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L'activation répétée des récepteurs à la neurotensine amplifie l'effet stimulant de l'amphétamine sur l'autostimulation intracérébrale.

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Sommaire

La méthode de déplacement des courbes <<curve shift paradigm>> a été utilisée en conjonction avec l'autostimulation intracérébrale pour (i) dissocier les effets sur la récompense des effets sur la performance induits par des drogues psychostimulantes, et (ii) de déterminer si la sensibilisation croisée entre la neurotensine (NT) ou la D-Tyr[11]neurotensine (D-Tyr[11]NT) et la d-amphétamine, qui produit une augmentation de la locomotion *in vivo*, produira également, une amplification de l'effet de récompense lorsque l'administration systémique de la d-amphétamine suit l'administration intracérébrale répétée d'un ou de l'autre neuropeptide. Comme prévu, la première injection, de NT ou de D-Tyr[11]NT, a diminué la performance et les seuils de récompense. Au Jour 7, après la quatrième et dernière injection, on a observé chez les animaux ayant reçu de la NT une tolérance par rapport aux effets de récompense de la première injection alors que les animaux ayant reçu de la D-Tyr[11]NT ont démontré une sensibilisation à ce même effet. Au septième jour, les injections de NT ont continué d'atténuer la performance alors que les animaux ayant reçu la D-Tyr[11]NT ont démontré une tolérance à cet effet. Une seule injection systémique de la d-amphétamine administrée une semaine plus tard (jour 14) a abaissé immédiatement le seuil d'autostimulation intracérébrale chez tous les animaux observés. Cet effet a commencé à diminuer vers la fin de l'expérience. Aucune sensibilisation croisée n'a été observée entre les peptides et la d-amphétamine sur les seuils d'autostimulation. Toutefois, le groupe qui a reçu le D-Tyr[11]NT a démontré une sensibilisation croisée qui a produit une augmentation de la performance induite par la d-amphétamine (relatif aux animaux ayant reçu un traitement préliminaire de saline). Le groupe contrôle a reçu en traitement préliminaire une injection de solution saline (0,9%) en quantité équivalente. En s'inspirant de la littérature existante, une hypothèse a été élaborée pour expliquer ces résultats. Selon ce modèle, la sensibilisation induite par les drogues psychostimulantes est composée de trois éléments distincts : la <<locomotion vers l'avant>> (<<forward locomotion>>), la stéréotypie et la récompense augmentée, que le système dopaminergique mésocorticolimbique sépare en réponses distinctes gérées principalement, mais pas exclusivement, par l'aire tegmentaire ventrale, la substance noire et le noyau accumbens.

Effects of repeated central injections of d-tyr[11]neurotensin and of neurotensin on amphetamine-induced potentiation of brain stimulation reward

Abstract

The curve shift method and the brain stimulation reward paradigm were used to dissociate reward and performance changes and to determine whether cross-sensitization to the locomotor stimulating effects of d-amphetamine (1 mg/kg, IP) extended to the reward-enhancing effects that this drug also produces when given subsequent to repeated unilateral ICV microinjections of neurotensin (18 nmol/10 μl) (NT) or D-Tyr[11]neurotensin (1.8 nmol/10 μl) (D-Tyr[11]NT). As expected, both acute NT and D-Tyr[11]NT attenuated reward threshold and performance. By Day 7, after the fourth and final peptide injection, tolerance had developed to the reward enhancing effect of acute NT while sensitization had developed to this effect by animals given D-Tyr[11]NT. NT injections continued to suppress maximal rates of responding on Day 7 while tolerance developed to this effect by animals given D-Tyr[11]NT. The acute systemic injection of d-amphetamine administered one week later (Day 14), immediately potentiated brain stimulation reward in all animals; an effect that began to subside towards the end of testing. No animals showed cross-sensitization on this parameter. In contrast, only the group that had been given D-Tyr[11]NT during peptide pre-treatment showed cross-sensitization to the d-amphetamine induced increase in maximal rates; this effect was statistically significant. Control animals had been pre-treated with an equal-volume injection (10 μl) of 0.9% saline. Results were discussed in relation to the current literature and were explained using a hypothetical model in which drug-induced sensitization is broken down into three distinct components: Forward locomotion, stereotypy, and reward enhancement, which are compartmentalized by the mesocorticolimbic DA system such that each component is mediated primarily, but not exclusively, by the VTA, SN and NAc, respectively.

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1.0 Introduction

Psychoactive drugs are drugs that affect brain functioning. There are many different classes of psychoactive drugs, several of which have been consumed for non-medicinal purposes for at least 1000 years. The ancient Greeks, for example, used opium, an extract of the poppy plant, for its recreational as well as its medicinal, analgesic effects, and the ancient Inca of Peru consumed coca leaves, the source of cocaine, for their mind-altering effects (Snyder, 1999). That which makes the consumption of certain drugs a subjectively pleasing recreational activity also instils within them the potential to become the objects of dependency by their users. Although most people who engage in psychoactive drug self-administration do not become addicts (Shaffer, 1997), many do.

Drug addiction is a phenomenon associated with frequent episodes of intense, compulsive drug-seeking and drug-taking behaviour that ultimately disrupts all aspects of an individual's life including work, family, friends, and leisure. Many habit-forming drugs are extremely powerful agents of abuse comprising addictive liabilities of enormous magnitude; this is not surprising when these drugs (i.e. cocaine or morphine) are compared to the sources from which they come (the coca or the poppy plant respectively). Cocaine and morphine are the chemically isolated psychoactive ingredients of the above-mentioned plants; this molecular refinement enables these drugs to exert their mood-enhancing effects immediately and at intensities never intended by nature.

The development of drug addiction can be swift depending on a variety of interacting factors including one's genetic disposition toward drug abuse, the biological changes that result from chronic (repeated, intermittent) drug intake, and the psychological as well as the social stability of the individual exposed to these drugs. There are as many approaches to understanding drug addiction as there are factors contributing to its development; although these approaches overlap, each focuses on a particular aspect of the problem. For example, while the social approach considers one's social status and one's ability to interact with others, the environmental approach focuses on one's home and work environments and the levels of stress associated with each. Consequently, both of these approaches predict who are at risk of becoming drug addicts, and what can be done to prevent this from happening. The behavioural approach, on the other hand, focuses on the changes in one's behaviour that result from taking habit-forming drugs, as well as on how these behavioural changes evolve as addiction to the drugs develops.

The objective of the biological approach, the approach of this paper, is to focus on the neural anatomical substrates that are responsible for the changes in behaviour that drug addiction is synonymous with.

I will begin by introducing the phenomenon of brain stimulation reward (BSR), an in vivo experimental model that has helped uncover much of what is known about the neurobiology of the reward-relevant pathways today. I will then review literature on the neural substrates of reward, describe the phenomenon of psychostimulant-induced reward, the role that amphetamine has played in the rodent model of self-administration, and summarise what is known about amphetamine's mechanism of action. Thereafter, I will present the phenomenon of behavioural sensitization and its relevance to drug addiction, which will be proceeded by a description of amphetamine-induced sensitization and what amphetamine has revealed through the rodent locomotor activity model of sensitization. I will then briefly review the roles that glutamate, glutamatergic efferents of the prefrontal cortex, neurotensin (NT), and the NT analogue, D-Tyr[11]neurotensin (D-Tyr[11]NT) play in the phenomenon of drug sensitization. Finally, I will introduce the main objectives of the present set of experiments:

(1) To compare the effects of NT and D-Tyr[11]NT on reward produced by BSR in the mesencephalic central grey, and on the ability of the animals administered either of these peptides to produce an operant response to obtain this reward.

(2) To determine the effects of repeated intermittent exposure to each of the peptides on measures of reward and performance.

(3) To determine whether repeated intermittent exposure to either peptide alters the effects of systemic amphetamine on measures of reward and performance.

1.1 Brain stimulation reward

Olds & Milner (1954) discovered that rats, of their own volition, would self-administer electrical stimulation conveyed through electrodes surgically implanted deep inside their brains; in fact, they did so enthusiastically, but only when the electrodes were placed in specific brain areas. That the electrical stimulation applied to only a limited number of brain loci sustained operant responding implies that anatomically specific circuits dedicated to transmission of a reward-relevant signal exist inside the brain. Olds and Milner also found that this electrical rewarding stimulation could selectively enhance any

behaviour coupled contingently to it, suggesting that as any naturally occurring stimulus, BSR act as a positive reinforcer. Furthermore, early experiments suggested that substances of abuse act on the neural circuitry that mediates BSR, which explains why these drugs can also act as potent positive reinforcers (Killam et al., 1957).

Problems with the notion that BSR and habit-forming drugs exert rewarding effects by acting on the same neural substrates existed. Firstly, if habit-forming drugs, themselves, are reinforcing because they act on common neural substrates, why are different classes of these drugs differentially self-administered by laboratory animals in a free environment (operationally defined as unlimited accessibility to these drugs)? Indeed, experienced animals consume opiates as a steady stream of modest, uniform doses (Deneau et al., 1969). In contrast, unlimited access to psychomotor stimulants shows a behavioural pattern of responding distinguished by alternating periods of bingeing and abstinence (Deneau et al., 1969; Pickens & Harris, 1968; Risner & Jones, 1976). Although the differences that exist in the overall pharmacodynamics and molecular configurations that characterize opiates and psychostimulants could explain the two distinct behavioural patterns just described, an equally plausible explanation may be that these drug self-administration patterns result from confounding produced by ad libitum availability. To illustrate, humans may behave differently when access to food is constant versus when it is available at variable intervals; in an ad libitum situation, humans may eat for many reasons other than nourishment (i.e. it tastes good or boredom). However, if they are uncertain of when they will eat again, humans eat even though they are neither hungry nor in the mood to "munch"; they may even eat things they do not like in order to avoid being hungry later, when food is no longer available. By manipulating the availability of stimulants so that different doses were available during different test sessions over the course of the entire experiment, Yokel & Pickens (1974) demonstrated a pattern of drug intake of psychomotor stimulants that resembled that of opiates. Actual drug intake by these animals, over the range of doses to which they were exposed, was uniform. Similar to the manner in which humans regulate their eating behaviour to keep from going hungry, the data of Yokel & Pickens (1974) showed that animals self-administer habit-forming drugs in such a way as to maintain a consistent blood and brain level, regardless of the class to which these drugs belong. Different classes of habit-

forming drugs, therefore, are able to promote similar self-administration patterns of consumption by laboratory animals.

Another problem with the viewpoint that BSR and habit-forming drugs activate the same reward-relevant substrates involves the differential patterns of responding that BSR and certain habit-forming drugs produce. Although the behavioural pattern of responding for BSR by unrestrained animals resembles that generated by animals working for ad libitum cocaine, it is quite different from that induced by habit-forming drugs in general. Unlike the behavioural patterns evoked by the drugs mentioned above, animals will work to acquire BSR to the point of exhaustion, without abstaining to negotiate even vital necessities such as food or water; they will also extinguish this behaviour extremely rapidly once BSR is no longer available (Gardner, 1997). That the rewarding effects of BSR are so immediate, and so quickly "forgotten" implies that they cannot be mediated by the same substrates mediating reward by drugs of abuse which, regardless of class, produce consistent and enduring patterns of extinction behaviour. Challenging this scepticism, Lepore & Franklin (1992) manipulated the manner in which BSR was administered to animals working to attain it and demonstrated that by mimicking the pharmacokinetics of self-administered drugs, BSR could evoke behavioural response patterns that were very similar to those seen in experiments using habit-forming drugs. Thus, BSR is also capable of reproducing the typical behavioural response pattern of extinction, which is marked by frustration-like responding (a preliminary, deliberate response rate increase), and followed thereafter by a gradual decay in responding that eventually leads to complete extinction.

In summary, Olds and Milner (1954) discovered that electrical brain stimulation can be rewarding, and in doing so, unveiled a tool capable of indicating exactly where in the brain reward-relevant substrates are located. Then, Killam et al. (1957) provided some experimental data which suggest that drugs of abuse act on the same neurocircuitry as BSR, which supported the existence of a brain reward system that, when activated by a given stimulus, signals the rewarding nature of that stimulus. Problems confronting the notion of a common neurocircuitry activated during BSR and by abusive drugs, including differential drug self-administration patterns evoked by opiates and psychostimulants, and differences in extinction patterns produced by both these classes of habit-forming drugs and BSR were then presented and resolved. In essence, when experiments are designed to match BSR and drugs of abuse for the

particular characteristics being measured, the behaviours that result are similar. Finally, to the extent that the similarities in the behaviours produced by BSR and abusive drugs are indicative of their activation of the same neural substrates, the potential for BSR to uncover the exact nature of the neurocircuitry mediating reward in general, and drug addiction in particular, is strengthened considerably.

1.2 Neural substrates of reward

The brain stem is the area of the central nervous system located between the spinal cord and the diencephalon. It contains cell bodies of three monoaminergic systems: dopamine (DA), norepinephrine (NE), and serotonin (5-HT) (Aston-Jones, 1984; Bowker et al., 1983; Roberts, 1978). Using histofluorescence microscopy, a method for mapping monoaminergic neurotransmitters developed during the 1960s, Dahlström & Fuxe (1964) began to uncover the monoaminergic anatomy within the brain, and in particular, that of DA, a reward-relevant neurotransmitter. One system, the nigrostriatal DA system, runs axons from the cell bodies of the substantia nigra (SN), thus named because of the dark pigmentation that distinguishes it, to the striatum (Kelly & Dodd, 1991). Another system, the mesocorticolimbic DA system, is a composite of two parallel subsystems. The first subsystem is the mesolimbic DA system. It runs axons from the ventral tegmental area (VTA) through the "medial forebrain bundle" (MFB) to various limbic structures including the amygdala, septum, olfactory tubercle, and the nucleus accumbens (NAc). The term "limbic", from the Latin word for "border", classifies a group of brain structures that surround the diencephalon, thus forming a border between it and the cerebral cortex (Kupfermann, 1991). Collectively, the limbic system is a constellation of brain structures devoted to memory, motivation, and the behavioural expression and regulation of emotion (Kupfermann, 1991). The second subsystem, the mesocortical DA system, like the mesolimbic system, begins in the VTA and runs through the MFB, but projects its axons to several cortical areas that belong to the limbic system, including the prefrontal cortex (PFC), as well as the cingulate and the entorhinal cortices (Kupfermann, 1991).

Since the discovery of Dahlström & Fuxe (1964), DA has proved to be a very important reward-mediating neurotransmitter. Corbett & Wise (1980), for instance, showed that current thresholds, which initiate operant responding for BSR, fluctuate as a function of the density of dopaminergic nerve elements

encompassing the tip of the stimulating electrode. Similarly, Miliaressis et al. (1982) revealed a similar (response rate / specific cell density) relationship in the midline raphé nuclei, a group of cell bodies in the brain stem from which some serotonergic neurones originate. Although serotonergic fibres ascend throughout the ventral midbrain and ventral diencephalon, a dense set of these projections synapses onto the mesolimbic DA system (Snyder, 1999), where brain reward is believed to be mediated. It is likely then, that this limbic-bound serotonergic projection relays reward-relevant information from the raphé to the mesolimbic DA system where it can be processed as such.

Further supporting a role for DA in the mediation of reward, is the fact that blocking DA neurotransmission can attenuate, and even completely abolish the rewarding efficacy of BSR (Boye & Rompré, 1996b, Morgenson et al., 1979; Wise, 1980a). In fact, DA blockade raises thresholds for BSR, thus imitating the consequence of reducing the strength of the electrical stimulation (Zarevics & Setler, 1979). Consequently, it is widely accepted today that DA neurotransmission, within the central mesolimbic DA systems described above, is critical for the induction of BSR.

Because of the close relationship between DA and BSR, it was once believed that BSR resulted from direct activation of the mesocorticolimbic DA axons that pass through the MFB (Moore & Bloom, 1978); electrophysiological data has since shown that this is not likely. The reward-relevant axons within the MFB directly stimulated during BSR have absolute refractory periods of 0.4-1.2 msec and are thought to be insulated by a fatty substance called myelin (Shizgal et al., 1980; Yeomans, 1979, 1989). In contrast, the absolute refractory periods of dopaminergic MFB axons range between 1.0 and 2.5 msec (Anderson et al., 1996; Yeomans et al., 1988). Because of this variance, it is now believed that MFB DA neurones likely constitute a "second", or later stage of the reward-relevant circuitry (Wise & Bozarth 1984). In as far as the MFB DA pathway is believed to be the target upon which habit-forming drugs exert their euphoric effects in humans as well as their reward-enhancing effects in laboratory animals (Wise, 1980a, 1980b), this "in-series" model of brain reward-circuitry supports the notion of a common reward-mediating substrate for BSR and abusive drugs, as well as the involvement of DA in this process. Indeed, microinjection studies have revealed that the VTA is the site responsible for the reward-enhancing and BSR-threshold-lowering effects of morphine (Bozarth & Wise 1984), and that the NAc is the site responsible for these effects when they are produced by amphetamine (Colle & Wise, 1988).

Furthermore, when microinjected directly into the NAc, neuroleptics (drugs that block DA receptors) attenuate the rewarding impact of both morphine and amphetamine (Wise & Rompré, 1989). Neuroleptics also reduce the rewarding efficacy of BSR (Gallistel & Karras, 1984; Gallistel & Freyd, 1987), and this reduction effect is potentiated by opiate antagonists (drugs that blocks opiate receptors) (Esposito et al., 1981). Finally, abusive drugs are known to lower BSR thresholds in laboratory animals (Wise, 1996), revealing the existence of a synergistic BSR-drug interaction for reward activation, which again substantiates the "in-series" model of reward-relevant neurocircuitry put forth by Wise & Bozarth (1984).

Murray & Shizgal (1996b) extended the known limits of the general reward system by uncovering a reward-relevant link between the anterior lateral hypothalamus and the VTA. Murray & Shizgal (1996a) also demonstrated that sufficiently large lesions of the anterolateral MFB could significantly and permanently elevate BSR thresholds at MFB sites posterior to (behind) the lesions. Together, these data implied that the efferent (descending) "first-stage" reward-relevant neurones connect the ventrolateral MFB to the VTA, and that these fibres likely make up a mixed population of axons that emerge from the anterior nuclei of the MFB. By placing two electrodes within the MFB, one in or near the VTA and the other near the hypothalamus, Gallistel et al. (1996) supported this view by demonstrating that only lesions of the MFB behind a given BSR site (relative to the VTA) significantly increased BSR threshold.

The anatomical mapping study performed by Rompré & Miliaressis (1985) revealed a group of BSR-positive sites projecting from the VTA back to the central grey region of the posterior mesencephalon (the area of the raphé). Using a behavioural model, collision tests were later performed by Boye & Rompré (1996b) who confirmed that these earlier data represented a previously undocumented segment of the reward-relevant pathway, and thus extended the known limits of the overall neural circuitry of reward. Finally, Moisan & Rompré (1998) provided electrophysiological evidence confirming that reward-relevant signals originating in the posterior mesencephalon activate a subset of midbrain DA neurones, thus serving as additional support for the hypothesis that DA neurones constitute a second stage of the reward-relevant circuitry.

