Striatal mechanisms of psychomotor stimulant reward, locomotor activation and aversion

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September 2006

A thesis submitted to McGill University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Your file Votre référence
ISBN: 978-0-494-27837-6
Our file Notre référence
ISBN: 978-0-494-27837-6

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ABSTRACT

Convergent evidence suggests that psychomotor stimulant drugs, such as amphetamine and cocaine, exert their rewarding and locomotor stimulant effects via increased dopamine (DA) transmission in the ventral striatum in rats. Additionally, recent evidence suggests that DA transmission importantly mediates the aversive effects of nicotine. The ventral striatum is not, however, a homogenous structure. Rather, anatomical and behavioural evidence suggests the presence of several discrete subregions. The most prominent of these are the core and medial shell regions of the nucleus accumbens, and the medial olfactory tubercle (OT). The objective of this thesis was therefore to examine the contribution of DAergic transmission in core, medial shell, and medial OT, to psychostimulant reward, locomotor stimulation and nicotine aversion.

The first experimental chapter examined the effects of 6-hydroxydopamine lesions of core vs. medial shell on amphetamine-stimulated locomotion and conditioned place preference (CPP). Core, but not medial shell lesions attenuated locomotor stimulation. Conversely, medial shell but not core lesions reduced CPP magnitude. Medial shell lesions likely affected reward processing rather than memory, since similar lesions failed to reduce morphine CPP. Taken together, these findings suggested a segregation of reward and locomotor stimulation, but left open the possibility that conditioned and unconditioned effects of amphetamine were the critical factor. Accordingly, in Chapter 4, amphetamine-conditioned activity was abolished by core, but not medial shell lesions,

suggesting that locomotor stimulation and reward processing, rather than conditioned vs. unconditioned drug effects, are segregated within the accumbens.

Chapters 5-7 examined whether the apparent separation of CPP and locomotor stimulation generalized to the psychostimulants cocaine and methylphenidate, and to nicotine. Locomotion stimulated by cocaine or methylphenidate was reduced by core lesions. Lesion effects on CPP, however, were more complex. Medial shell lesions reduced both i.v. cocaine and nicotine CPP, without affecting CPP for i.p. cocaine or methylphenidate. Lesions of the medial OT effectively reduced both i.v. cocaine and methyphenidate CPP. Unexpectedly, core lesions *increased* i.v. nicotine CPP and tended to do the same to i.v. cocaine CPP. In the case of nicotine, this appeared to result from reduced nicotine aversion, since core lesions abolished nicotine-conditioned taste aversion.

This work highlights the complex array of factors contributing to the behavioural sequelae of psychostimulant administration. Both the drug and route of administration appear to be important determinants of neurochemical and anatomical reward substrates. Such complexities must be considered in the search for more effective aids to reduce drug taking.

RÉSUMÉ

Plusieurs recherches suggèrent que les effets récompensant et locomoteur des drogues psychostimulantes comme l'amphétamine et la cocaïne favorisent la transmission de dopamine au striatum ventral des rats. Nous savons également que cette transmission de dopamine gère les effets opposés de la nicotine (les effets de répulsion à la nicotine). Toutefois, le striatum ventral n'est pas une structure homogène. En effet, l'analyse biologique (anatomique) et behavioriste montre qu'il est composé de plusieurs sous-régions. Les plus connues sont les parties core et shell médiale du noyau accumbens, et la partie médiale de l'olfactory tubercle. Restait à savoir comment la transmission de dopamine à la partie core, à la partie shell et à l'olfactory tubercle médial influençait les effets récompensants, locomoteurs et répulsifs des drogues psychostimulantes, et comment aussi elle influençait l'aversion à la nicotine. C'étaient là les objectifs de cette thèse.

Le premier étude est consacré aux lésions (employant 6-hydroxydopamine) des parties core et shell médiale du noyau accumbens. Comment ces lésions affectent-elles, chez le rat, les réponses locomotrices à l'amphétamine et la préférence d'un lieu (conditionnée par des drogues)? Des lésions de la partie core ont réduit l'effet locomoteur de l'amphétamine. Au contraire, des lésions de la partie shell médiale ont réduit la préférence. Il est peu probable que les lésions de la partie shell médiale aient affecté la mémoire parce que ces lésions n'ont pas réduit la préférence du lieu conditionnée par la morphine.

Ces résultats suggèrent un écart entre les effets récompensants et la stimulation locomotrice, mais laissent penser que les effets conditionnés et non conditionnés par l'amphétamine étaient un facteur primordial. Au quatrième chapître, les effets locomoteurs conditionnés par l'amphétamine étaient réduits par des lésions de la partie core, mais non par des lésions de la partie shell médiale. Nous en concluons que les stimulations locomotrices et les effets récompensants, plutôt que le conditionnement par les drogues, sont distincts au sein même de l'accumbens.

Dans les chapitres 5, 6 et 7, on a voulu savoir si l'écart entre la préférence de lieu conditionnée et l'effet locomoteur étaient pouvait être généralisée aux drogues stimulantes, telles la cocaïne, la méthylphenidate et la nicotine. L'effet locomoteur de la cocaïne ou de la méthylphénidate était réduit par les lésions de la partie core. Cependant, les effets des lésions sur la préférence de lieu conditionnée étaient plus complexes. Des lésions de la partie shell médiale ont réduit la préférence de lieu conditionnée pour la cocaïne (i.v.) et la nicotine, mais étaient sans effet pour la préférence de lieu conditionnée par la cocaïne (i.p.) ou la méthylphénidate. Des lésions de l' olfactory tubercle médiale ont réduit la préférence de lieu conditionnée par la cocaine (i.v.) et par la méthylphénidate. Contre toute attente, les lésions de la partie core ont augmenté la préférence de lieu conditionnée par la nicotine. Le même phénomène s'observe dans le cas de la préférence de lieu conditionnée par la cocaïne (i.v.). Quant à la nicotine, il semble que cela est dû à la réduction des effets répulsifs à la nicotine, car des lésions de la partie core rendent nulle la répulsion au goût conditionné par la nicotine.

Ces expériences mettent en lumière l'ensemble des facteurs qui influencent le comportement d'un corps sous l'effet de drogues psychostimulantes. Il apparaît que la drogue et le moyen d'administration sont deux facteurs importants quant aux substrats neurochimique et anatomique. Il faut tenir compte de tout cela dans l'élaboration de thérapies plus efficaces dans le traitement de la dépendance.

Ces expériences mettent en évidence les éléments complexes qui contribuent aux effets comportementaux occasionnés par l'administration de drouges stimulantes. Il apparaît que la drogue et le moyen d'administration sont deux facteurs importants quant aux contributions aux substrates neurochemique et anatomique. Il faut tenir compte de ces tout cela dans l'élaboration de thérapies plus efficaces dans le traitement de la dépendence.

ACKNOWLEDGEMENTS

Firstly, I would like to express my gratitude to my thesis supervisor Dr. Paul Clarke. His support, guidance, and his mentorship during the difficult moments of my thesis have been most appreciated. I am most thankful for his encouragement of independent thought and investigation.

Thank you to Brian Collier, my advisor, for telling me what I needed to hear, which did not usually coincide with what I expected to hear. Your guidance has been invaluable.

I would like to thank my colleagues, past and present – Golriz Baharnouri, Robert Biskin, Travis Chalmers, Diala Chehayeb, Annie Constantin, Lindsey McQuade, Rebecca Grant, Navneet Kaur and Amynah Pradhan, for their support and their company. I have learned much from these people.

To my mother, Heather Sellings and my grandparents, Edward and Phyllis Gelhorn – thank you for instilling me with the base to discern between right and wrong through critical thinking and analysis, and for instilling my desire to learn.

To Dr. Elissavet Kardami, thank you for introducing me to research.

I wish to thank the Natural Sciences and Engineering Research Council for four years of financial support, and to the Canadian Institute of Health Research, the Canadian

Tobacco Control Research Initiative and the Natural Sciences and Engineering Research Council for research funding. The studies presented in this thesis would not have happened without such support.

Thank-you to the support staff of the Department of Pharmacology, and the Animal Resource Centre for years of hard work that have helped me successfully complete my thesis work.

And finally, a sincere thank-you to my husband, Frédéric Boutin, for helping me to realize I have the strength to handle pretty much any challenge that comes my way.

CONTRIBUTION OF AUTHORS

This thesis is written in manuscript format, and is comprised of five manuscripts. Author contributions are listed below.

Chapter 3: Sellings LHL and Clarke PBS (2003) Segregation of amphetamine reward and locomotor activation between nucleus accumbens medial shell and core. *J Neurosci* **23**: 6295-6303.

All experimental work for this manuscript was performed by Laurie Sellings. The required techniques include stereotaxic infusion of 6-hydroxydopamine, behavioural testing for locomotor activity and conditioned place preference, cryosectioning, staining for Nissl substance using cresyl violet, autoradiography for the dopamine and serotonin transporters using the radioligand [^{125}I]RTI-55, quantitative analysis of autoradiographic film using the MCID 4 imaging system, and statistical analyses.

The manuscript was written and revised by Laurie Sellings and Paul Clarke.

Chapter 4: Sellings LHL and Clarke PBS (2006) 6-hydroxydopamine lesions of nucleus accumbens core, but not medial shell, attenuate amphetamine-induced conditioned activity. *Synapse,* **59:** 374-377.

Again, Laurie Sellings performed all experimental work. Techniques include those in Chapter 1, with the omission of conditioned place preference and the addition of conditioned locomotor testing.

The manuscript was written by Laurie Sellings and revised with Paul Clarke.

Chapter 5: Sellings LHL, McQuade LE, Clarke PBS (2006) Evidence for multiple sites within rat ventral striatum mediating cocaine conditioned place preference and locomotor activation. *J Pharmacol Exp Ther,* **317: 1178-1187**

Laurie Sellings performed the majority of the experimental work for this manuscript. Techniques are identical to those listed in Chapters 1 and 3, with the addition of catheterization of the jugular vein, and 6-OHDA lesions of the medial olfactory tubercle.

The manuscript was written by Laurie Sellings, and revised with Paul Clarke.

Lindsey McQuade performed the locomotor and conditioned place preference testing on one of the five experiments presented, as well as in preliminary work setting up the cocaine place preference paradigm.

Chapter 6: Sellings LHL, McQuade LE and Clarke PBS (2006) Characterization of dopamine-dependent rewarding and locomotor stimulant effects of intravenously-administered methylphenidate in rats. *Neuroscience,* **141: 1457-1468.**

Laurie Sellings performed the majority of the experimental work for this manuscript. Techniques in addition to those listed in Chapter 4 include dose-response analysis of cis-flupenthixol administration on locomotion, and both the acquisition and expression of conditioned place preference.

The manuscript was written by Laurie Sellings, and revised with Paul Clarke.

Lindsey McQuade performed the locomotor and conditioned place preference testing on two of the four experiments presented.

Chapter 7: Sellings LHL, McQuade LE and Clarke PBS (2006) Rewarding and aversive effects of nicotine are segregated within the nucleus accumbens. *Submitted to the Journal of Neuroscience, April 2006.*

Laurie Sellings performed the majority of the experimental work for this manuscript. Techniques are identical to those listed in Chapters 2, 4, and 5, in addition to conditioned taste aversion testing.

The manuscript was written by Laurie Sellings and revised in conjunction with Paul Clarke.

Lindsey McQuade performed the behavioural testing for the subcutaneous nicotine CPP experiment and the intravenous nicotine CPP dose-response experiment.

CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

The following list summarizes original work completed in this thesis:

Chapter 3:

- 6-hydroxydopamine lesions of the nucleus accumbens core, but not medial shell, reduce the locomotor stimulant effect of intraperitoneal amphetamine
- Conversely, 6-hydroxydopamine lesions of medial shell, but not core, given prior to conditioning, reduce conditioned place preference for amphetamine
- Medial shell, but not core, 6-hydroxydopamine lesions also reduce expression of amphetamine conditioned place preference
- Rats sustaining 6-hydroxydopamine lesions of medial shell do not differ from sham-operated rats for morphine conditioned place preference

Chapter 4:

- 6-hydroxydopamine lesions of the accumbens core given before training abolish amphetamine-conditioned locomotion in the absence of a complete block of the unconditioned locomotor stimulant effect
- Medial shell lesions using 6-hydroxydopamine have no effect on conditioned locomotion when given before training

Chapter 5:

- Core 6-hydroxydopamine lesions attenuate the locomotor stimulant effect of both intraperitoneal (5-20 mg/kg) and intravenous (0.5-1.5 mg/kg) cocaine over a wide dose range

- Medial shell lesions reduce intravenous cocaine CPP (0.5 mg/kg), but do not reduce intraperitoneal cocaine CPP (10 mg/kg)
- Both lesions of medial olfactory tubercle and medial shell reduce cocaine CPP
- When compared directly, the extent of dopaminergic denervation in medial olfactory tubercle relates significantly to intravenous cocaine CPP (0.5 mg/kg), whereas that in medial shell does not

Chapter 6:

- Rats can express a conditioned place preference for intravenous methylphenidate (5 mg/kg)
- The locomotor stimulant effect of methylphenidate is attenuated by systemic cis-flupenthixol administration (0.8 mg/kg)
- The acquisition of intravenous methylphenidate conditioned place preference is dose-dependently reduced by cis-flupenthixol administration (0.3 mg/kg)
- The expression of intravenous methylphenidate conditioned place preference is also reduced by cis-flupenthixol (0.3 mg/kg)
- 6-hydroxydopamine lesions of accumbens core reduce methylphenidate locomotor stimulation, but have no effect on conditioned place preference
- 6-hydroxydopamine lesions of medial shell affect neither locomotion nor conditioned place preference for intravenous methylphenidate
- 6-hydroxydopamine lesions of anteromedial olfactory tubercle, however, do significantly attenuate intravenous methylphenidate-induced conditioned place preference.

Chapter 7:

- Rats do not express a conditioned place preference for subcutaneous nicotine in a balanced CPP paradigm (0.1-0.6 mg/kg)
- Rats can express CPP for intravenous nicotine (0.015 mg/kg). However, this behaviour is extremely variable
- Rats reliably express conditioned taste aversion for intravenous nicotine (0.05 mg/kg)
- 6-hydroxydopamine lesions of the core increase CPP for i.v. nicotine
- Conversely, medial shell 6-hydroxydopamine lesions reduce CPP for i.v. nicotine
- Core 6-hydroxydopamine lesions abolish i.v. nicotine conditioned taste aversion
- Medial shell 6-hydroxydopamine lesions induce a slight, but non significant reduction in the magnitude of the conditioned taste aversion.

LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine, serotonin
6-OHDA	6-hydroxydopamine
7-OH-DPAT	7-hydroxy-N,N-di-n-propyl-2-aminotetralin
8-OH-DPAT	8-hydroxy-N,N-di-n-propyl-2-aminotetralin
alOT	anterolateral olfactory tubercle
amOT	anteromedial olfactory tubercle
AMPA	a-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid
AMPH	amphetamine
CeA	central nucleus of the amygdala
CLMA	conditioned locomotor activity
COC	cocaine
CPA	conditioned place aversion
CPP	conditioned place preference
CREB	cyclic AMP response element binding protein
CS	conditioned stimulus
CTA	conditioned taste aversion
DA	dopamine
DAergic	dopaminergic
DAT	dopamine transporter
DMI	desipramine
GABA	γ-aminobutyric acid
ICSA	intracranial self administration

ICSS	intracranial self stimulation
IVSA	intravenous self administration
MDMA	methylenedioxymethamphetaine
mOT	medial olfactory tubercle
MPD	methylphenidate
mSh	medial shell
NAcc	nucleus accumbens
NE	norepinephrine
NET	noradrenaline transporter
NIC	nicotine
NMDA	n-methyl d-aspartate
OT	olfactory tubercle
PPI	prepulse inhibition of the acoustic startle reflex
^{125}I-RTI-55	3β-(4-iodophenyl)tropan-2β-carboxylic acid methyl ester
SERT	serotonin transporter
SN	substantia nigra
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
US	unconditioned stimulus
USV	ultrasonic vocalization
vCP	ventral caudate putamen
vSh	ventral shell
VTA	ventral tegmental area

CHAPTER 1: Introduction and Comprehensive Literature Summary

Laurie H. L. Sellings

1.1 Drug use and addiction

Nearly all people experiment with potentially addictive substances, yet such use does not always lead to addiction. Addiction is characterized by compulsive drug-seeking and drug-taking at the expense of normal life activities (Robinson and Berridge, 2003). For example, a majority of people in Canada (80%) consume alcohol; however, only 10% of drinkers reported that alcohol consumption was having adverse effects on relationships, well-being and health (Canadian Executive Council on Addictions, 2004). Even after protracted drug abstinence, exposure to conditioned environmental cues, stress or the drug itself can re-instate compulsive drug-seeking and drug-taking behaviour.

Few pharmacotherapeutic options exist to treat drug addiction. Of those that are available, most either serve to replace the drug in the system or reduce drug taking by unknown mechanisms. Classic examples include methadone maintenance in opiate addiction, nicotine replacement therapy in smoking cessation, and treatment of alcoholism with the μ-opioid receptor antagonist naltrexone (Gottschalk et al., 1999; Kreek et al., 2002). A summary of common pharmacotherapies for addiction is given in Table 1. Drug-conditioned environmental cues are thought important in reinstating drug-seeking and drug taking (e.g. Robinson and Berridge, 2003; Hyman et al., 2006). Therefore, a better understanding of how the brain encodes the association between such environmental cues and the drug experience would likely facilitate development of more efficacious drugs in the treatment of addiction to several substances.

Table 1. Main pharmacotherapeutic interventions available in the treatment of drug addiction, broken down by drug.

Drug	Intervention	Efficacy	References
Nicotine	Nicotine replacement therapy	22% abstinent at 6 months (vs. 9% placebo)	(Fiore et al., 1994)
	Buproprion *blocker of DAT, NET and SERT*	One year quit rates: 12.4% placebo vs. ~20%	(Hurt et al., 1997) (Jorenby, 2002)
Opiates (e.g. heroin)	Methadone or LAAM maintenance *μ opioid receptor agonists*	Efficacy of >50% in reduction of heroin intake	(Kreek et al., 2002; Hubbard et al., 2003)
	Buprenorphine *partial μ receptor agonist and κ receptor antagonist* Buprenorphine/naloxone *μ receptor antagonist*	Efficacy similar to methadone maintenance	(Kreek et al., 2002)
Ethanol	Nalmefene or naltrexone *μ receptor antagonists*	Of moderate efficacy (20-50% quit rates)	(Kreek et al., 2002; Anton et al., 2006)
	Acamprosate *NMDA receptor antagonist*	Dependent on trial: some show efficacy. Others do not	(Kreek et al., 2002)
Cocaine, amphetamine	No approved interventions		(Kreek et al., 2002; Vocci et al., 2005)

1.2 Animal models of drug use

For ethical reasons, the scope of human studies examining drug-seeking and drug-taking is limited. Many human studies use neuroimaging methods to visualize drug-induced changes in the brain, and have proven useful in the identification of putative molecular and anatomical substrates of drug-taking in humans. However, such studies typically examine polydrug users with heterogeneous drug histories, and are by nature correlational (Gatley et al., 2005). Because of these constraints, animal models of the consequences of drug taking have been developed. These include behavioural activation, altered affective state, reinforcement of subsequent drug taking behaviour, behavioural sensitization, and exhibition of drug-conditioned behaviours upon exposure to drug environment cues, stress, or a drug prime. Such models are briefly described below.

1.2.1 Behavioural activation

Behavioural activation in animals can be measured in a relatively straightforward manner by examining changes in locomotor activity after administration of drugs, especially psychostimulants (Swerdlow et al., 1986). One caveat of locomotor activity measures is that at high drug doses, animals may exhibit stereotyped behaviour such as gnawing, sniffing and rearing; such behaviour may interfere with simple methods of locomotor activity quantification. Although studies examining behavioural activation have provided much insight into putative neural drug targets, how this measure represents more complicated drug-seeking and drug-taking behaviour is unclear.

