# THE INVOLVEMENT OF SUBSTANCE P IN RELAPSE TO COCAINE-SEEKING BEHAVIOUR IN RATS

by

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# THE INVOLVEMENT OF SUBSTANCE P IN RELAPSE TO COCAINE-SEEKING BEHAVIOUR IN RATS. M.A.. March 2001.

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## ABSTRACT

The aim of this thesis was to investigate the role of the neuropeptide substance P (SP) in mediating relapse to drug-seeking behaviour in rats. Experiment 1 showed that the SP analogue. DiMe-C7 (0. 1. 3. or 6μg/μl: 0.5μl/side) microinjected into the ventral tegmental area (VTA) significantly increased locomotor activity at all drug doses tested. Experiment 2 showed that intra-VTA injections of DiMe-C7 (0. 0.2. 1 or 5μg/μl) induced reinstatement of drug-seeking behaviour in a dose-dependent manner in animals previously trained to self-administer cocaine. Finally. experiment 3 showed that pre-treatment with the selective NK-1 receptor antagonist. Spantide II (0 or 1μg/0.5μl). by injection into the VTA. did not block DiMe-C7 (0 or 1μg/0.5μl)-induced reinstatement of cocaine-seeking behaviour. suggesting that DiMe-C7-induced reinstatement may not be dependent upon NK-1 receptor activation in the VTA. Together. these findings provide evidence for the role of SP and neurokinin receptors in drug relapse.

# ACKNOWLEDGEMENTS

# TABLE OF CONTENTS

# LIST OF FIGURES

# INTRODUCTION

Relapse to drug use is the primary problem in the treatment of drug addiction. The neurobiology of the rewarding actions of drugs of abuse is fairly well understood. However. what is not understood is why. when drugs are unavailable for extended periods. users experience persistent drug-craving and remain vulnerable to relapse (Stewart . 2000). In recent years. concerted efforts have been made to address the problem of relapse through investigations in the laboratory. Findings from animal studies have corroborated clinical reports which show that re-exposure to drugs (Meyer. 1988: Jaffe et al.. 1989) or to environmental stimuli previously associated with drug-taking (Davis and Smith. 1976: Childress et al.. 1999: Robbins et al.. 1999). are highly effective at inducing relapse in humans even efter extended periods of abstinence. Furthermore. relapse is more likely to occur in individuals exposed to high stress (Brown et al.. 1995). The animal studies which support these findings will be further described in a subsequent section.

The aim of this thesis is to investigate the role of the neuropeptide substance P (SP) in mediating relapse to drug-seeking. A number of neurochemical systems have been implicted in the neurobiology of relapse. most notably the mesolimbic dopamine (DA) system. However. differences between drug-induced and stress-induced relapse suggest that other neurochemical systems may also be involved (Shaham and Stewart. 1996). The SP system represents a potential novel neurochemical candidate for the mediation of relapse. and is therefore the focus of the present thesis. SP is a neuropeptide found in the brain which has been shown to be rewarding (Staubli and Huston. 1985:

Holzhauer-Oitzl and Huston. 1987). but to also play a significant role in the response to stress (Bannon et al.. 1983: Kramer et al.. 1998). Both of these characteristics are strongly implicated in drug relapse. Furthermore. numerous reports indicate significant interactions between SP and the mesolimbic DA system and will be reviewed in a subsequent section (Brownstein et al.. 1976: Kelley et al. 1979: Deutch et al.. 1985). Given the important role of the DA system in drug reward. these interactions provide further evidence for the potential involvement of SP in drug relapse.

In the present thesis. an animal model of relapse is used which was developed by Stewart and her colleagues (for review. refer to Shaham et al.. 2000). This model is based on the premise that drug-craving results in drug-seeking behaviour and relapse. The procedure involves training animals to press a lever in order self-administer drugs for a period of time. and then subsequently extinguishing this behaviour by allowing the animal to attempt to obtain the drug when it is no longer available. This eventually results in cessation of lever pressing or drug-seeking behaviour. The model. thus. allows one to study the ability of various events to reinitiate or reinstate drug-seeking behaviour (Stewart. 2000). This model further allows for the investigation of the neurobiological mechanisms of relapse.

## PHARMACOLOGY OF SUBSTANCE P

SP is an undecapeptide (11 amino acids) that acts as a neurotransmitter in both the peripheral and central nervous system (Pernow. 1983). SP belongs to a family of neuropeptides known as tachykinins. Tachykinins are peptides that share the C-terminal

sequence Phe–X–Gly–Leu–Met–NH$_2$ (Erspamer, 1981). Other peptides in the tachykinin family include neurokinin A, neurokinin B, and neuropeptide γ, which are all found in mammalian tissue. More recently, a number of nonmammalian tachykinins have been discovered such as eledoisin, which was isolated from octopus salivary glands, and the amphibian peptides physalaemin and kassinin (Maggio, 1988). Of all the tachykinin peptides, SP is the most abundant tachykinin found in the CNS, as well as in peripheral tissue (Arai and Emson, 1986).

Three classes of tachykinin receptors have been discovered in parallel with the identification of the various endogenous tachykinins. The tachykinin receptor subtypes have been classified as NK-1, NK-2 and NK-3 (Saria, 1999 for review). Recently, a fourth subtype has been identified and classified as NK-3$_B$ (Krause et al., 1997). The NK-3$_B$ receptor subtype is highly analogous to the originally identified NK-3 receptor. Each receptor subtype has a unique distribution in the CNS and in the periphery, as well as having a distinctive rank order of potency for the endogenous tachykinins (Saria, 1999). SP is the natural ligand with the highest affinity for the NK-1 receptor. However, neurokinin A and neurokinin B also exhibit a sizable affinity for the NK-1 receptor. (Quartara and Maggi, 1997). Neurokinin A exhibits the highest affinity for the NK-2 receptor and neurokinin B for the NK-3 receptors (Saria, 1999). The C-terminal sequence of SP (SP6-11) is essential for biological activity of the peptide. It is the minimum SP fragment retaining good affinity for the NK-1 receptor (Lee et al., 1986). The tachykinin NK-1 receptor is G-protein coupled (Guard et al., 1991) and upon stimulation, activates various second messenger systems (Mitsuhashi et al., 1992; Nakajima et al., 1992; Garcia et al., 1994; Mochizuki et al., 1994).

SP is found throughout the CNS and in a variety of peripheral organs (Otsuka and

Yoshioka. 1993 for review). There is a widespread distribution of SP-containing cell

groups in the CNS. SP-immunoreactivity is found in a subset of neurons projecting from

the striatum and the globus pallidus (Hong et al.. 1977: Kanazawa et al.. 1977). Other SP-

immunoreactive cell groups are found in the amygdaloid complex. habenular nucleus.

interpeduncular nucleus. and caudate putamen (Cuello and Kanazawa. 1978). In the

periphery. cell bodies and fibers displaying SP-immunoreactivity are found in autonomic

ganglia where SP acts as a sensory transmitter (Pernow. 1981 for review).

Tachykinin NK-1 receptors are also widely distributed within central and

peripheral tissue (Quartara and Maggi. 1998 for review). However. in the CNS there is

no apparent correlation between the density of SP innervation and the density of SP

binding sites (Herkenham. 1987). This "mismatch" is particularly evident in the

substantia nigra (SN). which receives the greatest SP innervation. but is nearly devoid of

NK-1 receptors (Mussap et al.. 1993). Various studies using different methodological

approaches such as immunohistochemistry. autoradiography. and mRNA expression.

(Quartara and Maggi. 1998 for review) have shown that the structures of the brain that

are particularly rich in tachykinin NK-1 receptors include the striatum. the nucleus

accumbens. the hippocampus. the lateral nucleus of the hypothalamus. the habenula. the

interpeduncular nucleus. the nucleus of the tractis solitaris. the raphe nuclei. and the

medulla oblongata (Otsuka and Yoshioka. 1993).

Autoradiographic and immunohistochemical studies have also shown that NK-1

receptors are found in several discrete sites in the spinal cord (Yashpal et al.. 1990:

Quartara and Maggi. 1998 for review). SP is present in high concentrations in terminals

of sensory afferent nerves in the spinal cord. The detection of a high concentration of SP in the dorsal horn of the spinal cord, was the first experimental observation implicating SP in the transmission of pain (Lembeck, 1953; Lembeck, 1988). Subsequent converging evidence has confirmed the finding that SP acts as a neurotransmitter at the spinal cord level, contributing to the processing of noxious stimuli (Quartara and Maggi, 1998 for review).

SP may often be colocalized with other tachykinins in the same neurons, as well as with classical neurotransmitters such as DA, acetylcholine (Ach), serotonin (5-HT), opiates, γ-aminobutyric acid (GABA) (Pernow, 1983; Hokfelt et al., 1987; Chang, 1988), and other neuropeptides such as corticotropin-releasing factor (CRF) (Shimada et al., 1989).