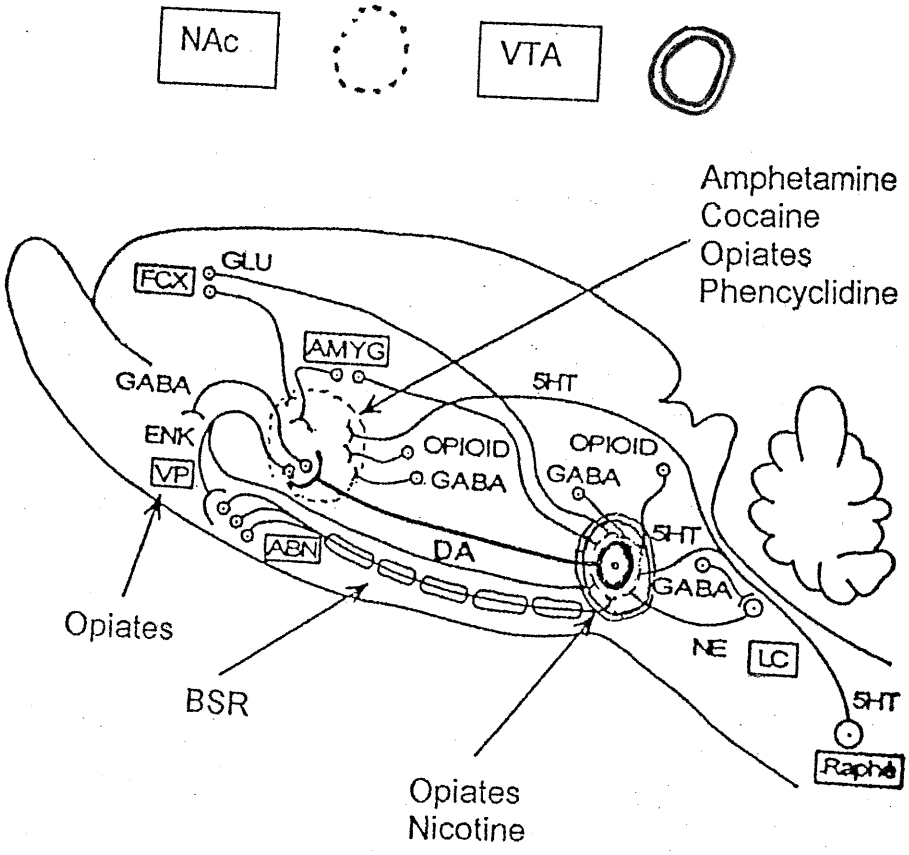
To summarise, the "first-stage" neurones of the reward-relevant circuit comprise a group of myelinated, non-DA, axons that transmit reward-relevant signals between at least the anterior portion of

the MFB and the VTA. Similarly, a group of axons, possibly serotonergic in nature, transmit reward-relevant signals from the raphé to the VTA. From the VTA, these signals are transferred to the "second-stage" mesolimbic and/or mesocortical MFB DA fibres, which transmit these signals to the terminal fields of the NAc and/or the PFC respectively. It is believed that drugs of abuse directly activate these mesocorticolimbic neurones. See Figure 1 for details.

Figure 1.

Schematic diagram of the presumed neurocircuitry of reward and sites at which various substances of abuse are thought to exert their reward-enhancing effects. Norepinephrine (NE) is a catecholaminergic neurotransmitter. Noradrenergic neurones originate in the locus coeruleus (LC) and synapse in the ventral tegmental area (VTA). GABA is an inhibitory neurotransmitter. GABAergic neurones form a fibre system that synapses onto the noradrenergic neurones of the LC and the cell bodies and terminal fields of the dopaminergic neurones that extend from the VTA to the nucleus accumbens (NAc). GABAergic neurones also project from the NAc to the ventral pallidum (VP). Opioids are endogenous peptides involved in a neural system that synapses in the VTA and NAc (OPIOID). Enkephalin (ENK) is an endogenous opioid pentapeptide. Enkephalinergic neurones emanate from the NAc forming a direct loop with the mesoaccumbal DA system via synapses from the NAc to the VTA, and an indirect loop via synapses onto the non-dopaminergic neurones of the anterior bed nuclei (ABN), which synapse onto dopaminergic cell bodies of the VTA. Reward-relevant neurones, thought to be serotonergic (5-HT) in nature, ascend from the raphé to the VTA and the NAc. Glutamate (GLU) is an excitatory neurotransmitter. Glutamatergic projections originate in the frontal cortex (FCX) and descend to the VTA and the NAc. Electrical brain stimulation (BSR) preferentially activates descending myelinated non-DA fibres that extend from the ABN to the dopaminergic cell bodies of the VTA. The dopaminergic cell bodies and axon terminals, located in the VTA and NAc respectively, are thought to be preferentially activated by various drugs of abuse. Although this figure indicates that amphetamine and cocaine act in the NAc only, these agents also exert their effects when injected directly into the VTA. Diagram taken from Gardner, E. L. (1997).

Figure 1


NAc
VTA
Amphetamine
Cocaine
Opiates
Phencyclidine
FCX
GLU
AMYG
GABA
5HT
ENK
VP
OPIOID
GABA
OPIOID
GABA
5HT
ABN
DA
GABA
NE
LC
5HT
Raphe
Opiates
BSR
Opiates
Nicotine

It is also likely that a group of "third-stage" neurones carry the reward signal from the terminal fields of the NAc to the ventral pallidum (VP) (Heimer et al., 1991; McAlonan et al, 1993; Mogenson et al., 1983; Wise & Bozarth, 1984). Chrobak & Napier (1993) obtained data suggesting that this hypothetical "third-stage" pathway use the endogenous opioid pentapeptide, enkephalin (ENK) as its principal neurotransmitter. They established that the majority of post-synaptic NAc neurones are sensitive to opiates. Interestingly, lesions to this NAc output pathway significantly reduce intravenous self-administration of both cocaine and opiates (Hubner & Koob, 1990), and significantly attenuate the acquisition of a conditioned place preference for these drugs (McAlonan et al., 1993). Furthermore, BSR applied to distinct rewarding brain loci including the basolateral amygdala, the medial dorsal thalamus and the posterior hypothalamus can also exert reward by releasing opioids in the VP (Stein, 1993).

γ–aminobutyric acid (GABA) is an inhibitory neurotransmitter (Kandel & Schwartz, 1991). Kalivas et al. (1993) revealed the existence of GABAergic synaptic connections between the NAc, the VP, and the VTA; Sesack & Pickel (1995) confirmed this, and both teams showed that this GABAergic pathway is co-localized with the previously described enkephalinergic projection from the NAc to the VP. It is thought that these two separate fibre systems co-regulate the same groups of dopaminergic neurones located in both the VP and VTA such that GABA hyperpolarizes (inhibits) them, while ENK disinhibits (excites) them, thereby providing both positive and negative feedback loops to this dopaminergic system. Supporting this hypothesis, Carlezon & Wise (1996) asserted that the crucial event for drug-induced reward is the inhibition of the GABAergic medium spiny output neurones located in the NAc. If ENK regulates rewarding DA transmission by disinhibiting the dopaminergic neurones it reaches, the proposed enkephalinergic neurones should synapse directly onto the mesotelencephalic dopaminergic axon terminals; this very type of (axo-axonic) synapse has been identified within the mesolimbic system (Gardner et al., 1980; Pollard et al., 1977, 1978). Furthermore, Kubota et al. (1986) found that a portion of the ascending mesotelencephalic dopaminergic fibres synapse directly back onto these enkephalinergic neurones.

In closing, the mesocorticolimbic DA system has been established as a crucial component of the brain's reward circuitry. Furthermore, BSR has been vital in revealing a series of interconnected fibre groups. These clusters of projection interneurones comprise at least three distinct interconnected

pathways, which together form an in-series circuit for reward that is fortified by various dopaminergic, GABAergic, and enkephalinergic feedback loops. Finally, the co-localization of the endogenous opioid peptide ENK with GABA, and the regulatory interactions between these neurones and the mesocorticolimbic DA fibres of the reward system, collectively, support the view that the same system mediating opiate reward also mediates psychostimulant reward.

1.3 Psychostimulant-induced reward and the rodent model of self-administration

Psychostimulant-induced reward is characterized as the feeling of euphoria felt by humans shortly after the ingestion of various classes of drugs. Laboratory animals are ordinarily used to investigate the neurobiology of the behavioural effects induced by drugs because they provide a comprehensive model that can be physically dissected so that direct correlations may be made between the drug-induced behaviours and the neurochemical and/or neurophysiological alterations that coincide with these drug-induced behaviours.

The rodent model of self-administration has revealed that virtually all drugs with abuse potential in humans are readily self-administered by laboratory animals. This is true, however, only for drugs that are abused for their positive reinforcing effects; anxiolytics for example, (i.e. valium), which are abused by humans, are not readily self-administered by laboratory animals (Gardner, 1997). In the self-administration paradigm, animals are trained to produce an operant response (i.e. press on a lever) to receive single injections of drug via an indwelling intravenous catheter; the number of lever presses by rats receiving drug is then compared with that by rats receiving either saline or nothing at all (via "dummy" lever-pressing). Although intraperitoneal and intramuscular routes are not effective in this paradigm, the intracerebral route of administration is. Furthermore, intracerebral injections are especially useful because, unlike injections via any other route, they enable molecules that do not readily pass through the blood-brain-barrier to be investigated.

1.4 Amphetamine

Amphetamine is a synthetic drug that mimics the psychoactive effects of the natural drug cocaine; like other drugs of abuse, it is readily self-administered by rats (Götestam & Andersson, 1975; Pickens &

Harris, 1968; Yokel & Pickens, 1973). Amphetamine was first used in the 1920s as a treatment for asthmatic wheezing because of its ability to dilate animal bronchia (see Snyder 1999). Its ability to suppress appetite (Booth, 1968, Cole, 1968; Leibowitz, 1975; Sanghvi et al., 1975) promptly transformed it into the remedy of choice for weight loss (Edwards & Swyer, 1950; Frisk, 1950; Howells,1955; Janut, 1952). However, its stimulating and euphoric effects quickly turned many of the people taking it for weight loss into amphetamine addicts (Bell, 1961; Kiloh & Brandon, 1962; McCormick, 1962; Oswald & Thacore, 1963).

Consistent with amphetamine use in humans, the rodent model of self-administration has revealed a behavioural pattern of responding for amphetamine characterized by alternating intervals of bingeing and abstinence (Pickens & Harris, 1968). Amphetamine self-administration in laboratory animals has since been established as an effective model for drug addiction.

Low to moderate doses of amphetamine evoke increases in forward locomotion and decreases in BSR threshold while larger doses produce periods of stereotypy, defined as species-specific, continuous and repetitive, exploratory-like behaviours that serve no apparent purpose (Ellinwood, 1971; Randrup & Munkvad, 1974). In the rat, sniffing, licking, biting, or gnawing typically characterizes stereotypy. To the extent that the divergent behaviours just mentioned are controlled by different neural destinations connected to the overall drug-activated neurocircuitry, these behaviours do not represent phase components of a pre-determined, unique composite of action. In fact, low stereotypy-inducing doses of amphetamine generate a behavioural pattern comprising mostly sniffing, and head and limb movements (Costall & Naylor, 1974; Segal & Kuczenski, 1997), whereas the administration of subsequently larger doses evokes completely different behaviours including biting, gnawing, and licking (Costall & Naylor, 1974). Furthermore, some components of stereotypy, such as limb movements and sniffing, can be induced by pharmacological agents that fail to evoke gnawing or biting (Braestrup et al., 1975; Costall & Naylor, 1975).

1.4.1 Amphetamine's mechanisms of action

Among the various classes of the amphetamines (i.e. levo-amphetamine and methamphetamine), dextro-amphetamine (d-amphetamine) acts by releasing monoamines (DA, NE and 5-HT) from the

terminal fields of monoaminergic neurones, by blocking the re-uptake of these monoamines, and by inhibiting monoamine oxidase; d-amphetamine shows higher selectivity for DA than it does for NE or 5-HT (Arnold et al., 1977; Carruba et al., 1977; Chiueh & Moore, 1974; Dyck et al., 1980; Leonard, 1976; Reches et al., 1977).

1.4.1.1 Dopamine

Consistent with the previously-noted data implicating the "second-stage" mesocorticolimbic DA system as the target of abusive drugs, in vivo microdialysis (Butcher et al., 1988; Zetterström et al., 1983) and brain voltammetry techniques (Gazzara et al., 1986; Knott et al., 1986) have demonstrated that amphetamine produces robust increases in extracellular DA in the neostriatum and NAc. Neither the pharmacologically induced abolishment of action potentials (Westerink et al., 1989), nor the reserpine-induced depletion of existing vesicular DA pools (Butcher, et al., 1988) significantly affects d-amphetamine's ability to enhance extracellular DA in these reward-relevant loci. It is therefore hypothesized that d-amphetamine preferentially releases pre-synaptic DA through a DA-carrier-controlled mechanism from the newly synthesised DA pools that it manufactures rather than release DA by action-potential-dependent exocytosis. Moreover, DA synthesis inhibitors completely abolish the behavioural effects of d-amphetamine (Weissman et al. 1966) as well as the enhanced DA release in forebrain reward loci (Butcher et al., 1988). Hence, further support for the notion that d-amphetamine acts by manufacturing and releasing newly formed DA as opposed to liberating previously packaged vesicular DA. Accordingly, Robinson et al. (1988) suggested that the increased efflux of DA observed in response to an amphetamine challenge by animals sensitized to d-amphetamine does not affect basal DA release; instead, sensitized neurones simply become more able to release more DA when more DA is available.

Notwithstanding, an active role for vesicular DA in mediating the effects of psychostimulants cannot be ruled out. Jaber et al. (1998) showed that "knock-out" mice missing the plasma membrane dopamine transporter (DAT) exhibited enhanced spontaneous locomotor activity similar to that seen by normal mice treated with amphetamine or cocaine. In addition, Fumagalli et al. (1999) demonstrated that by damaging vesicular function in mice heterozygous for vesicular monoamine transporter 2 (VMAT2 +/-), one could enhance methamphetamine-induced dopaminergic neurotoxicity in the striatum, relative to

wild-type control animals. Interestingly, Caron and co-workers also found that genetically removing the gene for DA transport in mice led to "persistent extracellular hyperdopaminergic tone that (was) functionally revealed as hyperactivity", (Gainetdinov et al., 1999). Finally, by isolating DA D_2 as the DA receptor subtype necessary for amphetamine-induced disruption of pre-pulse inhibition, a sensorimotor gating phenomenon, (there are at least four DA receptor subtypes known to date; see below for details), Ralph et al. (1999) emphasized, albeit indirectly, amphetamine's role as a DA re-uptake inhibitor. (It is widely accepted that DA re-uptake is a brain mechanism that relies extensively on autoreceptors, which are characterized as being exclusively of the DA D_2 receptor subtype; see below for details.)

At a molecular level, amphetamines may be defined as weak base psychostimulants (Sulzer et al., 1992, 1993). Weak-base agents spread across (vesicular) membranes according to the membranes' pH gradients (Maron et al., 1983). As the concentration of a weak-base agent increases to the point at which it surpasses the buffering capacity of the vesicle's interior, it causes the pH gradient across the vesicular membrane to breakdown (Sulzer et al., 1992). In the case of the amphetamines, which are the only extensively used class of drugs that promote transmitter release via a non-exocytic mechanism (Schuldiner et al., 1993), it is believed that an accumulation into synaptic vesicles diminishes vesicular DA reuptake, which, in turn, results in competition for protons between the amphetamine and vesicular DA. Consequently, the uncharged DA that results, diffuses out of the vesicle following its electrochemical concentration gradient, while the redistribution of vesicular DA leads to an increase in cytosolic DA; this increase, in turn, promotes reverse transport by increasing the concentration across the dopamine transporter (DAT) substrates (Amara & Kuhar, 1993; Pifl et al., 1995), thus leaving the amphetamine appropriated in the vesicles (Sulzer et al., 1992, 1993, 1995).

Several lines of evidence argue that the d-amphetamine-induced increase in NAc DA mediates the locomotor activating effect of amphetamine. For example, electrolytic lesion of the rostral hypothalamus, which interrupts mesolimbic dopaminergic input, abates amphetamine-induced locomotion (Costall & Naylor, 1974). Furthermore, 6-hydroxydopamine (6-OHDA), which, under specific conditions selectively destroys dopaminergic neurones, eliminates amphetamine-induced locomotion when it is applied to the NAc (Iversen et al., 1975; Joyce & Koob, 1981; Kelly & Iversen, 1976). In addition, Kelly & Roberts (1983) showed that electrolytic lesions of the NAc blocked the locomotor activating effects of d-

amphetamine. Consequently, it was concluded that the efferent fibres of the NAc serve to inhibit locomotor activity. Nevertheless, d-amphetamine injected directly into the NAc produces robust locomotor effects (Pijnenburg & van Rossum, 1973); these locomotor effects were profusely more intense than those following similar microinjections into the neostriatum. Furthermore, neuroleptics blocked the expression of locomotor behaviour only when they were injected into mesolimbic areas (i.e. the NAc); the same dose of neuroleptic injected into the caudate nucleus, for example, was unable to inhibit this behaviour (Pijnenburg et al., 1975). Finally, Thornburg & Moore (1973) demonstrated that blocking DA synthesis could attenuate d-amphetamine-induced locomotor activity, whereas blocking NE synthesis did not affect this behaviour at all. Taken together, these data confirm that the locomotor activating effects produced by d-amphetamine are the result of d-amphetamine-induced increases in extracellular DA in the NAc, and that similar drug-induced increases of DA in the neostriatum do not affect locomotor behaviour.

1.4.1.2 Norepinephrine

Although d-amphetamine's influence on NE is less vigorous than its influence on DA (Chiueh & Moore, 1974; Dingell et al., 1967; Weissman et al., 1966), the mechanisms by which d-amphetamine enhances NE neurotransmission are similar to those previously described for DA. Some believe that NE is very closely involved in the mediation of d-amphetamine-induced reward and locomotor-activation (Snyder, 1999). To the extent that this is true, it is nevertheless likely that NE plays a role secondary to that played by DA in d-amphetamine-induced behaviours. Notwithstanding, the extremely widespread synaptic connections between the ascending noradrenergic efferents of the locus coeruleus and the rest of the brain cannot be overlooked.

1.4.1.3 Serotonin

Although, less is known about amphetamine's influence on 5-HT and/or on serotonergic neurones *per se*, 5-HT is responsible for some of the neural changes that follow repeated d-amphetamine administration, and these alterations are likely to play a role in amphetamine-induced locomotor hyperactivity. Supporting this notion, Hotchkiss et al. (1979) demonstrated that chronic

methamphetamine treatment generates long-term alterations in the activity of tryptophan hydroxylase, the rate-limiting enzyme in the biosynthetic pathway of 5-HT; these changes are likely to affect 5-HT neurotransmission. Furthermore, Ricaurte et al. (1980) showed that repeated amphetamine treatment induces enduring changes in 5-HT content and in uptake sites. Finally, lesions to the raphé nuclei (Neill et al., 1972) or to the ascending serotonergic pathways (Green & Harvey, 1974) emanating from the raphé potentiate d-amphetamine-induced locomotion, as does co-administration of d-amphetamine with parachlorophenylalanine (a compound known to deplete 5-HT stores via inhibition of tryptophan hydroxylase) (Mabry & Campbell, 1973). Together, these findings suggest that 5-HT modulate drug-induced locomotor hyperactivity by exerting an inhibitory influence on it.

In closing, because d-amphetamine has been shown to have a much greater impact on the brain's dopaminergic systems than on its other monoaminergic systems, although both NE and 5-HT may mediate d-amphetamine-induced locomotion, they are likely to do so in concert with, but secondary to DA.

1.5 Behavioural sensitization

Behavioural sensitization is the phenomenon by which an organism becomes progressively more sensitive to one or many aspects of a particular stimulus because of repeated exposure to that stimulus. Behavioural sensitization is known to develop following repeated intermittent exposure to either stressful stimuli or various drugs of abuse including amphetamines (Kalivas & Stewart, 1991; Robinson & Becker, 1986). In fact, both stress and abusive drugs appear to act synergistically in their abilities to produce behavioural sensitization (Antelman et al., 1986; Hahn et al., 1986). Furthermore, this cross-sensitization is believed to be at least partly due to the increased DA-release in the terminal fields of the mesocorticolimbic DA system that both habit-forming drugs and stress produce (Clarke et al., 1988; Kalivas, & Stewart, 1991; Kelly & Iversen, 1976; Suzuki et al., 1997). Supporting the notion of a synergism between stress- and psychostimulant-induced behavioural sensitization, repeated administration of corticosterone (a hormone released by the adrenal cortex in response to stressful stimuli) can substitute for stress and thereby enhance the behavioural effects of amphetamine (Deroche et al., 1992b). Likewise, the suppression of stress-induced corticosterone secretion eliminates cross-

sensitization between stress and amphetamine- or morphine-induced behavioural potentiation (Deroche et al., 1992a).