1.2.2 Affective state

Rats are thought to communicate affective state through ultrasonic vocalizations (USVs). Two types of vocalizations have been characterized in adult rats - 50 kHz and 22 kHz. The 22 kHz call may represent a social alarm signal, and is thought to correspond to negative affect (Knutson et al., 2002). In contrast, 50 kHz calls have been compared to laughter in humans, and are thought to represent positive affective state (Knutson et al., 2002; Panksepp and Burgdorf, 2003). Rats emit 50 kHz calls after systemic amphetamine administration (Knutson et al., 2002). However, USVs are not well characterized in the context of psychoactive drugs. More specifically, it is unclear whether USVs are a pure measure of affective state, a form of social communication, or both (Knutson et al., 2002; Panksepp and Burgdorf, 2003).

1.2.3 Reinforcement

In operant responding paradigms, the delivery of a potential reinforcer is contingent on a response, such as a lever press or a nose poke. Such paradigms can serve to measure if a drug is reinforcing (White, 1989). Three common operant response paradigms used to examine potential substrates of drug taking behaviour are intravenous self-administration (IVSA), intracranial self-administration (ICSA) and intracranial self-stimulation (ICSS).

1.2.3.1 Intravenous self-administration and intracranial self-administration

IVSA and ICSA can serve as useful tools to examine multiple aspects of reinforcement in animals. In the case of IVSA, there is face validity with human drug-taking, since systemic drug administration is used. On the other hand, ICSA is useful for inspection of putative brain regions important in the reinforcing effects of drugs. In both cases, the

use of one of several schedules of drug delivery allows examination of different aspects of drug-taking. Three of the most common schedules are continuous reinforcement or fixed-ratio, progressive ratio, and second-order. In a continuous reinforcement or a fixed-ratio schedule, a constant number of responses results in administration of drug. This examines how much drug animals self administer in a set period of time. In a progressive ratio schedule, escalating responses are required to receive a drug infusion. This procedure measures how motivated animals are to take drugs, as evinced by the "break point" - the maximal number of responses the animal will make to receive the drug. In a second-order schedule, the control that drug-conditioned stimuli have on drug seeking behaviour is examined; this serves as a measure of conditioned reinforcement. The procedure combines two schedules – the first is a presentation of a neutral stimulus (e.g. a light) after a given number of responses. The second is an infusion of drug after a given number of presentations of the neutral stimulus. As such, IVSA and ICSA can be used to examine several aspects of drug-taking and drug-seeking behaviours in animal models.

However, there exist several drawbacks to the use of IVSA and ICSA. First, animals are not tested drug-free. As such, behavioural arousal may significantly affect operant responding. Second, effects of pharmacological or neurological manipulations on extinguishing drug taking behaviour may be influenced by an increase in *conditioned* behavioural activation in the test chamber. Third, responding may be the result simply of a drug's activating effect rather than a drug's reinforcing effect (Ikemoto and Wise,

2004). This problem can be circumvented by using either a second control lever on which responding reflects arousal state, or a yoked procedure.

1.2.3.2 Intracranial self-stimulation

Since the first demonstration that rats will self-administer current into specific parts of the brain (Olds and Milner, 1954), this technique has been exploited to pinpoint the brain loci supporting "rewarding" brain stimulation. As with drug self administration paradigms, ICSS can be made available on different schedules (Schaefer and Michael, 1992). Although it is unclear if or how ICSS mimics natural or chemical rewards, brain nuclei and fibre tracts implicated in drug reward processes also support ICSS (Wise, 1996). Additionally, drugs that are self-administered in animals, such as amphetamine, cocaine and nicotine, decrease the threshold of current that is self-administered (Schaefer and Michael, 1992; Wise, 1987). This has been interpreted as a summation of the rewarding effects of the drug and of the brain stimulation; the "rewarding" effect of the drug decreases the threshold of "rewarding" current required for self-administration. There are, however, several important limitations to ICSS. First, it lacks face validity – people self-administer drugs, not electrical current. Second, it is difficult to determine if the neurons activated by current application are also activated by self-administered drugs. Finally, when the operant response is the measure used to determine changes in 'reward thresholds', this measure could presumably be affected by changes in arousal state, or other non-'reward' events, although this can be problem circumvented by examining rate-intensity functions.

1.2.4 Behavioural sensitization

Drug effects may sensitize after repeated exposures. Locomotor activity after drug administration represents such a phenomenon (e.g. Li et al., 2004; Samaha et al., 2005). More specifically, rats treated repeatedly with a psychomotor stimulant drug exhibit increased locomotor activation when treated with the same drug after a period of protracted abstinence than do drug-naïve animals. This sensitization is accompanied by neuroadaptive changes, at molecular, cellular and systems levels (Robinson and Berridge, 2003; Ferrario et al., 2005). Since behavioural sensitization is observed well after drug administration has ceased, it has been proposed to model drug craving in humans (Robinson and Berridge, 2003).

1.2.5 Drug conditioned effects

The control that conditioned environmental cues have over drug seeking and drug taking is well documented (Le Foll and Goldberg, 2005; Weiss, 2005). Drug-associated cues are one of the most potent precipitators of craving and relapse in both animals and humans. Several drug effects are conditionable, including reward, locomotor stimulation, reinforcement and aversion. Because examination of conditioned effects of psychomotor stimulant drugs was the main focus of this thesis, these paradigms are now described in detail.

1.2.5.1 Conditioned place preference (CPP)

CPP is often used as an index of conditioned drug reward. It is studied in a learning paradigm in which an animal is taught to associate an unconditioned stimulus (US; e.g.

drug, food, sex) with a novel environment or cue (the conditioned stimulus, CS+). As a control, animals receive equal exposure to a second novel environment (the CS-), but in the absence of the US. After several CS+/US pairings, the animal is allowed access to both the CS+ and the CS-, and the time spent in contact with (or the number of approaches made to) the CS+ are measured in the absence of the US. If an animal prefers the CS+, the US is presumed to be rewarding. Conversely, if an animal prefers the CS-, the US is presumed to be aversive. Semantically, CPP may be more appropriately called conditioned *cue* preference, as animals are learning about environmental cues. Furthermore, spatial or place learning requires hippocampal memory systems (Silva et al., 1998), whereas cue learning appears dependent on the nucleus accumbens and amygdala (McDonald and White, 1993; McDonald and White, 1995; White et al., 2005).

There are several advantages of using CPP as a measure of drug reward. First, the CPP test is typically performed drug free (Bardo and Bevins, 2000). As such, state-independent associations made between the drug and the environmental cues are not responsible for the subsequently expressed CPP. Second, it is sensitive to both the rewarding and aversive effects of drugs (Bardo and Bevins, 2000). Third, as opposed to operant responding paradigms, drug dosing is controlled throughout the procedure (Bardo and Bevins, 2000). Finally, the procedure is sensitive to low doses of drug, and is often observed after one CS+/US pairing (Bardo et al., 1999; Bardo and Bevins, 2000; Spina et al., 2006).

CPP is not, however, without its pitfalls. A major disadvantage of CPP is the use of passive drug administration in training (Bardo and Bevins, 2000). This may be of concern, since gene transcription can differ after active vs. passive drug administration (Jacobs et al., 2002). Additionally, CPP has not been validated in human subjects (Bardo and Bevins, 2000). Importantly, it is difficult to design a paradigm in which both environments are equally preferred prior to pairing of one with the US (Bardo and Bevins, 2000). If one environment is inherently preferred, the CPP paradigm is said to be biased, which can lead to interpretational difficulties. For instance, if the drug in question can act as an anxiolytic, drug pairing with the initially unpreferred side may represent decreased anxiety rather than conditioned reward. However, this is only a valid criticism if the preference is not absolute (Tzschentke, 1998). Additionally, pairing of the drug with the initially preferred side may increase the incidence of negative results, since a ceiling effect could decrease the possibility of a statistically significant CPP from being observed (Bardo and Bevins, 2000). To avoid such challenges, an unbiased CPP paradigm was used in this thesis; details can be found in Chapter 2.

1.2.5.2 Conditioned locomotor activity (CLMA)

Locomotor stimulation is also a conditionable behaviour, as evinced by increased locomotor activity observed in drug-free animals in an environment where they had previously received repeated psychostimulant exposures (Beninger and Hahn, 1983; Gold et al., 1988; Brown and Fibiger, 1992). Like CPP, CLMA also measures a conditioned response to drug-associated stimuli. To examine CLMA, the drug (US) is paired repeatedly with a novel environment (CS+). Typically, a second group of animals

receives an equivalent number of US pairings in the home cage, and vehicle infusions in the CS+ environment. During the drug-free test, activity in the novel environment is compared to either baseline activity or to the group that received drug in the home cage. Increased activity in the group previously receiving novel environment drug pairings is interpreted as CLMA. The neural mechanisms underlying CLMA appear to differ at least in part from those of CPP. For example, the amygdala appears critical for cocaine CPP but not cocaine CLMA (Brown and Fibiger, 1993). Details of the CLMA paradigm used in this thesis can be found in Chapter 2.

1.2.5.3 Conditioned taste aversion (CTA)

In classical CTA learning, rats learn to associate a novel taste with a negative gustatory stimulus. This serves to prevent the animal from ingesting substances that could have negative consequences (Welzl et al., 2001). A classic example is lithium chloride, which induces robust CTA (e.g. Fenu et al., 2001). However, several drugs that are self-administered or that induce CPP also cause a CTA (Hunt and Amit, 1987). Examples include amphetamine (e.g. Wise et al., 1976), morphine (e.g. LeBlanc and Cappell, 1975) and nicotine (e.g. Kumar et al., 1983).

It is likely that different neural mechanisms underlie CTA for rewarding vs. malaise-inducing drugs (Hunt and Amit, 1987; Parker, 2003). First, emetic agents cause CTA either via stimulation of vagal afferents, or chemosensors in the area postrema (Hunt and Amit, 1987). Although the effect of vagal afferent manipulation on CTA for rewarding drugs has not been examined, the area postrema does not appear to critically mediate

such CTAs. For example, area postrema lesions do not affect CTA for apomorphine (van der Kooy et al., 1983) or high-dose amphetamine (Rabin et al., 1987 but see Carr and White, 1986), and actually *enhance* nicotine CTA (Ossenkopp and Giugno, 1990). Second, intracranial administration of reward-relevant drugs in extra-area postrema sites can produce CTA. Although this was not observed with amphetamine (Carr and White, 1986), nicotine delivery in the NAcc (Shoaib and Stolerman, 1995) or VTA (Laviolette et al., 2002) produced robust CTA. Finally, central administration of nicotinic receptor antagonists can reduce a CTA for nicotine (Reavill et al., 1986; Stolerman, 1988). For these reasons, it appears that the centrally-mediated, rather than the peripheral aversive properties of rewarding drugs are being examined in the CTA paradigm, and that it differs mechanistically from malaise-inducing drug CTA.

There exist some disadvantages to CTA as a measure of drug aversion. First, animals are in a state of water deprivation throughout conditioning and testing - it is unclear whether the aversive properties of drugs are altered as a result. Second, the procedure is commonly performed with solutions that are intrinsically preferred (e.g. saccharin solution) or avoided (e.g. NaCl solution), potentially complicating interpretation of changes in fluid consumption. Finally, at least one commentator has proposed that CTA for reward-relevant drugs provides a measure of drug *reward* rather than aversion (Di Chiara et al., 2004). This hypothesis posits that rats find the drug-paired flavour aversive as a result of comparison with a more rewarding stimulus (i.e. the drug). As such, increased CTA would indicate increased reward. This explanation, however, suffers from the following criticisms. First, animals can form conditioned taste *preferences* for

low doses of morphine (Mucha and Herz, 1986) or for intracerebroventricular ethanol (Crankshaw et al., 2003). Secondly, 6-hydroxydopamine (6-OHDA) lesions of the accumbens medial shell decreased nicotine CPP, but only minimally affected nicotine CTA (see Chapter 7). Finally, it is unclear why such a mechanism would be observed after conditioning to taste stimuli, but would not apply to conditioning in other modalities (tactile, odour or visual) as in CPP.

1.3 Examining the contribution of discrete brain regions to drug-induced behaviours

Convergent evidence using several experimental approaches has provided evidence that mesolimbic dopamine (DA) transmission, and more specifically within the nucleus accumbens (NAcc), is critical for psychostimulant reward and psychomotor activation. Most of the evidence derives from a few experimental approaches, which are discussed briefly below, with an emphasis on the 6-OHDA lesion since this was used extensively in this thesis.

1.3.1 Drug microinjection

One method of examining brain loci suspected to be involved in the behavioural consequences of drug taking is to infuse a small volume of the drug directly into the brain region of interest. This technique is advantageous in that the action of the drug in the periphery is not likely to contribute to the observed behaviour. There are, however, several concerns with this technique. These include drug diffusion away from the infusion site, difficulty in reliably infusing small volumes, lack of homogeneous drug

concentration at all sites of action, questionable relevance of drug concentrations (i.e. is it biologically relevant?), and anatomical selectivity (Ikemoto and Wise, 2004). Nevertheless, microinjection has provided valuable insight into the anatomical substrates of both conditioned and unconditioned drug effects.

1.3.2 Neurotransmitter measurement

There are two main methods of *in vivo* neurotransmitter measurement. The first of these is microdialysis followed by quantification of extracellular neurotransmitter levels. In this technique, small tissue regions are sampled to determine the concentration of molecules of interest. In the case of brain tissue, microdialysis is commonly used to measure extracellular concentrations of neurotransmitters and their metabolites. In short, intracerebral microdialysis is performed by infusing artificial cerebrospinal fluid through a hollow microdialysis probe, which is inserted into the brain area of interest, and consists of a semi-permeable membrane at the end of a stainless steel or silicon probe (Westerink, 1995). The technique is sensitive to low extracellular concentrations of catecholamines (3-20 fmol; Westerink, 1995; Fillenz, 2005). There are, however, several drawbacks to microdialysis. First, there is substantial blood brain barrier and neural tissue damage resulting from insertion of the 100-300 μm probe (Westerink, 1995; Fillenz, 2005). Second, it is insensitive to rapid changes in neurotransmitter concentrations (Westerink, 1995). Third, gliosis develops rapidly on the microdialysis probe, hence limiting the time of experiments to 3-4 days after implantation (Westerink, 1995), although this problem can be circumvented with an insertable probe. Finally, the

composition of the dialysate fluid can influence measured concentrations of neurotransmitter (Plock and Kloft, 2005).

The second technique is *in vivo* electrochemical monitoring. Compared to microdialysis, it offers improved spatial and temporal resolution. Electrochemical methods examine changes in current after application of a known voltage, from which the extracellular concentration of DA can be determined. Advantages of the technique are as follows. First, the small size of the electrodes allows their placement in brain tissue, and the measurement of neurotransmitter levels with relatively little damage to surrounding tissue (Michael and Wightman, 1999). Second, the short time period between scans (typically repeated every 100 ms; Fillenz, 2005) allows temporal resolution superior to that of microdialysis (Michael and Wightman, 1999). Drawbacks include the impossibility in differentiating DA from norepinephrine (NE), difficulty of distinguishing DA from other oxidizable species such as ascorbate and 3,4-dihydroxyphenyacetic acid, and sensitivity of background current to changes in Ca^{2+} and H^{+} ion concentrations (Michael and Wightman, 1999). In summary, both microdialysis and electrochemistry suffer some drawbacks, but each is useful to determine changes in DA concentrations.

1.3.3 Lesions

Although measures of neurotransmitter release in response to drugs and drug-related stimuli has added much to our knowledge of reward-relevant processing, it remains a correlative measure. More specifically, it is impossible to know whether neurotransmitter release is responsible for an observed behaviour, or whether it simply

accompanies the behaviour. As such, experiments examining such a parameter *in vivo* are, by necessity, correlational. However, if neurotransmission is disrupted at a site of interest, it can be said with some certainty that that region is mediating or enabling an aspect of the resultant behaviour. However, to determine whether a specific neurotransmitter or cell type is responsible, it is necessary to rule out non-selective lesion effects on behaviour. Physical lesion techniques, such as aspiration, radiofrequency and electrolysis, have been used frequently to destroy discrete brain nuclei. However, physical lesions are not cell-type selective, and damage both intrinsic neurons and fibres of passage. Additionally, they induce massive infiltration of reactive astrocytes to the lesion site, and typically cause extensive damage to adjacent structures (Willis and Smith, 1986; Willis et al., 1987; Jarrard, 2002). As such, a lesion with cell-type selectivity, resulting in minimal non-specific damage, would represent a significant improvement over such methods.

Chemical lesions represent a significant advance over physical lesion methods. Such lesions are induced by neurotoxins, including excitotoxins (e.g. kainic acid, ibotenic acid, quisqualate, N-methyl d-aspartate (NMDA)), and the catecholamine-selective neurotoxin 6-hydroxydopamine (6-OHDA). Chemical lesions can be more selective than physical lesions, both anatomically and neurochemically, because the extent of damage is controlled by both the volume and the concentration of toxin infused. However, excitotoxins do not exhibit cell-type selectivity, and have been demonstrated to transiently demyelinate fibres of passage (Ogawa et al., 1989; Brace et al., 1997; Arvanitogiannis and Shizgal, 1999; Jamin et al., 2001; Jarrard, 2002). In contrast, 6-

OHDA almost exclusively kills catecholaminergic cells. Since the interest of the present thesis was to examine the contribution of DA terminals in the ventral striatum to psychomotor stimulant-induced behaviours, the 6-OHDA lesion method was chosen.

6-OHDA selectivity and neurotoxicity have been examined *in vivo*. Early studies demonstrated that intravenous 6-OHDA denervated peripheral adrenergic nerve terminals by "degenerative destruction" of sympathetic nerves (Malmfors and Sachs, 1968). Since blockade of the norepinephrine transporter (NET) using desipramine (DMI) prevented peripheral neurotoxicity, it appeared that neuronal uptake was essential for the neurotoxic effect to take place (Malmfors and Sachs, 1968). Additionally, electron microscopic examination of peripheral adrenergic terminals demonstrated both degeneration and disappearence of NE terminals (Tranzer and Thoenen, 1968). 6-OHDA also substantially reduced whole-brain NE levels after intraventricular infusion (Uretsky and Iversen, 1969). Furthermore, DA levels were also reduced after intraventricular 6-OHDA infusion, to a similar extent as brain NE (Evetts et al., 1970; Uretsky and Iversen, 1970). Once inside the catecholaminergic terminal, 6-OHDA can induce cell death via formation of free radicals and inhibition of complexes I and IV of the mitochondrial electron transport chain (Glinka et al., 1997). Taken together, this evidence suggests that 6-OHDA induces degeneration of NE and DA terminals by several mechanisms in an uptake-dependent manner.

Compared to other lesion techniques, 6-OHDA induces relatively selective neurotoxic damage. Early studies employing intra-nigral infusion of 6-OHDA suggested that it was

fairly selective for DA neurons, excepting a region of necrosis immediately adjacent to the injection site (Sotelo et al., 1973). Additionally, after infusion into the substantia nigra (SN), DAergic cells degenerated whereas non-DAergic cells were spared (Maler et al., 1973). When directly compared to excitotoxic lesions, striatal infusion of 6-OHDA caused degeneration of catecholamine fibres while sparing intrinsic striatal cells. In contrast, the excitotoxin kainic acid caused degeneration of intrinsic neurons while sparing catecholaminergic fibers (Ogawa et al., 1989). As well, behaviourally equivalent radiofrequency vs. 6-OHDA lesions of the medial forebrain bundle revealed much greater non-specific damage after radiofrequency lesions (Willis and Smith, 1986; Willis et al., 1987). In addition, intra-striatal 6-OHDA infusions did not significantly affect tissue levels of choline acetyltransferase, glutamic acid decarboxylase, or phosphodiesterase activity (Kelly et al., 1977), suggesting that several non-DAergic neuronal markers are left intact after 6-OHDA infusion.