## SUBSTANCE P / DOPAMINE INTERACTIONS

Neuroanatomical studies have shown that SP is present in high concentration in terminals close to the DA-A10 cell bodies in the VTA (Brownstein et al., 1976; Kanazawa and Jessel, 1976). The VTA receives this SP innervation from SP-containing neurons originating from the medial habenular nucleus (Cuello and Kanazawa, 1978). Other studies have shown that SP also interacts with the DA-A9 cell bodies in the substantia nigra (SN) (Cheramy et al., 1977; Ljungdahl et al., 1978) which contains the highest concentration of SP in the brain (Brownstein et al., 1976; Kanazawa and Jessell, 1976). Innervation of SP to the SN originates from the striatum and globus pallidus (Hong et al., 1977; Kanazawa et al., 1977; Krause et al., 1984).

A wealth of neurochemical evidence has now accumulated suggesting that SP interacts with DA midbrain neurons in a facilitatory way (Kalivas. 1985). Injection of SP directly into the VTA activates DA metabolism in mesocortical and mesolimbic neurons (Deutch et al.. 1985: Cador et al. 1989). Similarly. injections of SP into the SN results in increases in striatal DA levels (Waldmeier et al.. 1978: James and Starr. 1979: Reid et al.. 1990).

Behavioural evidence for the interaction between SP and midbrain DA systems is consistent with a facilitatory effect of SP on DA. Injections of SP into the VTA stimulate locomotor and exploratory behaviour (Stinus et al.. 1978: Kelley et al.. 1979: Kelley et al.. 1985). These behavioural activating effects of SP are blocked by 6-OHDA lesions of the nucleus accumbens (NAcc) (Stinus et al.. 1978) or by pretreatment with a DA antagonist (Kelley et al.. 1979). Injections of SP into the SN shows a similar pattern to that seen following intra-VTA injections of SP (Kelley and Iversen. 1979).

Some studies have shown that SP levels may be regulated by the dopaminergic input from the SN. For instance. 6-OHDA lesions of the nigostriatal pathway or pre-treatment with DA receptor antagonists. result in decreased levels of SP in the SN (Cruz and Beckstead. 1989). whereas DA agonists increase levels of SP (for review. see Gerfen and Wilson. 1996). These findings suggest that striatal DA innervation is required for the maintenance of basal SP levels in the terminals of striatonigral SP neurons.

The interactions between SP and DA systems are consistent with some of the behavioural systems in which SP has been shown to be involved. As will be reviewed below. SP has been shown to be involved in both reward and stress responses. The involvement of SP in these behavioural systems appears to be DA-dependent. The

neuroantamical. neurochemical. and behavioural evidence supporting interactions between SP and DA systems. further support the involvement of SP in the reward and stress systems.

## SUBSTANCE P AND REWARD

A number of studies have shown that SP can have reinforcing properties (Staubli and Huston. 1985: Holzhauer-Oitzl and Huston. 1987: Holzhauer-Oitzl et al. 1987: Holzhauer-Oitzl et al.. 1988: Hasenohrl et al.. 1990) depending upon the dose of SP used and/or the site of action. For instance. Boix et al. (1995) found that injection of SP into the area of the nucleus basalis magnocellularis (NBM) in rats had reinforcing effects. as assessed by the conditioned place preference (CPP) task. Others have shown similar effects (Hasenohrl et al.. 1992). Yet others have shown reinforcing effects of SP in the CPP task when injected into the lateral hypothalamus / medial forebrain bundle. medial septum. and after systemic administration (Staubli and Huston. 1985: Holzhauer-Oitzl and Huston. 1987: Huston et al.. 1993).

These findings are consistent with studies showing interactions between SP and DA systems. The mesolimbic DA system is critically important in mediating the rewarding properties of psychostimulant drugs such as cocaine and amphetamine. and also natural rewards such as food (Koob and Bloom. 1988: Wise and Rompre. 1989). Rewarding drugs share in common the ability to increase extracellular DA in the NAcc. Boix et al. (1995) found that not only did injections of SP into the NBM induce CPP. they also increased extracellular levels of DA in the NAcc as measured by *in vivo*

microdialysis. Only those animals in which the administration of SP induced this increase in DA levels acquired a place preference. These findings suggest that SP's reinforcing effects in the NBM are DA-dependent. Studies have also shown that peripheral administration of SP increases DA levels in the NAcc which can last for hours (Boix et al.. 1992).

As previously mentioned, central administration of SP has been observed to induce behavioural excitation characterized by increases in locomotion (Barbeau et al.. 1980; Elliott and Iversen. 1986). Positive reinforcing drugs such as psychostimulants and opiates share in common the ability to activate locomotor behaviour. According to Wise and Bozarth (1987), the positive reinforcing effects and the locomotor activating effects of these drugs derive from activation of a common mechanism, that is activation of the dopaminergic system. SP appears to be yet another neurochemical system that shows this homology between locomotion and reward.

Given the interactions between SP and DA systems, and that the reinforcing effects of many drugs of abuse depend upon dopamingergic mechanisms within the ventral forebrain (Wise and Bozarth. 1987). a recent study examined whether the rewarding properties of abused drugs are dependent upon the SP system. Murtra et al. (2000) showed that disruption of the NK-1 receptor gene in mice resulted in the absence of the locomotor-stimulating and rewarding effects of morphine. These results provide evidence for the role of SP in opiate reward mechanisms and addiction. Although the effects found in this study were specific to opiates. they do suggest that SP is not only itself rewarding. but it may also be required for drug reward.

## SUBSTANCE P AND STRESS

One of the major neurochemical systems involved in the response to stress is the mesocorticolimbic DA system. The mesolimbic and mesocortical DA neurons projecting to the NAcc and the prefrontal cortex (PFC) respectively, have cell bodies originating in the ventral tegmental area (VTA) (Moore and Bloom, 1978). Acute stress induced by restraint or mild footshock have been reported to increase the turnover of DA in the frontal cortex and the NAcc (Thierry et al., 1976; Bannon and Roth, 1983). As previously described, neuroanatomical, neurochemical, and behavioural studies have demonstrated interactions between SP and the mesocorticolimbic DA system. These studies provide further evidence for the involvement of SP in the response to stress.

Studies show that stress-induced activation of DA may be mediated by SP. Injection of the stable SP agonist DiMe-C7 into the VTA produce a remarkably selective increase in levels of the DA metabolite 3,4-dihydroxyphenyl-acetic acid (DOPAC) in the PFC and the NAcc, a pattern resembling that seen after exposure to mild stress (Elliott et al., 1986). Furthermore, injection of a SP antibody directly into the VTA has been shown to prevent normal footshock-induced activation of mesocortical DA neurons (Bannon et al., 1983). Lisoprawski et al. (1981) have also shown that the stress-induced increase in DA release from the PFC and NAcc was accompanied by decreased levels of SP in the VTA, indicating that SP is being rapidly released during stress. Findings by Bannon et al. (1986) were consistent with those previously published. This study showed that SP depletion in the VTA following mild intermittent footshock occurred maximally by 10 minutes of footshock, and returned to basal levels 30 minutes after the cessation of shock, at which point DOPAC levels had also returned to normal.

As previously described. injections of SP into the VTA stimulate locomotor and exploratory behaviour. These behaviours are often seen following acute mild stressors (Stinus et al.. 1978: Kelley et al.. 1979: Kelley et al.. 1985). further showing that SP is involved in stress responses.

Finally. SP has also been implicated in various affective disorders which may involve stress as an underlying factor. For instance. chronic mild stress has been postulated to contribute to clinical depression. Animals chronically exposed to various stressors have been shown to display symptoms that are consistent with a depressive profile seen is humans (Wilner et al.. 1992). SP antagonists have been shown to act as putative antidepressants in an animal model of depression (stress-induced vocalizations in guinea pig pups following maternal separation) (Kramer et al.. 1998). Perhaps the most interesting finding suggesting the involvement of endogenous SP in depression came from a recent clinical study by Kramer et al. (1998) which found robust antidepressant effects of the SP antagonist MK-869 in patients with moderate to severe major depression. Both animal and human studies indicate that the endogenous SP system may be involved in affective disorders which may involve stress as a contributing factor. These studies. thus. provide further support for the involvement of SP in the stress system.

Together these studies suggest that SP innervation is involved in the stress-induced activation of mesocortical and mesolimbic DA systems. Neuroanatomical. neurochemical and behavioral studies indicate that endogenous SP may mediate stress-induced DA release. as well as stress-induced behavioural activation. Pre-clinical and

clinical studies implicating SP's involvement in stress-related disorders. further provide evidence for the role of SP in stress systems.

## NEUROBIOLOGY OF RELAPSE

As previously indicated. relapse to drug use can occur in response to re-exposure to drugs. exposure to drug-associated cues. and exposure to stressors. The neurobiology of relapse is not well understood. although a number of neurochemical systems have been implicated. One such system is the DA system. which is directly involved in the rewarding properties of drugs. but may also be involved in relapse to drug use. However. the neurobiological mechanims involved in relapse may be different for each type of trigger of relapse. For instance. a number of experimental findings suggest that the neurobiology of stress-induced relapse may be dissociable from that of drug-induced relapse. As such. neurochemical systems other than DA have been investigated and implicated in stress-induced relapse.