1.5.1 Drug addiction and behavioural sensitization

Behavioural sensitization is particularly relevant to drug addiction because it is causally linked to the increased propensity of amphetamine self-administration behaviour by laboratory animals with (Piazza et al., 1990; Stewart & de Wit, 1987), or without previous drug self-administration experience (Piazza & Le Moal, 1996). Its association with an increased propensity in humans toward drug addiction and relapse (the reinstatement of compulsive drug-seeking and drug-taking behaviour in abstaining individuals with prior histories of such behaviour) is further substantiated by the work of Robinson & Berridge (1993); they explained that because "the sensitization of DA systems is gated by associative learning", when these systems become sensitized by the repeated use of addictive drugs, they cause an exaggerated importance to be ascribed to the act of drug-taking as well as to stimuli associated with drug-taking. The sensitization of "incentive salience" just described, is what Robinson and Berridge (1993) believe transforms ordinary wanting into extreme drug craving. These researchers also asserted that the sensitization of the neural systems mediating incentive salience could transpire in the absence of changes to the neural systems responsible for either the subjective pleasurable effects of drugs or of drug withdrawal. To the extent that this is the case, as Koob & Le Moal (1997) have suggested, it may be concluded that drug-induced behavioural sensitization depicts the first twist in a downward spiral that marks the potential development of drug addiction.

1.5.2 Amphetamine-induced behavioural sensitization

Research on the effects of acute amphetamine injections has identified the multiphasic, dose-dependent response pattern that has served as a kind of building block upon which data pertaining to behavioural sensitization has been, and continues to be, accumulated. In most strains of rat, a low dose (up to approximately 1.0 mg/kg) of acute amphetamine results in a state of general hyperactivity characterized by locomotion and rearing (Robinson & Becker, 1986). Augmenting the dose to roughly 3.0

mg/kg causes this (initial) hyperactivity to be followed by a period of stereotypy shortly thereafter in which locomotion and rearing are no longer displayed. An ever larger acute dose (approaching 6.0 mg/kg) induces, yet, a triphasic response pattern in which early and late periods of locomotion are separated by intense, focussed stereotypy distinguished by chewing, sniffing, gnawing and repetitive movements of limbs (Segal et al., 1981).

The dose of amphetamine required to induce either locomotor hyperactivity (Leith & Kuczenski, 1982; Segal & Mandell, 1974) or stereotypy (Kilbey & Ellinwood, 1977; Leith & Kuczenski, 1981) decreases with repeated exposure, thus clearly indicating that repeated amphetamine administration leads to the development of sensitization with respect to both these behaviours. Importantly, because of the dichotomy that marks these two behaviours (both stereotypy and locomotion cannot be expressed simultaneously) the underlying neurobiological basis of the sensitization that develops with respect to both behaviours may not be accurately characterized by observation of these two behaviours alone. To illustrate, consider the findings of Segal & Mandell (1974) who demonstrated that when stereotypy was blocked by α–methyl-p-tyrosine, which inhibits tyrosine hydroxylase (the rate-limiting enzyme in catecholamine biosynthesis), an increase in locomotion emerged. Clearly, this implies a competitive relationship between these two elements of the behavioural response to amphetamine, and as such, the expression and duration of the described behavioural phases may, in fact, be the simple, confounding result of one behaviour masking the other. Consequently, both behaviours must be studied concurrently, and with an innovation that allows one to confer these observable behaviours with the corresponding neural alterations that are likely to be intrinsically responsible for them.

1.5.3 Dopamine and behavioural sensitization

As noted earlier, acute d-amphetamine causes increased DA-release in the terminal fields of the mesocorticolimbic DA system; this increase, particularly in the NAc, is believed to be responsible for the expression of the locomotor hyperactivity that this drug also produces. Chronic d-amphetamine administration, in turn, facilitates this drug-induced DA release, and thus, potentiates the ensuing

locomotor activating effect of the drug (Robinson & Becker, 1986). Indeed, the local activation of DA systems is sufficient to induce sensitization (Kalivas & Stewart, 1991; Robinson & Berridge, 1993).

Although we do not fully understand the mechanisms underlying the process of drug-induced behavioural sensitization, the facilitative responses to repeated exposure to d-amphetamine noted above are thought to be mediated, in part, by at least four different subtypes of DA receptors found within the mesocorticolimbic DA system.

Sustained sensitization of DA D_1 receptors located in the NAc has been shown following repeated administration of cocaine or d-amphetamine (Henry & White, 1991; Higashi et al., 1989; Wolf et al., 1994). Furthermore, Vezina (1996) showed that although a variety of DA D_2 antagonists do not block the development of d-amphetamine-induced locomotor sensitization, the DA D_1 receptor antagonist SCH23390 does. Vezina (1996) also demonstrated that pre-treatment with systemically injected SCH23390 blocks the locomotor sensitization typically observed following d-amphetamine microinjection directly into the VTA. Interestingly, co-administration of both SCH23390 and d-amphetamine into the VTA during pre-exposure blocks the sensitized NAc DA response normally observed following VTA injection of d-amphetamine alone (Vezina, 1996). However, this post-synaptic neuroadaptation, which causes enhanced neurotransmission at the junction between the "second-" and "third-stage " neurones of the previously-described reward-relevant neurocircuitry, is believed to develop secondary to the more transient desensitization of (pre-synaptic) DA D_2 autoreceptors located in the VTA (Kalivas & Stewart, 1991; Robinson & Becker, 1986; White, 1996).

DA D_2 autoreceptors are, by definition, a class of DA receptor found exclusively on dopaminergic neurones; they are activated by DA and DA agonists (drugs that activate DA receptors), and their activation causes the DA cells that express them to decrease neurotransmitter synthesis and release. Repeated intermittent exposure to amphetamine causes autoreceptors of VTA DA neurones to become tolerant to DA. In so doing, amphetamine diminishes VTA DA autoreceptors' effectiveness at suppressing DA synthesis and release, and thereby enables more DA to be released from the affected DA neurones (White & Wang, 1984); this autoreceptor sub-sensitivity is likely the consequence of modifications that this DA D_2 negative-feedback system undergoes. For instance, Seutin et al. (1991) demonstrated that acute amphetamine applied to VTA DA autoreceptors in vitro causes transient

desensitization. Based on this observation, these researchers suggested that amphetamine-induced behavioural sensitization is the result of an exacerbation, or perpetuation, of this desensitization.

Regardless of the degree to which both down-regulation and receptor-modulation account for the sub-sensitivity of VTA DA autoreceptors, the enhanced neurotransmission in the terminal fields of the mesocorticolimbic DA system that follows from these neuroadaptations appears to play a pivotal role in the expression of behavioural sensitization to psychomotor stimulants. Notwithstanding, while Khroyan et al. (1998) found that low doses of DA D_3 agonists selectively enhance the stereotypy response typically induced by larger doses of amphetamine, Feldpausch et al. (1998) demonstrated that co-administration of amphetamine with selective D_4 antagonists during pre-treatment completely abolishes the potentiated response to further amphetamine (challenge), thus implicating the DA D_4 receptor subtype in the development of sensitization as well.

Finally, evidence suggests that the dopaminergic mechanisms and substrates responsible for the initiation of sensitization are different from those responsible for the behavioural expression of sensitization (Kalivas & Stewart, 1991; Vezina, 1996). Substantiating this assertion, the locomotor activating effect following acute amphetamine, or morphine, does not sensitize with repeated microinjection into the NAc or the striatum (Dougherty & Ellinwood, 1981; Hitzemann et al., 1980; Kalivas & Weber, 1987; Vezina & Stewart, 1990; Vezina et al., 1987). However, when these same drugs are administered directly into the VTA, behavioural sensitization does develop (Joyce & Iversen, 1979; Kalivas & Weber, 1987; Vezina & Stewart, 1984; Vezina & Stewart, 1990). Taken together, these data strongly suggest that although DA neurotransmission in the terminal fields of the NAc underlie the behavioural expression of sensitization, the initiation of sensitization depends on processes initiated in the VTA.

1.5.4 Glutamate and behavioural sensitization

Glutamate is an amino acid that contributes to the development of behavioural sensitization (White, 1996). L-glutamate is the major excitatory neurotransmitter of the vertebrate CNS; it interacts with three major classes of receptors: NMDA and non-NMDA ionotropic receptors that gate ion channels

directly, and metabotropic, G-protein-coupled receptors that indirectly gate ion channels via activation of second-messengers (Kandel & Schwartz, 1991).

The NMDA receptor is activated by the amino acid analogue N-methyl-D-aspartate (NMDA), and is blocked by a variety of drugs including phencyclidine (also known as PCP or angel dust), and MK801 (Karler et al., 1989, 1990). It has been shown that the development of behavioural sensitization to amphetamine, cocaine, or morphine is prevented by blockade of NMDA receptors with MK-801 (Karler et al., 1989, 1990; Wolf & Jeziorski, 1993; Wolf & Khansa, 1991; Wolf et al., 1995). MK-801 also impedes the mesoaccumbal neuroadaptations that are associated with d-amphetamine-induced behavioural sensitization (Wolf et al., 1994).

White et al. (1995) revealed an enhanced responsiveness of dopaminergic systems to glutamate in animals sensitized to cocaine and amphetamine. Later, Zhang et al. (1997) determined that this increased responsiveness to glutamate is mediated by the non-NMDA AMPA receptors presumably located on VTA DA neurones. Finally, AMPA-receptor blockade has been shown to prevent the development of locomotor sensitization to cocaine or amphetamine without affecting the expression of sensitization to either drug (Li et al., 1997). Therefore, glutamate appears to be involved in the development (Karler et al., 1991), but not the expression of drug-induced locomotor sensitization, a notion upheld by Wolf et al. (1995) who demonstrated that NMDA receptor activation is, in fact, necessary for sensitization to develop.

1.5.4.1 Pathways of the PFC to the mesoaccumbal DA system

The mesoaccumbal DA system comprises dopaminergic neurones of the VTA projecting exclusively to the NAc; this system receives input from various parts of the brain including glutamatergic PFC efferents (descending projections). Indeed, the PFC innervates both the VTA and the NAc. In fact, the principal excitatory projections to the VTA and the NAc are glutamatergic PFC efferents (Sesack & Pickel, 1992). Furthermore, as mentioned before, these excitatory amino acid (EAA) pathways are also involved in the phenomenon of behavioural sensitization in that repeated exposure to amphetamine or cocaine potentiates the excitatory effects of glutamate on VTA DA neurones (White et al., 1995).

Ibotenic acid destroys the cell bodies of dopaminergic neurones causing them to fire repeatedly until death. Wolf et al. (1995) showed that ibotenic acid lesions of the prefrontal cortex or amygdala, and electrolytic lesions of the fornix block the development of sensitization to d-amphetamine-induced locomotor hyperactivity, but not to amphetamine-induced stereotypy. Interestingly, Dewar et al. (1997) revealed that ibotenic lesions of the PFC also diminish the number of DA D_1 receptors in the VTA. Consequently, they suggested that DA D_1 receptors expressed at PFC efferent terminals are relevant to the development, but not to the expression of d-amphetamine-induced sensitization (Karler et al., 1989), which supported Vezina (1996), who showed that the DA D_1 receptor antagonist SCH23390 also blocked the development of d-amphetamine-induced locomotor sensitization.

Finally, Robinson & Kolb (1997) showed that repeated intermittent exposure to amphetamine produces an enduring increase in the length of dendrites, in the density of dendritic spines, and in the number of branched spines of the medium spiny neurones, which constitute a major output pathway from the NAc. Repeated amphetamine was also found to induce similar changes in apical dendrites of layer III pyramidal cells of the PFC; these data are particularly interesting because, as noted earlier, the inhibition of medium spiny neurones in the NAc is believed to be critical for the expression of drug-induced reward (Carlezon & Wise, 1996). Taken together, these data demonstrate the ability of psychostimulant drugs to induce a variety of changes to the reward-relevant circuitry known to be involved in the development of behavioural sensitization. Furthermore, although the mesoaccumbens DA system is a major mediator of this phenomenon, other brain loci and neurotransmitters that interact with this system, such as glutamate and the glutamatergic PFC efferents, are also important and even necessary for behavioural sensitization to develop.

1.5.5 Neurotensin and amphetamine sensitization

Carraway & Leeman (1973) first detected neurotensin (NT) while attempting to isolate another neuropeptide (substance P) from bovine hypothalamic extracts. The extent to which NT exerts its influence as a neuromodulator within, and indeed, throughout the brain, is widespread. In fact, NT has been found to affect various neurotransmitter systems including those of NE, DA, and 5-HT (Jolas & Aghajanian, 1997; Rostène & Alexander, 1997).

High- and low-affinity NT receptors have already been discovered and characterized (Dubuc et al, 1994, 1999; Gully et al., 1993, 1997; Labbé-Jullié, et al., 1994; Mazella et al., 1996; Nalivaiko et al., 1998; Shotte et al, 1986; Tanaka et al., 1992; Vita et al., 1993). The high-affinity NT receptor (NTr1) is a G protein-coupled receptor that is agonised by NT, is insensitive to the antihistamine levocabastine, and is blocked by both known non-peptide NT receptor antagonists to date; SR48692 and SR142948A (Vincent et al., 1999). In contrast, the G protein-coupled low-affinity NT receptor (NTr2) does not bind well with NT, and is blocked by levocabastine and only one of the NT receptor antagonists; SR142948A (Vincent et al., 1999). Mazella et al. (1998) recently characterized a third NT receptor subtype that they referred to as "nts3". Interestingly, nts3 does not correspond to either the NTr1- or the NTr2 NT receptor subtypes previously cloned by Tanaka et al. (1990) and Mazella et al. (1996), respectively, nor does it resemble the NT receptor subtype identified earlier, in the amygdala, by Boudin et al. (1996).

It is noteworthy to mention here the possibility that one subtype of NT receptor be more susceptible to stimulation via ICV injection than other NT receptor subtypes; this possibility is succinctly illustrated by the discovery that while NT microinjected directly into the VTA increases DA-release in the NAc (Kalivas & Duffy, 1990) as well as behavioural hyperactivity in vivo (Rompré, 1997), NT administered directly into the NAc decreases amphetamine-induced locomotion, exerting anti-dopaminergic-like effects (Ervin et al., 1981; Kalivas et al., 1982, 1984). This is important because when NT is injected centrally, it also suppresses maximal rates of responding for BSR (Rompré, 1995), and attenuates amphetamine-induced locomotor activity (Nemeroff et al., 1983), suggesting then, that ICV NT preferentially activates an NT receptor subtype involved in blocking (amphetamine-induced) locomotor hyperactivity as opposed to enhancing this behaviour.

NT is co-localized (at least in rodents) with DA in the VTA (Seroogy et al., 1987; Studler et al., 1988), and NT receptors are distributed across the surface of VTA dopaminergic cell bodies (Dana et al., 1988; Dilts & Kalivas, 1989). In addition, acute VTA microinjection of NT, like amphetamine, significantly enhances BSR (Rompré & Boye, 1993; Rompré et al., 1992), elevates the firing frequency of VTA DA neurones (Seutin et al., 1989), and increases extracellular NAc DA, which is correlated with an increase in locomotor activity (Cador et al., 1985; Elliot et al., 1986; Kalivas & Duffy, 1990). Both the VTA NT-induced augmentation of DA-release in the NAc (Kalivas & Duffy, 1990) and the behavioural hyperactivity

that accompanies this augmentation are potentiated by repeated exposure of NT via intracerebroventricular (ICV) (Rompré, 1997) or direct VTA microinjections (Elliott & Nemeroff, 1986; Kalivas & Taylor, 1985). Thus, repeated intermittent administration of NT sensitizes NT systems; this sensitization, in turn, causes the potentiation of the peptide-induced increases of mesoaccumbal DA neurotransmission that the development of behavioural sensitization is associated with.

1.5.5.1 Neurotensin's mechanism of action

The activation of NT receptors depolarises DA cell membranes through an increase in cationic current (positive ions), or through the closure of P^+ channels. NT also increases the firing of dopaminergic neurones indirectly by reducing the sensitivity of DA D_2 autoreceptors located on the cell body (Seutin et al., 1989; Shi & Bunney, 1990, 1991). Indeed, Farkas et al. (1997) showed that the activation of DA D_2 terminal autoreceptors, which halts DA synthesis and secretion, causes the conductance of potassium ions across the cell membrane to increase, and thus, exerts an effect in complete opposition to that noted above for NT.

Interestingly, it has also been found that NT reduces the affinity of striatal DA D_2-like receptors in vivo, but only for their agonists; even large doses of NT failed to alter DA D_2 -antagonist binding under identical conditions (Von Euler et al., 1990a, b). It must be noted however, that we do not know for sure if the striatal DA D_2-like receptors characterized by von Euler et al. (1990a, b) were, in fact, terminal DA autoreceptors. Regardless of the molecular mechanism, the tight control over DA neurotransmission that NT exerts implies that NT is an important contributing factor to the occurrence of behavioural sensitization.

1.5.5.2 Effects of NT on behaviour

Using the curve-shift paradigm in conjunction with BSR, Rompré (1995) separated implied rewarding effects of centrally administered NT from any other (secondary) effects that might also result from ICV NT microinjection (Miliaressis et al., 1986); in doing so, he revealed two important results. Firstly, ICV NT, like neuroleptics, suppressed maximal rates of responding. Secondly, like psychomotor

stimulants, ICV NT significantly lowered the threshold for BSR responding in the caudal mesencephalic grey, but only for the highest dose tested (30.0 μg / 10 μl), and only after approximately 48 min post-injection. Rompré argued, therefore, that NT potentiated BSR responding in rats because it acts essentially as a psychostimulant. That this potentiation was delayed and apparent only in animals administered the highest dose tested was the result, he claimed, of the concurrent NT activation of another substrate mediating a performance deficit (diminished motor capacity). Furthermore, this second substrate, although more sensitive to NT than the one facilitating BSR responding, produced effects that were either shorter in duration, or of shorter latency than those mediated by the BSR-potentiating substrate. Supporting this viewpoint, NT exerts positive effects on various unrelated substrates that mediate hypothermia (Kalivas et al., 1985; Nemeroff et al., 1979) analgesia (Behbehani & Pert, 1984; Dubuc et al., 1992), hypotension (Kulinska Niedziela & Paluszak, 1997; Rioux et al., 1981), muscle relaxation (Kitabgi & Vincent, 1981; Osbahr 3rd, 1979), catalepsy (Snijders et al., 1982), and hyperglycaemia (Yawata et al., 1984). Rompré (1995) suggested that these two substrates might be located in the VTA and NAc respectively. Indeed, the NAc is more sensitive to the action of NT than the VTA is (Kalivas & Duffy, 1990). One possibility, therefore, is that larger doses of ICV NT compensate for this relative difference in NT sensitivity (Rompré, 1995). To the extent that this is true, the largest dose tested by Rompré (1995) appears to have been enough to override the neuroleptic-like effect of NT action in the NAc (Ervin et al., 1981) with the psychostimulant-like effects induced by NT in the VTA (Rompré & Gratton, 1992). Regardless of the exact mechanisms involved, a large enough dose of NT appears to exert psychostimulant-like effects in behaving rats when centrally administered.

1.5.5.3 Involvement of NT in the sensitization to psychostimulants

Given that neuropeptides are closely associated with the modulation of dopaminergic functioning (Bean & Roth, 1991; Blaha, & Phillips, 1992; Héaulme et al., 1997; Shi & Bunney, 1990, 1991), and that d-amphetamine influences the dopaminergic systems of the midbrain, d-amphetamine should affect neurotensinergic systems. To the extent that d-amphetamine does influence neurotensinergic systems, tests combining the use of both d-amphetamine and NT should further our understanding of the neural

circuitry mediating psychostimulant-induced behaviours, which in turn, should offer a more in-depth understanding of the behaviour and of the neurobiology underlying drug addiction. Rompré (1997) took this approach and reported that repeated intermittent administration of ICV NT sensitizes to the locomotor effects of d-amphetamine as measured by photocell crossings; this finding supported his earlier work (Rompré, 1995), which demonstrated that a large enough dose of NT exerts a psychostimulant-like effect on behaviour. Interestingly, D-Tyr[11]NT, an NT analogue that is more resistant to enzymatic degradation than its endogenous counterpart (NT), and therefore, presumably that much more potent (Checler et al., 1983), also produced this cross-sensitization effect. However, the degree to which it did was no different from the cross-sensitization that followed NT pre-treatment. Consequently, considering that locomotor activity following repeated D-Tyr[11]NT pre-test treatment was significantly higher than that following repeated NT pre-test treatment, this apparent cross-sensitization between d-amphetamine and either NT or D-Tyr[11]NT to the locomotor activating effects of d-amphetamine appears to be independent of NT's ability to invoke locomotor hyperactivity on its own. As Rompré (1997) pointed out, Elliot & Nemeroff (1986) showed that sensitization develops to repeated doses of locomotion-producing VTA NT, however this treatment fails to cross-sensitize to systemic amphetamine. Therefore, direct ventral tegmental stimulation of NT receptors cannot be the cause of the results noted above. Rompré (1997) attributed his results, instead, to indirect NT-induced VTA activation via the direct activation of the medial prefrontal cortex (mPFC), which is consistent with Rompré et al. (1998), who demonstrated that activation of NT receptors located in the mPFC enhances DA cell firing in the midbrain. Wolf et al. (1995), who revealed that lesions to the mPFC diminish amphetamine sensitization, provided further support for this interpretation. Accordingly, Rompré (1997) could explain his findings in a way that was congruent with his earlier work (1995), thus maintaining the notion that a centrally acting NT agonist is likely to influence DA-dependent behaviours in a manner resembling that of the psychostimulants.

