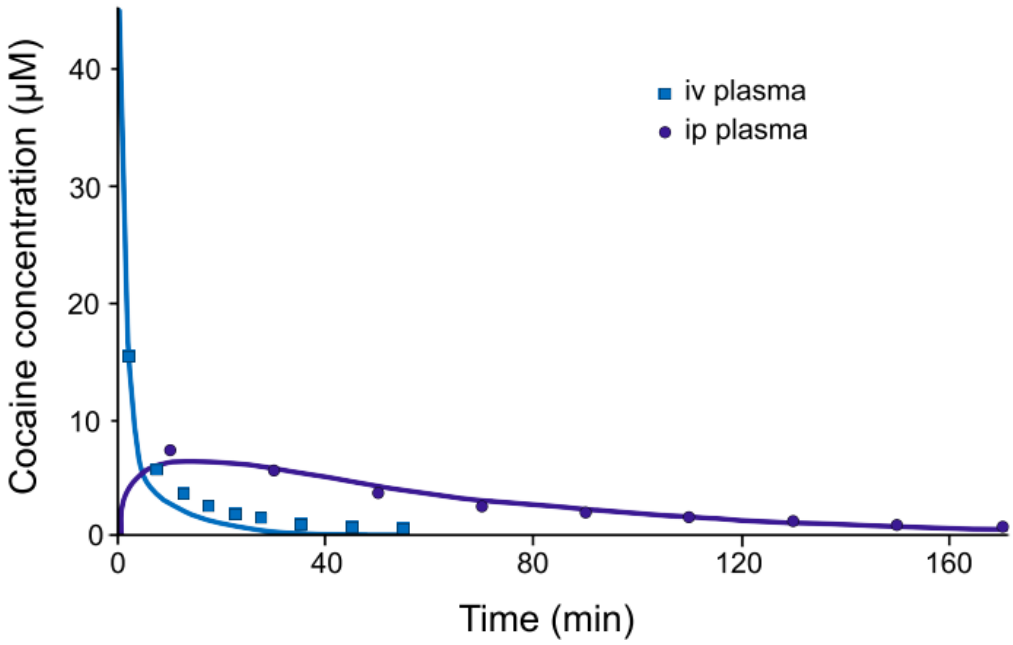

Cocaine concentration (μM)
40
30
20
10
0
0
40
80
120
160
Time (min)
iv plasma
ip plasma