### *Drug-Induced Relapse*

A powerful trigger of relapse is re-exposure to drugs of abuse. In the laboratory. experimenter-delivered priming infusions of the self-administered drug after extinction from drug self-administration will reliably reinstate drug-seeking behaviour (Gerber and Stretch. 1975: de Wit and Stewart. 1981: 1983). The ability of various drugs to induce relapse coincides with their ability to mimic the neurochemical effects of the self-administered drug (Self and Nestler. 1998). For example. opiates and psychostimulants.

which both activate the mesolimbic DA system (Wise and Bozarth. 1987: Di Chiara and Imperato. 1988). have been shown to reinstate responding in animals trained to self-administer psychostimulants (de Wit and Stewart. 1981). However. other drugs of abuse which do not require the DA system for their rewarding effects such as barbiturates. benzodiazepines and ethanol. all fail to reinstate reponding for psychostimulants (Gerber and Stretch. 1975: de Wit and Stewart. 1981: Slikker et al.. 1984: Comer et al.. 1993).

Evidence suggests that the ability of opiates and psychostimulants to induce relapse is dependent upon their ability to activate the mesolimbic DA system (Self and Nestler. 1998). For instance. microinfusions of amphetamine. as well as other dopaminergic agonists. directly into the NAcc. effectively reinstates heroin and cocaine-seeking behaviour (Stewart and Vezina. 1988: Wise et al.. 1990: de Wit and Stewart. 1983: Self et al.. 1996). Similarly. infusions of morphine directly into the VTA has been shown to reinstate both heroin and cocaine-seeking (Stewart et al.. 1984). Furthermore. DA antagonists have been shown to block the priming effects of heroin. amphetamine. and cocaine (Ettenberg. 1990: Shaham and Stewart. 1996: Weissenborn et al.. 1996). Together. these findings suggest that drug-induced DA release in the NAcc is both necessary and sufficient for opiate and psychostimulant drugs to induce relapse to drug-seeking behaviour (Self and Nestler. 1998).

*Cue-Induced Relapse*

Repeated drug exposure can result in the formation of powerful learned associations between the rewarding effects of drugs and specific environmental stimuli related to the drug-taking experience. These otherwise neutral environmental stimuli

acquire the ability to trigger both drug- and withdrawal-like responses in addicted subjects when subsequently presented in the absence of the drug (Wikler. 1973: Siegel. 1983: O'Brien et al.. 1992). Although the reports of cue-induced craving in humans are numerous. similar reports in animals are sparse (Self and Nestler. 1998). de Wit and Stewart (1981) showed that a tone that had been paired with drug infusions acquired the ability to induce reinstatement of drug-seeking. However. this effect was weak and was only present after the first test presentation of the tone.

The mechanism through which drug-associated stimuli can trigger drug-seeking behaviour has been hypothesized to be DA-dependent. In other words. drug-associated cues can induce relapse by activating the mesolimbic DA system (Stewart et al.. 1984: Robinson and Berridge. 1993: Wise. 1994). Some studies have reported enhanced DA release in the NAcc following presentation of drug-associated cues (Fontana et al.. 1993: Di Ciano et al.. 1995). providing evidence for the involvement of the DA system in cue-induced relapse.

The amygdala has previously been shown to play a role in the priming effects of drug-associated cues (Everitt et al.. 1999 for review). Meil and See (1997) recently reported that lesions of the basolateral nucleus of the amygdala attenuate the ability of cocaine-associated cues to induce relapse to cocaine-seeking behaviour. This finding indicates that the amygdala may be part of an important neural pathway through which drug-associated cues access and activate incentive motivational systems (Self and Nestler. 1998). which is consistent with the amydgala's role in conditioned emotional learning (McDonald and White. 1993 for review). Furthermore. the amygdala sends projections to VTA DA neurons (Gonzaless and Chesselet. 1990: Wallace et al.. 1992).

Stimulation of this pathway can excite VTA DA neurons. presumably leading to increased DA levels in the NAcc (Maeda and Mogenson. 1981). Thus. the amygdala represents a neural substrate through which drug-associated cues can activate the mesolimbic DA system to induce relapse to drug-seeking.

### Stress-Induded Relapse

Clinical reports indicate that relapse to drug use is more likely to occur in individuals exposed to high stress (Kosten et al.. 1986: Cooper et al.. 1992: McFall et al.. 1992: Brown et al.. 1995). Although these reports indicate that there is a relationship between exposure to stress and relapse. the exact nature of this relationship is not well understood. Furthermore. the correlational nature of these clinical studies does not allow for conclusions to be drawn about a causal link between stress and relapse to drugs (O'Doherty and Davis. 1987).

In recent years. support for these clinical findings has come from studies employing the reinstatement model previously discussed. Using this model. exposure to acute stress has been shown to be a powerful and reliable reinstator of drug-seeking behaviour in rats. Initial studies showed that exposure to 10-15 minutes of intermittent footshock reliably reinstates drug seeking behaviour after one to two weeks of extinction training in both heroin and cocaine-trained rats (Shaham and Stewart. 1995: Erb et al.. 1996). Erb et al. (1996) further showed that footshock stress reinstates drug seeking behaviour in extinguished animals after a prolonged drug-free period (4 – 6 weeks). These findings have been extended to other drugs of abuse such as nicotine (Buczek et al.. 1999) and alcohol (Lê et al.. 1998).

In all studies. footshock has been found to be at least as effective at inducing high levels of responding in tests for reinstatement as priming injections of the drug itself (Shaham et al.. 2000). This finding has led to the idea that stress contributes to relapse by activating neural systems in common with those activated by drugs of abuse. This idea is further supported by studies showing that stress and drugs of abuse do indeed activate some of the same neurochemical systems such as the mesolimbic DA system (Di Chiara and Imperato. 1988: Imperato et al.. 1992: Piazza and LeMoal. 1998). as well as the endogenous opioid system (Akil et al.. 1976: Terman et al.. 1984: Amit and Galina. 1986). These findings suggest that footshock stress may induce relapse by mimicking the effects of the drugs themselves. essentially acting as priming infusions. However. further experiments showed that manipulations that effectively blocked the effects of priming injections of heroin or cocaine. namely opioid and DA receptor antagonists. had no effect on stress-induced relapse (Shaham and Stewart. 1996). This finding suggests that although stress activates some of the same neurochemical systems involved in the rewarding efficacy of drugs of abuse. the ability of stress to induce relapse may be independent of its ability to activate these neurochemical systems.

Other neurochemical systems involved in the mediation of stress responses have been implicated in stress-induced relapse. For instance. the brain peptide corticotropin-releasing factor (CRF). which is involved in a variety of physiological and behavioural effects of stress (Dunn and Berridge. 1990). has been shown to reinstate heroin seeking behaviour following intracerebroventricular administration (Shaham et al.. 1997). Furthermore. a number of different CRF receptor antagonists have been shown to attenuate footshock-induced reinstatement in both cocaine and heroin trained rats

(Shaham et al., 1997; Erb et al., 1998; Shaham et al., 1998). It is interesting to note that

CRF receptor antagonists were shown to be relatively ineffective at blocking cocaine or

heroin-induced reinstatement (Shaham et al., 1997; Shaham et al., 1998). Given the

previous finding that opiate and DA antagonists block drug-induced but not footshock-

induced reinstatement, these findings suggest that the neurochemistry of stress-induced

relapse is not identical to that of drug-induced relapse.

The noradrenergic system is yet another neurochemical system involved in the

mediation of physiological responses to stress (Stanford, 1995; Bremner et al., 1996), and

has thus may play a role in stress-induced relapse. Clonidine, the *alpha-2* adrenoceptor

agonist, which reduces NE activity by acting on pre-synaptic *alpha-2* adrenoceptors, has

been shown to attenuate footshock-induced reinstatement of both cocaine and heroin

seeking (Erb et al., 2000; Shaham et al., 2000).

Taken together, these studies provide evidence to support the role of stress as a

factor in relapse to drug use. Clinical reports indicate that exposure to stress predisposes

individuals to relapse, even after long periods of abstinence. These reports are further

corroborated by animal studies which show that behavioural, as well as neurochemical

manipulations which induce stress, result in relapse to drug-seeking behaviour. Although

the exact nature of the relationship between stress and relapse is not well understood,

these findings point to stress as a major contributor of relapse.

## RATIONALE FOR EXPERIMENTS

SP has been shown to be rewarding (Staubli and Huston. 1985: Holzhauer-Oitzl and Huston, 1987), but to also play a significant role in the response to stress (Bannon et al., 1983: Kramer et al.. 1998). Both of these characteristics are implicated in drug relapse. As previously described, the mesolimbic DA system is also implicated in drug relapse, particularly during re-exposure to drugs (Stewart and Vezina, 1988: Shaham and Stewart, 1996). Thus, the interactions between SP and the mesolimbic DA system (Brownstein et al.. 1976: Kelley et al. 1979: Deutch et al.. 1985) provide further evidence for the potential role of SP in the mediation of relapse.