1.6 Furthering the prospect of peptide cross-sensitization to d-amphetamine-induced reward potentiation as measured via BSR thresholds.

The purpose of the present set of experiments was to advance our understanding of behavioural sensitization and drug addiction by further characterizing the involvement of NT and D-Tyr[11]NT in these

processes. More precisely, this study was aimed at comparing the effects of NT and D-Tyr[11]NT on reward produced by BSR in the mesencephalic central grey, and on the ability of the animals administered either of these peptides to produce an operant response to obtain this reward. In addition, we sought to determine the effects of repeated exposure to each of these peptides on measures of reward and performance. Finally, we wanted to determine whether repeated exposure to either of the peptides would alter the effects of systemic amphetamine on measures of reward and performance. With this said, it is important to note that although we did not measure locomotor hyperactivity via photo-beam crossings as did Rompré (1997), rates of responding to BSR in the present study were considered, within the context of this experiment, to be reflective of goal-oriented forward locomotion, and as such, were collectively recognised as a de facto index of drug-induced behavioural hyperactivity.

The theories of Glickman & Schiff (1967) and Wise & Bozarth (1987) posit that reward and drug-induced locomotion are governed by a common substrate within the MFB neural circuitry; they also suppose that midbrain DA neurotransmission is crucial for the expression of both these behaviours, and thus predict that the "extended" cross-sensitization alluded to above would occur.

Rompré (1995) attributed the decline in maximal rates of responding that he observed following ICV injection of NT to the activation of a substrate independent from the one believed to be responsible for the peptide-induced reward potentiation that he also simultaneously uncovered. Furthermore, the theory he developed at this time, that, "at high doses ICV, the (psychostimulant-like) action of NT in the VTA counteracts its (neuroleptic-like) action in the NAc", implies that he agreed with the two theories outlined above. As mentioned at the beginning of this section, Rompré revealed, as well, that repeated activation of NT receptors cross-sensitizes to the locomotor activating effects of d-amphetamine (1997). Furthermore, because the data reported by Rompré (1997) did not conflict with either Glickman & Schiff (1967) or Wise & Bozarth (1987), Rompré would likely predict that, in the present set of experiments, NT and D-Try[11]NT would cross-sensitize to the reward-activating effects of d-amphetamine.

Wise and co-workers, however, have gathered data since 1987 that would predict otherwise; in opposition to their earlier model (Wise & Bozarth, 1987), they suggest that drug-induced locomotor stimulating effects and reward facilitation are mediated by two separate mechanisms, and that

sensitization develops to the locomotor effects only (Bauco et al., 1993; Carlezon & Wise, 1993; Wise & Munn, 1993).

2.0 Hypotheses

1. It was hypothesized that both NT and D-Tyr[11]NT would produce effects of equal magnitude on both parameters being measured (reward threshold and maximal rate of responding) because the peptide concentrations used herein were adjusted, based on the findings of Kitabgi et al. (1980), Rompré (1995) and Del Vecchio et al. (1998), to produce equipotent behavioural effects.

2. Based on Rompré (1995), it was hypothesized that on Day 1, following a single ICV peptide injection, a gradual decrease over time would occur, relative to baseline values, for reward threshold, whereas an immediate decrease, followed by a gradual increase toward baseline rates would be observed for maximal rate of responding.

3. It was hypothesized that on Day 7, after the fourth and final intermittent central injection of either peptide, reward threshold would remain unchanged relative to the initial decrease observed on Day 1, while the peptide-induced effect on maximal rate would sensitise, and therefore, decrease further relative to the corresponding Day 1 rate.

4. Based on Wise and co-workers (1993), it was hypothesized that on Day 14, following a single IP injection of d-amphetamine neither NT nor D-Tyr[11]NT pre-treatment would cross-sensitize to the reward-potentiating effects of d-amphetamine, but that pre-treatment with either peptide would cross-sensitise, to the same degree, to the maximal rate increases induced by d-amphetamine, relative to control animals (pre-treated with saline and tested with d-amphetamine).

3.0 Method

3.1 Subjects

Subjects were adult, Long-Evans male rats, weighing between 275 g and-300 g upon their arrival to the animal colony. They were initially housed two per cage for approximately one to two weeks with free access to food and water. Temperature and humidity were maintained at 22^0 C and 50-60 % respectively, in an environment with a 13-h light /11-h dark cycle (lights on at 06:00).

3.2 Surgery

Following between one and two weeks of adaptation to the animal colony, animals were deeply anaesthetised with sodium pentobarbital [Somnotol, 65 mg/kg (IP)], administered an intramuscular injection of penicillin (0.1 ml Penlong XL, 150 000 UI/ml), and fixed on a stereotaxic frame. Approximately 20 min before the anaesthesia, atropine methylnitrate [0.4 mg/kg, (IP)] was administered to minimize bronchial secretions. Then, using the following coordinates, a stimulating electrode (Kinetrods, Ottawa, Ontario, model SME-01) was implanted within the caudal mesencephalic grey, and a 33 gauge single guide canula (Plastic One, model C315G), 11 mm in length was implanted above the left ventricle: 7.6 mm posterior to bregma, 0.0 mm lateral to the midline, 6.8 mm below the surface of the cranium for the stimulating electrode, and 0.3 mm posterior, 1.2 mm lateral and 2.4 mm vertical for the guide canula (Paxinos & Watson, 1986). The incisor bar was adjusted to maintain the surface of the skull horizontal between bregma and lambda. Five jeweller screws were threaded into the cranium and a bared stainless steel wire attached to a male amphenol connector was wrapped around them; the bared wire served as an indifferent electrode during electrical brain stimulation. The whole electrodes-canula assembly was secured to the skull with dental acrylic. The guide canula was closed with a removable stylet (Plastic One, Dummy Canula, model C315DC, length flush with the tip of the guide canula). Skin around the dental acrylic was sutured and immediately thereafter, treated with an antibiotic paste (Hibitaine Antibacterial-Antifungal Ointment, 1.0 % chlorlexidine acetate).

3.3 Training procedure and behavioural testing

After a minimum one-week postoperative recovery period, animals were screened for self-stimulation behaviour. They were placed individually into operant chambers, each chamber made from three plywood walls and one Plexiglas wall (front); a lever that activated a micro-switch was positioned 45 mm above the wire-mesh floor protruding 20 mm from the centre of one wall. Stimulation and indifferent electrodes were connected to the stimulator with flexible connectors attached to swivels, which allowed free movement of the animals within the chambers. The operant chambers were individually placed into larger foam-insulated boxes that were each equipped with a fan and a light (40 W); a front Plexiglas window allowed constant observation of each animal. Animals were given several minutes to adapt to their respective chambers before screening began. All tests, including the screening process just described, started between 1:00 PM and 2:30 PM.

Once an animal was adapted (at rest), the experimenter delivered several trains of stimulation and the animal's response was observed. Electrical stimulation was produced by a constant-current unit triggered by a pulse generator (Mundl, 1980); it consisted of a 200-ms train of cathodal rectangular pulses, each pulse 0.1 msec in duration. The stimulation intensity and frequency were initially set at 400 μA and 100 Hz respectively. If the animal displayed exploratory behaviour (i.e. sniffing and exploratory locomotion) in response to a few trains of stimulation, the experimenter attempted to initiate the self-stimulation response (lever press) using the standard shaping procedure. If, on the other hand, the animal failed to display any exploratory behaviour, then stimulation intensity was gradually raised, up to a maximum of 1000 μA. If the animal did not learn to self-stimulate following this initial screening procedure, the electrode was lowered by 0.4 mm and the new site was tested in the same way, on the following day. The electrode was lowered a maximum of 3 times (1.2 mm). Animals that failed to self-stimulate were sacrificed.

Once lever pressing was established, animals were trained to lever press with current intensity held constant at the level determined in the initial screening test. They were first allowed to self-stimulate for four consecutive 30-min sessions with stimulation frequency alternating between 17 and 15 pulses/train every 15 min. Then they were trained to respond during 40 discrete 60-sec trials, each trial separated by a 25-sec inter-trial interval during which time the stimulation lever was automatically

disconnected from the pulse generator. Each trial began with 5 priming stimulations that were delivered at a rate of 1 train/sec; the rate of lever presses was only recorded for the last 45 sec of each trial. Stimulation frequency alternated between 15 and 17 pulses/train on each consecutive, discrete trial. An 800-msec time-out period was introduced immediately after the end of the stimulation train to prevent delivery of stimulation at a rate higher than 1 train/sec, as proposed by Boye & Rompré (1996a). Animals were tested this way for two consecutive days. Rate/frequency functions were then determined daily by recording the rate of responding as a function of the log of several decreasing stimulation frequencies (23 to 5 pulses/train in 10 % steps) presented in descending order. Four rate/frequency functions were determined on a single day for each animal. From each of the last three functions, both a reward threshold and a maximal rate of responding were determined. Reward threshold was defined as the stimulation frequency eliciting a rate of responding equal to 50 % of the maximum. The current intensity was adjusted for each animal to yield a reward threshold near 11 pulses/train. Drug or vehicle testing began once reward threshold varied by less than 0.1 log unit for three consecutive days.

3.4 Drug or vehicle test

Animals were randomly assigned to one of three groups; these groups were tested with either NT, D-Tyr[11]NT, or with the vehicle. On a testing day, three rate/frequency functions were initially determined; the first was considered as a warm up and was therefore discarded; the remaining two were used to determine a mean baseline reward threshold and a mean baseline maximal rate of responding. Animals were then removed from the test chamber and were centrally injected with 1.8 nmol of D-Tyr[11]NT, 18 nmol of NT, or an equivalent volume of vehicle (0.9 % saline). Injections were administered in freely moving animals via insertion into the guide canula of a 26-gauge single internal injection canula (Plastic One, model C315I) that extended between 1.7 and 2.4 mm beyond the tip of the guide canula. The injection canula was connected to a 50-µl micro-syringe, which was activated by an infusion pump (Harvard Apparatus, model 11). The 10-µl-solution was injected over a period of 5 min, however the injection canula remained in place for a total of 6 min. Animals were then returned to their testing chambers, and six new rate/frequency functions were determined over a period of 119 min. Animals were tested this way on four occasions, once every second day (Days 1, 3, 5 and 7). Seven

days after the last day of the initial testing phase (Day 14), the effect of a single IP injection of d-amphetamine on self-stimulation behaviour was studied in all animals. First, three rate/frequency functions were determined. Then the animals were systemically injected with 1.0 mg/kg (IP) of d-amphetamine sulphate, and six new rate/frequency functions were determined.

3.5 Drugs

Both NT and D-Tyr[11]NT were dissolved in sterile saline at a concentration of 1.8 nmol/μl and stored frozen at -20^0 C in 50-μl aliquots. Drug solutions were thawed immediately before testing, mixed with sterile saline to the appropriate concentration whenever required, and used only once. The doses of NT and of its analogue, D-Tyr[11]NT, tested in this study were based on previous reports showing that these concentrations significantly diminished electrical self-stimulation thresholds (Rompré, 1995) and induced sensitization to the locomotor stimulant effect of d-amphetamine (Del Vecchio et al., 1998). D-amphetamine sulphate was dissolved in saline and injected in a volume of 1.0 ml/kg.

3.6 Histological analysis

Following the last behavioural test, animals were deeply anaesthetised with sodium pentobarbital [Somnotol, 65 mg/kg (IP)]. A direct anodal current of 100 μA was then passed for a period of 20 s though the stimulation electrode to mark the site of stimulation for each rat. Then animals were transcardially perfused with saline, followed by a 10 % formalin solution containing 3 % potassium ferricyanide, 3 % potassium ferricyanide trihydrate, and 0.5 % trichloroacetic acid. Afterward, animals' heads were detached and stored in cold 10 % formalin (4^0 C) for 24 h; the brains were subsequently removed and stored in 10 % formalin at room temperature. All brains were later frozen and 40-μm serial slices, that were mounted on gelatine-coated glass slides, were stained with a formal-thionin solution so that locations of the guide canula and of the tip of the stimulation electrode could be determined for each rat with a light microscope.

3.7 Data analysis

Group-mean reward thresholds and maximal rates of responding were calculated for each test day. Planned comparisons (LSD tests) were used to determine statistical significance between group means; results of these tests were significant when $p \leq 0.05$.

4.0 Results

Complete collections of data were gathered for 28 of the 52 rats originally prepared for this set of experiments. The remaining 24 rats were excluded for various reasons including complications leading to death during or soon after surgery, failure to self-stimulate, failure to self-stimulate at a stable rate, or loss of the stimulating electrode at some point during the course of the experiment.

Post hoc histology indicated that all but four stimulating-electrode sites were located along the midline between 4.4 mm and 7.1 mm below the surface of the cranium; these sites were also divergent along the rostral-caudal axis between 6.8 mm and 8.3 mm posterior to bregma according to the atlas of Paxinos & Watson (1986).

Figure 2 shows nine examples of the rate/frequency functions that were collected on Day 1 for all rats tested before, and at five time periods after ICV microinjection of 1.8 nmol D-Tyr[11]NT (top panel), 18 nmol NT (middle panel), or their vehicle (bottom panel). Most animals that received either D-Tyr[11]NT or NT showed shifts to the left (left column) following neuropeptide injections on Day 1. However, for some rats, the peptide produced weak changes (middle column) or rightward shifts (right column). Downward shifts, indicative of performance deficits, were also detected in most animals administered either peptide on Day 1.

Figure 3 shows the rate/frequency functions obtained on Day 7 from those animals that were depicted in Figure 2. Taken together, figures 2 and 3 show that, in contrast to rats of the D-Tyr[11]NT pre-treatment group, leftward shifts seen on Day 7 by animals given NT decreased in size relative to those seen on Day 1. As well, whereas all rats that had received D-Tyr[11]NT showed leftward shifts on Day 7, four of the eight rats tested with NT showed rightward shifts on this day.

Figure 2.

Illustrations of rate/frequency curves obtained from nine rats before, and at five time periods after ICV injections of either 1.8 nmol D-Tyr[11]NT (top panel), 18 nmol NT (middle panel), or their vehicle [10 μl, 0.9 % saline (bottom panel)] administered on Day 1. Responses to injections administered on this day were quite varied. Although animals that had received either D-Tyr[11]NT or NT showed shifts either to the left or to the right, in both groups the majority of rats tested showed leftward shifts that were reflective of the overall group-mean decreases in reward threshold for BSR that were also seen on this day. Note that downward shifts, indicative of performance deficits, were only detected in animals administered either peptide; no such shifts were significant in animals injected with an equal volume of saline.

Figure 2

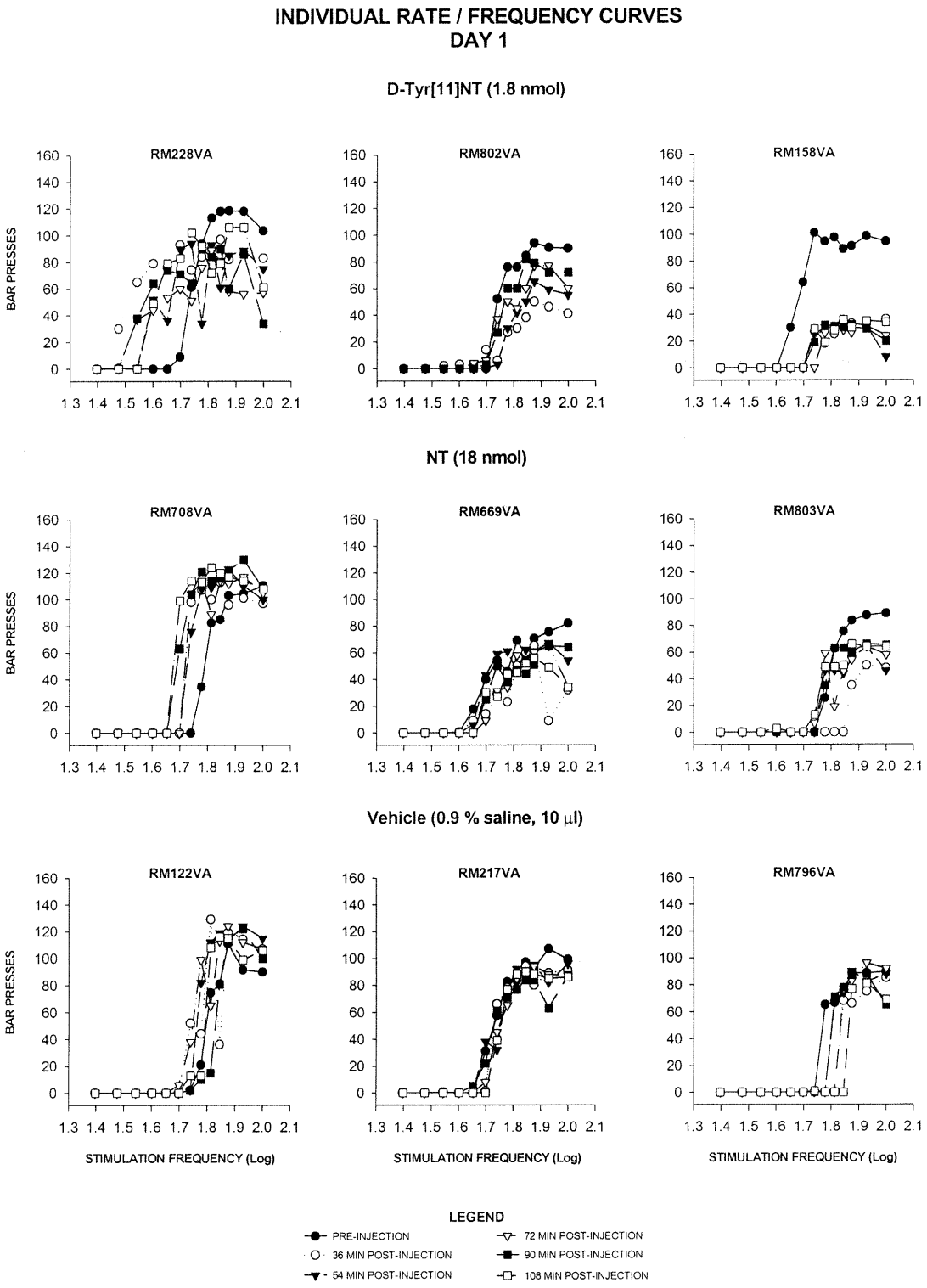
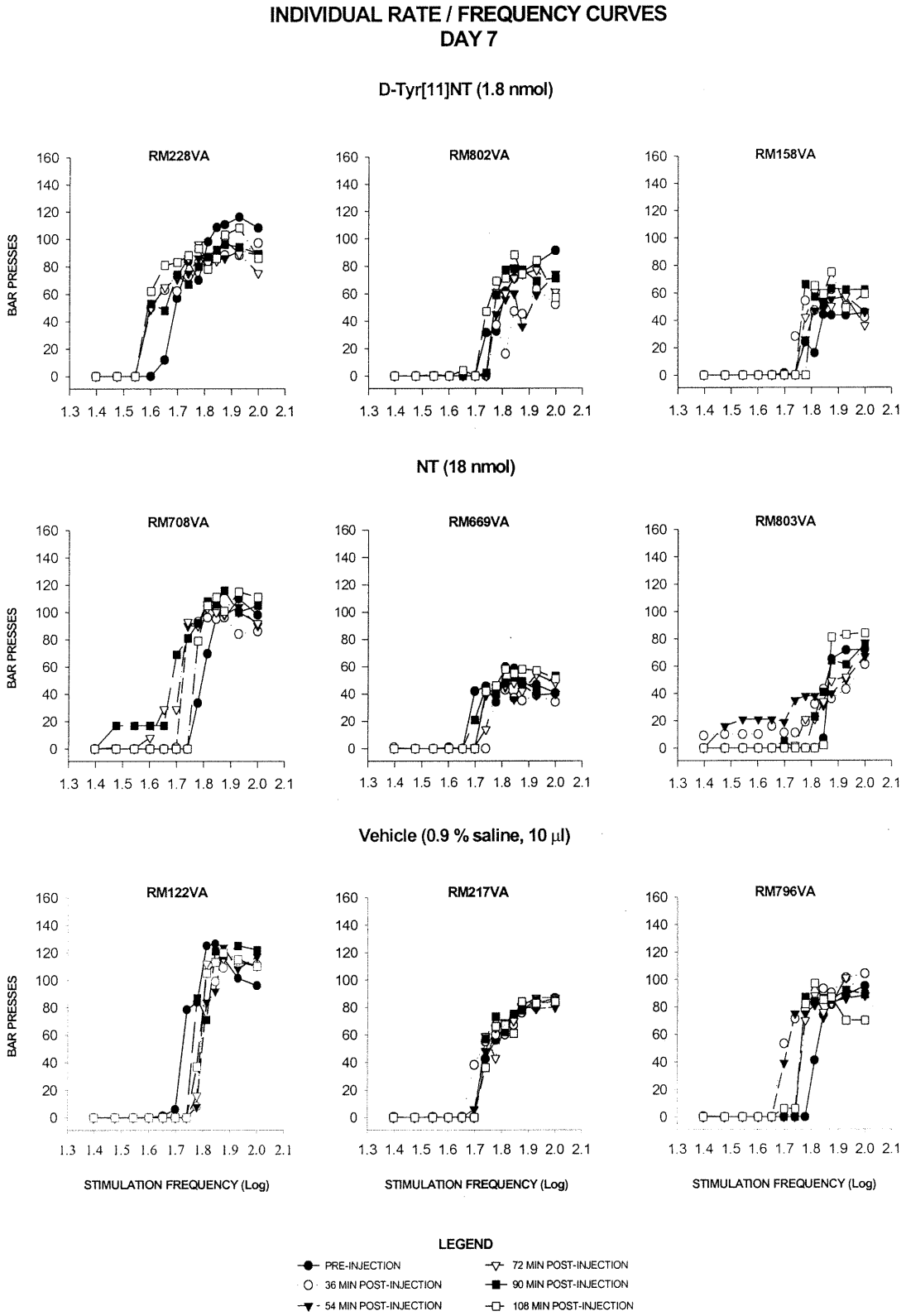