6-OHDA can, however, induce changes in non-DA or NE cells. Such changes are seen both at the receptor signalling and morphological levels. At the level of signal transduction, 6-OHDA infusion induced D1 receptor supersensitivity, which resulted from a switch to signalling through the MAP kinase ERK 1/2 (Gerfen et al., 2002). Postsynaptic remodelling of the predominantly γ-aminobutyric acid (GABA)ergic medium spiny neuron population in the striatum has also been reported, including changes such as an increased number of GABAergic boutons (Nitsch and Riesenberg, 1995), reduced spine density (Ingham et al., 1993), and a loss of asymmetric synapses (Ingham et al., 1998). In conclusion, 6-OHDA induces relatively selective lesions of

catecholaminergic neurons. However, neurons influenced by DAergic innervation may undergo neuroadaptations resulting from DAergic denervation.

1.4 Dopamine as a critical mediator of the effects of psychomotor stimulant drugs

Mesolimbic DA transmission has been implicated in several effects of drugs of abuse in animals. Evidence for this is discussed below.

1.4.1 Dopaminergic mechanisms in locomotor activation

Several investigations in the 1960s provided the first evidence that changes in catecholaminergic transmission accompanying psychostimulant administration were related to the locomotor stimulation. First, d-amphetamine administration dose-dependently changed brain amine content (Smith, 1965). Second, administration of reserpine, which transiently reduces DA and NE levels, dose-dependently reduced amphetamine-induced locomotor activation (Smith, 1963). Taken together, these results were suggestive of a central role for catecholaminergic transmission in the locomotor stimulant effect of amphetamine.

In the 1970s, it became clear that the catecholamine responsible for locomotor stimulation was the neurotransmitter DA, for several reasons. First, amphetamine-induced activity was attenuated by administration of DA (but not NE) antagonist (Pijnenburg et al., 1975). Second, 6-OHDA infused into either the soma (ventral tegmental area [VTA]; Le Moal et al., 1975; Koob et al., 1981) or the NAcc (Iversen et al., 1975; Koob et al., 1981; Joyce et al., 1983) of the mesolimbic DA system attenuated

amphetamine-induced locomotor stimulation. Third, microinjection of DA into the NAcc after reserpine-induced DA depletion was sufficient to reinstate normal locomotor activity in rats (Jackson et al., 1975). Fourth, both direct and indirect DA agonists elicited changes in locomotor activity microinjection into the NAcc or olfactory tubercle (OT; e.g. Pijnenburg et al., 1976; Delfs et al., 1990; Van Hartesveldt et al., 1992), whereas NE agonists were ineffective (Pijnenburg et al., 1976). As a whole, these manipulations suggested that DA is more important than NE for locomotor stimulation, especially in the ventral striatum.

The above studies examined unconditioned locomotion in response to drugs. Locomotor activity can also be conditioned by repeatedly pairing drug administration with a novel environment, and then examining an animal's drug-free activity level in that environment. DAergic mechanisms within the accumbens also appear to mediate *conditioned* locomotor activity. For example, 6-OHDA lesions of the accumbens either before or after conditioning with amphetamine attenuated subsequent conditioned activity measured in a drug-free test (Gold et al., 1988). It might be assumed that blunting the unconditioned locomotor response to drugs is sufficient to block the concomitant conditioned locomotor response. However, the literature is unclear on this point. For instance, a variety of manipulations that inhibit DA transmission reduce the unconditioned locomotor response, but do not reduce the conditioned locomotor response to amphetamine (Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson, 1991; DiLullo and Martin-Iverson, 1992a; DiLullo and Martin-Iverson, 1992b). In contrast, other DAergic manipulations during conditioning did block the development of

amphetamine-induced conditioned activity (Beninger and Hahn, 1983; Mazurski and Beninger, 1991; DiLullo and Martin-Iverson, 1992b). The literature is also unclear on whether DA is important in the *expression* of conditioned locomotion. More specifically, the DA receptor antagonist pimozide given on test day failed to block conditioned locomotor activity (Beninger and Hahn, 1983), whereas either intra-NAcc infusions of 6-OHDA (Gold et al., 1988) or a D3 receptor partial agonist (Aujla et al., 2002; Aujla and Beninger, 2004) blocked conditioned locomotor activity when administered before the CLMA test. Whereas several of these findings suggest a role for DA transmission in conditioned activity, it is unclear whether DA is critical for the development and/or expression of conditioned activity.

1.4.2 The dopamine hypothesis of reward/reinforcement

Psychomotor stimulant-induced locomotion and conditioned activity are not the only drug-induced behaviours influenced by changes in DA transmission. Indeed, both natural and chemical rewards are influenced by DAergic manipulations. The DA hypothesis of reward/reinforcement holds that DA transmission is critical for the rewarding and reinforcing effects of psychomotor stimulant drugs.

1.4.2.1 Dopamine transmission and drug reinforcement

Reinforcement refers to an increased probability that an animal will repeat an action if it results in reward receipt. As such, self-administration behaviour is a measure of reinforcement. As with locomotor activity, drug self-administration behaviour is dependent on DA transmission. For example, administration of DA, but not NE, receptor

antagonists caused an initial increase in responding for amphetamine followed by decreased responding in a manner consistent with extinction, indicating that the rewarding value of amphetamine was decreased (Yokel and Wise, 1975; Yokel and Wise, 1976). Additionally, administration of direct DA receptor agonists decreased responding for amphetamine, and were self-administered when substituted for amphetamine, suggesting that activation of DA receptors is sufficient to maintain responding (Yokel and Wise, 1978). In the case of cocaine, self administration was blocked by the DA receptor antagonist pimozide, but not by the NE blockers phentolamine or phenoxybenzamine (De Wit and Wise, 1977). The reinforcing properties of opiate drugs may also be dependent on DAergic transmission, as evinced by changes in responding for morphine after treatment with DA receptor agonists or antagonists (Glick and Cox, 1975). One potential confounding variable in all of these DA receptor antagonist studies, however, is the possibility that the drugs exerted inhibitory influences on locomotor activity. Arguing against this, lesion studies suggested that the NAcc appeared to be an important locus where DA transmission encodes psychostimulant reinforcement, without non-specific effects on the ability to respond. More specifically, 6-OHDA lesions of the accumbens were shown to reduce cocaine self-administration (Roberts et al., 1977; Pettit et al., 1984), without any major effects on responding for food (Roberts et al., 1977). Additionally, responding for heroin (Pettit et al., 1984) or morphine (Dworkin et al., 1988) was not affected by the 6-OHDA lesion. Taken together, this early evidence suggested a central role for mesolimbic DA in the motivational aspects of psychostimulant drug administration.

Interestingly, DA transmission may also be important in encoding natural rewards. Not only did DA blockade attenuate lever pressing for food (Wise et al., 1978a; Wise et al., 1978b), but also for water (Gerber et al., 1981). It is doubtful that antagonist effects on locomotor activity accounted for the reduction in responding, as pimozide-treated rats responded in an identical fashion to controls for food on the first test day (Wise et al., 1978a). In summary, not only does the self-administration of drug and natural rewards appear critically dependent on DA transmission, but this effect appears to result from reduced motivation to respond, since antagonist-treated animals were able to respond, but chose not to do so.

1.4.2.2 Dopamine transmission and drug reward

A substance is said to be rewarding if it promotes approach behaviour (White, 1989). Conditioned place preference (CPP) serves as a measure of conditioned reward, and is an attractive way to examine drug reward since it is not vulnerable to the motor impairing effects of drugs to the same extent as is IVSA. Several lines of convergent evidence also implicate DAergic transmission in drug CPP. First, treatment with neuroleptic drugs attenuated the acquisition of CPP for amphetamine (Spyraki et al., 1982b; Mackey and van der Kooy, 1985) and i.v. cocaine (Spyraki et al., 1987), but not for morphine (Mackey and van der Kooy, 1985), heroin (McFarland and Ettenberg, 1999 but see Spyraki et al., 1983) or i.p. cocaine (Spyraki et al., 1982a; Spyraki et al., 1987). The NAcc appears to be a critical anatomical locus for CPP, since 6-OHDA lesions of the accumbens reduced CPP for amphetamine (Spyraki et al., 1982b) and increased CPP for apormorphine (van der Kooy et al., 1983); in the latter case, this was presumably by

action on supersensitized postsynaptic DA receptors. Additionally, rats formed CPP after intra-accumbens infusion of amphetamine (Carr and White, 1983; Carr and White, 1986). Although CPP is a memory-dependent measure, antagonist and lesion effects appear to result from reward reduction rather than from general memory deficits (see Section 1.6.4 and Chapters 3 and 5). Hence, as seen with intravenous self administration, psychostimulant CPP also appeared to be critically dependent on DA transmission in the accumbens. However, route of administration, at least in the case of cocaine, may be an important variable.

1.4.3 Mechanisms of dopamine release by psychomotor stimulants and nicotine

Convergent evidence strongly implicated DA release in the rewarding and locomotor stimulant effects of psychostimulants and nicotine. All of these drugs act as indirect DA receptor agonists, by increasing extracellular DA levels. The mechanism by which these drugs increase DA levels, however, differs from drug to drug, and shall be outlined here.

Amphetamine can promote DA release by several mechanisms, as recently reviewed by Sulzer and colleagues (2005). Methylphenidate, a structural analogue of amphetamine, releases DA in a manner similar to amphetamine. These drugs can bind both to plasma membrane DAT and vesicular monoamine transporters. Hence, these lipophilic molecules are first transported into the cell terminal, either through DAT or by diffusion through the plasma membrane, and subsequently into the synaptic vesicle where they become protonated. This causes drug to accumulate within synaptic vesicles, and to displace DA, which is reverse transported from the synaptic vesicle into the cytosol. DA

then leaves the cell terminal by reverse transport through DAT. Additional mechanisms may include monoamine oxidase inhibition and regulation of tyrosine hydroxylase activity. Importantly, amphetamine also has affinity for NET and SERT, and methylphenidate for NET.

Cocaine is also an indirect DA receptor agonist, but increases extracellular monoamine levels in a different manner from amphetamine and methylphenidate. More specifically, cocaine increases extracellular DA, NE and serotonin (5-HT) levels by competitive blockade of plasma membrane DAT, NET and the 5-HT transporter (SERT) respectively (Greco and Garris, 2003). Hence, DA, NE and 5-HT accumulate in the extracellular milieu. In addition to its monoamine-releasing capabilities, it also has important local anesthetic properties derived from blocking voltage gated Na^+ channels (Matthews and Collins, 1983).

Nicotine is an agonist at nicotinic acetylcholine receptors. These receptors are located in most brain regions. Accordingly, nicotine can act through multiple mechanisms to promote or modulate mesolimbic DA release, as shall be outlined here. First, nicotinic acetylcholine receptors are located both on the soma of mesolimbic DA neurons (Champtiaux et al., 2003), and presynaptically on glutamate terminals originating from the prefrontal cortex and the pedunculopontine and laterodorsal tegmental nuclei (Mansvelder and McGehee, 2000; Jones and Wonnacott, 2004; Wonnacott et al., 2005). Hence, after acute nicotine administration, accumbal DA release may be increased either

directly by nicotine or indirectly by glutamate acting on mesolimbic DA soma. Second, DA terminals in the NAcc possess β2 subunit-containing nicotinic receptors (Zhou et al., 2002). It is thought that activation of these receptors on mesolimbic terminals may enhance DA release from mesolimbic DA terminals (Zhou et al., 2002), and nicotine's action here could modulate DA release in the NAcc.

1.4.4 Dopamine receptor signalling

A brief overview of DA receptor signalling shall be presented here. This is by no means an exhaustive list of all potential signalling transduction cascades that these receptors may engage; such a discussion is beyond the scope of this thesis. Rather, the signalling mechanisms that lead to molecular neuroadaptations that are thought to influence drug reward mechanisms are the focus of this section.

A growing list of molecular neuroadaptations has been observed after administration of rewarding drugs. These molecular changes can be influenced by DA receptor signalling. DA receptors are G-protein coupled receptors that fall into two classes – D1-like receptors, comprising D1 and D5 receptors, and D2-like receptors, comprising the D2 receptor, as well as D3 and D4 receptors (Missale et al., 1998; Bonci and Hopf, 2005). These two receptor classes exert opposing influences on adenylyl cyclase activity; D1-like receptors activate adenylyl cyclase by coupling to $G_{\alpha s}$ or $G_{\alpha olf}$ proteins, whereas D2-like receptors inhibit adenylyl cyclase through $G_{\alpha i/o}$ (Bonci and Hopf, 2005). Adenylyl cyclase catalyzes the formation of cAMP from ATP, which can then activate protein kinase A. Protein kinase A phosphorylates multiple enzymes, ion channels and other

targets, one of which is the transcription factor cAMP response element binding protein (CREB). In its phosphorylated form, CREB can dimerize and bind to cAMP response elements, where it can influence the transcription of several genes including fos-related antigens (e.g. ΔFosB) and dynorphin (Nestler, 2004; Carlezon, Jr. et al., 2005).

Manipulations of accumbal CREB are sufficient to exert effects on motivated behaviours. More specifically, infusions of viral vectors encoding CREB, or a mutant form of CREB that cannot be phosphorylated, into the accumbens shell have been shown to produce effects on cocaine CPP, sexual activity, anxiety, and several other behaviours in the rat (Carlezon, Jr. et al., 1998; Nestler, 2001; Barrot et al., 2002; Carlezon, Jr. et al., 2005; Barrot et al., 2005). Drug-induced increases in CREB phosphorylation result in higher expression of the endogenous κ opioid receptor agonist dynorphin (Carlezon, Jr. et al., 1998), and increased protein levels of the stable fos-related antigen ΔFosB (Nestler, 2001; Nestler, 2004). Whereas dynorphin decreases the rewarding properties of cocaine, higher levels of ΔFosB that accumulate with repeated drug administration make animals more sensitive to the rewarding effects of several drugs, including cocaine and morphine (Kelz et al., 1999; Nestler, 2001).

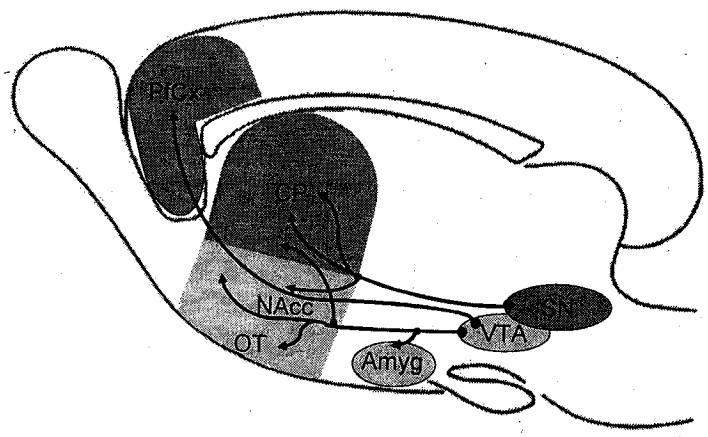
1.5 Anatomy of the ventral striatum

A rich literature has examined the contribution of ventral striatal subregions to several aspects of motivated responding for drug and natural rewards, as well as locomotor activation. Before a critical discussion of such studies is possible, a brief description of the anatomy of the ventral striatum is fitting.

The mesotelencephalic DA system in the rat can be broadly divided into three different pathways. The first of these is the mesostriatal DA system, which consists of a projection from the substantia nigra pars compacta (SNc) to the caudate-putamen, with small numbers of fibers projecting to mesocortical and mesolimbic terminal fields. The second of these, the mesocortical DA system, projects from the ventral tegmental area (VTA) to several cortical regions including the medial prefrontral, anterior cingulate and suprarhinal cortices. The third of these pathways is the mesolimbic DA system (Gardner and Ashby, Jr., 2000). This pathway consists of a projection from the VTA and medial SNc to several forebrain terminal areas, including the amygdala, bed nucleus of stria terminalis, lateral septal area, lateral hypothalamus, olfactory tubercle and nucleus accumbens (Gardner and Ashby, Jr., 2000). A simplified diagram of the three mesotelencephalic DA systems is shown in Figure 1.

The extended striatum can be viewed as an anatomical and functional continuum, with medioventral portions subserving more limbic functions, and dorsolateral portions supporting locomotor activity (Voorn et al., 2004). The dorsal striatum is important in normal locomotor activity, and substantial depletions in DA levels here are believed to be responsible for the motor deficits observed in Parkinson's disease. In contrast, the ventral striatum, comprising the NAcc and striatal portions of the OT, appears more important in drug-induced locomotor activation, reward and reinforcement. The contribution of subregions of the *ventral* striatum to conditioned reward, aversion, and both unconditioned and conditioned locomotion was the focus of this thesis. The

Figure 1. Schematic of the three mesotelencephalic dopamine systems. The first of these is the mesostriatal (nigrostriatal) DA system, consisting of a projection arising predominantly from the substantia nigra pars compacta, and terminating in the caudate-putamen. The second is the mesocortical projection. This arises predominantly from the VTA, and terminates in various regions of the frontal cortex. The final pathway is the mesolimbic projection, which arises predominantly in VTA, and terminates in several regions, including the nucleus accumbens. See Section 1.5 for details. Abbreviations: Amyg, amygdala; NAcc, nucleus accumbens; OT, olfactory tubercle; PfCx, prefrontal cortex; SN, substantia nigra; CP, caudate putamen; VTA, ventral tegmental area.



accumbens was long viewed as a specialized portion of the striatum, as it sent projections to not only basal ganglia structures such as the globus pallidus, entopeduncular nucleus, and motor thalamus, but also to limbic structures such as the extended amygdala and the lateral hypothalamus (Zahm and Brog, 1992). The accumbens is divisible into at least two prominent structures in both rodent and primate brain, namely a dorsolateral core and a ventromedial shell region, on the basis of differential histochemical staining and anatomical projection patterns (Zaborszky et al., 1985; Zahm and Brog, 1992; Jongen-Relo et al., 1994; Pennartz et al., 1994). The anatomy of the *rat* ventral striatum will be the focus of this section.

It is noteworthy that, within the accumbens, there exist potential subregions other than core and shell. In the rat, for example, as many as five sub-subregions of the shell have been proposed on the basis of differential tyrosine hydroxylase immunoreactivity after repeated cocaine treatment (Todtenkopf and Stellar, 2000). Two of the better characterized are the septal pole or cone region, and the rostral pole. The septal pole represents the caudalmost portion of the shell. One of the more striking attributes of this region is the presence of dopamine β hydroxylase, the synthetic enzyme for NE synthesis (Berridge et al., 1997). The rostral pole comprises the rostral third of the accumbens, and represents a region of the accumbens where it is difficult to discern core from shell (Zahm and Heimer, 1993; Tan et al., 1995). Within the rostral pole region, medial regions have "shell-like" projection patterns and histochemical marker distributions, whereas lateral portions of the rostral pole more closely resemble the core (Zahm and Heimer, 1993). The olfactory tubercle has not been as well characterized as the NAcc;

however, recent behavioural evidence suggests that discrete functional subunits also exist within the OT. More specifically, medial but not lateral OT sites support robust self-administration of amphetamine and cocaine in the rat (Ikemoto, 2003; Ikemoto et al., 2005).