Figure 3

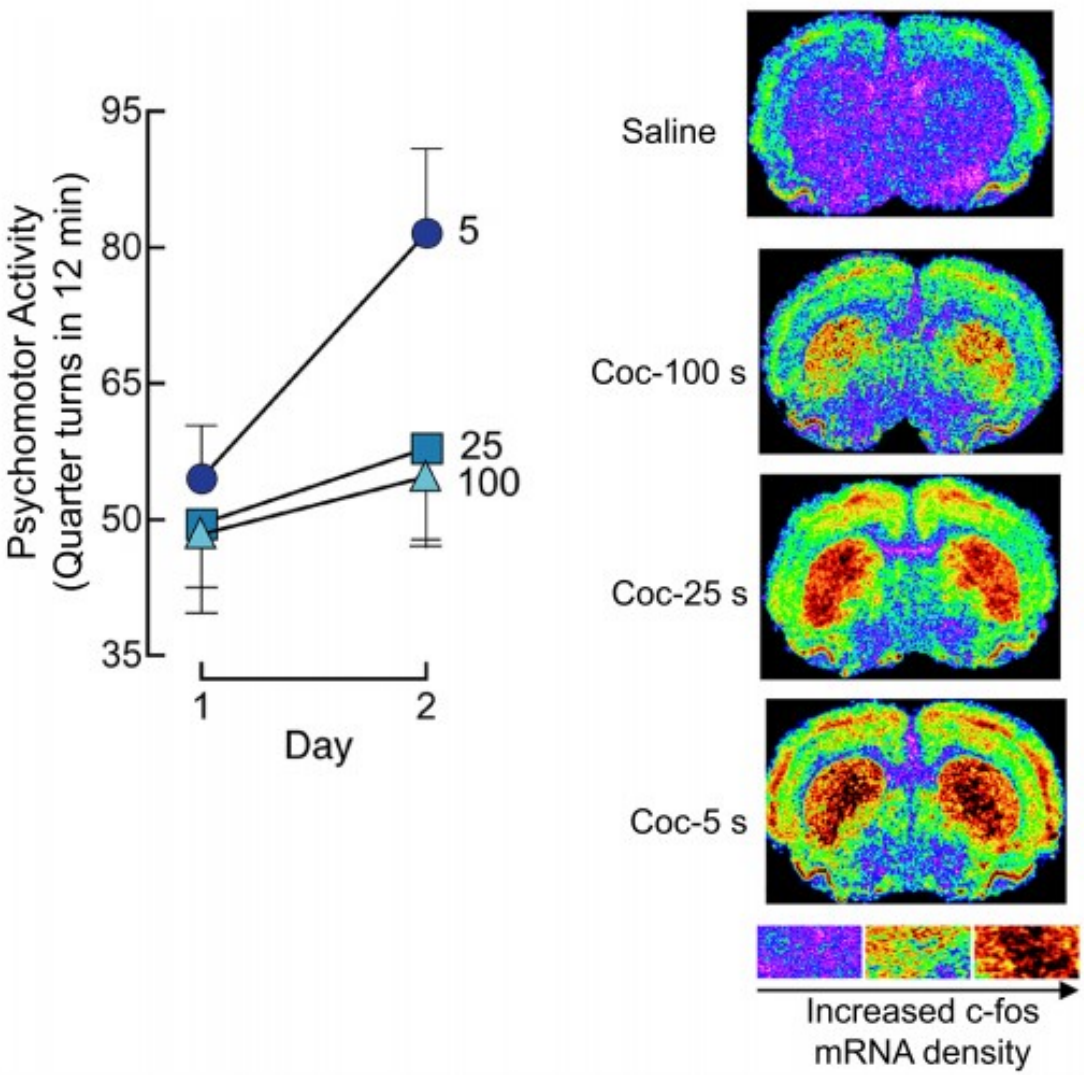

Psychomotor Activity
(Quarter turns in 12 min)
95
80
65
50
35
1
2
Day
5
25
100
Saline
Coc-100 s
Coc-25 s
Coc-5 s
Increased c-fos
mRNA density