INDIVIDUAL RATE / FREQUENCY CURVES
DAY 1
D-Tyr[11]NT (1.8 nmol)
RM228VA
RM802VA
RM158VA
NT (18 nmol)
RM708VA
RM669VA
RM803VA
Vehicle (0.9 % saline, 10 µl)
RM122VA
RM217VA
RM796VA
BAR PRESSES
STIMULATION FREQUENCY (Log)
LEGEND
PRE-INJECTION
36 MIN POST-INJECTION
54 MIN POST-INJECTION
72 MIN POST-INJECTION
90 MIN POST-INJECTION
108 MIN POST-INJECTION

Figure 3.

Illustrations of rate/frequency curves obtained from nine rats before, and at five time periods after ICV injections of either 1.8 nmol D-Tyr[11]NT (top panel), 18 nmol NT (middle panel), or their vehicle [10 μl, 0.9 % saline (bottom panel)] administered on Day 7. Responses to peptide injections administered on this day were more consistent than those observed on Day 1 (see Figure 2). Note that following peptide injections, leftward shifts were seen in the data compiled on Day 7 for animals that showed rightward shifts on Day 1 (see Figure 2). Note, as well, that downward shifts were considerably smaller on this day than those seen on Day 1, indicating that for some animals tolerance has developed to the performance deficit peptide injections seemingly produced when administered acutely (see Figure 2).

Figure 3


INDIVIDUAL RATE / FREQUENCY CURVES
DAY 7
D-Tyr[11]NT (1.8 nmol)
RM228VA
RM802VA
RM158VA
NT (18 nmol)
RM708VA
RM669VA
RM803VA
Vehicle (0.9 % saline, 10 µl)
RM122VA
RM217VA
RM796VA
BAR PRESSES
STIMULATION FREQUENCY (Log)
LEGEND
PRE-INJECTION
36 MIN POST-INJECTION
54 MIN POST-INJECTION
72 MIN POST-INJECTION
90 MIN POST-INJECTION
108 MIN POST-INJECTION

Mean changes in frequency thresholds expressed as percentages of pre-injection assessments measured on Day 1 (top panel) and on Day 7 (bottom panel) following ICV injections of vehicle (n = 6), NT (n = 8 on Day 1 and n = 10 on Day 7), or D-Tyr[11]NT (n = 8 on Day 1 and n = 9 on Day 7) are shown in Figure 4. Because NT completely suppressed responding in half of all rats tested on the first training trial (0-15 min post-injection) of Day 1, only the data obtained during the last five threshold determinations are shown, and were analysed. On Day 1, NT and D-Tyr[11]NT enhanced the rewarding effect of BSR as reflected by a decrease in threshold; this effect reached statistical significance 90 min after the injection of NT (+, $p < 0.05$; ++, $p < 0.01$) and 108 min following the injection of D-Tyr[11]NT (***, $p < 0.001$). At 90 min, the size of this effect for animals given NT was 88.6 % +/- 3.0 % (SEM) relative to the corresponding group mean for BSR threshold (expressed as 100%). Similarly, for animals given D-Tyr[11]NT, this effect was 88.6 % +/- 7.1 % (SEM) at 108 min relative to the corresponding group mean for BSR threshold by animals of this group. The reward-enhancing effect produced by NT was at no time statistically different from that produced by D-Tyr[11]NT on Day 1; this, however, was not the case for the corresponding data obtained on Day 7. Instead, on this day, the group that had received repeated injections of NT showed BSR thresholds that were no different, at any time during testing, from to the group that had received the vehicle (bottom panel). Furthermore, the reward enhancing effect of D-Tyr [11]NT, in contrast to that observed by NT, increased with repeated injections, such that on Day 7 threshold measures for this group were significantly lower than those of the vehicle, (and therefore of the NT) group between 36 and 90 min post-injection (**, $p < 0.01$; ***, $p < 0.001$).

Rate/frequency functions depicted in Figure 5 illustrate examples of performance deficits induced by acute ICV injection of either D-Tyr[11]NT or NT. The large downward shifts in maximal rates of responding immediately following the administration of either peptide represent declines in maximal rates ranging from 60 to 80 bar presses. In general, maximal rates increased gradually toward baseline levels, presumably as the effects of the peptides dissipated. However, more extreme performance deficits, which did not follow this time-dependent post-injection upward trend, were also evident; these extreme cases appeared subsequent to acute ICV D-Tyr[11]NT injections only. As expected, no performance deficits comparable to the magnitudes seen following peptide injections were apparent in any animal that

had received vehicle; downward shifts seen in this group did not exceed 40 bar presses, and were most likely to have occurred as a result of fatigue.

Mean changes in maximal rate of responding expressed as percentages of pre-injection assessments measured on Day 1 (top panel) and on Day 7 (bottom panel) following ICV injections of vehicle (n = 6), NT (n = 8 on Day 1 and n = 10 on Day 7), or D-Tyr[11]NT (n = 8 on Day 1 and n = 9 on Day 7) are shown in Figure 6. Because NT completely suppressed responding in half of all animals tested on the first training trial (0-15 min post-injection) of Day 1, only the data obtained during the last five threshold determinations are shown, and were analysed.

The planned comparisons revealed that on Day 1, NT lowered maximal rates of responding for BSR, an effect that reached significance from 0 to 72 min post-injection (++, $p < 0.01$; +++, $p < 0.001$). Likewise, D-Tyr[11]NT produced a lasting decrease in maximal rates of responding on this day that reached significance between 36 and 90 min post-injection (**, $p < 0.01$; ***, $p < 0.001$). Note, however, that the suppression of maximal rates of responding by D-Tyr[11]NT was larger than that produced by NT between 72 and 90 min post-injection.

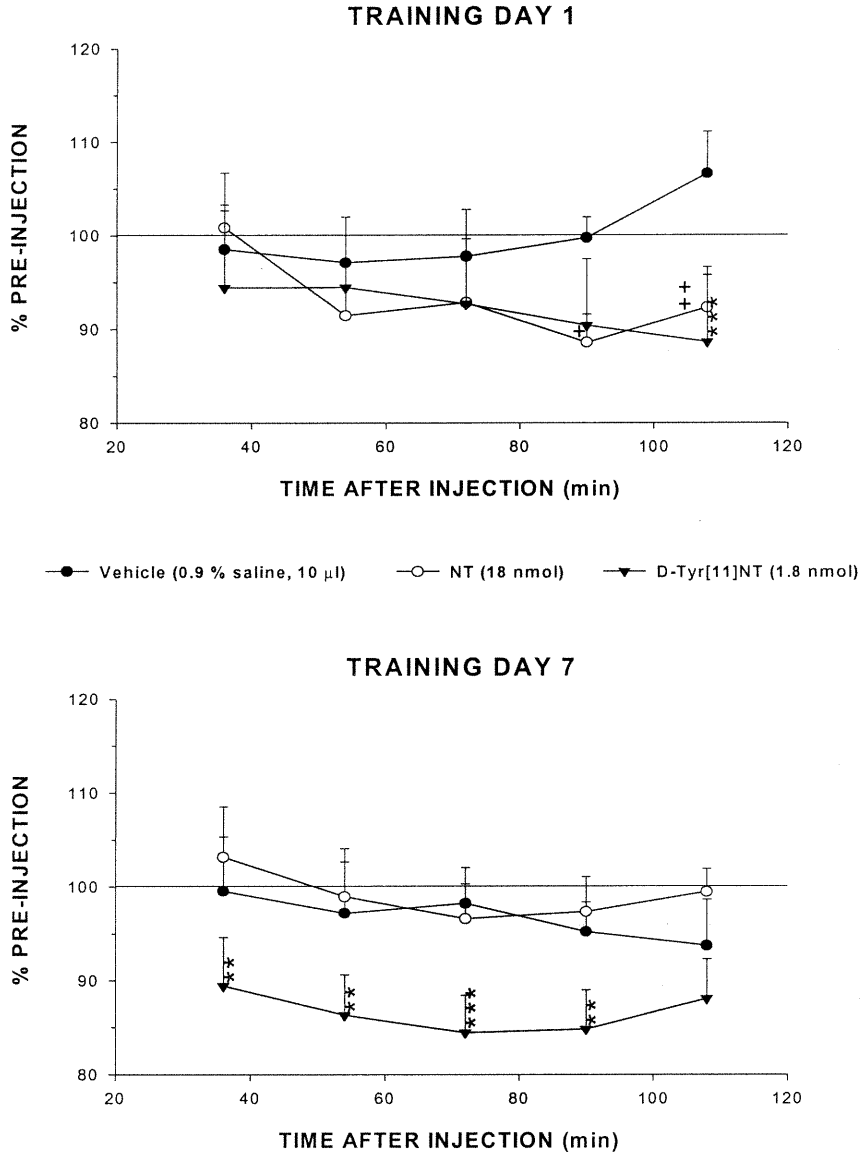
The suppression of maximal rates of responding by NT was also observed on Day 7 but only between 36 and 54 min post-injection ($p < 0.001$ relative to vehicle). In addition, as the bottom panel of Figure 6 shows, in contrast to the sustained suppression of maximal rates of responding following repeated NT administration, tolerance developed to this effect on Day 7 after repeated injections of D-Tyr[11]NT. Furthermore, a significant increase in maximal rates of responding relative to vehicle-treated animals was also apparent on this day between 72 and 108 min post-injection (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Importantly, maximal rates of responding for D-Tyr[11]NT-treated animals rose to approximately 110% of this group's mean baseline level.

Rate/frequency functions obtained following a single injection of d-amphetamine, administered on Day 14, in rats pre-exposed to D-Tyr[11]NT (top panel), NT (middle panel), or vehicle (bottom panel) are shown in Figure 7. In the vehicle-pre-exposed rats, d-amphetamine produced leftward shifts of the rate/frequency functions, as it did in the rats pre-exposed to either peptide. Figure 7 also shows that as time elapsed and the effects of the drug began to wear off, the curves began to shift back to the right, hence the decay of this reward-enhancing effect.

Figure 4.

Mean changes in reward threshold (expressed as percentages of pre-injection estimates) measured on Training Day 1 (top panel), and again on Training Day 7, (bottom panel) after ICV microinjection of saline (n = 6); 18 nmol of NT (n = 8 on Day 1 and n = 10 on Day 7); or 1.8 nmol of D-Tyr[11]NT (n = 8 on Day 1 and n = 9 on Day 7). Because NT completely suppressed responding during the first 15 min post-injection on Day 1, only the last five threshold determinations were analysed on this day. On Day 1, reward threshold was significantly altered by peptide treatments relative to saline from 90 min post-injection onward. By Day 7, D-Tyr[11]NT had decreased reward threshold between 36 and 90 min post-injection, whereas NT had an opposite effect at 36 min post-injection. Asterisks and crosses indicate statistical significant differences between the group that received vehicle and those that received either D-Tyr[11]NT or NT respectively (** $p < 0.01$; *** $p < 0.001$; + $p < 0.05$, ++ $p < 0.01$).

Figure 4



+, $p < 0.05$; ++, $p < 0.01$ for NT versus vehicle
**, $p < 0.01$; ***, $p < 0.001$ for D-Tyr[11]NT versus vehicle

Figure 5.

Motor deficits following acute ICV peptide injection. Some animals in both the NT and the D-Tyr[11]NT groups showed large downward shifts in asymptotic rates of responding immediately following the administration of either peptide on Day 1. These decreases in maximal rates, which ranged from between 70 and 80 bar presses, gradually increased toward baseline as time passed. More extreme motor deficits such as those expressed by rats RM158VA and RM804VA were also evident. Noteworthy is that these latter shifts did not follow the time-dependent post-injection upward trend of the former vertical shifts, and were preceded by acute ICV D-Tyr[11]NT injections only. Animals injected with saline did not display motor deficits of these magnitudes; downward shifts by rats treated with saline did not surpass 40 bar presses.

Figure 5

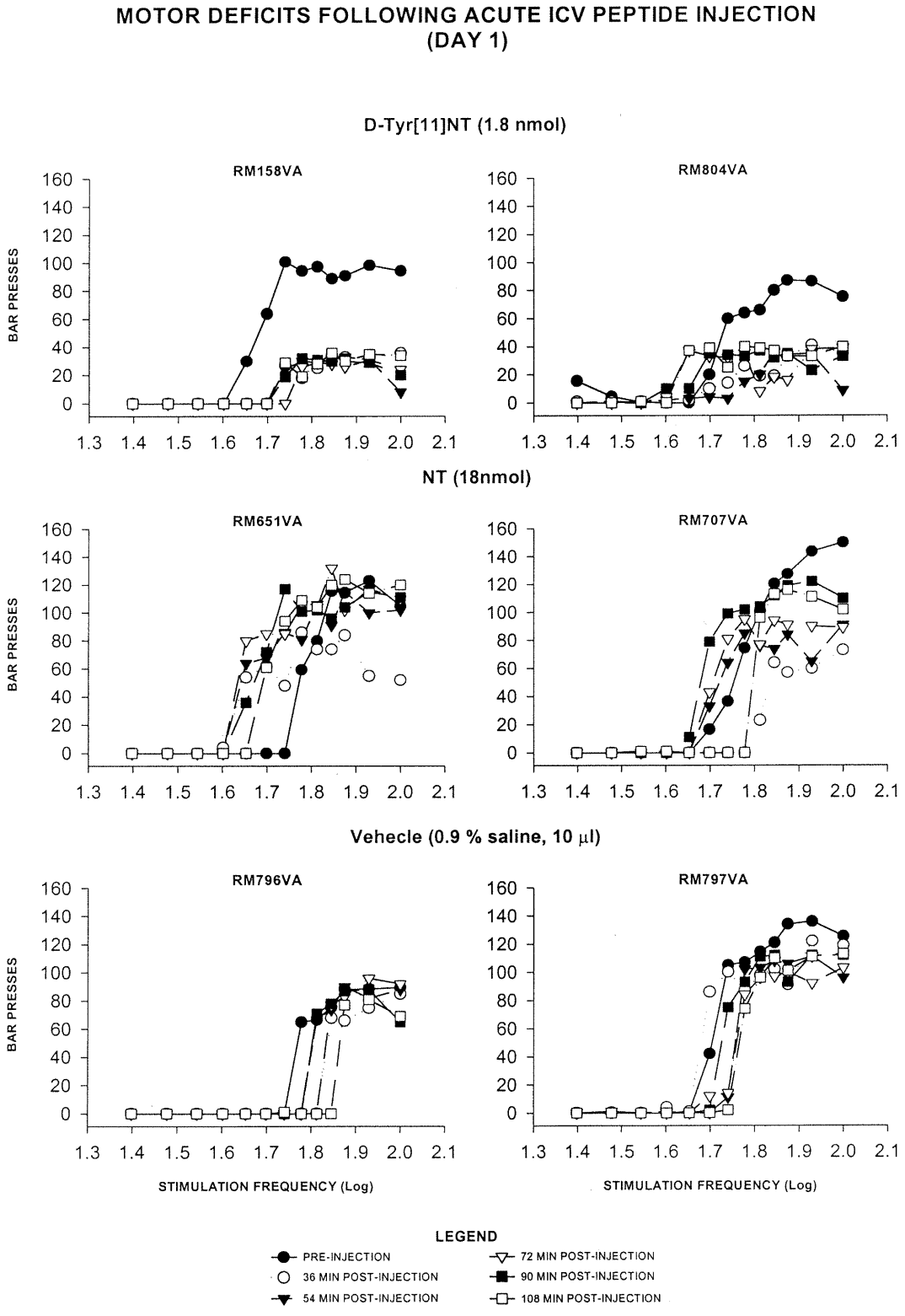
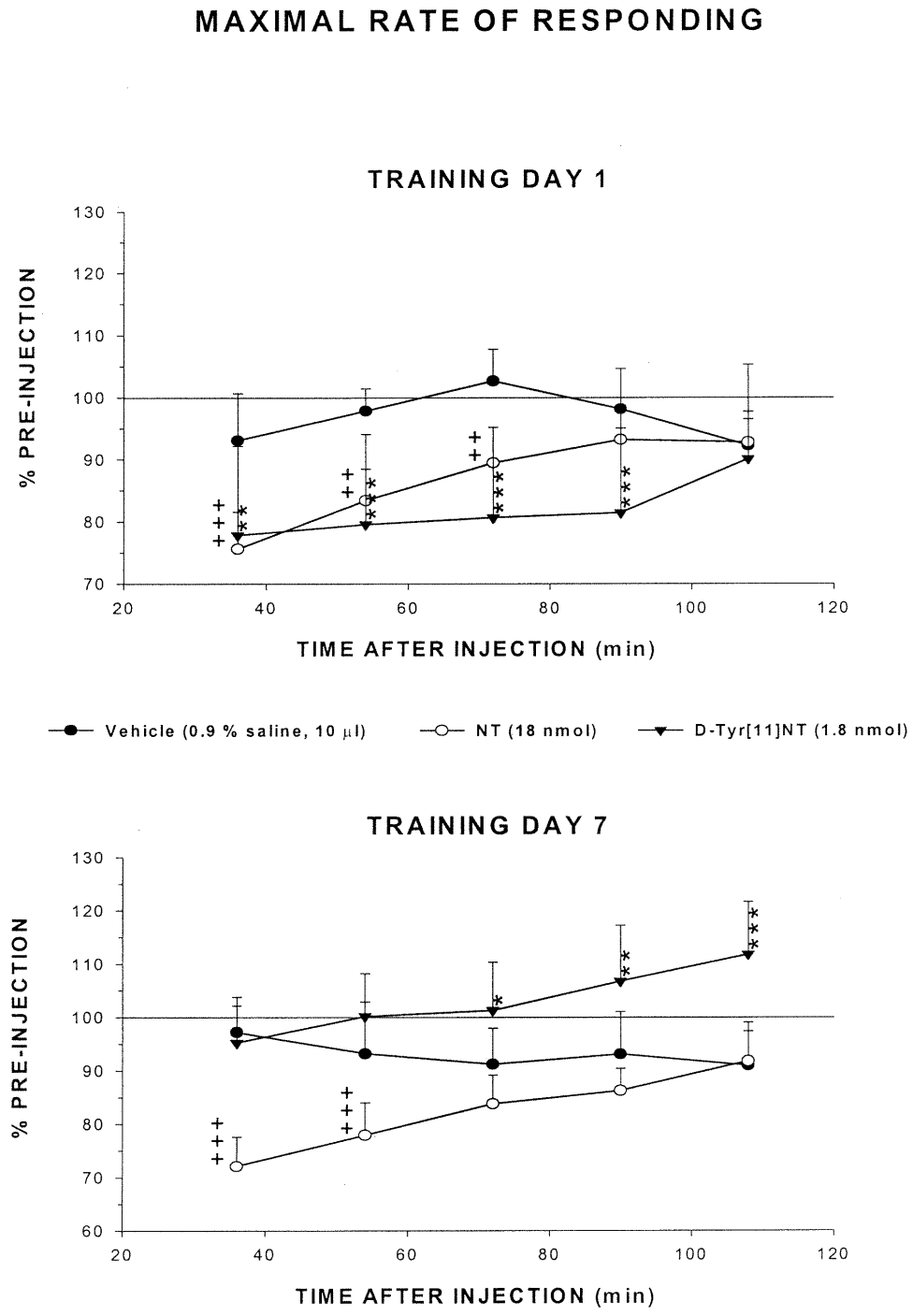

MOTOR DEFICITS FOLLOWING ACUTE ICV PEPTIDE INJECTION
(DAY 1)
D-Tyr[11]NT (1.8 nmol)
RM158VA
RM804VA
NT (18nmol)
RM651VA
RM707VA
Vehecle (0.9 % saline, 10 µl)
RM796VA
RM797VA
BAR PRESSES
STIMULATION FREQUENCY (Log)
LEGEND
PRE-INJECTION
36 MIN POST-INJECTION
54 MIN POST-INJECTION
72 MIN POST-INJECTION
90 MIN POST-INJECTION
108 MIN POST-INJECTION

Figure 6.