1.5.1 Afferent and efferent projections of the ventral striatum

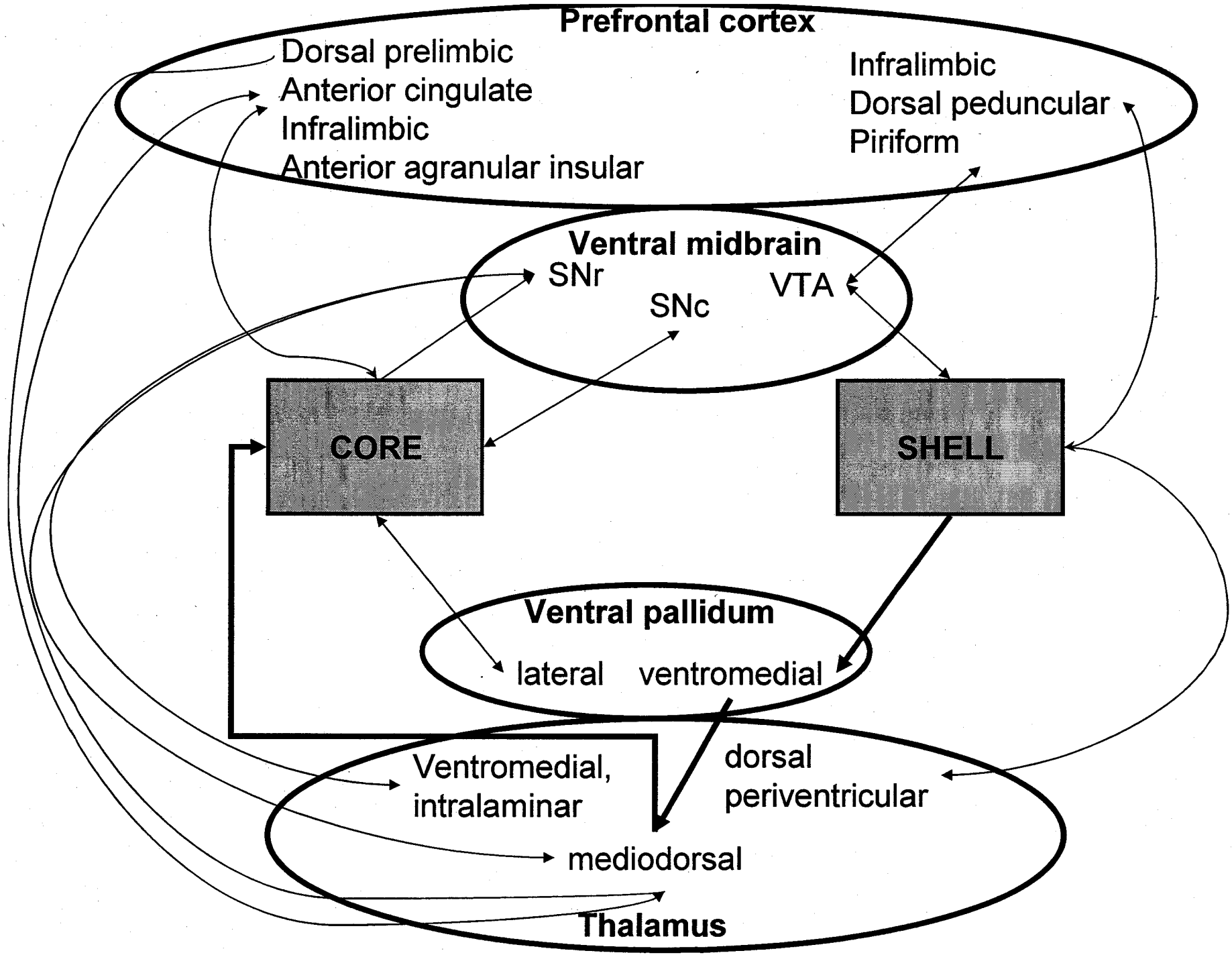
Generally speaking, the core and shell are innervated by and send afferent projections to similar brain nuclei. For example, both receive major DAergic input from the VTA, and project to the ventral pallidum, the VTA, and to prefrontal cortex (e.g. Zahm, 1999). However, core and shell receive and send projections to distinct subregions of such brain nuclei (Zahm, 1999). The OT shares several characteristics with the accumbens in terms of afferent and efferent projections, and receives an additional input from the olfactory bulb (Alheid et al., 1990). Although differences between efferent and afferent projections of the core and shell have been extensively examined, comparative studies examining accumbens and OT subregion projection patterns are lacking.

Most accumbens afferents and efferents are arranged in a topographical manner. For example, it has long been known that the projections from the VTA and SNc to the NAcc are topographically arranged in both the mediolateral and anteroposterior axes, and that the senses are reversed in the dorsoventral plane (Fallon and Moore, 1978). This implies that the VTA projects to the medial NAcc, and the medial SNc to the lateral and ventral NAcc (Fallon and Moore, 1978). The reverse also appears to be true, as the medial shell rather than the core projects to the medial VTA , and the core more strongly innervates

the lateral VTA, SNc and SN pars reticulata (SNr; Brog et al., 1993; Kalivas et al., 1993). However, it remains unknown whether overlapping or distinct neuronal populations project to core vs. shell of the rat, although there is evidence in the primate that different populations of DA neurons innervate these areas (Haber and McFarland, 1999). A similar topographical arrangement is observed in the case of subicular projections to the NAcc. More specifically, the ventral subiculum projects to the shell and medial OT, and the dorsolateral subiculum projects to the core (Groenewegen et al., 1987). In the case of the raphe, bilateral projections from the dorsal raphe nucleus innervate medial shell, lateral shell and core, whereas median raphe projections innervate medial shell exclusively (Brog et al., 1993). Taken together, these connections suggest that the core and shell are poised to differentially influence, and to be differentially influenced by, several brain nuclei.

There exist few feedback loops allowing communication between the core and the shell. The best defined of these in the rat is the relay through the mediodorsal thalamus, which may allow the shell to modulate responses in the core (Groenewegen et al., 1999; see Figure 2). The accumbens is a part of an intricate circuit including several thalamic nuclei, the VTA and SN, the ventral pallidum and cortical regions (Kalivas et al., 1999). Additionally, in the primate, the striato-nigro-striatal projection consists of a series of projections, in which efferent projections of the ventral striatum to the VTA/SN are more dorsal than are afferent projections. This allows ventral portions of the ventral striatum to communicate with more dorsal regions via "ascending spirals" through the VTA/SN (Haber et al., 2000). Whether this is also true for the rat has not been determined.

Figure 2. Schematic of corticothalamostriatal circuits through the core and the shell. The shell can indirectly influence the core subregion through the mediodorsal nucleus of the thalamus. References: Zahm and Heimer, 1990; Brog et al., 1993; Freedman and Cassell, 1994; Ding et al., 2001; Wang and Shyu, 2004; Vertes, 2004.


Prefrontal cortex
Dorsal prelimbic
Anterior cingulate
Infralimbic
Anterior agranular insular
Infralimbic
Dorsal peduncular
Piriform
Ventral midbrain
SNr
SNc
VTA
CORE
SHELL
Ventral pallidum
lateral
ventromedial
Ventromedial,
intralaminar
dorsal
periventricular
mediodorsal
Thalamus

In the rat, it was recently demonstrated that ventral striatal subregions send reciprocal projections, hence allowing direct communication between core, shell and OT. For example, such projections are observed between the core and shell, rostral and caudal shell, rostral pole and core, rostral pole and shell, and medial shell and medial OT (van Dongen et al., 2005). Whereas core efferents largely avoid the medial OT, in general, its efferent projections are more robust than its afferent projections (van Dongen et al., 2005). Hence, the core is poised to directly influence the shell, but not the medial OT, via a direct projection. Additionally, the shell may either directly influence both medial OT and core, in addition to indirectly modulating the core.

Although most accumbal afferent and efferent targets are different subregions of the same nucleus, the medial shell subregion projects to and receives projections from several loci that largely avoid the core. The two primary structures that send afferent and efferent projections to the medial shell but not the core are the lateral hypothalamus and several nuclei of the extended amygdala (Heimer et al., 1991; Brog et al., 1993). Several midbrain and brainstem nuclei, including the medial parabrachial nucleus, retrorubral field, periaqueductal grey, pedunculopontine nucleus and locus coerelus, also project predominantly to the medial shell (Brog et al., 1993).

These differences in projection patterns to and from core and shell are suggestive of distinct functional roles for these subregions in motivated and drug-related behaviours. Several studies have examined potential functional differences, and these are discussed in the following sections.

1.6 Reward-relevant processing and the ventral striatum

DA transmission in the ventral striatum is an important mediator of drug reward and reinforcement (see Section 1.4). Considering the anatomical differences between core and shell, it is no surprise that functional investigations of the core vs. shell have revealed different functional roles for ventral striatal subregions in reward processing.

1.6.1 The ventral striatum in drug self-administration

Convergent evidence suggests that the portion of the medial ventral striatum is of importance in the primary reinforcing effects of abused drugs. For example, rats will self-administer cocaine or amphetamine directly into the medial shell and the medial OT, but not into the core (Rodd-Henricks et al., 2002; Chevrette et al., 2002; Ikemoto, 2003; Ikemoto et al., 2005). The self-administration of cocaine into the medial shell and medial OT was prevented by co-administration of the D1 receptor antagonist SCH 23390 and the D2 receptor antagonist sulpiride, suggesting that cocaine self-administration was dependent on DA receptor activation (Ikemoto, 2003). DA receptor stimulation in the medial shell appears sufficient to support ICSA, as both the DAT blocker nomifenisine (Carlezon, Jr. et al., 1995) and a mixture of D1 and D2 receptor agonists (Ikemoto et al., 1997) were self-administered into the medial shell and medial OT, but not into the core. This was prevented by co-administration either SCH 23390 or sulpiride (Carlezon, Jr. and Wise, 1996b; Ikemoto et al., 1997). Taken together, these studies provide evidence for a direct effect of stimulation of DA receptors in the medial shell and medial OT, but not the core, in intracranial self-administration of psychomotor stimulants.

DA transmission in the medial shell not only supports intracranial self-administration of psychomotor stimulant drugs, but also underlies intravenous self-administration of several systemically-administered drugs. For example, intra-shell infusions of SCH 23390 dose-dependently *increased* the rate of cocaine self-administration in a manner resembling extinction behaviour, but were without effect on responding for food (Bari and Pierce, 2005). In contrast, intra-core infusions of the same drug reduced responding for both cocaine and food, suggesting that the antagonist was affecting operant responding (Bari and Pierce, 2005). Taken together, this suggests that medial shell DA transmission shell is selective for *drug* reinforcement. Other evidence supporting a preferential role for medial shell DA transmission in intravenous self-administration is more indirect. For instance, indices of neuronal activation are increased selectively in the medial shell after drug self-administration. For example, c-fos immunoreactivity was significantly higher in the shell than the core of rats trained to self-administer nicotine (Pagliusi et al., 1996) or cocaine (Berlanga et al., 2003); indeed, c-fos expression in cholinergic interneurons of the shell correlated with the amount of self-administered cocaine (Berlanga et al., 2003). Additionally, cerebral glucose utilization was increased in the shell during ethanol consumption, but not in the accumbens core (Porrino et al., 1998a; Porrino et al., 1998b). Taken together, these studies suggest a central role for the shell in the self-administration of systemically-administered drugs.

Although DA transmission in the medial ventral striatum (medial shell and medial OT) appears critical for reinforcement mediated by direct and indirect DA agonists, the medial

shell also supports non-DA-dependent reinforcement. For instance, the NMDA receptor antagonists phencyclidine and dizoclipine are self-administered into the accumbens medial shell, but not the core (Carlezon, Jr. and Wise, 1996b). Since this was not prevented by co-administration of sulpiride (Carlezon, Jr. and Wise, 1996b), NMDA receptor antagonists appear to be self-administered into the shell independently of D2 receptor activation. Glutamatergic and DAergic mechanisms may interact in drug self-administration, as cocaine self-administration was significantly *reduced* by systemic dizocilpine administration, without affecting extracellular DA levels (Pierce et al., 1997). In summary, glutamatergic transmission in the medial shell can influence reinforcement independently of DA transmission.

Taken together, these findings support a critical role for the accumbens shell in primary drug reinforcement, with contributions from both DAergic and glutamatergic transmission. However, since both intra-shell and intra-core administration of antisense directed against the transcription factor cAMP response element binding protein (CREB) reduced responding for intravenous cocaine (Choi et al., 2006), signal transduction in both subregions may be required to support operant responding for self-administered drugs.

1.6.2 The ventral striatum in reinstatement of drug seeking

A hallmark of addiction is relapse to drug taking, even after protracted abstinence. This aspect of addiction can be modelled in the rodent using a reinstatement procedure. In such procedures, the animal is trained to self-administer a drug. Once stable responding

for the drug is achieved, the drug is taken away, and the operant response behaviour is extinguished. At a later time point (days to months) after drug cessation, the drug-seeking behaviour can be "reinstated" by administration of a drug prime, a stressor, or a drug-associated cue. Reinstatement of drug seeking behaviour can be affected by manipulation of either shell or core, depending on the stimulus triggering relapse and the transmitter system examined. One approach that has been used to examine the relative contribution of accumbal subregions to reinstatement of drug seeking is to temporarily inactivate neuronal activity in core or shell by co-infusing the $GABA_A$ and $GABA_B$ receptor agonists baclofen and muscimol. This manipulation has implicated the shell in cue- (Fuchs et al., 2004), both the shell and the core in stress- (McFarland et al., 2004), and the core but not the shell in drug prime- (McFarland and Kalivas, 2001) induced reinstatement of cocaine seeking.

In contrast, DAergic transmission in the shell, but not in the core, appears to influence reinstatement. In the case of drug prime-induced reinstatement, infusion of D1 or D2 receptor antagonists into the shell, but not the core, reduced drug seeking behaviour (Anderson et al., 2003; Anderson et al., 2005 but see Bachtell et al., 2005). Additionally, intra-shell infusions of D1/D5 or D2 DA receptor agonists effectively reinstated cocaine seeking, whereas core infusions were ineffective (Schmidt et al., 2006). Taken together, these studies suggest that stimulation of DA receptors in the shell is sufficient to induce reinstatement.

Changes in glutamatergic signalling in the shell subregion have been shown to affect both cue- and stress-induced relapse of cocaine seeking. First, extinction training increased α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA) receptor expression in the accumbens shell but not the core (Sutton et al., 2003). Second, infusion of viral vectors encoding the AMPA receptor subunits GluR1 and GluR2 into the shell made rats more resistant to stress-induced reinstatement of cocaine seeking (Sutton et al., 2003). Third, antagonism of metabotropic glutamate receptors were found to reduce cue-induced relapse to heroin seeking preferentially after infusion into the shell (Caine et al., 1995; Bossert et al., 2005). Taken together, these results suggest that DAergic and glutamatergic transmission is important in the shell, but not in the core, in reinstatement of drug seeking.

1.6.3 The ventral striatum in conditioned reinforcement

Conditioned reinforcement occurs when a neutral stimulus becomes a secondary reinforcer after repeated pairing with a primary reinforcer. DA transmission in the nucleus accumbens appears important in the control that conditioned reinforcers exert over responding, as intra-accumbens infusions of amphetamine increased responding for a conditioned reinforcer, whereas accumbal 6-OHDA lesions reversed this enhancement of responding (Taylor and Robbins, 1984; Taylor and Robbins, 1986). Interestingly, extracellular DA levels remained unchanged in both core and shell under control of a cocaine-associated cue (Ito et al., 2000), although this does not exclude a possible enabling role for accumbal DA transmission. Indeed, systemic administration of a

selective D3 receptor antagonist effectively reduced responding for a conditioned reinforcer associated with cocaine administration (Di Ciano et al., 2003).

It is possible that glutamatergic mechanisms within the accumbens are more important in conditioned reinforcement. For example, infusion of the AMPA receptor antagonist LY 293558, but not the NMDA receptor antagonist AP-5, into the core subregion reduced responding for the cocaine-associated cue during the first interval of a second order schedule, suggesting that the core is important for conditioned reinforcement (Di Ciano and Everitt, 2001). These findings accord with neuronal inactivation studies, as both temporary (baclofen and muscimol infusion) and permanent (excitotoxic lesion) inactivation of the core, but not the shell, reduced responding for cocaine-associated cues (Ito et al., 2004; Di Ciano and Everitt, 2004). Additionally, permanent inactivation of the core also reduced responding for the secondary reinforcer associated with food (Parkinson et al., 1999; Hutcheson et al., 2001). Taken together, these lines of evidence suggest that the core is a more important mediator or enabler of the control of drug-conditioned cues over drug seeking. However, whether DA transmission in either core or shell per se is important has not been examined.

1.6.4 The ventral striatum in conditioned place preference

Manipulations of DA transmission within the nucleus accumbens affect CPP for a wide array of drugs of abuse (Koob, 1999; Ikemoto and Wise, 2004). Surprisingly, few studies to date have directly compared the effects of manipulations of medial vs. lateral ventral striatum on CPP. Instead, most studies have examined the effects of manipulations of

only the shell on the establishment of CPP. For example, both intra-shell amphetamine (Schildein et al., 1998) and intra-anteromedial OT cocaine (Ikemoto, 2003) produce CPP, but the effects of intra-core infusions were not examined in these studies. Additionally, either NMDA or AMPA receptor blockade in the shell during CPP acquisition blocked 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT) CPP (Biondo et al., 2005), but it is unclear if this was an effect on associative memory or drug reward. Similarly, although viral-mediated gene transfer of the glial glutamate transporter GLT-1 into the shell significantly reduced methamphetamine CPP (Fujio et al., 2005), the results of this study were equally consistent with a reduction in associative memory as with a reduction in drug reward. In one final study, infusions of a viral vector expressing the 5-HT_{1B} receptor into the shell *increased* low-dose cocaine CPP in a balanced paradigm, but abolished higher-dose cocaine CPP (Neumaier et al., 2002). Since low-dose cocaine CPP was enhanced by this manipulation, the alterations observed on CPP magnitude are unlikely to have resulted by affecting associative memory. Rather, the results are more consistent with a dose-response curve shift to the left, indicative of increased sensitivity to the rewarding effects of cocaine. In summary, since none of these manipulations in the shell were compared to identical manipulations in the core, a role for the core in CPP cannot be excluded. Additionally, most of these studies have not convincingly ruled out the possibility that associative memory rather than reward mechanisms were affected.

It is unclear how DA transmission in the medial shell affects CPP induced by opiate drugs. More specifically, although systemic morphine administration increased DA release in the shell (Huang et al., 2003; Huang et al., 2004), neither intra-shell nor intra-

core morphine infusions produced CPP (Olmstead and Franklin, 1997; Schildein et al., 1998). However, CPP for systemic morphine was decreased by over-expression of CREB in the shell, and increased by over-expression of a dominant negative mutant form of CREB (Barrot et al., 2002), implying that gene transcription in the shell can influence morphine CPP. It is unlikely that this resulted from non-specific effects on associative memory, since CREB over-expression also eliminated the preference for sucrose in a simple preference test that was independent of associative memory (Barrot et al., 2002). It is possible that morphine acting on μ or κ opioid receptors differentially influences CPP. For example, endomorphin-1 infusions into the shell produce CPP, whereas intra-shell endomorphin-2 caused conditioned place *aversion*; the aversion, but not the preference, was prevented by microinjection of antisera against dynorphin, an endogenous κ opioid receptor agonist (Terashvili et al., 2004). In other words, it is possible that microinjections of morphine into the shell did not produce CPP because morphine was acting on both μ and κ opioid receptors, resulting in a motivationally neutral state. In further support of a role for the shell in morphine CPP, both drug-prime and stress-primed reinstatement of morphine CPP were prevented by electrolytic lesions of the shell, but not of the core (Wang et al., 2002), suggesting that the shell can influence morphine CPP.

In addition to mediolateral gradients, rostrocaudal gradients within the accumbens may be of importance in CPP. For example, infusion of the histamine H1 receptor blocker clorpheniramine into the caudal but not rostral accumbens induced CPP (Zimmermann et

al., 1999), and the reverse was observed for muscimol (Reynolds and Berridge, 2002). Whether such gradients are pertinent for CPP for rewarding drugs has not been examined.

Of the few studies that have directly compared medial and lateral ventral striatum, manipulations of the medial shell more consistently affect CPP than do similar manipulations of the core. For instance, testosterone implants produced CPP when placed into accumbens shell, but not core (Frye et al., 2002). In the case of nicotine, CPP was prevented by intra-shell, but not intra core infusions of the D1 receptor antagonist SCH 39166, suggesting that transmission at D1 receptors in the shell is critical in mediating nicotine CPP (Spina et al., 2006). In summary, psychomotor stimulants, direct DA agonists and several other types of drugs appear to exert shell-dependent CPP. However, comparative studies of medial and lateral ventral striatum are lacking.

1.6.5 The ventral striatum in natural rewards

It is doubtful that the mesolimbic dopamine system developed to encode the motivational effects of psychoactive drugs. More likely, its intended role is to reinforce actions that lead to the consumption of natural rewards, such as food and sex. Accordingly, a rich literature has examined whether neurotransmission in NAcc core vs. shell may differentially influence such behaviours, especially in the case of feeding.