The involvement of SP in both reward and stress responses suggests that SP may induce relapse for one of two reasons: it may act as a drug primer. thereby mimicking the neurochemical effects of the self-administered drug, or it may act as a stressor by activating neurochemical stress response systems. Both of these events have been shown to trigger relapse. and they both involve the activation of the DA system. Given the extensive interactions between SP and the DA system, the aim of the following experiments was to examine the role of SP in drug relapse.

Three experiments were conducted to this end. The first experiment consisted of a locomotor study which was conducted in order to determine effective drug doses of the stable SP analogue DiMe-C7 (Eison et al.. 1982). The locomotor-activating effects of DiMe-C7 have previously been reported (Elliott et al.. 1990) and have been shown to be reliable and quite significant. Thus. the locomotor activating property of DiMe-C7 was used as a behavioural measure of drug efficacy. Three doses of DiMe-C7 were selected according to previously published findings. Injections of this drug were made into the

VTA where it stimulates DA neurons, thus resulting in increases in locomotor behaviour. The results from this experiment were used to select appropriate doses of DiMe-C7 that would be used for the second experiment.

In the second experiment, the reinstatement procedure previously discussed was used to investigate whether DiMe-C7 would reinstate drug-seeking behaviour in rats previously trained to self-administer cocaine. Following cocaine self-administration training and extinction, animals were injected with three doses of DiMe-C7 into the VTA. Reinstatement to cocaine-seeking following this manipulation was then examined.

The third experiment was conducted in order to investigate the specific neurokinin receptor subtype(s) in the VTA involved in SP-induced reinstatement of drug-seeking. Since SP has been shown to preferentially activate the NK-1 receptor, the selective NK-1 receptor antagonist, Spantide II (Maggi et al., 1991), was selected in an effort to examine the involvement of the NK-1 receptor subtype in SP-induced reinstatement of drug-seeking. In this experiment, animals underwent the reinstatement procedure as described above. During the reinstatement phase of the experiment, animals received an intra-VTA injection of Spantide II immediately prior to DiMe-C7 injection. DiMe-C7-induced reinstatement to cocaine-seeking following blockade of NK-1 receptors by Spantide II was then assessed.

## MATERIALS AND METHODS

### General methods

*Subjects*

Experimentally naïve male Wistar rats (Charles River. Montreal. Canada) weighing 300-350g were used in these experiments. Animals were individually housed in plexiglas cages in a temperature controlled room maintained on a 12hr light/dark cycle (lights off at 8:30am). Animals had free access to food and water. All testing was carried out in the dark phase of the cycle. Upon arrival. animals were given one week to acclimatize to the new housing conditions prior to any surgical or testing procedures. All procedures were carried out with due regard for the Animals for Research Act. the Guidelines of the Canadian Council on Animal Care. and relevant University of Toronto policy.

### Surgery

*Anesthesia and Analgesia*

Animals were anesthetized with sodium pentobarbital (65mg/kg: i.p.). immediately following an injection of 0.15ml of atropine sulfate (0.6 mg/ml: i.p.). Following surgery. each animal received a subcutaneous injection of buprenorphine hydrochloride (0.025mg/kg).

*Intravenous catheterization*

A chronic indwelling intravenous catheter was implanted into the external jugular vein. Each catheter was constructed from silastic tubing that was inserted into the right

jugular vein and anchored with a series of non-absorbable sutures. The portion of tubing exiting the jugular vein, constructed from polyethylene tubing and connected to a threaded guide cannula, was tunneled subcutaneously and secured between the skin and fascia via polypropylene mesh to the animal's back at the mid-scapular region. The opening of the guide cannula was sealed with a plastic cap made from microbore tubing, so as to prevent clogging. Catheters were flushed twice daily for the first 10-days post-surgery with a 0.15ml solution of saline containing heparin (30 units/ml) to inhibit blood clotting, streptokinase (700 units/ml) to dissolve fibrinogen, plasminogen, and thrombin clots, and penicillin G benzathine (250,000 units/ml) to treat and prevent infection. Following these first 10-days, catheters were flushed once daily with the same solution, but which lacks penicillin G. Animals that appeared to be infected, underwent rigorous flushing with the original post-surgical solution. Throughout the experiment, catheters were tested for patency on a regular basis. Animals which appeared to have clogged or leaking catheters were omitted from the study.

*Intracranial cannulation*

Animals were implanted with bilateral guide cannulae (22 gauge) aimed 2mm above the VTA. The flat skull coordinates for the VTA were -5.9mm posterior from bregma, + /-0.6mm from the midline, and -6.3mm ventral to the skull surface (Paxinos and Watson, 1982). Cannulae were secured to the skull with dental acrylic and jeweler's screws fixed to the skull. Dummy guides were placed into the cannulae extending 0.1mm below the tip in order to prevent blockage, as well as debris entering the brain.

## *Apparatus*

### *Locomotor activity boxes*

All locomotor activity testing was conducted with in-house constructed activity-monitor boxes (34 x 33cm). Boxes were constructed of metal sides, wire mesh floor, wire mesh front covered with cardboard, and plexiglas top and back. Locomotor activity was measured via two horizontal infrared emitters and corresponding detectors positioned 11cm apart and 3cm above the grid floor. Boxes were interfaced with a computer via in-house designed software which measured both total beam breaks and crossovers (defined as a consecutive front and back beam break).

### *Operant chambers*

Self-administration testing was conducted in ventilated and sound-attenuated operant chambers measuring 22cm$^3$ (Med Associates Inc., Georgia, Vt., USA). There were two retractable response levers (4.5cm wide) in each of the chambers, mounted 7cm above a steel rod floor. Stimulus lights were positioned 5cm above each lever and on the opposite wall, there was a dim houselight centered 2cm from the top of the chamber. For each chamber, a liquid infusion assembly was connected to an infusion pump located immediately outside of the chambers.

At the start of each self-administration session, the retractable levers were introduced, and the white light above the active lever was illuminated for 30sec., as well as the houselight, which remained on for the entire session. When the active lever was depressed, the Med PC software was programmed such that the infusion pump was activated, delivering a 100µl infusion. During the infusion, the light located above the active lever was illuminated for 20sec. During this 20sec. period, further responses of the

active lever were recorded, but did not lead to further infusions (time-out period).

Depression of the inactive lever did not at any time result in the activation of the pump.

*Drugs*

The SP analogue DiMe-C7 (pGlu-Gln-Phe-N-Methyl-Phe-Sar-Leu-Met-NH$_2$),

obtained from Sigma (Toronto, ON, Canada), was dissolved in acid saline (pH 6.0). The

SP antagonist Spantide II (H$_2$N-N-ε-Nicotinoyl-D-Lys-Pro-3-Pyridyl-Ala-Pro-di-Cl-D-

Phe-Asn-D-Trp-Phe-D-Trp-Leu-Nle-NH$_2$), obtained from Bachem (California, USA),

was dissolved in 2-hydroxypropyl-γ-cyclodextrin (45g/100ml). For the training phase of

the relapse experiments, cocaine hydrochloride (1mg/kg/infusion), obtained from BDH

Chemicals (Toronto, ON, Canada), was dissolved in sterile physiological saline (0.9%

NaCl) prior to each daily training session.

*Histology*

Upon completion of each experiment, animals were anesthetized and perfused

transcardially with saline (0.9%) and then formalin (10%). Brains were removed and

stored in a formalin and sucrose solution (25%) for at least 24 hours prior to sectioning.

Brains were then blocked and frozen to -17°C, and 40μm coronal sections were cut of the

VTA and any other tissue which contained cannula tracts. Sections were mounted on

gelatin-coated slides for cresyl violet staining, and then coverslipped. Verification of

cannula placements was done under a light microscope.

**Procedures**

*Experiment 1: Effects of intra-VTA microinjections of DiMe-C7 on locomotor activity*

In this experiment, the effects of intra-VTA injections of DiMe-C7 on locomotor

activity were examined in order to determine an appropriate range of DiMe-C7 doses for

Experiment 2.

Following 7 days of post-surgical recovery, animals ($n$ = 8) were habituated to the

locomotor boxes during three 1-hour daily sessions. On the third day of habituation,

animals received bilateral sham injections into the VTA immediately prior to the

habituation session. A 30 gauge injector was lowered 2mm below the tip of each cannula

guide and was then immediately removed. The dummy guides were then placed back into

each cannula.

During drug testing days, animals were habituated to the locomotor boxes for 1-

hour prior to receiving an injection. Animals were then removed from the locomotor

boxes following which they received a bilateral injection of DiMe-C7 (0, 1, 3, or 6μg/μl;

0.5μl/side) into the VTA. Drug was delivered over a 45sec. period with a microinjection

pump. The injectors remained in place for an additional 60sec to allow for further drug

diffusion. Animals were then placed back into the locomotor boxes for an additional 2-

hour period. Each animal received all drug doses in a counterbalanced order and each

injection was separated by 3 days.