Figure 4

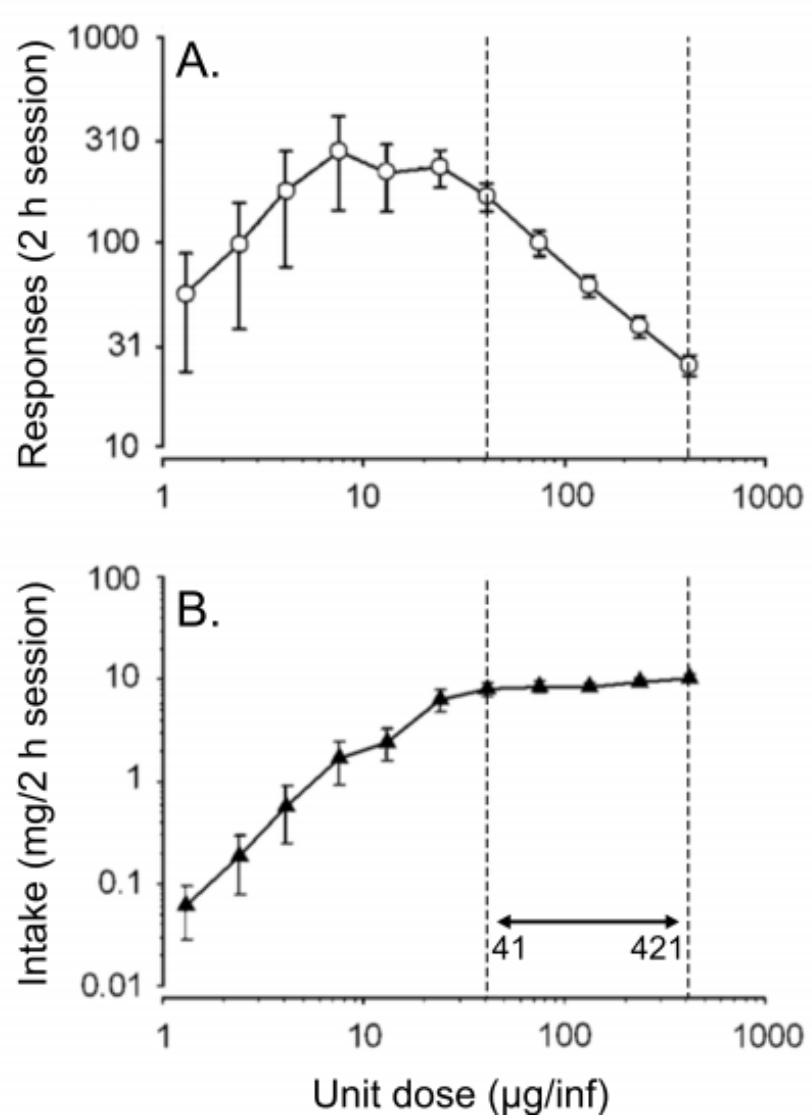

A.
Responses (2 h session)
1000
310
100
31
10
1
10
100
1000
B.
Intake (mg/2 h session)
100
10
1
0.1
0.01
41
421
1
10
100
1000
Unit dose (µg/inf)