1.6.5.1 The ventral striatum in feeding

The contribution of several neurotransmitter systems in the accumbens to feeding has been extensively investigated. GABAergic signalling in the shell rather than in the core

appears to play a central role. For example, activation of either $GABA_A$ or $GABA_B$ receptors in the medial shell increased feeding behaviours (Stratford and Kelley, 1997; Basso and Kelley, 1999; Reynolds and Berridge, 2001; Reynolds and Berridge, 2002), whereas core infusions, and infusions in ventral or lateral shell, were ineffective (Stratford and Kelley, 1997; Basso and Kelley, 1999). This medial-shell dependent feeding response was blocked by $GABA_A$ and $GABA_B$ receptor antagonists, respectively, suggesting that GABA receptor activation was critical (Stratford and Kelley, 1997). GABAergic signalling appears to interact with other neurotransmitter systems to induce feeding, as feeding induced by $GABA_A$ receptor activation, but not by $GABA_B$ receptor activation, was antagonized by naltrexone (Znamensky et al., 2001). In addition, infusions of agonists for several other receptor types into the shell are sufficient to induce feeding. More specifically, intra-shell infusions of DA (Swanson et al., 1997), the μ receptor agonist DAMGO (Ragnauth et al., 2000), the δ receptor agonist deltorphin (Ragnauth et al., 2000), the cannabinoid receptor 1 agonist 2-arachidonoyl glycerol (Kirkham et al., 2002), and NMDA (Echo et al., 2001) all effectively increased feeding behaviour. In contrast, AMPA receptor activation in the accumbens shell appears to reduce feeding. For example, AMPA infusions decreased (Stratford et al., 1998), whereas infusions of the AMPA antagonist DNQX into the rostral shell (but not in the core) induced feeding (Maldonado-Irizarry et al., 1995; Reynolds and Berridge, 2003). In summary, neurotransmission in the accumbens medial shell rather than in the core appears to importantly regulate feeding behaviour.

The connections between the medial shell and both the lateral hypothalamus and the central nucleus of the amygdala (CeA) appear to regulate feeding behaviour. In the case of the lateral hypothalamus, first, infusions of the NMDA receptor antagonist AP-5 into the lateral hypothalamus reduced feeding induced by intra-shell muscimol (Stratford and Kelley, 1999). Second, inhibition of $GABA_A$ receptors in the lateral hypothalamus inhibited the feeding response elicited by intra-shell administration of the AMPA receptor antagonist DNQX (Maldonado-Irizarry et al., 1995). Third, administration of the orexigenic neuropeptide orexin A in the lateral hypothalamus both activated c-fos in the accumbens shell (Mullett et al., 2000), and induced feeding that was blocked by intra-shell naltrexone treatment (Sweet et al., 2004). Taken together, these studies suggest that the connection between the lateral hypothalamus and the medial shell is important in control of the feeding response.

In the case of the CeA, first, infusion of the μ opioid receptor agonist DAMGO into the CeA increased both feeding and c-fos expression in the shell (Levine et al., 2004), suggesting that intrinsic medial shell neurons were activated by this manipulation. Second, feeding elicited by intra-accumbens DAMGO administration was blocked by intra-CeA infusion of naltrexone (Kim et al., 2004). Finally, intra-shell muscimol induced feeding was antagonized by intra-CeA administration of muscimol, suggesting that activation of $GABA_A$ receptors in the CeA inhibits feeding induced by activation of shell $GABA_A$ receptors (Baldo et al., 2005). Taken together, these observations suggest a functional link between the shell and both the lateral hypothalamus and the CeA in the control of feeding behaviour.

In contrast to feeding behaviours, the core but not the shell appears critical in operant *responding* for food (Kelley, 1999). For example, learning of an operant task to obtain food was disrupted preferentially by infusion of AP-5 into the core rather than the shell (Kelley et al., 1997), and responding for sucrose was prevented by intra-core infusions of the muscarinic receptor antagonist scopolamine (Pratt and Kelley, 2004). In addition, infusions of either D1 or D2 antagonists into the core were more effective than shell infusions at reducing lever presses to obtain a palatable food (Nowend et al., 2001). The core also appears to mediate the anticipation of food receipt in food restricted animals, as excitotoxic core (but not shell) lesions reduced anticipatory arousal prior to food receipt in rats (Mendoza et al., 2005). Since exposure to appetitive food stimuli increased DA release in the core (Bassareo and Di Chiara, 1999), whereas consumption of palatable food increased DA in shell (Bassareo and Di Chiara, 1997), it is possible that the core subregion controls anticipation, whereas the shell controls consummatory behaviour related to food.

1.6.5.2 The ventral striatum in hedonic reactions to palatable food

Both opioid and GABA receptor activation in the shell appear important for expression of positive hedonic reactions to palatable food. For example, either morphine or muscimol infusions into the shell increased positive hedonic reactions to sucrose (Pecina and Berridge, 2000). The effect of morphine into the shell was probably a result of μ receptor activation in the rostral shell by morphine, since infusions of the μ receptor agonist DAMGO into the rostral shell increased positive hedonic reactions to the taste of sucrose

(Pecina and Berridge, 2005). A potential convergent molecular target of μ and $GABA_A$ receptors may be CREB, as its over-expression in the shell decreased sucrose preference, whereas dominant negative CREB over-expression increased sucrose preference (Barrot et al., 2002). Since increased extracellular DA levels observed in the shell upon consumption of a palatable food were blocked by systemic opioid receptor antagonism (Tanda and Di Chiara, 1998), DA transmission in the shell may modulate or enable positive hedonic reactions.

1.6.5.3. The ventral striatum in sex and mating behaviours

There is some indirect evidence that the core and shell may participate differentially in mating behaviours. For example, sensitized c-fos expression was observed in the shell, but not the core of sexually experienced male rats after exposure to females in oestrous (Lopez and Ettenberg, 2002). In contrast, exposure to a previously neutral odour paired with copulation increased c-fos expression selectively in the core (Kippin et al., 2003), suggesting different parts of the accumbens may be activated by pheromones vs. odours conditioned to sexual activity.

The core region appears to be important in promoting physical proximity between partners during sexual contact, whereas the shell may be important in monogamous partner formation. More specifically, excitotoxic lesions of the core, but not the shell, increased the probability that a female would withdraw after a mount from a male rat (Guarraci et al., 2002). In the case of partner formation, either blockade of D2 transmission or activation of D1 receptors in the rostral shell of male prairie voles

prevented the expression of aggressive behaviours towards unfamiliar females indicative of monogamous pair formation (Aragona et al., 2006). These studies suggest that physical proximity and long-term partner formation are likely mediated by separate mechanisms.

1.6.6 The ventral striatum in intracranial self-stimulation

Many drugs that can cause addiction in humans also decrease ICSS thresholds in rats (Gardner, 2002). Drug-induced decreases in ICSS thresholds may engage accumbal mechanisms, since self-stimulation of the medial forebrain bundle induced c-fos expression both core and shell (Hunt and McGregor, 1998). The threshold of responding for lateral hypothalamic stimulation is decreased by several pharmacological manipulations, including NMDA receptor antagonism, DAT blockade, and μ or δ opioid receptor activation (Carlezon, Jr. and Wise, 1996a; Johnson et al., 1995). Although in the cases of NMDA receptor antagonist or the DAT blocker only medial shell infusions were examined (Carlezon, Jr. and Wise, 1996a), these manipulations were more effective after infusion into medial shell than into the core for the opioid receptor agonists (Johnson et al., 1995). This suggests that the shell is a more important mediator of drug-induced decrease in ICSS thresholds in the lateral hypothalamus than is the core. Whether this is also true for other sites that support ICSS remains to be determined.

1.6.7 The ventral striatum in production of ultrasonic vocalizations

Although the emission of 50 kHz USVs is thought to represent positive affective state, they have not been well characterized in the context of reward-relevant processing.

Nevertheless, intra-NAcc infusion of amphetamine dose-dependently produced 50 kHz USVs (Burgdorf et al., 2001). Additionally, intra-shell amphetamine infusions produced calls more efficiently than did intra-core infusions (Burgdorf et al., 2001; Thompson et al., 2006). This effect was blocked by co-infusion of D1 or D2 receptor antagonists (Thompson et al., 2006). Although this result is suggestive of a role for DA transmission in the accumbens shell in the production of 50 kHz USVs, more studies are needed to characterize this as an index of drug reward.

1.7 Responses to salient, non-reward stimuli and the ventral striatum

It is widely accepted that administration of psychomotor stimulant drugs increases DA transmission in the ventral striatum (Schultz, 1998; Wise, 2004; Ikemoto and Wise, 2004; Di Chiara, 2005). However, DA release in the ventral striatum is also altered after exposure to salient and aversive stimuli (Horvitz, 2000). Accordingly, several investigations have examined how manipulations of core vs. shell affect behaviour in response to salient and aversive stimuli.

1.7.1 The ventral striatum in stress

As seen after exposure to rewarding stimuli, both extracellular DA levels and DA utilization are increased selectively in the shell vs. core after exposures to acute stressors such as footshock (Kalivas and Duffy, 1995; Wu et al., 1999) and restraint stress (Deutch and Cameron, 1992). Stress-induced DA release may be dependent on glucocorticoid action (Marinelli and Piazza, 2002). More specifically, these hormones selectively increased shell DA release in response to an acute mild stressor (injection stress; Barrot et

al., 2000). In contrast, both basal DA levels and drug-induced increases in extracellular DA in the shell are decreased after exposure to *chronic*, unavoidable stress. For example, basal DA levels in the shell were blunted up to 21 d after receipt of chronic footshock (Mangiavacchi et al., 2001), as was the cocaine-induced increase in extracellular DA in the shell (Gambarana et al., 1999). The blunted DA levels are accompanied by decreased DAT and increased DA D1 receptor binding sites (Scheggi et al., 2002). Taken together, these results suggest that changes in DA tone in the shell accompany acute and chronic stressful events.

The core subregion may also play a role in exposure to certain types of stressful or aversive stimuli. For example, exposure to predator odour in mice increased fos-reactive antigen immunoreactivity in the shell, and decreased it in the core (Hebb et al., 2004). Additionally, exposure to either aversive taste stimuli or predator odour increased DA levels in the core more than in the shell (Bassareo et al., 2002). This suggests that DA responses or neuronal activation in the core subregion may also importantly mediate responses to a subset of aversive or stressful stimuli.

1.7.2 The ventral striatum in pain and analgesia

The NAcc may modulate the affective component of pain stimuli. Most rodent studies examining the effects of accumbal manipulations have used the formalin test as a model for tonic pain. In this test, infusion of amphetamine directly into the accumbens produced an analgesic effect (Altier and Stewart, 1993) that was reversed by DA receptor antagonism (Altier and Stewart, 1998). Additionally, 6-OHDA lesions of the accumbens

reduced amphetamine-induced analgesia (Clarke and Franklin, 1992). However, few studies to date have examined whether discrete ventral striatal compartments contribute differentially to nociception, and existing evidence is indirect. For instance, DA transmission in the shell subregion was decreased after an acute painful stimulus (tail pinch; Di Chiara et al., 1999), whereas injection of formalin into the multifidus muscle of the lower back induced significant elevations in c-fos expression in accumbens core (Ohtori et al., 2000). Whether different aspects of nociception may be handled differently by the core vs. shell of the accumbens remains to be determined.

1.7.3 The ventral striatum in conditioned taste aversion

CTA can be induced not only by agents that produce malaise such as LiCl, but also by drugs that are self-administered (e.g. amphetamine, morphine, nicotine). Learning of a taste aversion for the malaise-inducing drug LiCl appears to be at least partially mediated by DAergic mechanisms, since LiCl CTA was strengthened by systemic amphetamine given after consumption of the LiCl-paired fluid during the conditioning phase (Fenu and Di Chiara, 2003). An important site of action may be the NAcc, since decreased DA levels were observed during expression of LiCl CTA (Mark et al., 1991). Furthermore, the shell appears to be the critical locus of action, for the following two reasons. First, both the acquisition of LiCl CTA (Fenu et al., 2001), and its potentiation by amphetamine (Fenu and Di Chiara, 2003) were prevented by intra-shell, but not intra-core, infusion of a DA D1 receptor antagonist. Second, learning of a CTA for lithium is attenuated by intra-shell, but not intra-core infusions of the NMDA receptor antagonist APV, and the $GABA_A$ receptor agonist muscimol (Ramirez-Lugo et al., 2006).

In contrast, little is known about the contribution of the accumbens to CTA for reward-relevant drugs. The brain structures contributing to this type of CTA may depend on the drug in question, as CTA was formed to intra-NAcc nicotine (Shoaib and Stolerman, 1995), but not amphetamine (Carr and White, 1986). Nicotine CTA may be DA-dependent, in that systemic administration of DA receptor antagonists prevented acquisition of nicotine CTA (Di Chiara et al., 2004). No studies had, to the best of my knowledge, examined the contribution of DA transmission in different ventral striatal subregions to CTA for reward-relevant drugs prior to work presented in this thesis (see Chapter 7).

1.7.4 The ventral striatum in conditioned place aversion (CPA)

The NAcc, and especially its shell compartment, has an established role in the processing of conditioned reward (Di Chiara et al., 2004). This may also be true of conditioned aversion. Indeed, κ opioid receptor agonists infused into the accumbens induce CPA (Bals-Kubik et al., 1993). CPA for κ agonists appears to be DA-dependent, as intra-accumbens infusion of either 6-OHDA or a D1 receptor antagonist abolished κ opioid receptor agonist-induced CPA (Shippenberg et al., 1993). Additionally, this may be dependent on the caudal shell, since CPA was induced by caudal shell infusions of the endogenous opioid ligand endomorphin-2 (Terashvili et al., 2004). However, other infusions sites were not examined in this study. Several other studies have also examined the effects of caudal shell drug infusions on induction of CPP. For example, CPA was produced by caudal shell infusions of the $GABA_A$ receptor agonist muscimol (Reynolds

and Berridge, 2002) or the AMPA/kainite receptor antagonist DNQX (Reynolds and Berridge, 2003). Here, rostral shell muscimol infusions produced CPP (Reynolds and Berridge, 2002) and core infusions of DNQX had no effect (Reynolds and Berridge, 2003), suggesting that CPA was dependent on the caudal shell. The transcription factor CREB may be critical in CPA acquisition, since CREB over-expression in the shell decreased CPA induced by the μ receptor antagonist naloxone (Barrot et al., 2002). Taken together, these results suggest that neurotransmission in the caudal shell mediates CPA.

1.7.5 The ventral striatum in conditioned fear

It is unclear whether the core or the shell plays a more prominent role in conditioned fear. Whereas exposure to contextual cues associated with footshock induced more c-fos expression in the accumbens shell than in either core or OT (Beck and Fibiger, 1995), animals sustaining excitotoxic insult of the core but not the shell failed to exhibit a conditioned freezing response to presentation of a discrete tone stimulus previously paired with footshock (Levita et al., 2002; Cassaday et al., 2005). This apparent discrepancy may represent a difference between conditioning to discrete vs. contextual cues, since extracellular DA levels were increased in the core upon exposure to aversively-conditioned *contextual* cues, whereas extracellular DA levels in the shell are increased during exposure to aversively conditioned *discrete* cues (Pezze et al., 2001). In summary, although lesion evidence suggests that the core is important in mediating conditioned responses to discrete stimuli, this does not accord with measures of neuronal activation or increases in extracellular DA. More studies are required to determine if any

important core/shell differences exist, whether they are related to cue type, and how extracellular DA levels influence the acquisition and expression of conditioned fear.

1.7.6 The ventral striatum in novelty

Reactions to novel, salient stimuli appear intimately connected with other motivational responses. In addition to rewarding or stressful stimuli, changes in DA transmission in the shell also accompany exposure to novel stimuli. The evidence for this is as follows. First, DA levels measured by cyclic voltammetry are increased in the shell subregion upon first entry into a novel environment (Rebec et al., 1997a; Rebec et al., 1997b). Second, levels of the DA metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid were increased in shell and decreased in the core after exposure to a novel environment (Noguchi et al., 2001), suggesting increased DA turnover in the shell. Finally, exposure to a pair of novel, but not familiar, juvenile rats increased extracellular DA in the shell, but not the core (De Leonibus et al., 2006). The increase in DA may be motivationally significant, since 6-OHDA lesions of the ventral striatum reduced CPP for a novel environment (Pierce et al., 1990).

Considered as a whole, the presented evidence suggests that DA transmission in the accumbens shell accompanies exposures to a wide range of novel stimuli. Taken with the evidence suggesting that shell DA transmission is also consistently changed after exposures to both rewarding and stressful stimuli, this suggests that tonic changes in extracellular DA in the shell may represent a general response to presentation of salient stimuli (see Chapter 8).

1.8 Ventral striatal mediation of behavioural sensitization

Sensitization to the locomotor stimulant effects of psychomotor stimulant drugs is a well-characterized phenomenon that is observed subsequent to repeated drug challenge. The rewarding effects of psychomotor stimulants can also sensitize with repeated exposures; this has been observed in the CPP paradigm (e.g. Lett, 1989; Shippenberg and Heidbreder, 1995a). Although the phenomenon of reward sensitization has not been well characterized, locomotor sensitization appears to depend on DA transmission in the nucleus accumbens, with contributions from both shell and core.

1.8.1 The ventral striatum in locomotor sensitization

Neurotransmission is altered in both the core and the shell during the development of locomotor sensitization. For example, repeated pre-treatment with nicotine or morphine sensitized DA release stimulated by these drugs in the core, but not the shell (Cadoni and Di Chiara, 1999; Cadoni and Di Chiara, 2000; Balfour, 2002). Interestingly, evidence suggests that increased extracellular DA release in the core may be behaviourally significant, since MDMA-induced locomotor sensitization was blocked by intra-core infusions of the D1 receptor antagonist SCH 23390 (Ramos et al., 2004). However, this study did not examine the effect of intra-shell antagonist infusions. DA is not the only neurotransmitter in the NAcc altered in response to sensitization. More specifically, glutamate levels were increased in the core but not the shell of animals sensitized to the locomotor stimulant effect of cocaine (Pierce et al., 1996a). Additionally, antagonism of non-NMDA glutamate receptors in the core blocked the augmented locomotor response

to cocaine (Pierce et al., 1996a). The glutamatergic input to the core from the dorsal prefrontal cortex may be of importance in cocaine sensitization, since ibotenic acid lesions of this area both blocked the augmented locomotor stimulation in response to cocaine, and reduced glutamate levels in the core (Pierce et al., 1998). Synaptic remodelling in the core also accompanies cocaine locomotor sensitization, as evinced by increased dendritic spine density on medium spiny neurons of the core, but not of the shell of cocaine-sensitized animals (Ferrario et al., 2005). Taken together, this evidence suggests that both sensitized neurotransmitter release and synaptic remodelling in the accumbens core accompany locomotor sensitization.

Several manipulations of the shell subregion also appear to influence both the acquisition and the expression of locomotor sensitization. For example, locomotor sensitization was observed after intra-shell, but not intra-core infusions of either cocaine (Filip and Siwanowicz, 2001) or amphetamine (Pierce and Kalivas, 1995) in rats that previously received systemic cocaine injections, suggesting that DA transmission in the shell may underlie the expression of locomotor sensitization. Serotonergic transmission in the shell may also influence this expression. More specifically, intra-shell, but not intra-core infusions of a 5-HT_{1b} receptor antagonist attenuated, whereas a 5-HT_{1b} receptor agonist increased the expression of cocaine locomotor sensitization (Przegalinski et al., 2002a; Przegalinski et al., 2002b). The shell subregion may also be important in the acquisition of locomotor sensitization, as evinced by the following two studies. First, electrolytic lesions of the shell prior to sensitizing drug treatments prevented subsequent expression of locomotor sensitization two weeks after cessation of cocaine treatment (Brenhouse et

al., 2006). Second, since neither ibotenic nor volkensin lesions after repeated cocaine treatment reduced sensitization to the locomotor effects of cocaine (Todtenkopf et al., 2002), the effect of electrolytic lesions on locomotor sensitization appears to result from disrupted acquisition of the behaviour.

Taken together, this evidence suggests that both the core and shell subregions influence sensitization to the locomotor stimulant effect of reward-relevant drugs. The relative importance of each in the acquisition vs. expression of locomotor sensitization has yet to be clarified.