***Experiment 2: Effects of intra-VTA microinjections of DiMe-C7 on reinstatement of cocaine-seeking***

In this experiment, the effects of intra-VTA injections of DiMe-C7 on

reinstatement of cocaine seeking in cocaine trained rats was studied using a paradigm

which consisted of three phases: self-administration training, extinction, and test for

reinstatement.

*Self-administration training*

Following 7 days of post-surgical recovery, animals ($n$ = 8) were trained to self-

administer cocaine (1mg/kg/infusion; i.v.) during daily 90-minute sessions for a period of

15 days on a fixed ratio schedule of reinforcement (FR1).

*Extinction*

During extinction, conditions remained the same as those in training except that

saline was substituted for cocaine, and each daily extinction session was 3-hours in

duration. These extinction conditions remained in place until the rats made 15 or fewer

responses on the active lever (saline infusions + timeout responses). Animals then

received daily bilateral sham injections whereby a 30 gauge injector was lowered 2mm

below the cannula tip and then immediately removed. Sham injections were administered

in order to habituate the animals to the injection procedure. These extinction conditions

were repeated until the animals once again reached a criterion level of responding of 15

or fewer responses on the active lever. Once animals reached this criterion level of

responding, the reinstatement procedure began. The extinction phase of the experiment

lasted on average 15 days.

*Test for reinstatement*

Reinstatement conditions were the same as those in extinction (saline substituted for cocaine). except that each session was 90-minutes in duration. During each reinstatement session. animals received a bilateral injection of DiMe-C7 (0. 0.2. 1 or 5μg/μl: 0.5μl/side) into the VTA in a counterbalanced order. Drug was delivered over a 45sec. period with a microinfusion pump. The injectors remained in place for an additional 60sec. Animals were then placed into the boxes and the self-administration session was commenced. Each animal received all drug doses in a counterbalanced order and each injection was separated by at least 3 days. Following each reinstatement session. animals were placed under the extinction conditions stated above on subsequent daily sessions. Extinction conditions were run for at least 3 consecutive days or until animals reached a criterion level of responding of 15 or fewer responses on the active lever.

## Experiment 3: Effects of intra-VTA microinjections of Spantide II on DiMe-C7-induced reinstatement of cocaine seeking

In this experiment. the effects of intra-VTA injections of Spantide II on DiMe-C7 induced reinstatement of cocaine seeking in cocaine trained rats ($n = 6$) was studied using the same procedures as Experiment 2.

*Self-administration training*

Training procedure was identical to that used in Experiment 2.

*Extinction*

Extinction procedure was identical to that used in Experiment 2.

*Test for reinstatement*

Reinstatement procedure was identical to that used in Experiment 2 except for the following. During each reinstatement session, animals received a bilateral injection of Spantide II (0 or 1µg/0.5µl; 0.25µl/side) into the VTA. Drug was delivered over a 23sec. period and injectors remained in place for an additional 60sec. Immediately following the injection, animals received a second injection of DiMe-C7 (0 or 1µg/0.5µl; 0.25µl/side) into the VTA. Drug was delivered over a 23sec. period and injectors remained in place for an additional 60sec. Following this injection, animals were placed into the boxes for a 90-minute reinstatement session. Each animals received each dose of Spantide II and DiMe-C7 in a counterbalanced order. All other reinstatement conditions remained identical to those in Experiment 2.

**RESULTS**

*Experiment 1: Effects of intra-VTA microinjections of DiMe-C7 on locomotor activity*

Figure 1 shows the location of injection sites in a representative section selected

from slices taken from animals in experiments 1, 2 and 3.

Figure 2 shows the mean locomotor activity scores (crossovers) during the entire

3 hour testing period. Intra-VTA DiMe-C7 (0, 1, 3, and 6μg/ul: 0.5μg/μl) injections

occurred 60 minutes post-habituation. All statistical analyses were performed on the post-

injection data. Intra-VTA infusions of DiMe-C7 significantly increased locomotor

activity levels at all drug doses tested. A repeated measures ANOVA revealed a

significant main effect of time, $F(23,161) = 5.98$, $p < 0.000$, as well as a significant dose

by time interaction, $F(69,483) = 2.53$, $p < 0.000$. The main effect of dose was not

significant. Post hoc analysis using Fisher's Least Significant Difference (LSD) multiple

comparisons showed that locomotor activity was significantly greater at the two

intermediate doses of DiMe-C7 (1μg and 3μg) as compared to vehicle from 20 minutes

post-injection to 55 minutes post-injection. The analysis also showed that locomotor

activity was significantly greater at the highest dose of DiMe-C7 (6μg) as compared to

vehicle from 30 minutes post-injection to 80 minutes post-injection.

*Figure 1.* Location of injection sites in a representative section selected from slices

taken from animals in experiments 1. 2 and 3 represented by dark circles. Section based

on the atlas of Paxinos and Watson (1982) representing -6.3 mm from bregma.

VTA
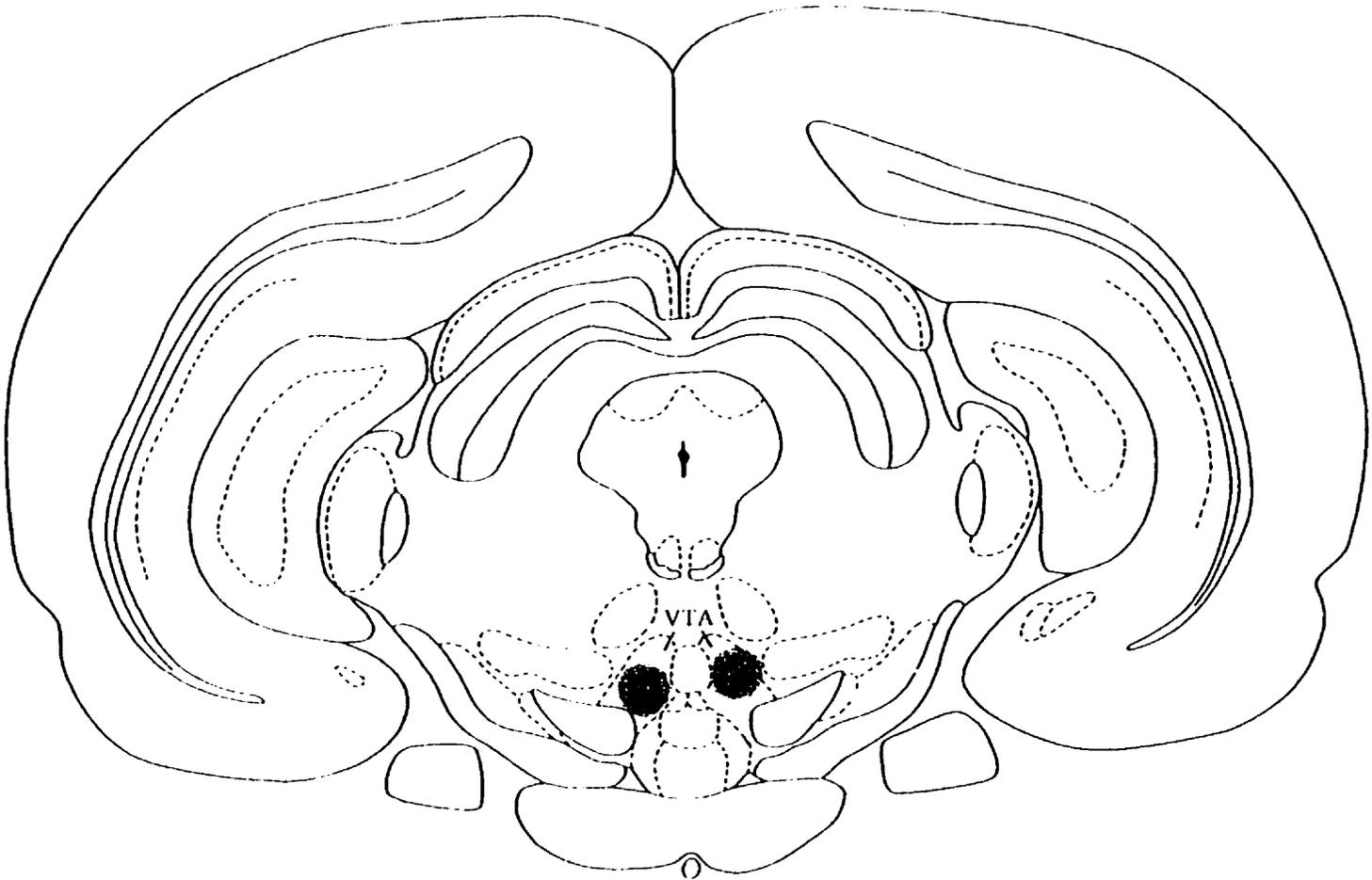
*Figure 2.* The mean (± S.E.M.) locomotor activity scores (crossovers) summed over 5

minute intervals. for the entire 3 hour testing period. in animals receiving intra-VTA

injections of DiMe-C7 (0. 1. 3. and 6μg/ul: 0.5μg/μl). Injections occurred 60 minutes