Mean changes in maximal rates of responding (expressed as percentages of pre-injection estimates) measured on Training Day 1 (top panel), and Training Day 7, (bottom panel) after microinjection of saline (n = 6); 18 nmol of NT (n = 8 on Day 1 and n = 10 on Day 7); or 1.8 nmol of D-Tyr[11]NT (n = 8 on Day 1 and n = 9 on Day 7). On Day 1, both NT and D-Tyr[11]NT produced a strong and significant suppression of responding; this effect remained stable with repeated injections of NT. In contrast, repeated injections of D-Tyr[11]NT produced an increase in maximal rate of responding on Day 7. Asterisks and crosses indicate statistical significant differences between the group that received vehicle and those that received either D-Tyr[11]NT or NT respectively (* $p < 0.05$,** $p < 0.01$; *** $p < 0.001$; ++ $p < 0.01$, +++ $p < 0.001$).

Figure 6



++, $p < 0.01$; +++, $p < 0.001$ for NT versus vehicle
*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ for D-Tyr[11]NT versus vehicle

Figure 7.

Rate/frequency curves obtained from nine rats before and at five time-periods after systemic injections of d-amphetamine (1.0 mg/kg) on Day 14. All animals were pre-exposed to either D-Tyr[11]NT, NT or their vehicle (10 μl, 0.9 % saline) during the training phase of the experiment (see Figures 2 & 3). As expected, d-amphetamine produced leftward shifts in the rate/frequency functions of all animals tested. Interestingly, these leftward shifts were more or less of the same magnitude regardless of pre-treatment, thus indicating that the reward-enhancing effect produced by injections of d-amphetamine on this day was similar for all animals tested.

Figure 7

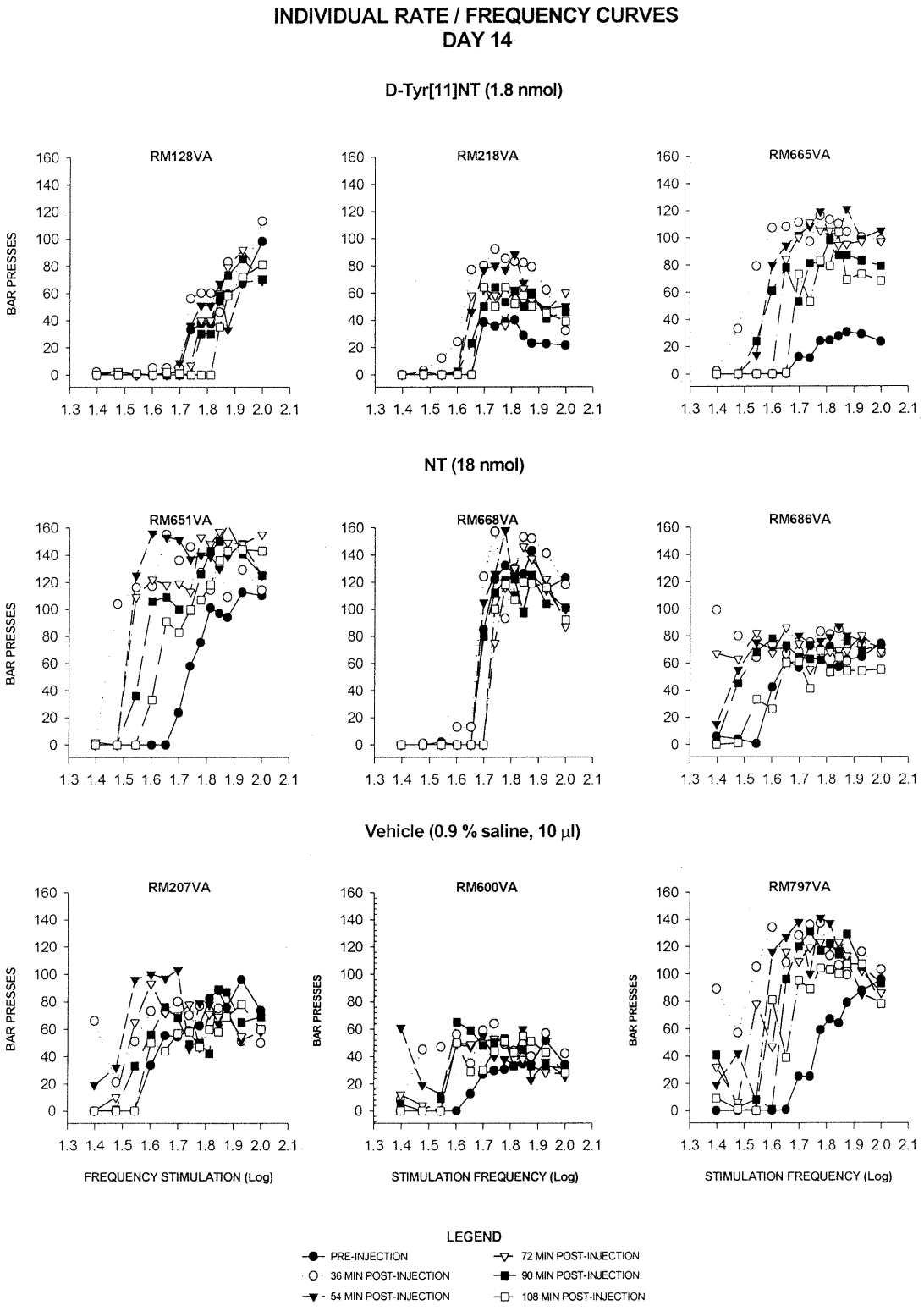

INDIVIDUAL RATE / FREQUENCY CURVES
DAY 14
D-Tyr[11]NT (1.8 nmol)
RM128VA
RM218VA
RM665VA
NT (18 nmol)
RM651VA
RM668VA
RM686VA
Vehicle (0.9 % saline, 10 µl)
RM207VA
RM600VA
RM797VA
BAR PRESSES
FREQUENCY STIMULATION (Log)
STIMULATION FREQUENCY (Log)
LEGEND
PRE-INJECTION
36 MIN POST-INJECTION
54 MIN POST-INJECTION
72 MIN POST-INJECTION
90 MIN POST-INJECTION
108 MIN POST-INJECTION

Figure 8 shows the mean changes in frequency threshold expressed as percentages of pre-injection assessments measured on Day 14 immediately after a single systemic injection of 1.0 mg/kg d-amphetamine (see legend for descriptions of the three groups). No significant effects resulting from the combinations of pre-exposure to either peptide or their vehicle and later acute IP injections of d-amphetamine were revealed on Day 14. In fact, all animals tested responded to the d-amphetamine injection in a very similar manner characterized by an immediate 30% decrease in frequency threshold that gradually returned to baseline levels. At the end of the test session, these group-mean measures of frequency threshold, for all three groups, reached 90% of their corresponding pre-injection baseline values.

Mean changes in maximal rate of responding (expressed as percentages of pre-injection rates) measured on Day 14 after systemic injections of d-amphetamine (1.0 mg/kg) are shown in Figure 9. As Figure 9 illustrates, although previous central injections of NT had not differentially affected the ability of animals subsequently exposed to d-amphetamine to bar press (relative to vehicle), d-amphetamine injection following pre-exposure to D-Tyr[11]NT had markedly raised maximal rates of responding to between 150 % and 162 % of this group's mean pre-injection baseline rate. Furthermore, planned comparisons revealed that this effect was statistically significant for the entire duration of the test ($p < 0.001$).

Within the context of this experiment, a ceiling effect would be said to have occurred if animals responded so robustly to BSR during training, that it would be physically impossible for them to respond at a rate any higher, despite psychostimulant administration of even extremely high doses. In contrast, a floor effect denotes the opposite situation, one in which animals respond at such low levels prior to a given treatment, that any effect elicited by this treatment, however slight, would be significant relative to the aforementioned baseline.

In order to better understand the significant increase in maximal rates revealed on Day 14 by D-Tyr[11]NT-pre-treated animals only, correlations and linear analyses of group-mean maximal rates of responding to BSR before and after the injection of d-amphetamine on this day are shown in Figure 10; these tests assessed the likelihood that this increase in maximal rates of responding following D-Tyr[11]NT pre-treatment (see Figure 9) was revealed as a result of the floor effect phenomenon. That the

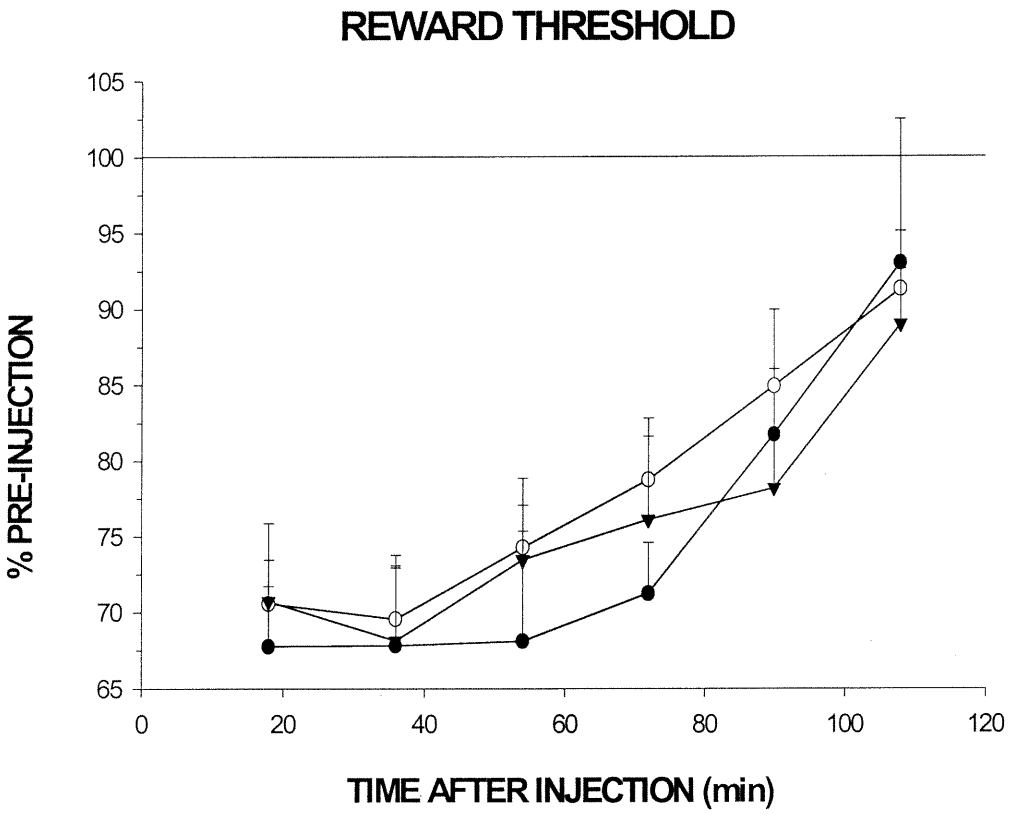
same group showing a significant increase in maximal rates of responding in Figure 9 is the only group that also showed a significant negative correlation in Figure 10 ($r = -0.71$, $p < 0.05$) supports the possibility that a floor effect had indeed occurred regarding the measurement of this parameter on Day 14.

Figure 8.

Mean changes in reward threshold (expressed as percentages of pre-injection estimates) measured on Day 14 immediately following a systemic injection of d-amphetamine (1.0 mg/kg); all animals showed a lower threshold for BSR responding in response to this injection. Furthermore, the magnitude of this decrease in reward threshold was more or less the same for all animals tested regardless of pre-exposure condition. The legend describes pre-treatment groups.

Figure 8

SENSITISATION TEST
DAY 14



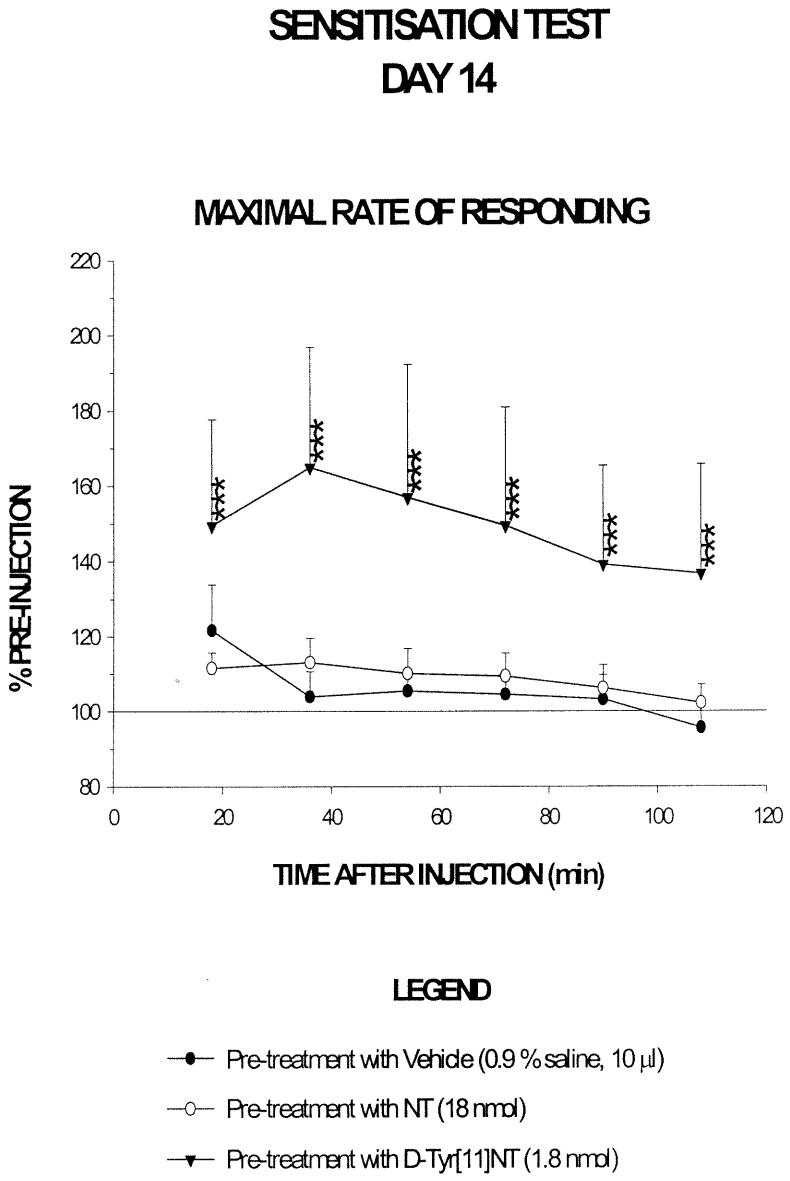
LEGEND

- Pre-treatment with Vehicle (0.9 % saline, 10 µl)
- Pre-treatment with NT (18 nmol)
- Pre-treatment with D-Tyr[11]NT (1.8 nmol)

Figure 9.

Mean changes in maximal rate of responding (expressed as percentages of pre-injection rates) measured on Day 14 after a systemic injection of d-amphetamine (1.0 mg/kg). Rats pre-exposed to D-Tyr[11]NT showed a large and significant increase in maximal rate of responding, an effect not seen in rats pre-exposed to NT or saline. Rats pre-exposed to saline showed a small increase in maximal rate at 18 min post-injection but this effect was not statistically different from the corresponding time point for NT. The legend describes pre-treatment groups. (***, $p < 0.001$ relative to vehicle).

Figure 9



***, $p < 0.001$ for D-Tyr[11]NT versus vehicle

Figure 10.

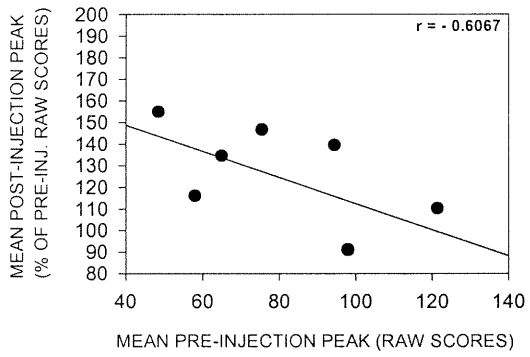
Correlations and linear analyses of group-mean maximal rates of responding to BSR before and after systemic injection of d-amphetamine (1.0 mg/kg). The negative correlation seen for the group that was chronically pre-exposed to D-Tyr[11]NT prior to the d-amphetamine injection suggests that animals pre-exposed to D-Tyr[11]NT were generally low responders for BSR, and that this (group) trait was responsible for the trend toward a significant increase in maximal rate of responding revealed by this group of rats relative to the other two groups. Rats that respond robustly to BSR before the administration of psychostimulant drugs are not as able as low responders are to demonstrate the drug's ability to increase hyperactivity. Similarly, rats that respond minimally to BSR are able to demonstrate very subtle drug-induced effects that "normal" rats cannot. Thus, this figure suggests that a floor effect allowed rats of the D-Tyr [11]NT group to show a trend that was, in essence, an exaggeration of the effect produced by the d-amphetamine administered on Day 14, because all animals of this group were low-responders for BSR.