1.8.2 The ventral striatum in reward sensitization

Locomotor stimulation is not the only behavioural consequence of rewarding drugs that sensitizes with repeated drug exposures. Indeed, conditioned reward has also been shown to sensitize. More specifically, repeated amphetamine, morphine or cocaine administration enhanced subsequent CPP for these drugs compared to untreated controls (Lett, 1989). The mechanisms of locomotor and reward sensitization likely differ, since μ receptor knockout mice show reduced cocaine CPP, but enhanced cocaine locomotor sensitization (Hall et al., 2004). The phenomenon of reward sensitization appears critically dependent on opioid transmission, at least in the case of psychomotor stimulants. For example, administration of a κ receptor agonist in conjunction with either cocaine or morphine during the induction of sensitization prevented the expression of enhanced cocaine, but not morphine CPP compared to non-pretreated controls (Shippenberg et al., 1996; Shippenberg et al., 1998). This appears to be centrally

mediated, since intracerebroventricular administration of the κ agonist was also effective in preventing cocaine CPP sensitization (Shippenberg et al., 1996). Systemic administration of a δ receptor antagonist prior to cocaine during pre-treatment also effectively prevented the development of sensitization to cocaine CPP (Shippenberg and Heidbreder, 1995b).

A handful of anatomical localization studies haves suggested that mesolimbic DA transmission is important in reward sensitization. For example, one injection of cocaine sensitized both CPP for morphine, and CPA for κ receptor agonists; these enhancements were blocked by intra-VTA infusions of the NMDA receptor antagonist MK-801 prior to the sensitizing cocaine treatments, suggesting that the VTA is important for the development of sensitization (Shippenberg and Heidbreder, 1995a). Additionally, the increased responding for conditioned reward observed after intra-NAcc amphetamine was potentiated in animals sensitized to cocaine (Taylor and Horger, 1999), suggesting that activation of ventral striatal DA receptors potentiated conditioned reward. However, since amphetamine infusion sites were divided evenly between shell and medial core, no conclusion can be drawn with respect to core vs. shell contributions. Studies determining the anatomical localization within the ventral striatum of reward sensitization are lacking. Additionally, it is unclear what role, if any, DA transmission plays in the establishment or expression of this behaviour.

1.9 Ventral striatal mediation of locomotor activity

1.9.1 The ventral striatum in basal locomotor activity

Although mesostriatal DA has been more clearly implicated in the control of locomotor activity than has mesolimbic DA, manipulations of the ventral striatum can affect locomotor activity. However, it is unclear whether manipulations of the core or shell subregion preferentially affect basal locomotion. For example, although infusion of either corticotrophin releasing factor (Holahan et al., 1997) or AP-5 (Pulvirenti et al., 1994) into the shell, but not the core, increased locomotor activity, infusions of DA have been effective after either core or shell infusions. More specifically, in one study, infusion of DA into the accumbens revealed a "hotspot" of locomotor mediation in the dorsomedial core/ventromedial caudate-putamen (Campbell et al., 1997). In contrast, Swanson et al. (1997) reported that shell infusions of DA were more effective in eliciting locomotor stimulation. This apparent contradiction may be reconciled by considering that the shell infusion sites in the latter study were largely localized in the dorsomedial shell, suggesting that dorsomedial NAcc, rather than core or shell, may mediate this locomotor stimulant effect. In agreement with a role for dorsomedial accumbens in locomotion, intra-core infusions of the DA D2 receptor antagonist eticlopride were more effective at decreasing basal locomotor activity than were intra-shell infusions (Boye et al., 2001). In this study, core infusions were largely localized in the dorsal part of the structure, whereas medial shell infusions were largely aimed at the ventromedial shell (Boye et al., 2001).

Lesion studies have also been inconclusive, with electrolytic or excitotoxic lesions of the shell (Weiner et al., 1996; Jongen-Relo et al., 2002) or excitotoxic lesions of the core (Parkinson et al., 1999) increasing baseline activity in a novel field. This apparent

contradiction – shell or core excitotoxic lesions increasing baseline activity – may result from different excitotoxins (NMDA vs. quinolinic acid respectively) or from different lesion extents. In contrast, 6-OHDA lesions of neither core nor shell appreciably affected basal locomotor activity (Boye et al., 2001), suggesting that DA transmission in the accumbens is not necessary for locomotor activity. Although these studies suggest that the ventral striatum can influence normal locomotor activity, this is not always the case. Additionally, a specific subregion of the ventral striatum is not clearly implicated in basal locomotion.

1.9.2 The ventral striatum in turning behaviour

Unilateral infusions of drugs acting as direct or indirect DA receptor agonists into the shell, but not the core, elicit contralateral turning behaviour. Accordingly, intra-shell infusions of amphetamine (Schildein et al., 1998; Bernstein and Beninger, 2000), morphine (Schildein et al., 1998), and a combination of direct D1 and D2 receptor agonists (Koshikawa et al., 1996a; Koshikawa et al., 1996b) produced turning when infused into the shell, but not the core. In addition, turning was prevented by co-infusion of D1 or D2 receptor antagonists in conjunction with D1 and D2 receptor agonists into the shell (Koshikawa et al., 1996a; Koshikawa et al., 1996b), suggesting that stimulation of both D1-like and D2-like receptors is necessary to produce DA-dependent turning behaviour. Further supporting a critical role of shell DA transmission, *ipsilateral* turning was induced by systemic amphetamine paired with intra-shell, but not intra-core, infusions of eticlopride (Bernstein and Beninger, 2000). Other transmitter systems may also influence DA agonist-induced turning. More specifically, DA agonist-induced

turning was prevented when co-infused with either the nicotinic antagonist mecamylamine (Moribe et al., 2005), the μ opioid receptor antagonist CTOP (Matsuzaki et al., 2004), the AMPA receptor antagonist NBQX, or the NMDA receptor antagonist dizocilpine (Ikeda et al., 2003). Taken together, these studies suggest that transmission at several receptor types in the accumbens shell interact to influence DA agonist-induced turning behaviour.

1.9.3 The ventral striatum in oral movements

As with turning behaviour, oral movements also appear to be dependent on DA receptor stimulation in the shell. For instance, infusion of a mixture of the D1 receptor agonist SKF 82958 and the D2/D3 receptor agonist quinpirole (but not the D3 receptor agonist PD 128 907; Koshikawa et al., 1996a) produced jaw movements only after infusion into the shell (Cools et al., 1995; Adachi et al., 1997). Such movements were prevented by co-administration of D1 and D2 receptor antagonists (Koshikawa et al., 1996a). Taken together, all of these studies suggest that some forms of motor activity may be dependent on DA transmission in the shell, but not the core.

1.9.4 The ventral striatum in psychomotor stimulant-induced locomotion

In contrast to turning and oral movements, the locomotor stimulant effect experienced after administration of psychomotor stimulant drugs appears dependent on transmission in core or shell, depending on the drug and the experimental method used. This is illustrated clearly for amphetamine. First, direct infusion of amphetamine into core, medial shell or medial tubercle elicited similar levels of locomotor activity and rearing

(Ikemoto, 2002). It is unlikely that this was a result of drug diffusion, since a small infusion volume was used, a threshold dose of amphetamine was infused, and locomotor activation was examined at early time points after drug infusion. Second, rats sustaining excitotoxic lesions of the core exhibited hyperactivity (Parkinson et al., 1999), whereas electrolytic *shell* lesioned rats exhibited hyperactivity compared to sham-lesioned controls after amphetamine administration (Weiner et al., 1996). However, excitotoxic core and electrolytic shell lesions also increased locomotor activity after saline administration compared to sham-lesioned animals, hence complicating the interpretation of lesion effects on locomotor stimulation. Third, 6-OHDA infusion into core more effectively reduced the locomotor stimulant effect of amphetamine than did intra-shell infusions (Boye et al., 2001). Taken together, these studies leave no clear picture as to which structure may be a more important mediator of amphetamine-induced locomotor stimulation.

In the case of cocaine, both core and shell sites may contribute to the locomotor stimulant effect of the drug. Microinjection of cocaine elicited an increase in locomotor activity after infusion into medial shell (Filip and Siwanowicz, 2001; Ikemoto, 2002) or tubercle (Ikemoto, 2002), but not core (Filip and Siwanowicz, 2001; Ikemoto, 2002). Importantly, this may reflect the greater susceptibility of core neurons to local anaesthesia, as procaine infusions into the core but not the medial shell or medial OT reduced spontaneous locomotor activity (Ikemoto and Witkin, 2003). In support of a role for the core in cocaine-induced locomotor activity, c-fos expression in the core correlated positively with cocaine-induced locomotor stimulation (Szucs et al., 2005). Cocaine can exert

several pharmacological actions, and it is unlikely that cocaine acts solely through DA transmission to exert locomotor stimulation. More specifically, alteration of glutamatergic transmission in the core or serotonergic transmission in the shell can alter cocaine-induced locomotor activity. More specifically, intra-core infusion of the NMDA receptor antagonist AP-5 prevented cocaine-stimulated locomotion (Pulvirenti et al., 1994). In addition, cocaine-stimulated locomotor activity was reduced by intra-shell infusions of a 5-HT_4 receptor partial agonist or a 5-HT_{2c} receptor antagonist (McMahon and Cunningham, 1999; McMahon et al., 2001), and increased by a serotonin-selective reuptake inhibitor (Bubar et al., 2003). Taken together, these results suggest that action on 5-HT receptors in the shell, or NMDA receptors in the core, can influence cocaine-stimulated locomotion. What role accumbal DA transmission in the core or the shell plays in mediating the locomotor stimulant effects of cocaine remains unknown.

It is unclear whether DA transmission in core, shell or olfactory tubercle is more effective in producing locomotor stimulation. Supporting a role for the medial shell, co-infusion of the D1 receptor agonist SKF 38393 alone (Pierce et al., 1996b; Swanson et al., 1997) elicited locomotor activation more effectively after intra-shell than intra-core infusions. In another study, co-infusion of SKF 38393 and quinpirole into the medial shell or medial tubercle, but not the core, elicited locomotor activity and rearing (Ikemoto, 2002). Supporting a role for core DA transmission, infusion of the DA receptor antagonist eticlopride into core but not medial shell reduced the locomotor stimulant effect of both nicotine and amphetamine (Boye et al., 2001). However, the antagonist alone reduced locomotor activity after saline administration. Additionally, infusion of the mixed D2/D3

receptor agonist 7-OH-DPAT into the core elicited locomotor activity (Barik and de Beaurepaire, 2005), whereas shell infusions were without effect (Koshikawa et al., 1996a). In summary, it is unclear whether DA transmission in one ventral striatal subregion is of primary importance in mediating locomotor activity. Critical factors may include the drug examined and the experimental methods employed.

1.9.5 The ventral striatum in conditioned locomotor activity (CLMA)

Conditioned locomotor activity is a well-documented phenomenon following treatment with psychostimulant drugs (Brown and Fibiger, 1992). As with CPP, conditioned increases in activity upon contact with drug-associated cues likely represent anticipation of drug receipt (Beninger and Hahn, 1983; Vezina and Stewart, 1987; Gold et al., 1988) that appears dependent on accumbens DA transmission (e.g. Beninger and Hahn, 1983; Gold et al., 1988; see Section 1.4.1 for more detailed discussion). However, studies examining the relative contribution of core and shell to CLMA are lacking. More specifically, no previous published studies had, to my knowledge, examined potential differential mediation of the conditioned activity by discrete ventral striatal subregions prior to the work presented in this thesis (see Chapter 4).

Statement of Purpose

The general objective of this thesis was to examine the contributions of discrete ventral striatal subregions, namely the accumbens medial shell, the accumbens core, and the anteromedial olfactory tubercle, to several behavioural aspects of acute psychomotor stimulant administration.

To this end, the **first specific objective** examined the effects of core vs. medial shell DA denervation on amphetamine-induced locomotor stimulation and CPP. Although unilateral infusions of on amphetamine into the shell had been shown to produce CPP, no comparison had been made with the core (Chevrette et al., 2002). Additionally, differential mediation of behavioural effects of systemically-administered amphetamine by core vs. medial shell had been examined only for locomotor activity, with conflicting results (Weiner et al., 1996; Parkinson et al., 1999; Boye et al., 2001). We hypothesized that 6-OHDA infusion into the core would affect amphetamine's stimulant effect, while medial shell infusions would likely decrease amphetamine CPP.

The **second specific objective** arose out of an interest in identifying a potential anatomical locus where DA transmission underlies conditioned drug effects. If such a region existed, it could represent a target to prevent cue-induced relapse. As such, the aim was to determine if the functional segregation observed in the first objective resulted from a difference between locomotion and reward, or conditioned and unconditioned drug effects. Hence, rats sustaining 6-OHDA lesions of the medial shell or the core were examined for amphetamine-conditioned and unconditioned activity. Since core DA

transmission can mediate certain conditioned associations (e.g. conditioned reinforcement), we hypothesized that our initial interpretation (i.e. segregation of locomotion vs. reward) was more plausible.

The **final goal** was to investigate if the functional segregation observed for amphetamine generalized to other psychomotor stimulant drugs. The first drug examined was cocaine. It became clear, however, during the course of experimentation, that the anteromedial portion of the olfactory tubercle was also of potential importance in mediating cocaine ICSA and CPP (Ikemoto, 2003). Hence, initial experiments examined core vs. medial shell mediation of both i.p. and i.v. cocaine CPP and locomotor stimulation, whereas a further experiment directly compared medial shell and anteromedial olfactory tubercle in i.v. cocaine CPP.

The second drug examined was methylphenidate (Ritalin^{TM}), a stimulant structurally related to amphetamine used in the treatment of attention deficit hyperactivity disorder. First, it was determined that blockade of DA transmission prevented i.v. methylphenidate CPP and locomotor activity. This established that both conditioned i.v. methylphenidate reward and locomotor stimulation were dependent on DA transmission, and laid the foundation for subsequent lesion experiments. Second, the contributions of core vs. medial shell DA transmission to i.v. methylphenidate CPP and locomotor activity were examined. Finally, since neither subregion clearly mediated CPP, the hypothesis that the anteromedial OT may contribute to i.v. methylphenidate CPP was investigated.

The final drug examined was nicotine. The intravenous route of administration was chosen, since this more closely models the pharmacokinetics of cigarette smoking. Since the nicotine dose chosen did not elicit appreciable locomotor stimulation, only nicotine CPP was examined. Unexpectedly, DA-depleting lesions of the accumbens core were found to increase nicotine CPP. Nicotine is known to produce aversive effects, suggesting core lesions may have reduced this component of nicotine's action. Accordingly, lesion effects on nicotine aversion were directly examined in the CTA paradigm.

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CHAPTER 2: Technical summary

Laurie H. L. Sellings

2.1 Conditioned place preference

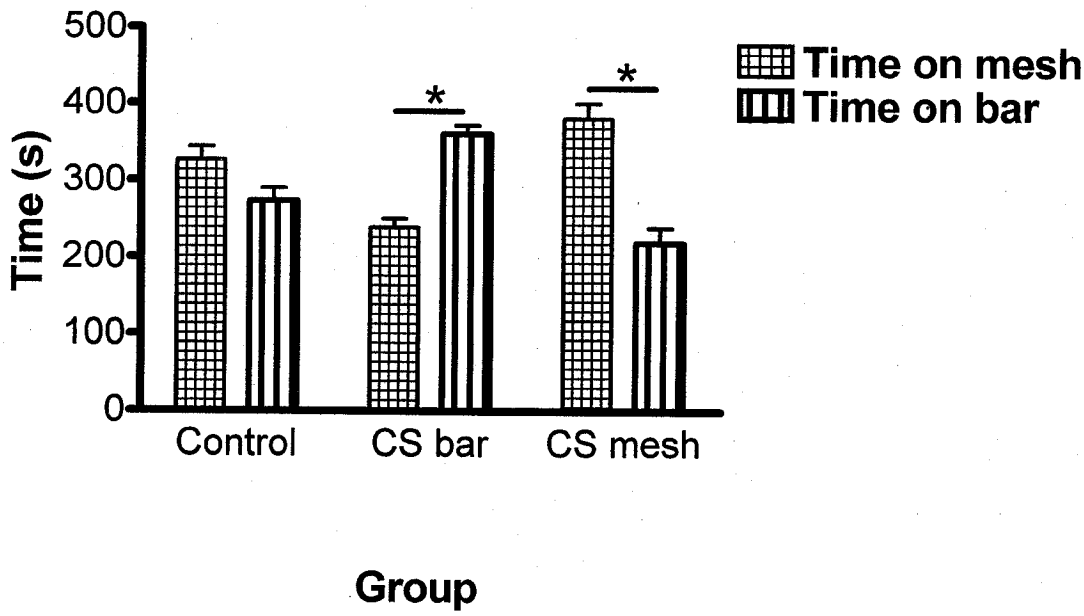
The CPP paradigm used in this thesis was unbiased. This was shown in an initial pilot study, in which animals conditioned with saline on both textures did not significantly prefer either texture. In addition, amphetamine was conditionable to either texture (Figure 1). Details of the CPP paradigm are illustrated in Figure 2A.

In this CPP paradigm, the CS+ and CS- were represented only by tactile cues. Since all testing was done under far red wavelength (650 nm) light, the impact of the visual cues was negligible, although subtle olfactory differences between the two textures cannot be fully ruled out. CPP can measure both conditioned approach behaviour and conditioned reward. The first of these measures the motivation of the animal to approach the cues previously paired with drug. The latter measures the tendency of the animal to remain in contact with the drug-paired cues. Since no distal visual cues were available in the CPP paradigm used in the present thesis, conditioned approach behaviour is probably not responsible for the observed CPP. As such, this paradigm is likely measuring pure conditioned drug reward.

2.2 Conditioned activity

The CLMA paradigm used in the present thesis represents a modification from what is normally observed in the literature, in order to make the CLMA configuration more comparable to the CPP studies. Instead of one group of animals receiving drug injections in the novel environment, and a second group receiving home cage injections, all animals received amphetamine injections on one novel texture (CS+; bar or mesh), and saline

Figure 1. Rats express CPP for amphetamine in an unbiased paradigm. Rats were conditioned in one of three groups: those receiving saline on both textures (control, n=8), those receiving amphetamine on bar texture (CS+ bar, n=8), and those receiving amphetamine on mesh texture (CS+ mesh, n=8). Rats conditioned with saline on both textures did not significantly prefer either bar or mesh ($p>0.05$; one-sample t-test with Bonferroni correction). Rats expressed a preference for the drug-paired texture, regardless of whether drug was previously paired with bar or mesh ($p<0.001$ for both; paired t-test with Bonferroni correction).



injections on a second novel texture (CS-; mesh or bar). The difference in locomotor activity observed drug-free on test day in each of these contexts was calculated, and represented conditioned locomotor activity. The paradigm used in this thesis is illustrated in Figure 2B.

2.3 Conditioned taste aversion (CTA)

The CTA paradigm used in this thesis represented a modification of a previously published protocol (Laviolette et al., 2002). In this CTA paradigm, two novel unsweetened flavours (unsweetened cherry and grape Kool-aid™), neither of which was spontaneously preferred, served as conditioned taste stimuli (Figure 3). This allowed circumvention of a common problem in CTA – namely, the spontaneous preference or avoidance of one of the CS flavours. The details of the paradigm are described in Figure 3.