post- habituation with injection being depicted by an arrow. (* denotes a significant
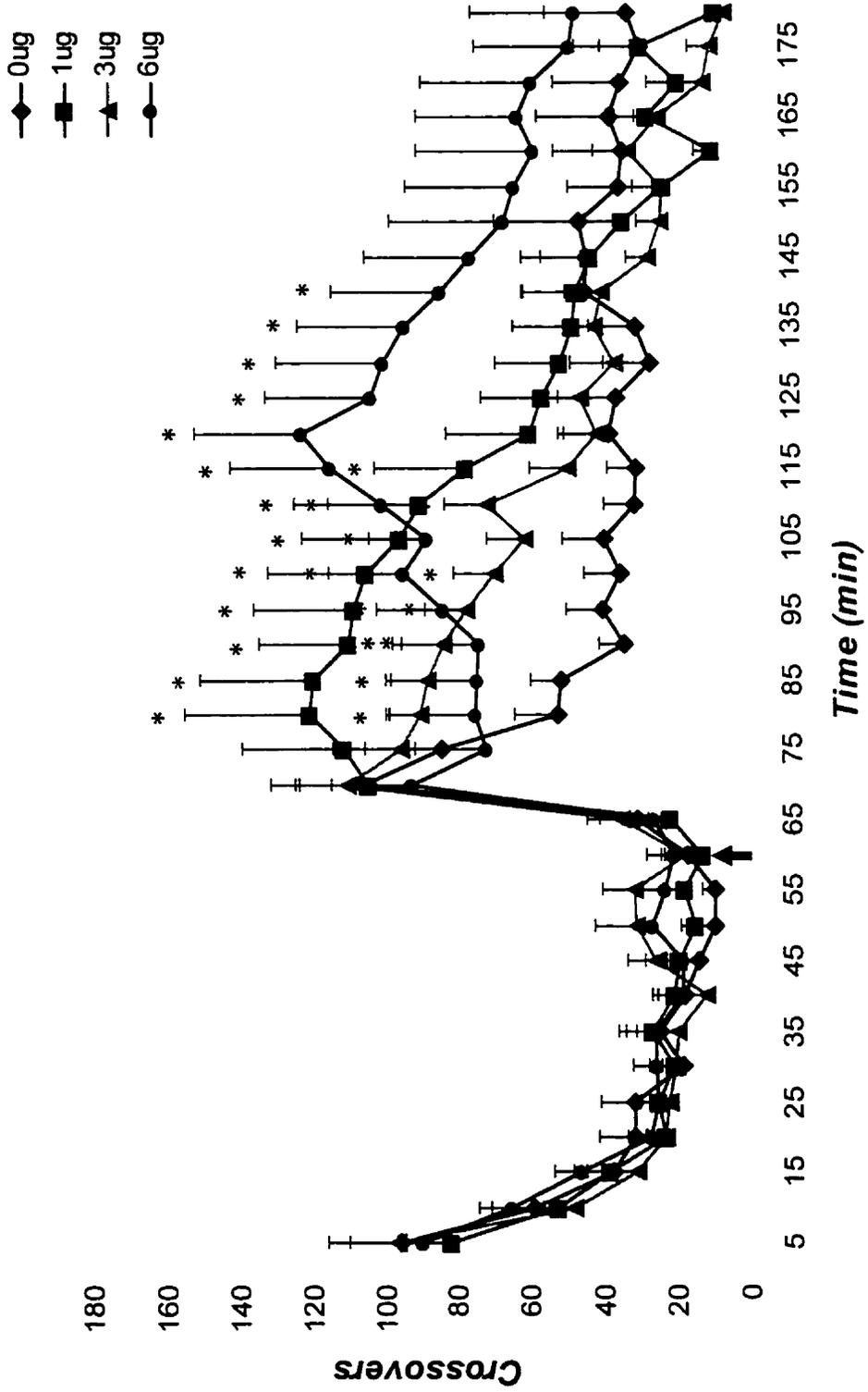
difference from vehicle condition. $p < 0.05$)

*Figure:* Crossovers versus Time (min), with curves for 0ug (♦), 1ug (■), 3ug (▲), and 6ug (●).

***Experiment 2: Effects of intra-VTA microinjections of DiMe-C7 on reinstatement of cocaine-seeking***

Figure 3 shows the mean number of responses on the active and inactive levers following intra-VTA injections of DiMe-C7 (0, 0.2, 1 and 5μg/μl: 0.5μl/side). Intra-VTA infusions of DiMe-C7 significantly increased responding on the active lever in a dose-dependent manner. A repeated measures ANOVA revealed significant main effects of dose. $F(3,21) = 4.43$, $p < 0.05$. lever. $F(1.7) = 12.73$, $p < 0.01$. as well as a significant dose by lever interaction. $F(3.21) = 4.86$, $p < 0.05$. Post hoc analysis using Tukey's Honestly Significant (HSD) multiple comparison procedure showed that there was significantly greater responding on the active lever following injections of the highest dose of DiMe-C7 (5μg) as compared to vehicle ($p < 0.0005$), as well as the lowest dose of DiMe-C7 (0.2μg) ($p < 0.05$). The analysis also showed that there was significantly greater responding on the active lever as compared to the inactive lever at the highest dose of DiMe-C7 (5μg) ($p < 0.001$).

*Figure 3.* The mean (± S.E.M.) number of responses on the active and inactive levers in animals receiving intra-VTA injections of DiMe-C7 (0. 0.2. 1 and 5µg/µl: 0.5µl/side). (* denotes a significant difference for vehicle condition. $p < 0.05$: (** denotes a significant difference from vehicle condition and from inactive lever for that dose. $p < 0.001$)
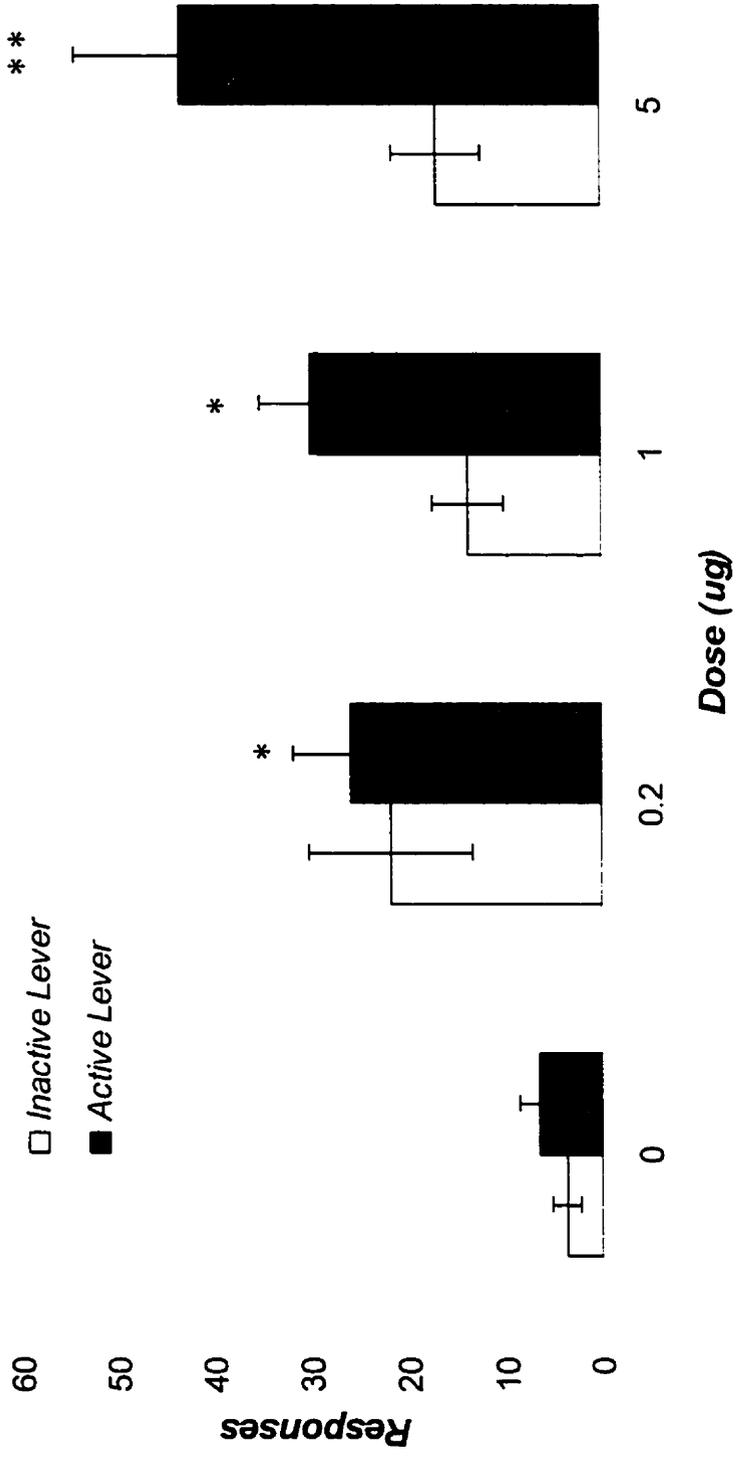
***Experiment 3: Effects of intra-VTA microinjections of Spantide II on DiMe-C7-induced reinstatement of cocaine seeking***

Figure 4 shows the mean number of responses on the active lever following intra-VTA injections of Spantide II (0 and 1μg/0.5μl: 0.25μl/side) immediately followed by an injection of DiMe-C7 (0 and 1μg/0.5μl: 0.25μl/side). A repeated measures ANOVA revealed a significant main effect of DiMe-C7. $F(1.5) = 25.7$. $p < 0.005$. indicating that DiMe-C7(1μg) co-administered with Spantide II (0 or 1μg ) significantly increased responding on the active lever as compared to vehicle. The main effect of Spantide II and the Spantide II by DiMe-C7 interaction were not significant.