Figure 10

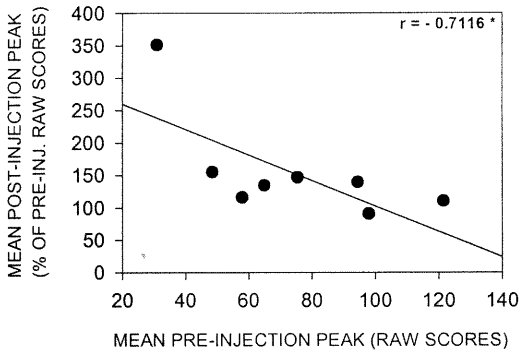
CORRELATIONS AND LINEAR ANALYSES OF MAXIMAL (PEAK) RATES OF RESPONDING ON DAY 14

D-Tyr[11]NT (1.8 mnol)

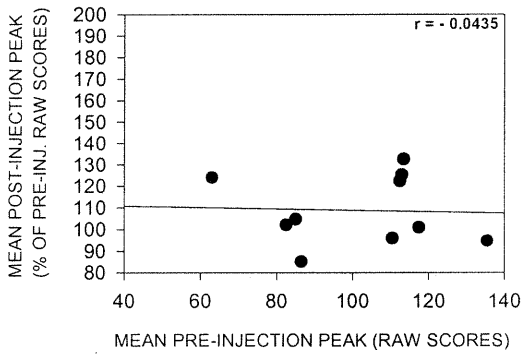
ALL SUBJECTS EXCLUDING RM665VA



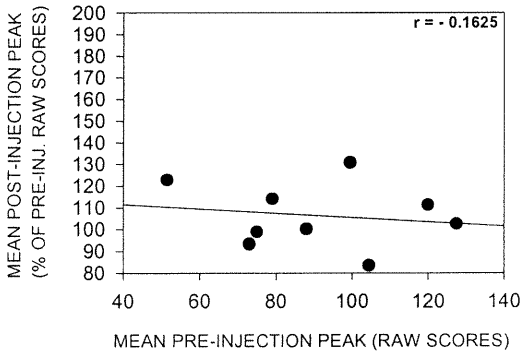
ALL SUBJECTS INCLUDING RM665VA



NT (18 nmol)



Vehicle (0.9 % saline, 10 μl)



*, $p < 0.05$

5.0 Discussion

This study was aimed at (i) comparing the effects of NT and D-Tyr[11]NT on reward produced by electrical stimulation in the mesencephalic central grey, and on the ability of the animal to produce an operant response to obtain this reward; (ii) determining the effects of repeated exposure to each of the peptides on measures of reward and performance and (iii) determining whether repeated exposure to the peptides alters the effects of systemic d-amphetamine on measures of reward and performance. Significance of the results obtained in reference to the current literature is discussed in the following sections along with hypothetical mechanisms of action on the relevant neural circuitry.

While for most animals ICV peptide injections induced leftward shifts of the rate/frequency functions calculated for BSR testing on Day 1, for some rats these injections produced shifts to the right. Notwithstanding, by Day 7 most NT-injected rats also showed leftward shifts following injections. Because histological analysis revealed that all but four sites were located along the midline between 4.4 mm and 7.1 mm below the surface of the cranium, and because stimulating electrode sites also varied along the rostral-caudal axis from 6.80 mm -8.30 mm posterior to bregma (Paxinos & Watson, 1986), one explanation for the differential effects on reward threshold observed on Day 1 is that they occurred as a result of stimulating electrode location-differences, which may have caused the activation of distinct neurones that were less sensitive to direct electrical stimulation than those of the intended site (the central mesencephalic grey). Regardless, because no clear trend was found, more data is needed to test this theory.

5.1 H_1 NT and D-Tyr[11]NT will produce effects of equal magnitude on both reward threshold and maximal rate of responding.

It was hypothesized that the two peptides (NT and D-Tyr[11]NT) used in the present set of experiments would produce effects of equal magnitude on both parameters (reward threshold and maximal rate of responding) tested because D-Tyr[11]NT is an NT analogue that interacts with NT systems. However, D-Tyr[11]NT differs from NT in important ways. For instance, D-Tyr[11]NT has a higher resistance to enzymatic degradation than NT in vivo, which makes it a relatively more effective and longer lasting peptide (Checler et al., 1983; Rivest et al., 1991b). Indeed, Jolicoeur et al. (1984) reported

that replacement of the amino acid residue L-tyrosine in position 11 of the amino acid sequence of endogenous NT with its d-isomer (D-Tyr[11]) significantly increases the structural analogue's (D-Tyr[11]NT's) duration of action. Hence, smaller concentrations of D-Tyr[11]NT can produce physiological effects of comparable magnitudes to those resulting from larger concentrations of NT because alterations of its eleventh (amino acid) position decrease the affinity of the peptide for the enzymes that normally work to break it down. Kitabgi et al. (1980) also reported that the biological potency of D-Tyr[11]NT is a tenfold greater than that of NT when measured in vivo, which is precisely why, in the present study, we chose the concentration variations between NT (18 nmol) and D-Tyr[11]NT (1.8 nmol) that we did.

First, there is the data obtained on Day 1 (at 108 min); here, both peptides significantly lowered reward threshold. In addition, the ANOVA for this data revealed no significant differences between (peptide) treatment groups. Notwithstanding, H_1 was not supported because by Day 7, while tolerance to the reward enhancing effect of NT had developed with repeated injections such that no difference on this measure was observable at any time on Day 7 relative to the group that had received the vehicle, the reward enhancing effect of D-Tyr [11]NT increased with repeated injections such that threshold measures for this group were significantly lower than those of the vehicle, (and therefore of the NT) group between 36 and 90 min post-injection.

The data compiled on Day 1 and Day 7 for maximal rate of responding followed a similar trend. While both peptides significantly lowered maximal rates on Day 1, by Day 7, tolerance had developed to this effect by the group given NT, whereas some sort of sensitization had developed to this effect by the animals given D-Tyr [11]NT.

5.2 H_2 On Day 1, following a single ICV peptide injection, a gradual decrease over time will occur, relative to baseline rates, for reward threshold, whereas an immediate decrease, followed by a gradual increase over time toward baseline rates, will occur for maximal rate of responding.

This hypothesis was supported by the data obtained in the present set of experiments. Grouped data showed that acute NT administration decreased thresholds for BSR; this was reflected by leftward shifts of the rate/frequency functions calculated on Day 1. These leftward shifts signify an NT-induced potentiation of BSR, thus implying that NT was rewarding in its own right. Indeed, drugs of abuse that

induce feelings of euphoria in humans decrease thresholds for BSR when given to laboratory animals just before BSR testing (Wise, 1996). The NT-induced reward-enhancing effect detected in the present study was expected because of a similar effect reported by Rompré (1995) who tested the same brain location for BSR against the same NT concentration used herein (18 nmol).

Central injections of NT are known to elevate DA cell firing and to produce robust and enduring augmentations of DA metabolism in the NAc (Blaha et al., 1990; Steinberg et al, 1995). Indeed, BSR itself is associated with increased activity of the mesolimbic DA system (Wise, 1996; Wise & Rompré, 1989), and particularly, with increased levels of DA in the NAc (Blaha & Phillips, 1990; Gratton et al., 1988). Furthermore, habit-forming drugs such as amphetamine, cocaine, and μ and δ opiates, which are rewarding in their own right, lower thresholds for BSR by increasing dopaminergic activity in the NAc (Wise, 1996). Taken together, these three facts suggest that the mechanism of action by which NT elicits the reward-enhancing effect revealed in the present study likely involves an NT-DA interaction within the limbic system between the VTA and the NAc. This interpretation is strengthened by the fact that mesocorticolimbic DA is a critical component in the expression of reward-mediated behaviours (Koob, 1992; Wise & Rompré, 1989). Furthermore, microinjection studies have revealed that NT administered directly into the VTA facilitates BSR responding (Rompré et al., 1992; Rompré & Gratton, 1993) and stimulates forward locomotion (Cador et al., 1985; Kalivas & Duffy, 1990), another behaviour that is motivated by positive reinforcers (Wise & Bozarth, 1987). Finally, NT microinjection into the VTA has also been shown to establish a conditioned place preference (Glimcher et al., 1984) and to reinforce self-administration (Glimcher et al., 1987).

While NT receptor activation of dopaminergic neurones located in the VTA is likely to mediate the acute NT-induced decrease in reward threshold seen in this study, the validity of this hypothesis is, nonetheless, compromised by the fact that maximal rates of responding did not increase following the NT injection administered on this day. Again, although we did not measure (locomotor) hyperactivity via photo-beam crossings as did Rompré (1997), rates of responding to BSR were thought, within the context of this experiment, to be reflective of goal-directed forward locomotion, and as such, were considered as a de facto index of drug-induced hyperactivity. To the extent that this is so, for the NT-activated dopaminergic elements of the VTA to be mediator of the acute NT-induced reward potentiation seen in

the present study, maximal rates would have been expected to increase following the peptide injection because increased locomotor hyperactivity is also associated with VTA NT microinjections (Kalivas & Duffy, 1990). Therefore, other mesolimbic brain sites that are equally devoted to motivation and the behavioural expression of emotion, such as the amygdala (Kupfermann, 1991), cannot be overlooked. Indeed, the common brain-reward circuit upon which abusable drugs are hypothesized to produce their rewarding effects includes the central nucleus of the amygdala (Leshner & Koob, 1999), an area rich in NT receptors (Alexander. & Leeman, 1998; Walker et al., 1998) that is associated with enhanced stimulus-reward learning (Hitchcott et al., 1997).

Other possible sites of action that cannot be ruled out as mediators of the NT-induced reward-enhancing effect found in this experiment on Day 1 include the PFC (the terminal field of the mesocortical DA system) and the neostriatum, which receives DA input from the SN. Not only does the activation of PFC NT receptors stimulate midbrain DA firing (Rompré et al, 1998), but also, lesions of the PFC diminish DA D_1 receptor concentration in the VTA (Dewar et al., 1997), which affects subsequent DA neurotransmission in the NAc. In support of a role for the neostriatum, on the other hand, "the route of the nigrostriatal projection supports strong self-stimulation effects from the substantia nigra to the entopeduncular nucleus" (Prado Alcalá et al., 1975). Furthermore, striatal NT release is thought to coordinate activity of the two major efferent projection systems of the striatum (the striatopallidal and striatonigral projections), both of which are regulated primarily by DA D_1 and DA D_2 receptors.

Acute D-Tyr[11]NT, like NT, caused rate/frequency curves to shift leftward in the majority of animals tested; this effect, in both peptide treatment conditions, was marked by a 10 % reduction in group-mean reward thresholds, which implied that the concentrations of both neuropeptides used in this study were equipotent, at least in their abilities to produce reward-enhancing effects in behaving animals following acute administration. Notwithstanding, D-Tyr[11]NT differs from NT in its affinities for various NT receptor subtypes. High- and low-affinity NT receptors have been discovered, cloned, and characterized (Dubuc et al., 1994, 1999; Gully et al., 1993, 1997; Labbé-Jullié, et al., 1994; Mazella et al., 1996; Nalivaiko et al., 1998; Shotte et al., 1986; Tanaka et al., 1990; Vita et al., 1993). As mentioned in the introduction, the high-affinity NT receptor (NTr_1) is a G protein-coupled receptor that is activated by NT, is insensitive to the antihistamine levocabastine, and is blocked by both known non-peptide NT

receptor antagonists to date; SR48692 and SR142948A (Vincent et al., 1999). In contrast, the G protein-coupled low-affinity NT receptor (NTr_2) has a lower affinity for NT, and is blocked by levocabastine and only one of the NT receptor antagonists: SR142948A (Vincent et al., 1999). Interestingly, D-Tyr[11]NT has been shown to bind to the NTr_2 receptor with greater affinity than does NT (Labbé-Jullié et al.,1994). Indeed, D-Tyr[11]NT is more effective at eliciting the analgesic NTr_2-mediated effect of NT (which is antagonized by SR142948A only) than NT itself is when these drugs are administered and compared in vivo (Dubuc et al., 1999; Jolicoeur et al, 1984).

Although D-Try[11]NT likely exerts its acute reward-enhancing effect via mesocorticolimbic DA, the mechanisms involved appear to be more complex than those previously described for NT. For example, significantly higher doses of D-Try[11]NT are required to produce the same level of DA release in the NAc (the site believed to be responsible for the reward-enhancing effect of BSR) than is needed by NT, in vivo (Steinberg et al. 1995). Yet, D-Try[11]NT is clearly more potent than NT at inducing behavioural excitation (Steinberg et al. 1995), another classic reward-relevant DA-dependent behaviour.

To the extent that BSR potentiation is due to increased extracellular NAc DA, the acute reward-enhancing effect produced by D-Tyr[11]NT in the present study is not likely to result from VTA NT receptor activation as was first hypothesized for the matching effect observed in this experiment by NT. Instead, it is proposed that this acute D-Tyr[11]NT-induced effect be mediated by DA-releasing NTr_2.receptors located in other brain loci that also project to the NAc. While NTr_1 receptors, which exist in greater numbers in the VTA and the SN than in any other region of the central midbrain DA systems (Hermans & Maloteaux, 1998) show a greater affinity for NT than they do for D-Tyr[11]NT, NTr_2 receptors, which are not found in the VTA (Hermans & Maloteaux, 1998; Kasckow & Nemeroff, 1991), show a greater affinity for D-Tyr[11]NT than they do for NT (Labbé-Jullié et al., 1994; Vincent et al., 1999). Therefore, if the acute peptide-induced decrease in reward threshold seen here were mediated by the activation of NT receptors located in the VTA, then both peptides would have interacted with NTr_1 receptors exclusively. However, if this were the case, then because of the higher affinity for NT relative to that for D-Tyr[11]NT by the NTr_1 receptors in this brain region, even if equal concentrations of NT and D-Tyr[11]NT were used in this study, the effect elicited by NT should have been greater than that produced by D-Tyr[11]NT. Nevertheless, just the opposite was observed. Indeed, "D-Tyr[11]NT is a poor inducer

of DA-release in the NAc" when injected directly into the VTA (Steinberg et al., 1995), likely because there are no NTr_2. receptors located there. Thus, although NT could have, and likely did exert its reward-enhancing effect in the VTA, it appears that D-Tyr[11]NT did not.

D-Tyr[11]NT likely exerted its reward-potentiating effect via activation of NTr_2 receptors because of the high affinity these receptors show for this peptide. This assertion is supported by the fact that the D-Tyr[11]NT concentration used in this set of experiments, despite it being 1/10 the size of the concentration used for NT, produced a reward-enhancing effect of equal magnitude to that produced by NT. Furthermore, Mazella et al. (1996) reported that NTr_2 receptors are maximally expressed in a select group of areas of the adult rat brain that includes the neocortex. Therefore, the PFC, which synapses onto catecholamine terminals in the NAc (Sesack & Pickel, 1992) is an excellent candidate for the mediation of the acute D-Tyr[11]NT-induced reward-potentiating effect reported in the present study, especially considering that PFC NT receptor activation stimulates midbrain DA release (Gariano & Groves, 1988; Rompré et al., 1998).

It was also predicted that on Day 1, following a single ICV injection of either peptide, maximal rates would decrease immediately and then gradually increase toward pre-injection baseline levels, which is what was observed. NT produced decreases in maximal rates of responding that were reflected by downward shifts of the rate/frequency functions of animals to which this peptide was administered. It is well known that central injections of NT cause muscle relaxation (Kitabgi, & Freychet, 1978; Kitabgi & Vincent, 1981; Ohashi et al., 1994; Osbahr 3rd et al., 1979). Therefore, the simplest explanation for this downward shift, generally produced by manipulations that alter performance (Miliaressis et al., 1982, 1986), is that aside from potentiating BSR-induced reward, NT induced muscle relaxation that made it more difficult for the animals to press for electrical stimulation. Importantly, this vertical shift does not imply that the reward associated with BSR was, in any way, lessened. Indeed, the leftward shifts discussed above suggest the opposite; acute NT administration enhanced the reward associated with BSR.

As mentioned earlier, because the independent variable in this experiment was operant responding for BSR and not locomotor activity, maximal rates of responding observed in this experiment are said to measure drug-induced performance effects rather than drug-induced forward locomotion.

However, it is worth restating that rates of responding may be regarded as reflecting goal-directed forward locomotion, and as such, may be used as a de facto index of drug-induced hyperactivity. To the extent that this is the case, maximal rate measures may provide some insight as to the possible site of action for the performance deficits observed on Day 1. Indeed, if the downward shift of the rate/frequency functions seen on Day 1 following ICV NT injection is the result on an NT-induced suppression of locomotion rather than the result of muscle relaxation, then this peptide-induced effect may be due to activation of NT receptors located in the NAc. Recall that although microinjection of NT into the VTA stimulates locomotor activity (Kalivas & Duffy, 1990), NT microinjected either centrally (Rompré, 1997) or directly into the NAc induces DA-antagonist-like effects on DA function, that essentially suppress expression of DA-dependent behaviours believed to be associated with reward; this includes forward locomotion (Ervin et al., 1981; Kalivas et al., 1982, 1984).

The major problem with the hypothesis mentioned above is that, as previously discussed, acute ICV NT was suggested to have exerted its action on NTr_1 receptors located on VTA dopaminergic perikarya (cell bodies), thus leading to a potentiation of reward. Because of the dichotomy of effect observable when NT is microinjected directly into the NAc versus when it is injected into the VTA, NT-induced activation of both sites simultaneously would likely have caused the effects of one site to cancel out the effects of the other site. For this reason, if NT did specifically suppress locomotor activity, likely sites of action for the initiation of this effect must exist outside the mesolimbic DA pathway.

The NT-receptor-rich nigrostriatal DA system (Palacios & Kuhar, 1981; Quirion, 1983) has been implicated in response selection and performance (Eagle et al., 1999). The nigrostriatal DA system is also involved in mediating motor activity and motoric responses to pharmacological manipulations. Indeed, amphetamine injection following lesions to the SN is known to produce circling behaviour in the direction ipsilateral to (toward) the lesions (Costall et al., 1976). Finally, Matsumoto et al. (1999) demonstrated that the striatum and its nigrostriatal afferents act in the initial learning that transforms the performance of sequences of movements into single motor patterns. Taken together, these findings reinforce the possibility that the NT-induced performance deficit observable on Day 1 in the present study may be due to the activation of NT receptors located in the nigrostriatal DA system.

To the extent that the NT-induced performance deficit seen here is due to suppression of locomotion as opposed to the muscle relaxant effect alluded to earlier, another possible site of action for this effect is the PFC, which projects to both the mesolimbic and the nigrostriatal midbrain DA systems (Gerfen, 1984; Wolf et al., 1995).

Like NT, D-Tyr[11]NT produced a decrease in maximal rates of responding on Day 1. This decrease in maximal rates was strikingly similar in magnitude for both peptides tested, which implies that maximal rate suppression exerted by both peptides was mediated by a common substrate. Indeed, if rate suppression following injection of either peptide is due to the activation of a common substrate, then the likeliest explanation for this finding is that both peptides produced muscle relaxant effects via activation of a substrate that binds both peptides with equal affinity. Again, because the literature has not yet uncovered possible sites of action within the brain for the NT-induced muscle relaxant effect, no more can be said now about this possibility. Nevertheless, NT receptors have been found in the spinal cord (Jennes et al., 1982; Gibson et al., 1981; Reinecke, 1985); these receptors may play a contributing role to this peptide-induced effect.

If, however, the attenuation of maximal rates is the result of a D-Tyr[11]NT-induced suppression specific to locomotion, then the likeliest site of action for this effect is the NAc because of the DA-antagonist-like effect that NT and its analogues exert there [i.e. suppression of spontaneous and drug-induced locomotion (Ervin et al., 1981; Kalivas et al., 1982, 1984)]. Although this may be the case, to the extent that NT and D-Tyr[11]NT both activate NTr_1 receptors, the sites just suggested to be mediating this effect following NT injection may, for the same reasons, be considered for the mediation of the corresponding D-Tyr[11]NT-induced affect as well.

Regardless of the mechanisms involved, it appears that only acute administration of the unequal concentrations of the two peptides used in this study produced equipotent effects on reward enhancement as well as on maximal rates of responding. Nevertheless, the time courses of both acute effects show considerable divergence. While maximal rates were suppressed maximally by both peptides immediately following peptide injection, and reached baseline levels by the end of testing, a significant peptide-induced decrease in reward threshold following the injection of either peptide was not apparent until at least 90 min post-injection. This finding is consistent with that of Rompré (1995) who also

reported a rapid suppression in maximal rates of responding coupled with a delayed decrease in reward threshold following various acute doses of centrally injected NT. The differential effects between frequency thresholds and maximal rates of responding suggested then (Rompré, 1995) as they do now, that drug-induced locomotion and reward be mediated independently of each other. In addition, Robledo et al. (1993) showed that NAc NT attenuates the locomotor but not the rewarding effect of cocaine, thus further reinforcing this position.