2.4 6-hydroxydopamine mini-lesion technique

The approach used to examine the contributions of DA transmission in discrete ventral striatal subregions to psychomotor stimulant-induced behaviours was a 6-hydroxydopamine mini-lesion technique. This required selective lesioning of small, adjacent structures. In order to do this successfully, minimizing the diffusion of the toxin to subregions adjacent to the target site was essential. A commonly infused dose of 6-OHDA used to lesion the accumbens is 8 μg of toxin infused in 2 μl of infusate (e.g. Spyraki et al., 1982; Spyraki et al., 1983; Powell et al., 2003); most studies use similar

Figure 2. Illustration of the conditioned place preference (CPP) and conditioned activity (CLMA) paradigms used. A) CPP consisted of three phases (pre-exposure, conditioning and test) and took place over eight consecutive days. In the pre-exposure phase (day 1), which served to habituate animals to the testing procedure, rats received saline injection prior to placement into the CPP cage in the absence of tactile cues. The conditioning phase lasted six days (days 2-7). On alternate days, rats received three pairings between the CS+ texture and drug, and three pairings between the CS- texture and saline on alternate days. Experiments were as fully counterbalanced as possible, in that half of the animals received drug on bar, and half on mesh texture. Additionally, half the rats received drug on conditioning days 1, 3 and 5, and the other half on days 2, 4 and 6. Locomotor activity was also tracked during the conditioning phase. On the drug-free test day (day 8), rats were placed into the CPP cage, which contained one bar and one mesh tile. The amount of time rats spent on either texture was tracked by a commercial tracking system. B) CLMA testing also consisted of three phases (pre-exposure, conditioning and test). The pre-exposure phase (day 1) was identical to that described for CPP. The conditioning phase (days 2-11) consisted of five drug CS+ pairings, and five saline CS- pairings, on alternate days. As with CPP, all experiments were as fully counterbalanced as possible with respect to drug-texture pairing and the order of drug administration. The test phase (day 12) consisted of two separate sessions of ten minutes each. In one session, the bottom of the page was covered with bar tiles in the other session with mesh. Textures were presented in a counterbalanced order. The two test sessions were separated by two hours.

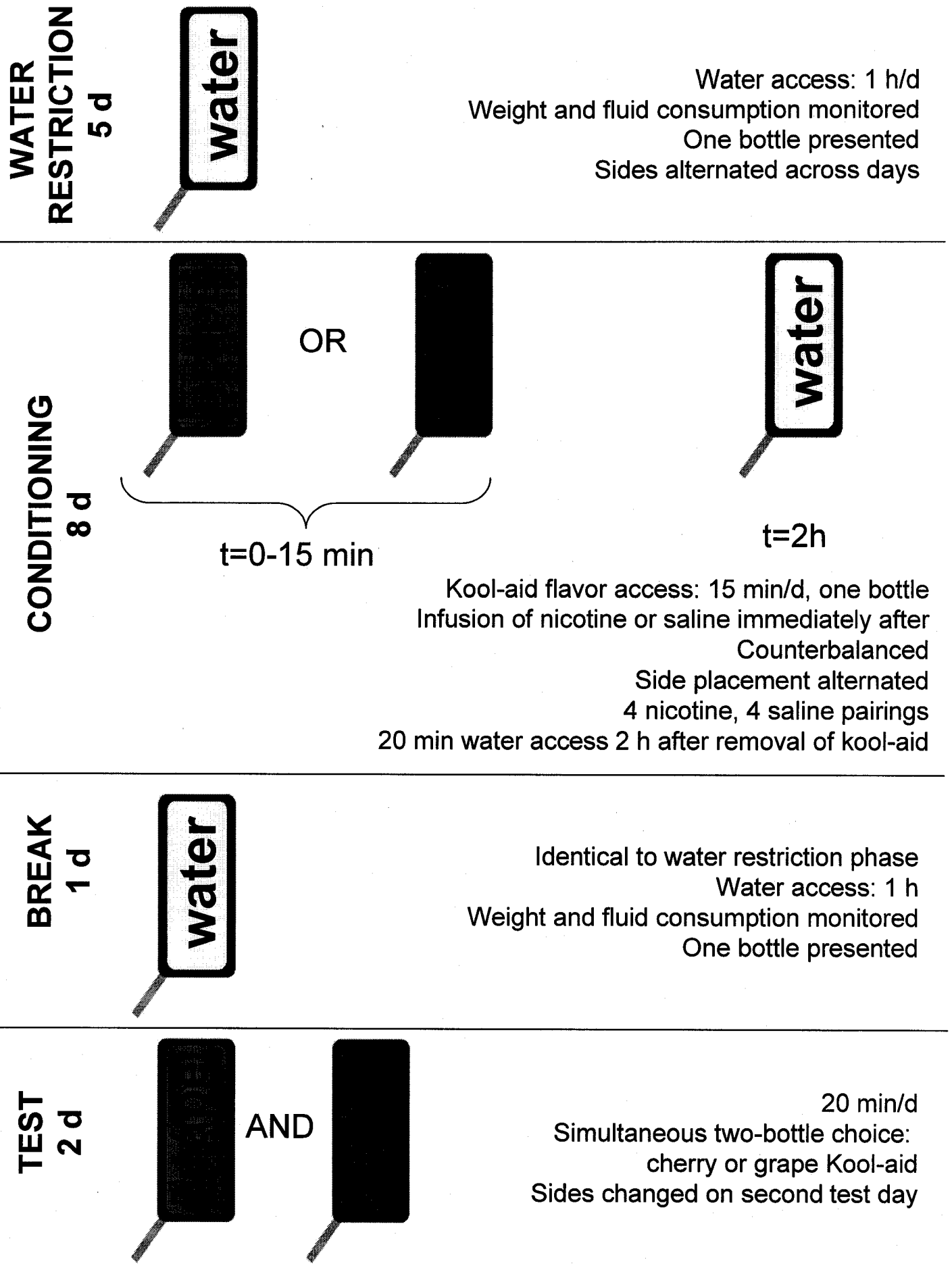
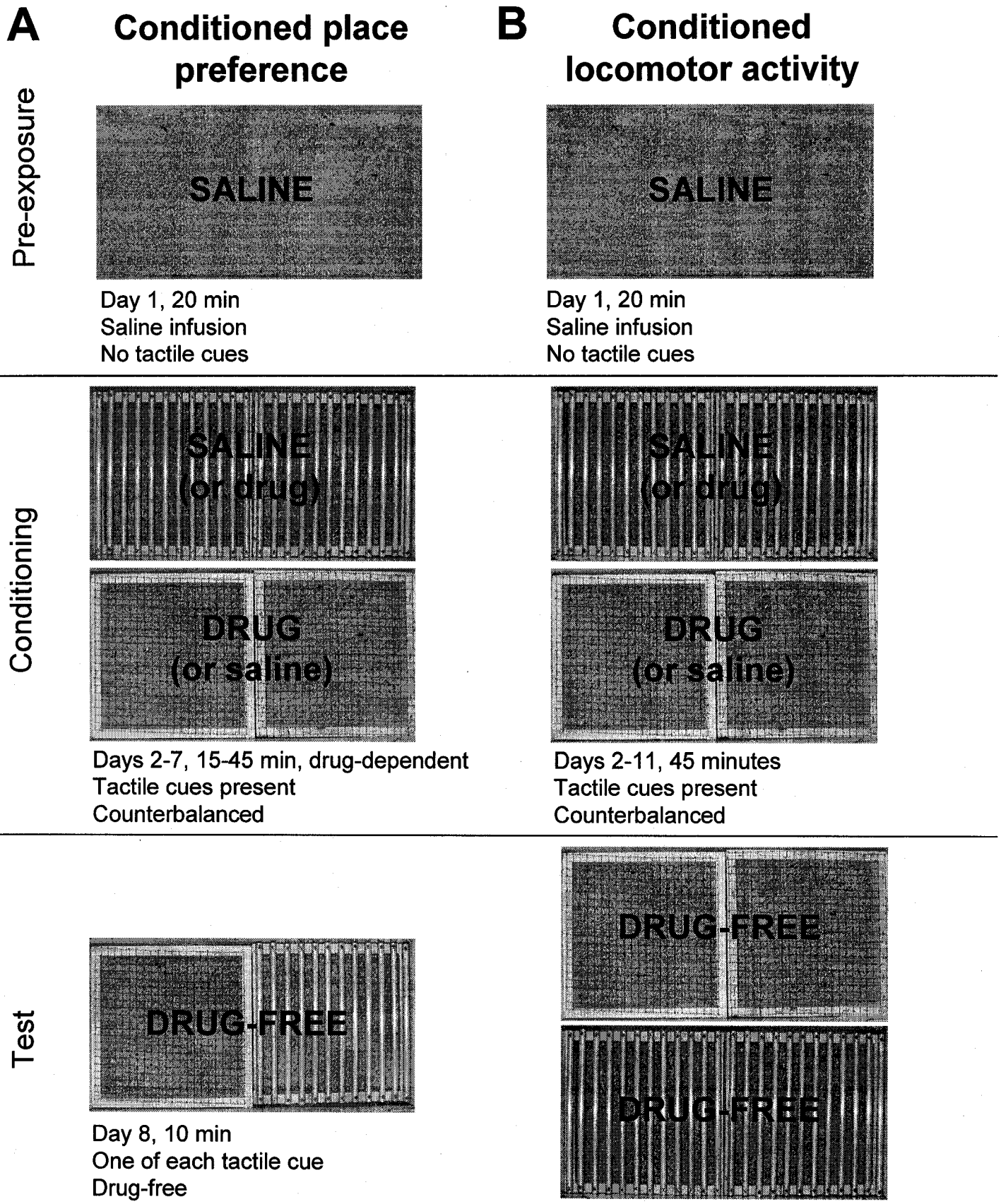

WATER RESTRICTION 5 d
water
Water access: 1 h/d
Weight and fluid consumption monitored
One bottle presented
Sides alternated across days
CONDITIONING 8 d
OR
water
t=0-15 min
t=2h
Kool-aid flavor access: 15 min/d, one bottle
Infusion of nicotine or saline immediately after
Counterbalanced
Side placement alternated
4 nicotine, 4 saline pairings
20 min water access 2 h after removal of kool-aid
BREAK 1 d
water
Identical to water restriction phase
Water access: 1 h
Weight and fluid consumption monitored
One bottle presented
TEST 2 d
AND
20 min/d
Simultaneous two-bottle choice:
cherry or grape Kool-aid
Sides changed on second test day

Figure 3. Illustration of the balanced conditioned taste aversion paradigm used. Conditioned taste aversion testing consisted of four phases: water restriction, conditioning, break and test, occurring on 16 consecutive days. During the water restriction phase (5 d), rats were permitted access to ad libitum water for 1 h/d. During conditioning (8 d), rats were exposed to one of two novel flavours (unsweetened cherry or grape Kool-Aid) for fifteen minutes, and received an intravenous injection of nicotine or saline immediately after. Both the Kool-Aid flavour paired with nicotine injection and the order of drug administration were fully counterbalanced. Two hours post-injection, rats were allowed 15 minutes access to water, to ensure adequate fluid consumption. During the break phase (1 d), rats were allowed 1 h access to water (as in the water restriction phase). During the test phase (2 d), rats had 20 min access to both cherry and grape Kool-Aid in a two bottle choice paradigm. The position of the bottles was reversed on the second day, to account for any effect of cage side preference. The volume of each cherry and grape fluid consumed on both days was measured, and a mean over the two days was taken. All four phases were performed in the home cage.


A
Conditioned place preference
B
Conditioned locomotor activity
Pre-exposure
SALINE
SALINE
Day 1, 20 min
Saline infusion
No tactile cues
Day 1, 20 min
Saline infusion
No tactile cues
Conditioning
SALINE (or drug)
DRUG (or saline)
SALINE (or drug)
DRUG (or saline)
Days 2-7, 15-45 min, drug-dependent
Tactile cues present
Counterbalanced
Days 2-11, 45 minutes
Tactile cues present
Counterbalanced
Test
DRUG-FREE
DRUG-FREE
DRUG-FREE
Day 8, 10 min
One of each tactile cue
Drug-free
Day 12, 10 min each
Separated by 2 h
Drug-free

Textures were presented in a counterbalanced order. The two test sessions were separated by two hours. Therefore, the amount of toxin commonly infused to lesion the NAcc, and the quantity used in the mini-lesions used in this thesis are approximately equivalent.

Since a high concentration of 6-OHDA was infused, there is a possibility for non-specific damage to occur that is greater in magnitude than after a conventional 6-OHDA lesion. However, this seems not to be the case, for the following two reasons. First, Nissl staining revealed only slight non-selective damage in a subset of medial shell- and medial olfactory tubercle-lesioned animals (see Chapters 3 and 5). Second, SERT autoradiography revealed only minimal changes in lesioned vs. control animals (see Chapters 3-7). When analyzed, none of these changes were statistically significant (see Chapter 5).

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CHAPTER 3: Segregation of amphetamine reward and locomotor activation between nucleus accumbens medial shell and core

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J Neurosci, **23**: 6295-6303

ABSTRACT

Convergent evidence suggests that amphetamine (AMPH) exerts its rewarding and locomotor stimulating effects via release of dopamine in the nucleus accumbens. However, there is no consensus as to the relative contributions of core and medial shell subregions to these effects. Moreover, the literature is based primarily on intracranial administration, which cannot fully mimic the drug distribution achieved by systemic administration. In the present study, the effects of bilateral 6-hydroxydopamine lesions of the accumbens core or medial shell on rewarding and locomotor stimulating effects of systemically administered amphetamine (0.75 mg/kg, i.p.) were examined in a conditioned place preference (CPP) procedure relying solely on tactile cues (floor texture). Residual dopamine innervation was quantified by [^{125}I]-RTI-55 binding to the dopamine transporter. When lesions were performed before the conditioning phase, AMPH-induced locomotor stimulation and CPP magnitude were positively correlated with residual dopamine transporter binding in core and medial shell, respectively. Medial shell lesions did not affect morphine CPP, arguing that a sensory or mnemonic deficit was not responsible for the lesion-induced reduction in AMPH CPP. Medial shell lesions performed between the conditioning phase and the test day reduced the expression of amphetamine CPP. These results suggest that after systemic amphetamine administration, rewarding and locomotor stimulating effects of the drug are anatomically dissociated within the nucleus accumbens: the medial shell contributes to rewarding effects, whereas the core contributes to behavioral activation.

Key words: nucleus accumbens core; nucleus accumbens medial shell; amphetamine; 6-hydroxydopamine; locomotion; reward; conditioned place preference; morphine

INTRODUCTION

Convergent evidence suggests that the rewarding and behavioral activating effects of psychomotor stimulant drugs are initiated by increased dopaminergic transmission in the nucleus accumbens (NAcc). Evidence is perhaps strongest for the prototypic psychomotor stimulant, amphetamine (AMPH). For example, the locomotor stimulant effect of systemic AMPH is mimicked by intra-accumbens infusion of AMPH or dopamine (DA) (Pijnenburg et al., 1976; Campbell et al., 1997) and is inhibited by intraaccumbens administration of DA antagonists (Pijnenburg et al., 1975; Roberts et al., 1975; Phillips et al., 1994) or 6-hydroxydopamine (6-OHDA) (Kelly et al., 1975; Joyce et al., 1983; Clarke et al., 1988). Similarly, the rewarding effects of AMPH are either mimicked or inhibited by the same types of manipulations (Yokel and Wise, 1976; Lyness et al., 1979; Spyraki et al., 1982; Carr and White, 1991; Phillips et al., 1994; Izzo et al., 2001).

The NAcc is a heterogeneous structure, as evinced by immunohistochemical staining and neuronal projection patterns (Zahm and Brog, 1992). The major subdivisions are a medioventral shell and a dorsolateral core. These subregions are functionally distinct (Maldonado-Irizarry and Kelley, 1995; Weiner et al., 1996; Kelley et al., 1997; Parkinson et al., 1999; Boye et al., 2001; Ikemoto, 2002), but their precise roles in reward and locomotor activation are uncertain. For example, the locomotor stimulant effect of AMPH has been attributed to an action in the core (Weiner et al., 1996; West et al., 1999; Boye et al., 2001) or in medial shell (Heidbreder and Feldon, 1998; Parkinson et al., 1999) or in both structures (Pierce and Kalivas, 1995; Ikemoto, 2002). In contrast, certain dopaminergic drugs have been shown to maintain responding when infused into (medial)

shell but not core (Carlezon and Wise, 1996; Ikemoto et al., 1997). Intra-shell self-administration of AMPH has also been reported (Hoebel et al., 1983; Chevrette et al., 2002), but in these studies intra-core infusions were not examined.

A feature of almost all the behavioral studies using AMPH was that the drug was given directly into the NAcc; after intracranial administration, drug distribution and local concentration differ markedly from that achieved after systemic administration. Recently, we combined systemic AMPH administration with 6-OHDA lesions and found that locomotor stimulation was blunted by dopaminergic denervation of core and not medial shell (Boye et al., 2001).

The present study aimed to establish the relative involvement of NAcc core and medial shell subregions in systemic AMPH-induced behavioral activation and reward. Rats that had sustained 6-OHDA lesions of NAcc core or medial shell were assessed for AMPH-induced locomotor activation and conditioned place preference (CPP). To assess the possibility that decreased CPP indicated a deficit not in reward but in learning, memory, or sensory function, morphine CPP was also tested.

MATERIALS AND METHODS

Subjects

Subjects were 142 male Long–Evans rats (Charles River, St. Constant, Quebec) weighing 270–310 gm at time of surgery. Rats were housed in groups of three in clear Plexiglas cages in a temperature- and humidity-controlled animal colony that was lit from 7 A.M. to 7 P.M. Food and water were available *ad libitum* except during behavioral testing. All

experiments were approved by the McGill Faculty of Medicine Animal Care Committee in accordance with Canadian Council on Animal Care guidelines.

Stereotaxic infusion of 6-OHDA

Rats were anesthetized with ketamine HCl (90 mg/kg, i.p.) and xylazine HCl (16 mg/kg, i.p.) 15 min after pretreatment with atropine methyl nitrate (0.05 mg/kg, s.c.). The rat was placed in a stereotaxic apparatus (Kopf, Tujunga, CA) with the incisor bar set at -3.9 mm. Rats received bilateral infusions of either 6-OHDA or vehicle into either NAcc core or medial shell. Infusions were made via a 30 gauge stainless steel cannula attached by polyethylene tubing to a 10 μl Hamilton syringe driven by a model 5000 Micro Injection Unit (Kopf). For greater accuracy, coordinates for both the core and the medial shell were derived from the mean of two coordinate systems. Thus, anterior-posterior coordinates were +10.3 mm from interaural zero and +1.3 mm from bregma for both core and shell. Lateral coordinates were ±0.6 mm (shell) and ±2.4 mm (core). Ventral coordinates for shell (three injections) were +2.0, +2.4, and +2.8 mm from interaural zero and -8.0, -7.6, and -7.2 mm from bregma. Ventral coordinates for core were +2.9 mm from interaural zero and -7.1 mm from bregma. All coordinates are based on the atlas of Paxinos and Watson (1997). 6-OHDA or vehicle was infused on each side in a volume of 0.1 μl (core) or as three infusions of 0.06 μl each (shell) at a rate of 0.1 μl/min. The concentration of 6-OHDA used was 80 μg/μl (core) or 48 μg/μl (shell). The cannula remained at the final infusion site for 5 min. Dipyrone (100 mg/kg, s.c.) provided analgesia after surgery. Animals were allowed 7–11 d recovery before conditioning (experiments 1 and 3) or testing (experiment 2). Four animals died after surgery in experiment 3.

Conditioned place preference testing

General procedure. The method was modified from that of Vezina and Stewart (1987). Eight CPP cages [58.1 cm (length) x 28.8 cm (width) x 53.0 cm (height)] were used, each comprising four outer walls made of white plastic-coated particle board (Melamine) and an open top. Cages sat on linoleum flooring covered with a thin layer of Beta Chip bedding. There was no wall dividing the cage into two compartments. Two removable square floor tiles [28.5 cm (length) x 28.5 cm (width) x 5.5 cm (height)] were inserted into each cage; these served as tactile cues. Floor tiles were of two types: mesh and bar. These two textures were provided, respectively, by a stainless steel grid with squares of 1 x 1 cm and by 12 stainless steel bars of 1.2 cm diameter separated by 1.5 cm edge to edge. Both floor types were mounted on square Melamine frames. All behavioral testing was performed in a room lit with a Kodak GBX-2 safelight filter (Vistek, Toronto, Ontario, Canada) providing far-red illumination (wavelength >650 nm) to minimize visual cues. The location and movements of rats during behavioral testing were monitored by a closed circuit television video camera (Panasonic) linked to a commercial tracking system (EthoVision v3.0, Noldus Information Technology, Leesburg, VA).