*Figure 4.* The mean (± S.E.M) number of responses on the active lever in animals

receiving intra-VTA injections of Spantide II (0 or 1μg/0.5μl: 0.25μl/side) + DiMe-C7 (0

or 1μg/0.5μl: 0.25μl/side).

DiMe-C7 (ug)

Responses on Active Lever

Spantide II (0ug)
Spantide II (1ug)
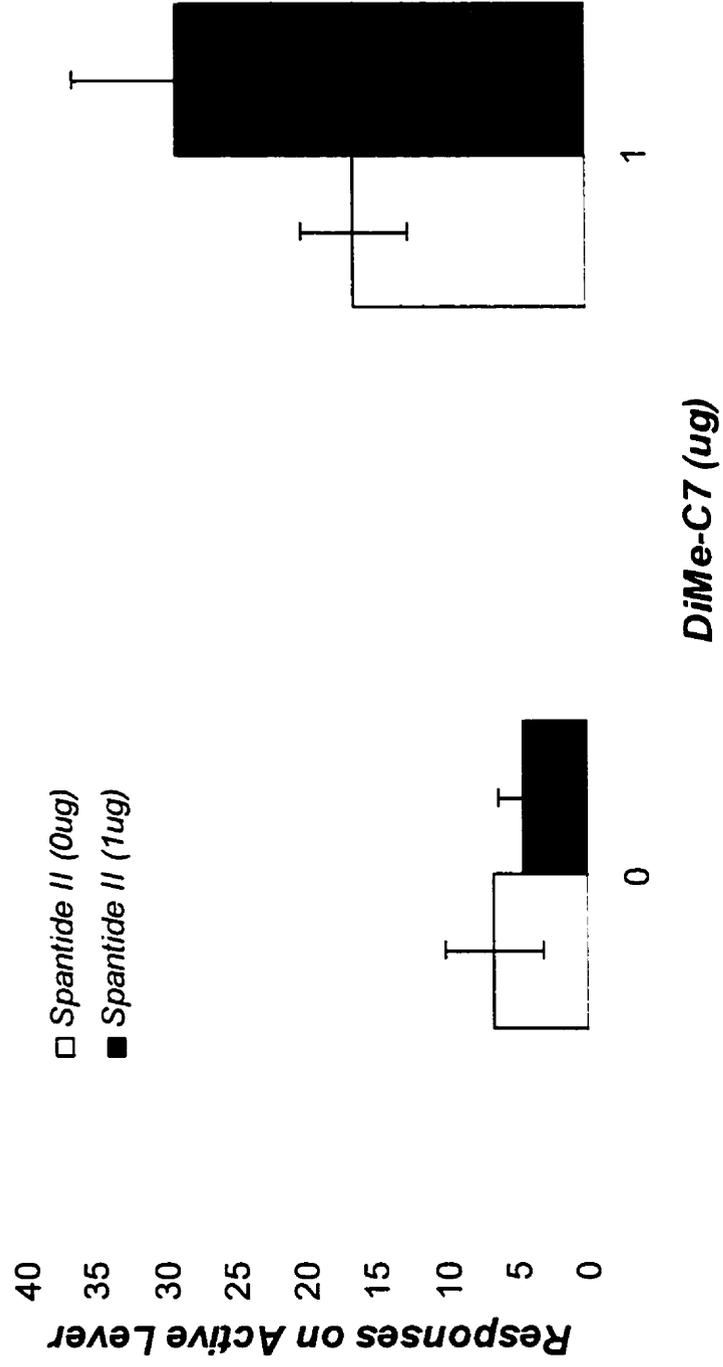
## DISCUSSION

The aim of the present study was to examine the role of the neuropeptide SP in relapse to cocaine-seeking behaviour in rats. Experiment 1 examined the locomotor-stimulating properties of a range of doses of the SP analogue. DiMe-C7. microinjected into the VTA. Experiment 2 then examined the ability of intra-VTA administration of DiMe-C7 to reinstate drug-seeking behaviour in animals previously trained to self-administer cocaine. Finally. in order to investigate the specific neurokinin receptor subtype in the VTA involved in DiMe-C7-induced reinstatement. experiment 3 examined whether the selective NK-1 receptor antagonist. Spantide II. would block DiMe-C7-induced reinstatement of cocaine-seeking.

The first experiment in this study showed that injections of DiMe-C7 into the VTA significantly increased locomotor activity at all doses tested. This finding is consistent with studies demonstrating the locomotor-activating effects of SP and its various agonists (Kelley et al.. 1979: Eison et al.. 1982: Barnes et al.. 1990: Elliott et al.. 1990). Given that injections of DiMe-C7 into the VTA have been shown to increase the release of DA and its metabolites from the NAcc (Elliott et al.. 1986: Barnes et al.. 1990: Boix et al.. 1992: Huston et al.. 1993). the locomotor-activating effects of DiMe-C7 are thought to be DA-dependent. This notion is further supported by a study showing that pre-treatment with the DA receptor antagonist. haloperidol. blocked the locomotor-activating effects of intra-VTA administered DiMe-C7 (Eison et al.. 1982).

The most interesting finding from this study. however. was demonstrated in experiment 2 which showed that DiMe-C7 injected into the VTA induces reinstatement

to cocaine-seeking. This novel finding is consistent with the ability of DiMe-C7 to stimulate locomotor activity. Given that DiMe-C7-induced locomotor activation is likely dependent upon stimulation of DA neurons in the VTA. it suggests that DiMe-C7-induced reinstatement may also depend upon the activation of the DA system. A number of studies have shown that DA is critically involved in drug relapse. particularly drug-induced relapse (Stewart and Vezina. 1988: Wise et al.. 1990: de Wit and Stewart. 1993: Self et al.. 1996). Thus. it seems plausible that DiMe-C7 may induce relapse by mimicking the neurochemical effects of the self-administered drug. thereby acting in much the same way as a priming injection of a DA agonist would. for instance.

Another mechanism by which DiMe-C7 could possibly induce reinstatement may also depend upon activation of DA systems. One of the initial reasons for conducting this study was to further understand the SP system. with particular emphasis on understanding its role in stress responses. Although this issue is not specifically addressed in the present set of experiments. it is possible that DiMe-C7 induces reinstatement via its stress-inducing effects. Much of the evidence for the involvement of SP in stress responses comes from studies which show extensive interactions between SP and the mesocorticolimbic DA system. The mesocorticolimbic DA system is one of the major neurochemical systems involved in the response to stress. Acute stress induced by restraint or mild footshock have been reported to increase the turnover of DA in the frontal cortex and the NAcc (Thierry et al.. 1976: Bannon and Roth. 1983). Studies have shown that stress-induced activation of these DA neurons may be mediated by the SP system. For instance. injection of a SP antibody directly into the VTA has been shown to prevent footshock-induced activation of mesocortical DA neurons (Bannon et al.. 1983).

which are preferentially activated during exposure to acute stressors (Thierry et al.. 1976:
Bannon and Roth. 1983). Furthermore. injections of DiMe-C7 into the VTA produces a
remarkably selective increase in levels of DA metabolites in the PFC and the NAcc.
thereby resembling that seen after exposure to mild stress (Elliott et al.. 1986). Thus.
there is very good reason to believe that SP may be involved. or perhaps may mediate.
stress-induced DA release.

A number of studies have shown that footshock stress is a powerful and reliable
reinstator of drug-seeking behaviour in rats (Shaham and Stewart. 1995: Erb et al.. 1996:
Shaham et al.. 1997: Erb et al.. 1998: Shaham et al.. 1998). The involvement of the SP
system in DA-mediated responses to stress. may suggest that perhaps DiMe-C7 induces
reinstatement not by acting as a drug primer. but rather by acting as a stressor. Given the
involvement of DA in both drug-priming effects and SP-related stress responses. it is not
possible to determine the mechanism by which DiMe-C7 induces reinstatement. This
problem further uncovers some of the issues yet to be understood about relapse. For
instance. it is not quite clear whether stress induces relapse by acting as a primer itself.
This would suggest that stress-induced and drug-induced relapse are mediated via the
same mechanisms. However. there is some evidence to suggest that this is not the case.
Shaham and Stewart (1996) found that opioid and DA receptor antagonists. which
effectively blocked the effects of priming injections of heroin and cocaine. had no effect
on footshock-induced relapse. This study suggests that neurochemical systems other than
DA are involved in stress-induced relapse.