5.3 H_3 On Day 7, after the fourth and final intermittent central injection of either peptide, reward threshold will remain unchanged relative to the initial decrease observed on Day 1, while the peptide-induced effect on maximal rate will sensitise in such a way that maximal rate will increase relative to the corresponding Day 1 rate.

Although this hypothesis was not supported by the data gathered in the present study, it was based on a variety of reports in the literature, all of which tested repeated intermittent injections of various rewarding drugs against BSR. In every case but two (Kokkinidis & Zacharko, 1980; Predy & Kokkinidis, 1984), neither sensitization nor tolerance to the drug-induced reward-potentiating effect these drugs initially produced was found (Bauco & Wise, 1994; Bauco et al., 1993; Carlezon & Wise, 1993; Wise & Munn, 1993).

Interestingly, repeated intermittent ICV injections of NT or D-Tyr[11]NT, in the present set of experiments, led to differential effects by Day 7 at which time all animals had received a total of four central injections of either peptide or their vehicle. While tolerance had developed to the reward-enhancing effect produced by acute NT, sensitization had developed to the matched effect produced by acute D-Tyr[11]NT. One explanation for this finding is that, as already hypothesized, these two peptides act at different sites along the reward-relevant pathway. That NT and D-Tyr[11]NT produced tolerance and sensitization to the reward threshold response for BSR respectively, while habit-forming drugs tested in the same manner produced neither effect, may be tied to the neuromodulatory function of the NT receptors that these two peptides activate.

As previously discussed, the acute NT-induced reward-enhancing effect uncovered in this experiment was hypothesized to be due to the activation of NTr_1 receptors localized on the DA perikarya

of the VTA. Assuming that NT receptors of the same subtype respond to NT exposure in an identical fashion regardless of where in the brain these receptors are located, the desensitization found here following repeated NT administration is in line with the data of Faggin & Cubeddu (1990) and Faggin et al. (1990), who reported rapid NT-induced desensitization to the DA-releasing effect of NT in the SN. To substantiate this last statement, it is worth recalling that the SN, like the VTA, is recognized for its exceptionally high density of NTr_1 receptors relative to any other mesotelencephalic DA system component (Hermans & Maloteaux, 1998).

In accordance with the literature supporting the concept of NT receptor heterogeneity (Gully et al., 1997; Héaulme et al., 1997; Labbé-Jullié, et al., 1994; Steinberg et al., 1995), the most tempting explanation for these two findings is that the NTr_2 receptors proposed to mediate the acute ICV D-Tyr[11]NT-induced reward-enhancement sensitized with repeated exposure, while the NTr_1 receptors, presumed to mediate the acute NT-induced reward-enhancing effect, desensitized with repeated exposure. Indeed, the NTr_1 receptor "undergoes potent desensitization by repeated application of neurotensin" (Tanaka et al., 1990). However, there exists no evidence that would support NT-induced sensitization of NTr_2 receptors. A more likely hypothesis then, is that the sensitized reward-enhancing effect induced by D-Tyr[11]NT on Day 7 is the result of alterations occurring to the neurones projecting from either the amygdala or the PFC to the NAc. From a neural systems perspective, this hypothesis is consistent with the suggestion that locomotor sensitization be mediated by amphetamine-induced modifications to the neurones of the PFC and/or amygdala that project to the VTA (Wolf et al., 1995). In essence, Wolf et al. (1995) showed that lesions to the PFC or amygdala selectively prevented amphetamine-induced sensitization of post-stereotypy locomotion. They also demonstrated that co-administration of NMDA receptor antagonists with amphetamine prevented the development of sensitization to both stereotypy and post-stereotypy locomotion. Given that stereotypy intensity is more closely correlated to the amount of DA released in striatum than in the NAc (Sharp et al., 1987), Wolf et al. (1995) argued that while locomotor sensitization is affected by amphetamine-induced changes to VTA-bound projections from the PFC and/or amygdala, "by analogy, there is an EAA input to the SN that is required for sensitization of stereotyped behaviours". Indeed, Leith & Kuczenski (1982) demonstrated that locomotion and stereotypy are dissociable components of amphetamine-induced behavioural

sensitization. Similarly, Wise and co-workers have provided evidence supporting the dissociation between drug-induced forward locomotion and reward potentiation (Bauco et al., 1993; Bauco & Wise, 1994, 1997; Carlezon Jr. & Wise, 1993; Wise & Munn, 1993). Finally, Carlezon & Wise (1996) proposed that the crucial event for drug-induced reward is the inhibition of the GABAergic medium spiny output neurones of the NAc, which suggests a specific role for the NAc in drug-induced reward, in particular. Taken together then, drug-induced sensitization may be broken down into three distinct components: forward locomotion, stereotypy, and reward enhancement that are compartmentalized by the mesocorticolimbic DA system such that each component is mediated primarily by the VTA, SN and NAc, respectively. Nonetheless, this does not preclude the notion that some degree of interactivity between these and other mesocorticolimbic sites is also involved in the development of drug-induced sensitization. Indeed, virtually all sites within the mesocorticolimbic system are interconnected one way or another (Kupfermann, 1991).

It was also hypothesized that the peptide-induced decrease in maximal rate of responding observed on Day 1 would sensitise following repeated intermittent exposure of either peptide, but that this sensitization would be expressed as an increase in maximal rates relative to the rate measured on Day 1, and relative to the group measure for animals given vehicle on Day 7. This prediction was based on Wise and coworkers (Bauco et al., 1993; Carlezon Jr. & Wise, 1993; Wise & Munn, 1993), who revealed that although drug-induced reward facilitation did not sensitise with repeated drug exposure, drug-induced locomotor activating effects did. Nevertheless, Rompré (1997) would not likely agree with this prediction because unlike the studies just listed, he tested NT and D-Tyr[11]NT and found no change in the level of locomotor activity seen following repeated intermittent ICV injections of either peptide relative to corresponding levels observed following acute peptide treatments. Rompré (1997) did reveal, however, that relative to control animals, rats administered repeated injections of D-Tyr[11]NT, but not NT showed greater levels of locomotor activity during the second hour of testing as measured by photobeam crossings. The simplest explanation as to why Rompré's results do not coincide with those of Wise and coworkers (1993) involves at least two factors: (1) intrinsic property differences between the peptides used by Rompré and the drugs of abuse used by Wise's laboratory; and (2) Rompré tested only

photobeam crossings whereas the others also measured thresholds for BSR which appears to affect the development of locomotor sensitization (Bauco et al., 1993; Wise & Munn, 1993).

The data collected for maximal rates of responding over the first week of testing resembled those compiled during the same time period for reward threshold in that similar effects were produced by acute ICV injections of either NT or D-Tyr[11]NT administered on Day 1, and differential effects were observed following repeated intermittent injections of these same peptides administered on Day 7.

The performance suppression induced by NT on Day 1 grew slightly stronger by Day 7. In contrast, the performance suppression produced by D-Tyr[11]NT on Day 1 disappeared by Day 7, and was, in fact, replaced by a statistically significant increase in maximal rate of responding from 72 min post-injection until the end of testing. In conjunction with the work of Wise and coworkers (1993), this D-Tyr[11]NT-induced maximal rate increase, which approached 110 % of the corresponding mean pre-injection (baseline) rate supports the notion of a homology between performance and locomotion alluded to above. It also suggests the possibility that as tolerance developed to the acute D-Tyr[11]NT-induced performance-suppression effect, repeated D-Tyr[11]NT exposure led to the behavioural expression of previously masked, peptide-induced locomotor activating effects that may have even become sensitized due to the repeated peptide exposure. Indeed, as already indicated, the locomotor stimulating effects of drugs that are rewarding sensitize following repeated intermittent exposure to them (Bauco et al., 1993; Bauco & Wise, 1994; Carlezon Jr. & Wise, 1993; Wise & Munn, 1993).

Thus far in this manuscript, it has become clear that while acutely, NT and D-Tyr[11]NT produced comparable effects on both parameters studied herein (reward threshold and maximal rate of responding), repeated injection of D-Tyr[11]NT significantly lowered and raised measures for both parameters respectively, while repeated NT treatment produced subtle increases in these measures, at best, relative to those recorded following the initial injection. Regardless of the mechanisms involved, these data appear to be more congruent with the notion that reward and performance are mediated by separate neural substrates than by a common substrate as proposed earlier by Glickman and Schiff (1967) and Wise & Bozarth (1987).

5.4 H_4 On Day 14, following a single IP injection of d-amphetamine, neither NT nor D-Tyr[11]NT pre-treatment will cross-sensitize to the reward-potentiating effects of d-amphetamine, but pre-treatment with either peptide will cross-sensitise, equipotently, to the maximal rate increases induced by d-amphetamine, relative to control animals (pre-treated with saline and tested with d-amphetamine).

The major objective of the present study was to determine whether repeated intermittent exposure to either NT or D-Tyr[11]NT would significantly alter the neural circuitry of the brain reward system specifically targeted by d-amphetamine. It was hypothesized that on Day 14, following a single IP injection of d-amphetamine, reward threshold would decrease relative to baseline levels, but that neither NT nor D-Tyr[11]NT pre-treatment would cross-sensitize to the reward-potentiating effects of d-amphetamine. Consistent with the literature (Bozarth et al., 1980; Gallistel & Karras, 1984; Gardner, 1997; Wise, 1996; Wise & Rompré, 1989) d-amphetamine did decrease reward thresholds. Furthermore, this effect did not differ between groups, which indicates that repeated exposure to either NT or D-Tyr[11]NT did not alter the reward-enhancing response to subsequent acute systemic injections of d-amphetamine.

The lack of effect caused by pre-treatment with either peptide is consistent with the literature arguing that, when tested against BSR, the rewarding properties of abusive drugs including nicotine (Bauco & Wise, 1994), morphine and cocaine (Bauco et al., 1993), phencyclidine (Carlezon Jr. & Wise, 1993), and amphetamine (Wise & Munn, 1993) do not change with chronic administration (but see Kokkinidis & Zacharko, 1980 and Predy & Kokkinidis, 1984 for exceptions).

It was also hypothesized that on Day 14, following a single IP injection of d-amphetamine, maximal rates of responding for BSR would increase relative to baseline levels, and that pre-treatment with either peptide would equipotently cross-sensitise to the maximal rate increases induced by d-amphetamine, relative to control animals (pre-treated with vehicle and tested with d-amphetamine).

D-amphetamine induced a small increase in maximal rates of responding by control animals as well as by those pre-exposed to NT. In contrast, pre-exposure to D-Tyr[11]NT led to a statistically significant increase in maximal rates of responding following the acute systemic d-amphetamine injection,

hence the development of cross-sensitization between D-Tyr[11]NT and d-amphetamine on performance enhancement.

It is unclear why only D-Tyr[11]NT showed a sensitized performance effect in conjunction with systemic d-amphetamine treatment. Other differences between NT and D-Tyr[11]NT have been reported, and in most cases, these differences are characterized by a greater potency by D-Tyr[11]NT relative to NT to exert its influence on the selected NT receptor-agonist attributes being measured. These attributes include a greater potency of D-Tyr[11]NT to produce hypothermia (Checler et al., 1983; Jolicoeur et al., 1984), to attenuate muscle rigidity and tremors (Rivest et al., 1991), and to induce analgesia (Dubuc et al., 1999; Jolicoeur et al, 1984). Some researchers believe that these particular differences may be due to the fact that, in vivo, D-Tyr[11]NT is less sensitive to enzymatic degradation than NT is. Hence, because D-Tyr[11]NT stays intact longer than NT does, it produces effects that are more potent than those induced by NT (Checler et al., 1983; Kitabgi et al., 1980). Still, others have explored the relationships between the structure and duration of NT's central actions and concluded that, "no clear correlation (exists) between (the) relative potency of (NT) analogues and their duration of action", (Jolicoeur et al., 1984).

Regardless of why, exactly, D-Tyr[11]NT, but not NT, cross-sensitized to d-amphetamine-induced performance-enhancement, the findings of Castel et al. (1989) are particularly interesting because they showed that ICV injections of NT induced hypomotility while ICV injections of D-Tyr[11]NT induced hypermotility. Although the peptide injections performed by Castel et al. (1989) were acute, and as such, appear not to be relevant to material presented in this section of the discussion, they are mentioned because they demonstrate a difference between the induced effects of NT and D-Tyr[11]NT that relate specifically to the expression of behaviours affecting performance, and in doing so, they offer some reconciliation for the cross-sensitization observed in the present experiment on d-amphetamine-induced performance-enhancement by D-Tyr[11]NT alone. Equally important to this discussion, they also portray, yet, another instance of opposing effects between NT and D-Tyr[11]NT.

Clearly, despite the absence of cross-sensitization by NT to d-amphetamine-induced performance-enhancement, the significant effect on this measure produced by D-Tyr[11]NT strengthens the growing consensus that reward potentiation is mediated separately from other behaviours elicited by

habit-forming drugs in general. Moreover, because the case has been made that performance and locomotion both represent goal-directed behaviours characterized by motoric activity that is initiated by animals in a front-forward position, maximal rates measured in this study may be reflective of a homology between both these parameters. Furthermore, to the extent that this is so, the present results are in line with the recent literature demonstrating that although drug-induced forward locomotion sensitizes with repeated drug exposure, reward potentiation as measured by BSR, for the most part, does not (see Kokkinidis & Zacharko, 1980 and Predy & Kokkinidis, 1984 for exceptions).

As previously noted, maximal rates of responding in the present set of experiments were enhanced following repeated D-Tyr[11]NT exposure suggesting that D-Tyr[11]NT-induced sensitization had developed by Day 7. Perhaps then, on Day 14, d-amphetamine activated the same receptors presumed to have been sensitized by D-Tyr[11]NT on Day 7. Considering that the amygdala may contain a previously uncharacterized NT receptor subtype that does not correspond to any of the three NT receptor subtypes already cloned (Boudin, 1996), it is possible that this new NT receptor subtype resembles the NTr_2 receptor in its binding affinities for NT and D-Tyr[11]NT. Following from this line of thought, perhaps the NT receptors in question became sensitized by the centrally-injected D-Tyr[11]NT that had reached the amygdala upon injection. This speculation is somewhat congruent with the demonstration by Horger (1994) that pre-exposure to SR48692, which blocks the vast majority of NT receptors in the VTA (Szigethy & Beaudet, 1989), attenuates the development of behavioural sensitization to cocaine. Further supporting this hypothesis is the discovery that locomotor sensitization results following treatment with D-Trp[11]NT (an NT analogue in which the Tyr residue in position 11 of endogenous NT is been replaced with D-Trp) after repeated injections of GBR12783, which like d-amphetamine, is a DA-uptake inhibitor (Boulay et al., 1996).

There is also support in the literature suggesting that the PFC mediate psychostimulant-induced sensitization. Firstly, Tzschentke & Schmidt (1998) showed that quinolinic lesions of the pre-limbic mPFC affects the development of cocaine-induced behavioural sensitization in rats by decreasing sniffing and increasing grooming behaviour. Secondly, DA depletion in the mPFC attenuates the amphetamine-induced increases in extracellular NAc DA as well as the increased potentiation of locomotor activity that is typically coupled with this DA increase (King et al, 1997). Thirdly, excitotoxic lesions of the PFC have

been shown to diminish the development of amphetamine-induced amphetamine sensitization (Wolf et al., 1995). The findings just mentioned are relevant to the results for maximal rates measured on Day 14 of the present study because, in addition, Rompré and co-workers demonstrated that activation of PFC NT receptors enhances midbrain DA impulse flow (Rompré et al., 1998) and that endogenous NT is, in fact, required for the initiation of amphetamine sensitization (Rompré & Perron, 2000). It may be then, that the development of sensitization, observed for maximal rates, that occurred following repeated intermittent ICV injections of D-Tyr[11]NT on Day 7, and the significant cross-sensitization for this parameter seen on Day 14, following the IP injection of d-amphetamine, may be mediated by the PFC.

Notwithstanding the possibilities outlined above, the correlation analyses that were performed on Day 14 to better understand the significant maximal rate increase observed by the animals pre-exposed to D-Tyr[11]NT on Day 14, suggested the occurrence of a floor effect that had, in essence, amplified the magnitude of the increase in maximal rates of responding that had occurred for this group.

6.0 Conclusions

The present set of experiments has uncovered information about the involvement of NT and D-Tyr[11]NT in the phenomenon of behavioural sensitization. It has also demonstrated the involvement of D-Tyr[11]NT, as well as the lack of involvement of NT, in the cross-sensitization of d-amphetamine-induced performance enhancement (and perhaps d-amphetamine-induced hyperactivity), a behaviour that has been used to speculate on hypothetical mechanisms of action within the relevant neural circuitry.

Interestingly, although similar effects by both peptides were seen following acute ICV injections on Day 1, which suggested that the concentrations of both peptides used in this study were equipotent, differential effects were seen for both parameters measured by Day 7. For both parameters investigated, on Day 7 NT-injected rats resembled control animals administered vehicle, whereas animals injected with D-Tyr[11]NT showed significant decreases in reward threshold and significant increases maximal rates.

Although it was not expected that the peptides used in this set of experiments would differ in the behavioural effects they produced, reconciliation for these differences was achieved through a hypothetical model inspired by Wolf et al. (1995) who argued that while locomotor sensitization is affected by amphetamine-induced changes to VTA-bound projections from the PFC and/or amygdala, "by analogy, there is an EAA input to the SN that is required for sensitization of stereotyped behaviours". In essence, the model proposed herein suggests that the mesocorticolimbic DA system breaks down and compartmentalizes drug-induced sensitization into three separate components. These components (forward locomotion, stereotypy and reward potentiation) are then each mediated primarily by one specific zone of the mesotelencephalic DA systems (the VTA, SN and NAc respectively). Thus, in the case of reward potentiation, it is theorized that NT activates the high affinity NTr_1 receptors located in the VTA while D-Tyr[11]NT activates NTr_2 receptors, to which it binds with high affinity, in areas, excluding the VTA, that send excitatory projections to the NAc.

It is unclear why only D-Tyr[11]NT showed cross-sensitization with d-amphetamine on maximal rates of responding measured on Day 14. Nevertheless, other differences between NT and D-Tyr[11]NT have been reported, and in most cases, these differences are characterized by a greater potency by D-Tyr[11]NT relative to NT to exert its influence on the selected NT receptor-agonist attributes being measured. These attributes include a greater potency of D-Tyr[11]NT to produce hypothermia (Checler

et al., 1983; Jolicoeur et al., 1984), to attenuate muscle rigidity and tremors (Rivest et al., 1991), and to induce analgesia (Dubuc et al., 1999; Jolicoeur et al, 1984).

If the decrease in maximal rates produced by both peptides seen on Day 1 was due to a suppression effect specific to locomotor activity, then NT receptors in either the SN or the PFC, which projects to the striatum as well as to the VTA and NAc, may be responsible for this effect. Nevertheless, the muscle relaxant effect that both these peptides are well known for (Kitabgi, & Freychet, 1978; Kitabgi & Vincent, 1981; Ohashi et al., 1994; Osbahr 3rd et al., 1979) may be the simplest explanation available for this effect.

The alternative to the viewpoint above, that the measure of performance in experiments like this one is homologous with forward locomotion, makes the case that Wise and colleagues (1993) were correct in their assumption that drug-induced locomotor stimulating effects and reward facilitation are mediated by two separate mechanisms. However, future research will be needed to confirm this perspective.

In conclusion, the data collected herein have proven to be consistent with the view that NT-receptor activation within the dopaminergic elements of the mesocorticolimbic system are involved in the modulation of behaviours known to be expressed following the administration of (psychostimulant) drugs of abuse. However, the extent to which this NT-DA interaction contributes to the expression of behaviours related to habit-forming-drug-induced performance and reward is still unclear at this time. Nevertheless, the knowledge brought forth by the present set of experiments, that NT receptors are, indeed, involved in various components of behavioural sensitization and even cross-sensitization, essentially complements the work of Rompré (1997). In addition, these data bring us that much closer to understanding drug abuse, and more importantly, the greater problem of widespread drug addiction that has continued to plague us since the early 1800s.

7.0 References

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