Figure 5

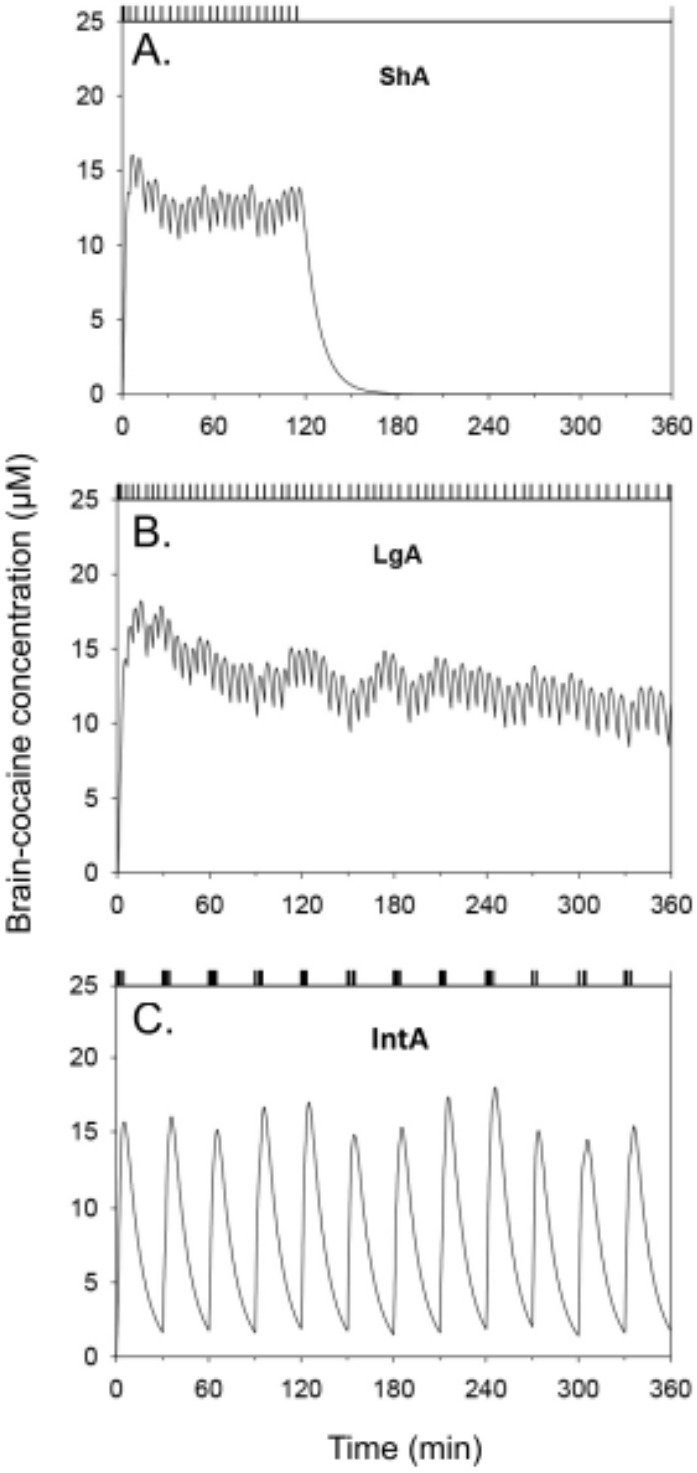

A.
ShA
B.
LgA
C.
IntA
Brain-cocaine concentration (μM)
Time (min)
0
5
10
15
20
25
0
60
120
180
240
300
360

Figure 6

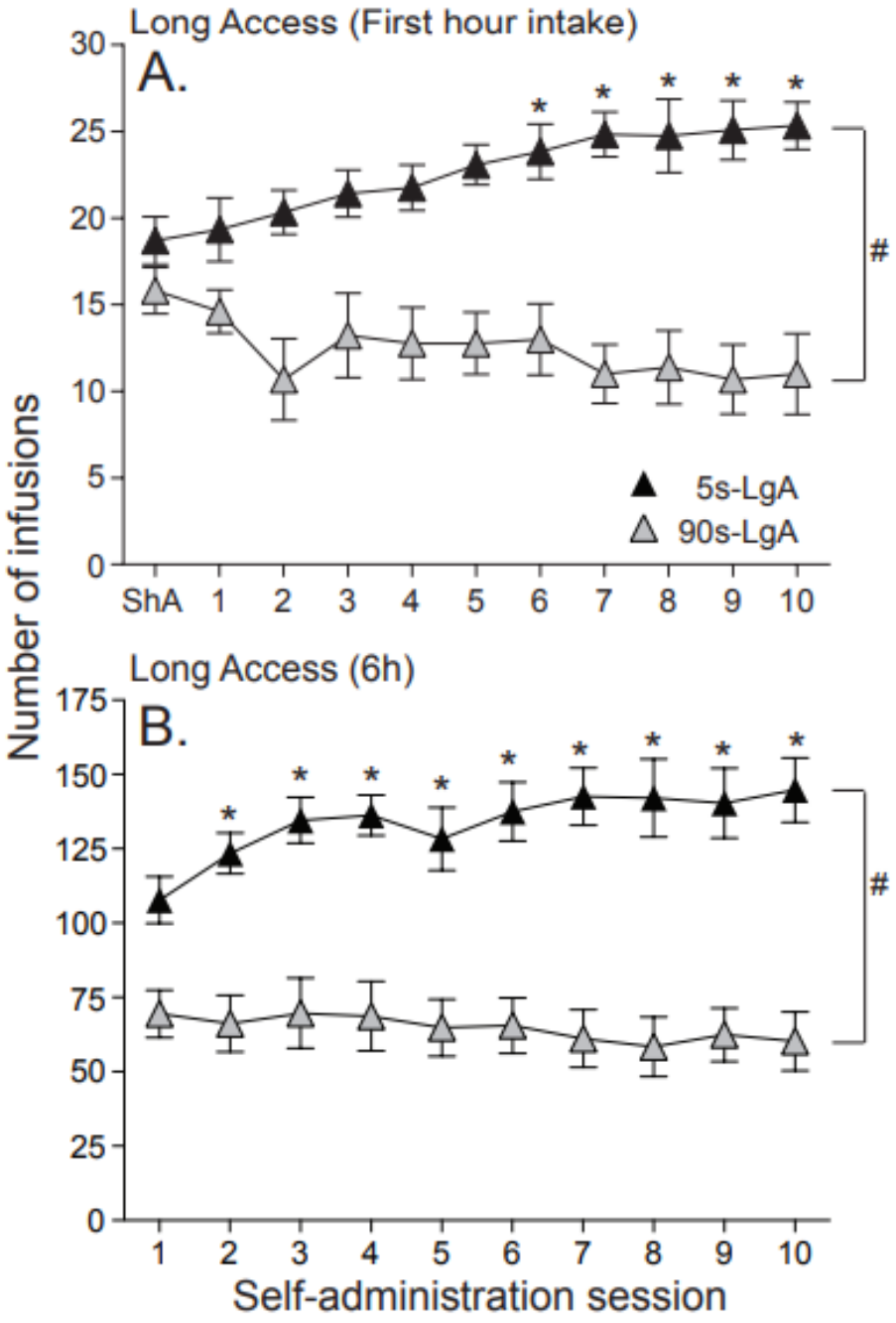
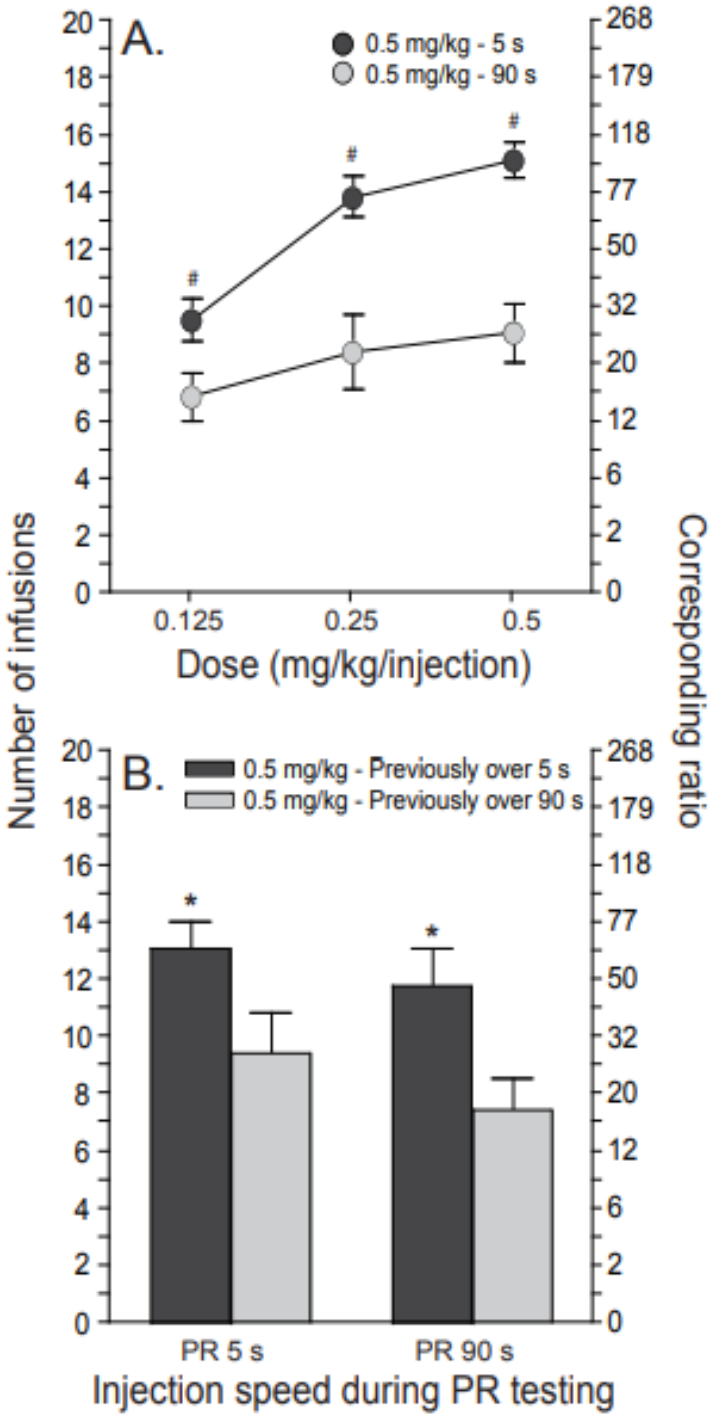

Long Access (First hour intake)
A.
30
25
20
15
10
5
0
*
*
*
*
*
#
5s-LgA
90s-LgA
ShA
1
2
3
4
5
6
7
8
9
10
Long Access (6h)
B.
175
150
125
100
75
50
25
0
*
*
*
*
*
*
*
*
#
1
2
3
4
5
6
7
8
9
10
Number of infusions
Self-administration session

Figure 7


A.
0.5 mg/kg - 5 s
0.5 mg/kg - 90 s
#
#
#
Number of infusions
Corresponding ratio
0.125
0.25
0.5
Dose (mg/kg/injection)
B.
0.5 mg/kg - Previously over 5 s
0.5 mg/kg - Previously over 90 s
*
*
PR 5 s
PR 90 s
Injection speed during PR testing

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