SP may be one of those neurochemical systems. particularly since it is not clear
whether its involvement in stress responses is exclusively DA-mediated. Injections of SP

into certain brain sites have been shown to produce aversive effects. For instance. injections of SP into the bed nucleus of the stria terminalis (BNST). the basolateral nucleus of the amygdala. the dorsal periaqueductal gray. and the lateral septal nucleus. all have been shown to produce anxiogenic effects on the elevated plus-maze (Aguiar and Brandao.1996: de Lima and Ribeiro. 1996: Teixeira et al.. 1996: Gavioli et al.. 1999). Furthermore. intraventricular injections of SP also tend to produce aversive effects as demonstrated in a place conditioning paradigm and on the elevated plus-maze (Elliott. 1988: Gavioli et al.. 1999). Thus. in order to further examine the mechanisms by which DiMe-C7 induces reinstatement. injections of DiMe-C7 into some of these brain sites would allow us to further examine whether SP may play a role in stress-induced relapse.

However. given that in the present study DiMe-C7 was injected into the VTA. it is very likely that it induced reinstatement exclusively via DA-dependent mechanisms. Intra-VTA administration of DiMe-C7 has clearly been shown to activate mesolimbic DA neurons (Elliott et al.. 1986: Barnes et al.. 1990: Boix et al.. 1992: Huston et al.. 1993). and thus the most plausible explanation for the resultant reinstatement effect is that DiMe-C7 acted in essence like a drug-primer. A priming infusion of heroin or cocaine induces reinstatement by activation of mesolimbic DA neurons. Activation of these neurons is absolutely critical for opiate and psychostimulant drugs to induce relapse to drug-seeking behaviour (Self and Nestler. 1998). Thus. intra-VTA DiMe-C7 may act in a similar manner to induce reinstatement.

As previously mentioned. the mesolimbic DA system does not appear to mediate stress-induced relapse. DA and opiate receptor antagonists have been shown to be ineffective at blocking footshock-induced reinstatement (Shaham and Stewart. 1996). It

appears that CRF may be involved in the mediation of stress-induced relapse. A number of different CRF receptor antagonists have been shown to attenuate footshock-induced reinstatement (Shaham et al.. 1997: Erb et al.. 1998: Shaham et al.. 1998). However. it is also evident that CRF may not be the only neurochemical system involved in stress-induced relapse. Blockade of noradrenergic activity has also been shown to attenuate footshock-induced reinstatement (Erb et al.. 2000; Shaham et al.. 2000). Thus. it is plausible that the SP system may be yet another neurochemical system involved in the mediation of stress-induced relapse. Blockade of footshock-induced reinstatement by a SP receptor antagonist. for instance. would provide evidence for this idea. It would also demonstrate that the DiMe-C7-induced reinstatement shown in Experiment 2 of this study. may not be an exclusively DA-mediated phenomenon.

The final goal of the present study was to examine the specific neurokinin receptor subtype in the VTA involved in DiMe-C7-induced reinstatement of drug-seeking. Experiment 3 showed that intra-VTA injections of the NK-1 receptor antagonist. Spantide II. did not block or attenuate DiMe-C7-induced reinstatement of cocaine-seeking. This finding may suggest that SP-induced relapse is not mediated via NK-1 receptors in the VTA. Although this is certainly one possibility. there are a number of other possible explanations for this lack of effect. Perhaps the most likely explanation is that DiMe-C7 is not an NK-1 selective agonist. DiMe-C7 was selected as a SP agonist for use in these experiments on the basis of its demonstrated metabolic stability and prolonged central actions when compared to exogenously administered SP (Eison et al.. 1982). However. DiMe-C7 and other C-terminal SP analogues have also been reported to display a greater affinity for the NK-3 receptor than for the NK-1 receptor and show a

higher NK-3/NK-1 receptor affinity ratio (Stoessl et al.. 1991; Regoli et al.. 1994 for

review). DiMe-C7-induced reinstatement to drug-seeking is thus quite possibly NK-3

receptor mediated. Autoradiographic studies have shown that there are specific NK-3

binding sites in the VTA (Dam et al.. 1990; Stoessl and Hill. 1990). Furthermore. NK-3

selective agonists injected into the VTA have been shown to increase locomotor

behaviour in much the same way as SP or other NK-1 agonists (Elliott et al.. 1986:

Stoessl et al.. 1991; Overton et al.. 1992). These findings support the hypothesis that

DiMe-C7-induced locomotor activation and reinstatement to drug-seeking might be NK-

3 receptor mediated.

Spantide II has been shown to be a fairly selective antagonist for the NK-1

receptor subtype. Furthermore. it has been shown that its actions are unlikely to involve

blockade of NK-3 receptors (Maggi et al.. 1991). Therefore. the inability of Spantide II to

block DiMe-C7-induced reinstatement is not surprising. If DiMe-C7 preferentially

activates the NK-3 receptor subtype. blockade of NK-1 receptors by Spantide II would

not be expected to block DiMe-C7-induced reinstatement. However. the present

experiment cannot confirm this explanation. Given that there was no independent

measure of the efficacy of the Spantide II at the dose used in this experiment. it is not

possible to determine whether in fact the administered drug was active. The dose of 1μg

was selected based on a study which showed that at this dose. intra-nigral injections of

Spantide II co-administered with SP. was able to block SP-induced increases in striatal

DA levels (Reid et al.. 1990). This study showed that not only was Spantide II able to

block SP-induced striatal DA release. it also showed that it selectively antagonized the

effects of the NK-1 natural ligand. SP. and not neurokinin A. which is the natural ligand

for the NK-2 receptor subtype. Nonetheless. the possibility still remains that in the

present study. Spantide II at the selected dose. was not active and therefore was not able

to block DiMe-C7-induced reinstatement. A dose-response study would be necessary to

test this hypothesis.

The potential involvement of the NK-3 receptor subtype in drug relapse is of

particular interest given the strong interaction between the NK-3 receptor and

mesencephalic DA neurons. As previously mentioned. there are specific NK-3 binding

sites in the VTA (Dam et al.. 1990: Stoessl and Hill. 1990). Behavioural studies have

shown that NK-3 selective agonists injected into the VTA increase locomotor activity

(Elliott et al.. 1986: Stoessl et al.. 1991: Overton et al.. 1992). Furthermore. an in vivo

microdialysis study has shown that the selective NK-3 agonist. senktide. injected into the

SN or in the VTA. increased the extracellular DA content in target areas such as the

striatum. NAcc. and PFC (Marco et al.. 1988). Thus.the picture emerging from these and

other studies is that NK-3 receptor activation closely resembles that of NK-1 receptor

activation. As with the NK-1 receptor. NK-3 binding sites are localized in areas of the

brain reward circuitry including the olfactory tubercle. diagonal band of Broca. prefrontal

and frontal cortex. medial nucleus of septum. VTA. amygdala. BNST. hippocampus.

lateral hypothalamus. and ventral pallidum (Massi et al.. 2000 for review). A study by

Ciccocioppo et al. (1998) has also shown that intraventricular injections of a selective

NK-3 receptor agonist induced pronounced CPP in rats. suggesting that NK-3 receptor

stimulation may have rewarding properties. These findings suggest that the NK-3

receptor may also play a role in the control of reward processes associated with drugs of

abuse. Although there is limited published data on the psychopharmacology and

behavioural properties mediated by NK-3 receptors. the present study may provide further evidence for the involvement of the NK-3 receptor in drug relapse.

The NK-1 receptor was hypothesized to potentially be involved in stress-induced relapse. given its involvement in stress responses. However. to date. there is not much evidence to implicate the NK-3 receptor in similar responses to stress. Although there is some evidence to suggest that NK-3 receptor stimulation may actually have an anxiolytic and antidepressant profile (Ribeiro and De Lima. 1998: Ribeiro et al.. 1999: Panocka et al.. 2000). which is the opposite of that associated with NK-1 receptor stimulation. Given that SP itself has been shown to be both anxiogenic and anxiolytic depending upon the dose and route of administration (Aguiar and Brandao. 1996: Hasenohrl et al.. 1990: Hasenohrl et al.. 1998). it is possible that this apparent disparity can be accounted for by the neurokinin receptor subtype that SP is activating. Perhaps at certain dose ranges. SP preferentially activates one receptor subtype over the other. for instance.

In conclusion. the present study has shown that the SP analogue. DiMe-C7. injected into the VTA produces locomotor activation and induces reinstatement of drug-seeking behaviour in rats previously trained to self-administer cocaine. Furthermore. it was shown that the selective NK-1 receptor antagonist. Spantide II. injected into the VTA. did not block DiMe-C7-induced reinstatement of cocaine-seeking. These novel findings provide evidence for the role of SP and neurokinin receptors. in drug relapse. Further studies should focus on the mechanisms and brain regions through which SP mediates these effects. and possible interactions with neurochemical responses to stress.

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