Behavioral testing took place over 8 consecutive days and consisted of three phases: preexposure, conditioning, and testing. During all three phases, animals were habituated to the test room in home cages for 15 min before placement into test CPP cages. The preexposure phase served to habituate each animal to the CPP cage itself. This phase comprised a single 20 min session performed in the absence of floor tiles. The conditioning phase took place on days 2–7. It comprised six daily sessions of 45 min each: three sessions with drug and three sessions with saline administration. Drug and

saline were administered on alternating days. After injection, each rat was immediately placed in the middle of a CPP cage. During the conditioning trials, rats had access to the entire cage, which provided a single tactile floor cue (either two mesh tiles or two bar tiles). On the day immediately after the final conditioning trial, a single 10 min test session was given. Here, the CPP cages contained one bar tile and one mesh tile. Animals in a drug-free state were placed in the middle of the cage and given free choice between the half of their cage with the bar texture and that with the mesh texture. Before a new test or conditioning session was started, half of the soiled Beta Chip was removed and replaced with new bedding, and the cage walls and tiles were wiped with 40% ethanol and allowed to dry. Groups of animals were counterbalanced as fully as possible, not only with respect to the texture that was paired with drug but also with respect to the position of that texture within the test cage on test day and the order of drug versus saline administration during conditioning.

On the test day, the time spent on each side of the apparatus was recorded. The location of a rat was defined as its center, as determined by the tracking system. During conditioning trials, locomotor activity was recorded as total horizontal distance moved. All testing was done between 8:30 A.M. and 5:30 P.M. A pilot study in which rats received saline paired with both floor textures showed that rats had no significant preference for either texture on test day (our unpublished observations). Thus the procedure can be considered unbiased.

Experimental procedures. In experiment 1, rats received bilateral infusion of 6-OHDA or vehicle into either core or medial shell 7–11 d before preexposure. Rats were then

conditioned with 0.75 mg/kg AMPH intraperitoneally. In experiment 2, rats received bilateral 6-OHDA or vehicle infusions into medial shell. Half of the rats in each surgery group received 0.75 mg/kg AMPH intraperitoneally; the other half were conditioned with 10 mg/kg morphine intraperitoneally. Experiment 3 is similar in design to experiment 1 except that rats underwent stereotaxic infusion surgery after conditioning but before testing (Figure 1).

Quantitative [^{125}I]RTI-55 autoradiography

The extent of the 6-OHDA lesion was quantified by autoradiographic labeling of the plasmalemmal DA transporter (DAT) using a nonsaturating concentration of [^{125}I]RTI-55 (2200 Ci/mmol; NEN-Mandel, Guelph, Ontario), because it has been shown previously that percentage loss of DAT accurately represents tissue DA loss (Joyce, 1991a,1991b). [^{125}I]RTI-55 binds selectively to DAT provided the serotonin transporter (SERT) is inhibited (Boja et al., 1992; Coulter et al., 1995). Conversely, SERT can be selectively labeled via occlusion of DAT (Pradhan et al., 2002). The day after CPP testing, rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and decapitated. Brains were removed rapidly and frozen in 2-methylbutane at -50°C for 30 sec and stored at -40°C. Coronal sections (20 μm) were taken on a cryostat at four rostrocaudal levels through the nucleus accumbens: +11.2, +10.7, +10.2, and + 9.7 mm anterior to interaural zero (Paxinos and Watson, 1997). At each level, five adjacent sections were collected: four for autoradiography and one for Nissl staining with cresyl violet. Sections were thaw mounted onto gelatin-subbed slides, air dried at room temperature for 20–30 min, and stored with desiccant at -40°C.

Sections were thawed at room temperature for 10 min and then placed in a staining dish containing an aqueous buffer solution of 120 mM NaCl, 0.1 M sucrose, 10 mM sodium phosphate buffer, and 10 pM [^{125}I]RTI-55. To assay for DAT binding, 50 nM citalopram hydrobromide was used to occlude SERT; nonspecific binding was determined by addition of 10 μM GBR 12909. To measure SERT binding, 1 μM GBR 12935·2HCl was added to occlude DAT; nonspecific binding was determined by addition of 50 nM citalopram HBr (Pradhan et al., 2002). Slides were incubated at room temperature for 2 hr and then washed three times in cold buffer solution (once for 1 min, twice for 20 min) and for 1 sec in distilled and deionized water. They were then blow dried and placed in an x-ray film cassette. Kodak BioMax MS film (Amersham Biosciences, Baie d'Urfé, Québec) was exposed to slides for 48 hr (DAT) or 120 hr (SERT) with [^{125}I] autoradiographic standards (Amersham Biosciences). Films were then processed with Kodak D19 developer and Kodak GBX fixer (Amersham Biosciences). DAT and SERT binding were quantified using an MCID M4 imaging system (Imaging Research, St. Catherines, Ontario).

Histological examination

Tissue was stained with cresyl violet to assess nonspecific damage, as follows. Sections were thawed at room temperature for 10 min and then placed in 0.5% cresyl violet (Sigma-Aldrich, Oakville, Ontario) in distilled water for 20 min. They were rinsed in 95% ethanol twice for 2 min and then in 100% ethanol three times for 15 sec and were dehydrated in xylene three times for 5 min. Slides were coverslipped with Permount and examined under a light microscope (40–200 x magnification).

Drugs

Drug sources were as follows: morphine sulfate (gift from Sabex 2002 Inc., Boucherville, Quebec); D-amphetamine sulfate (Bureau of Drug Research, Ottawa, Ontario); citalopram HBr (gift from H. Lundbeck A/S); dipyrone (Vetoquinol, Quebec, Quebec); ketamine HCl (Vetalar, Vetrepharm, London, Ontario); xylazine HCl (Anased, Novopharm, Toronto, Ontario); atropine methyl nitrate, 6-OHDA HBr, GBR 12909, and GBR 12935·2HCl (Sigma-Aldrich, Oakville, Ontario). All other chemicals were obtained from Fisher Scientific (Montreal, Quebec).

Morphine sulfate and D-amphetamine sulfate were dissolved in sterile 0.9% saline and injected at 1 ml/kg. 6-OHDA HBr was dissolved in sterile 0.9% saline containing 0.3 mg/ml sodium metabisulfite (Sigma-Aldrich) as an antioxidant and protected from light. Both 6-OHDA and vehicle solutions were made to pH 7.3 ± 0.1 with NaOH. Doses of all drugs except 6-OHDA HBr are expressed as the salt. 6-OHDA HBr doses are expressed as free base.

Data analysis

A commercial software program (Systat v10.2, SPSS Inc., Chicago, IL) was used for all data analyses. Locomotor response to AMPH was calculated as the difference of locomotor counts between AMPH and saline conditioning sessions; baseline saline scores were calculated as the mean activity over all three conditioning sessions with saline on test day. CPP magnitude was calculated as the difference between time spent on the drug-paired and vehicle-paired sides. The relationship between locomotor and reward measures versus [^{125}I]-RTI-55 labeling was analyzed by multiple linear regression (experiments 1

and 3) or Mann–Whitney *U* test (experiment 2). Activity scores (experiment 1) were analyzed by ANOVA. A p value of <0.05 (two-tailed) was considered significant.

RESULTS

Histological and autoradiographic characterization of lesions

Minimal neuronal loss was evident at the site of injection in both vehicle groups and in the core lesioned group in all three experiments. A representative coronal section of the medial shell vehicle-infused group is shown in Figure 2A. In the medial shell lesioned group, tissue damage was more extensive but was nevertheless confined to 0.3 mm from the infusion site, sparing most of the structure (Figure 2B).

[^{125}I]RTI-55 autoradiographs of DAT binding are shown in Figure 3 at four anterior-posterior levels. Sampling locations for DAT binding density are indicated in Figure 4. Absolute values for [^{125}I]RTI-55 binding to DAT and SERT are given in Tables 1 and 2. In all experiments, core lesions were less anatomically selective than shell lesions (Figure 5). Pooled across experiments, core 6-OHDA animals showed a mean decrease in DAT binding of 68% in core, 29% in medial shell, 30% in ventral shell, 37% in ventral caudate-putamen, and 30% in olfactory tubercle (OT). In contrast, medial shell-infused 6-OHDA reduced DAT binding in medial shell by 62%, but only by 13, 7, 1, and 12% in core, ventral shell, ventral caudate-putamen, and OT, respectively. SERT binding was virtually unchanged (89–111% of control) by the 6-OHDA lesions in all three experiments (Tables 1,2).

NAcc core and medial shell lesions before conditioning inhibited AMPH-mediated locomotor activation and CPP, respectively

In experiment 1, lesions were performed before drug conditioning. Overall, the AMPH locomotor stimulant effect differed across successive conditioning sessions (SESSION: $F_{(2,84)} = 4.47, p < 0.02$; mean ± SEM; AMPH-saline difference score 40 ± 5, 63 ± 6, and 53 ± 8 m). However, locomotor data were pooled across sessions, because an initial three-way ANOVA revealed no significant interactions between SESSION and either AREA or LESION ($F_{(2,84)} < 1.31, p > 0.2$). Saline session locomotor scores did not differ significantly between surgery groups (AREA: $F_{(1,42)} = 1.01, p > 0.25$; LESION: $F_{(1,42)} = 0.70, p > 0.25$; AREA x LESION: $F_{(1,42)} = 0.95, p > 0.25$) (Figure 6, legend). Because lesions were not anatomically specific (Figure 4), multiple linear regression analysis was performed to assess contributions of core and shell DA innervation to the AMPH-induced locomotor response. Figure 6, A and B, shows the relationship between locomotor responses to AMPH during conditioning versus DAT binding in core and medial shell. The locomotor stimulant response was significantly correlated with DAT binding in NAcc core ($p < 0.01$) but not NAcc medial shell ($p > 0.25$) (Figure 6A, B). Conversely, the magnitude of AMPH CPP was significantly correlated with residual DAT in the medial shell ($p < 0.0001$) but not in the core ($p > 0.5$) (Figure 6C, D).

NAcc medial shell lesions did not prevent acquisition of a CPP for morphine

In experiment 2, the effects of preconditioning lesions of medial shell were tested in rats conditioned with either morphine (10 mg/kg, i.p.) or AMPH (0.75 mg/kg, i.p.). As in experiment 1, AMPH CPP magnitude was reduced or abolished by medial shell 6-OHDA infusion (lesion vs sham: Mann–Whitney $U = 90; p < 0.02$) (Figure 7). In contrast, lesioned rats did acquire a morphine CPP, and this was of similar magnitude to that of sham controls (lesion vs sham: Mann–Whitney $U = 63; p > 0.5$) (Figure 7).

Expression of a conditioned place preference for AMPH was abolished by NAcc medial shell, but not NAcc core, lesions

In experiment 3, lesions were performed after conditioning but before testing. Figure 8, A and B, shows the relationship between DAT binding in NAcc core or medial shell and the CPP magnitude. Two extreme outliers, as defined by the Systat software, were excluded before data analysis. Multiple linear regression analysis showed that CPP magnitude correlated significantly with residual DAT binding in NAcc medial shell ($p < 0.0005$) but not in NAcc core ($p > 0.25$).

DISCUSSION

Methodological aspects

Dopaminergic denervation in core or medial shell has rarely been achieved with any anatomical selectivity (Boye et al., 2001). The present study incorporated several methodological improvements. First, stereotaxic lesion coordinates were improved. Second, multiple infusion sites were used for medial shell lesions. Third, diffusion of 6-OHDA from the infusion site was minimized by administering a high concentration in a small volume. Consequently, core and medial shell DAT binding were largely independent (Pearson $r = 0.30$), which was not the case in our previous study (Pearson $r = 0.84$) (Boye et al., 2001). In addition, nonspecific tissue damage was reduced by neutralizing the 6-OHDA solution before infusion. Thus, despite the unusually high concentration of 6-OHDA used, Nissl staining and SERT autoradiography revealed minimal nonspecific damage.

The present CPP procedure possesses several attractive features. First, latent inhibition can be avoided during the initial preexposure phase by omitting the conditioned stimuli. Second, rats conditioned with saline on both textures showed no significant preference for either texture on test day (our unpublished observations). Hence, our procedure is balanced and avoids the interpretational difficulties inherent in "biased" procedures (Bardo and Bevins, 2000). The current study is the first to show an AMPH CPP using solely tactile cues.

Mechanisms of amphetamine-induced locomotor activation

The present findings suggest that after systemic AMPH administration, locomotor stimulation is dependent on transmission in NAcc core and not medial shell. To date, only three published studies have examined this question using systemic rather than intracranial AMPH (Weiner et al., 1996; Parkinson et al., 1999; Boye et al., 2001). Two of these studies showed that core rather than shell lesions reduced AMPH-induced locomotor activation (Weiner et al., 1996; Boye et al., 2001). In contrast, Parkinson et al. (1999) reported that excitotoxic lesions of the NAcc core enhanced locomotor stimulant responses to systemic AMPH, whereas medial shell lesions had the opposite effect. On this basis, these authors attributed a critical role to the shell; however, in the latter study, shell lesions attenuated AMPH locomotion to only a modest extent, and core lesions increased baseline locomotion, complicating the interpretation of drug effects. On balance, therefore, the available evidence suggests that NAcc core plays an important role in the locomotor stimulant effect of systemically administered AMPH.

In the present study, medial shell DA innervation was not related to AMPH locomotor stimulation. In contrast, we previously observed a significant negative correlation ($p < 0.02$), such that DA denervation in the medial shell was associated with greater locomotor responses (Boye et al., 2001). This discrepancy may reflect differences in lesions coordinates or functional gradients within each NAcc subregion (Essman et al., 1993; Campbell et al., 1997).

Other striatal regions, notably ventromedial striatum (Dickson et al., 1994), OT (Cools, 1986; Ikemoto, 2002), and anteromedial caudate (Fink and Smith, 1979, 1980), have also been implicated in AMPH-induced locomotion. It is doubtful that denervation of ventromedial striatum played a significant role in the present study, because lesions were restricted to the anterior portion, which appears not to contribute to AMPH locomotor activation (Dickson et al., 1994). On the basis of intracranial infusion studies (Cools, 1986; Ikemoto, 2002), the OT has been proposed as a key structure mediating the locomotor stimulant effects of AMPH. In contrast, locomotor stimulation after systemic AMPH administration was unaffected by 6-OHDA lesions of OT, despite substantial loss of tissue DA (Clarke et al., 1988). The anteromedial caudate has been proposed to mediate AMPH-induced locomotion (Fink and Smith, 1979), but this area was probably spared by our lesions. The ventral shell subregion was partially depleted by our core 6-OHDA infusions and, to our knowledge, has not been studied with respect to AMPH locomotion.

Our 6-OHDA infusions almost certainly destroyed noradrenaline (NA) as well as DA terminals in the ventral striatum (Robbins et al., 1983). Disruption of noradrenergic

transmission tends to inhibit AMPH-induced locomotion (Ogren et al., 1983; Archer et al., 1986; Dickinson et al., 1988; Blanc et al., 1994; Darracq et al., 1998; Harro et al., 2000; Drouin et al., 2002a,b; Auclair et al., 2002) [but see Ventura et al. (2003)], with the medial prefrontal cortex identified as a potential site of action (Blanc et al., 1994; Darracq et al., 1998). In contrast, noradrenergic transmission in the NAcc appears not to contribute directly to locomotor stimulation (Pijnenburg et al., 1975; Roberts et al., 1975; Kelly and Iversen, 1976; Joyce et al., 1983). Thus, our 6-OHDA lesion effects on AMPH-induced locomotor activation probably reflect decreased DA rather than NA transmission.

Mechanisms of AMPH-induced reward

Considerable evidence suggests that AMPH exerts its rewarding effects via DA release in the NAcc (Di Chiara, 1995; Koob et al., 1998), with little if any contribution from NA in this structure (Yokel and Wise, 1975; Roberts et al., 1977). In the present study, medial shell DA denervation was associated with attenuated AMPH CPP. It is unlikely that neighboring structures contributed to this effect, because they were only slightly denervated (Tables 1, 2). Moreover, substantial 6-OHDA lesions of OT did not alter a CPP for systemic AMPH (Clarke et al., 1990). Our findings therefore support a role for NAcc medial shell DA in the rewarding effect of AMPH. This conclusion accords with intracranial self-administration studies using other dopaminergic drugs (Carlezon and Wise, 1996; Ikemoto et al., 1997).

The inhibition of AMPH CPP caused by preconditioning 6-OHDA lesions could reflect impaired acquisition or expression, or both. It is well established that acquisition and expression of CPP are mediated by different dopaminergic mechanisms (Hiroi and White,

1990, 1991a,b; Acquas and Di Chiara, 1994; Bardo et al., 1999). For example, DA antagonist studies show that DA D1 and D2 receptors are required for acquisition, but only DA D1 receptors are required for expression (Hiroi and White, 1991a,1991b; Acquas and Di Chiara, 1994; Bardo et al., 1999). Because our 6-OHDA lesions presumably impaired transmission at both DA receptor types, both acquisition and expression are likely to be affected.

In the present study, morphine served as a positive control. The finding that morphine CPP was unaffected by medial shell lesions (experiment 2) suggests that lesion-induced reduction of AMPH CPP did not result from impaired sensory, motor, or mnemonic function. The present findings also accord with evidence that morphine CPP occurs via a DA-independent mechanism when drug exposure is minimized (Mackey and van der Kooy, 1985; Bechara and van der Kooy, 1992; Bechara et al., 1992; Nader and van der Kooy, 1997; Laviolette et al., 2002). In contrast, 6-OHDA lesions of the NAcc have been found to reduce opiate CPP in nondependent rats (Spyraki et al., 1983; Shippenberg et al., 1993). Several factors could account for this discrepancy. First, these authors denervated the entire NAcc. It is possible that the NAcc medial shell subregion is neither necessary nor sufficient to mediate opiate reward. Second, although our lesions eliminated AMPH CPP, they may not have decreased DA transmission sufficiently to affect morphine CPP. Third, it is possible that different neural mechanisms underlie morphine CPP depending on whether multiple sensory cues or solely tactile cues are used.

Dissociation of locomotion and reward

The current findings demonstrate a double dissociation in NAcc core versus shell with

regard to AMPH-induced locomotor activation and reward. They extend evidence from other behavioral paradigms that also suggest that locomotion and reward are dissociable (Burns et al., 1993; Robledo et al., 1993; Kelley et al., 1997; Ventura et al., 2003). Burns et al. (1993) performed lesions of the ventral subiculum or basolateral amygdala and demonstrated a double dissociation of the locomotor stimulation and conditioned reinforcement produced by intra-NAcc AMPH. However, it is not clear whether the lesion affected reward processes or produced a memory or sensory deficit. Robledo et al. (1993) showed that neurotensin administered into the NAcc core decreased the locomotor stimulant effect of cocaine but did not affect intravenous self-administration of the drug. In this study, cocaine was given intraperitoneally in the locomotor tests, making interpretation difficult. Kelley et al. (1997) found that administration of an NMDA receptor antagonist into NAcc core, but not shell, disrupted the acquisition of food-reinforced responding without affecting spontaneous locomotor activity. However, this study did not examine the effects of psychostimulants. Last, Ventura et al. (2003) demonstrated that in mice, NA-depleting lesions of the medial prefrontal cortex blocked both AMPH-induced NAcc DA release and CPP while preserving the locomotor stimulant response. It would be interesting to determine whether core and medial shell DA release are differentially affected by these lesions and whether this result extends to rats.

In conclusion, the present study provides the first clear anatomical dissociation between the rewarding and locomotor-activating effects of the prototypic psychostimulant drug AMPH in rats. These acute behavioral effects were mapped onto NAcc medial shell and core, respectively. The experimental approach used here should help to further define

mechanisms underlying acute and chronic behavioral effects of other drugs of abuse. Finally, the present core/shell dissociation may be relevant to the role of DA in reward anticipation versus consumption (Wise, 2002), incentive salience (Berridge and Robinson, 1998), and other forms of learning (Redgrave et al., 1999; Schultz, 2002).

Figure 1. Experimental design of experiments 1, 2 and 3. Vehicle or 6-OHDA infusions were given at the time indicated by the arrows. In experiments 1 and 3, rats received infusions into either core or medial shell, depending on group (filled arrows). In experiment 2, only medial shell was targeted (white arrow). During the conditioning phase, each rat received saline and a drug (AMPH or morphine, dose as indicated) on alternating days (see Materials and Methods). IP, Intraperitoneal.

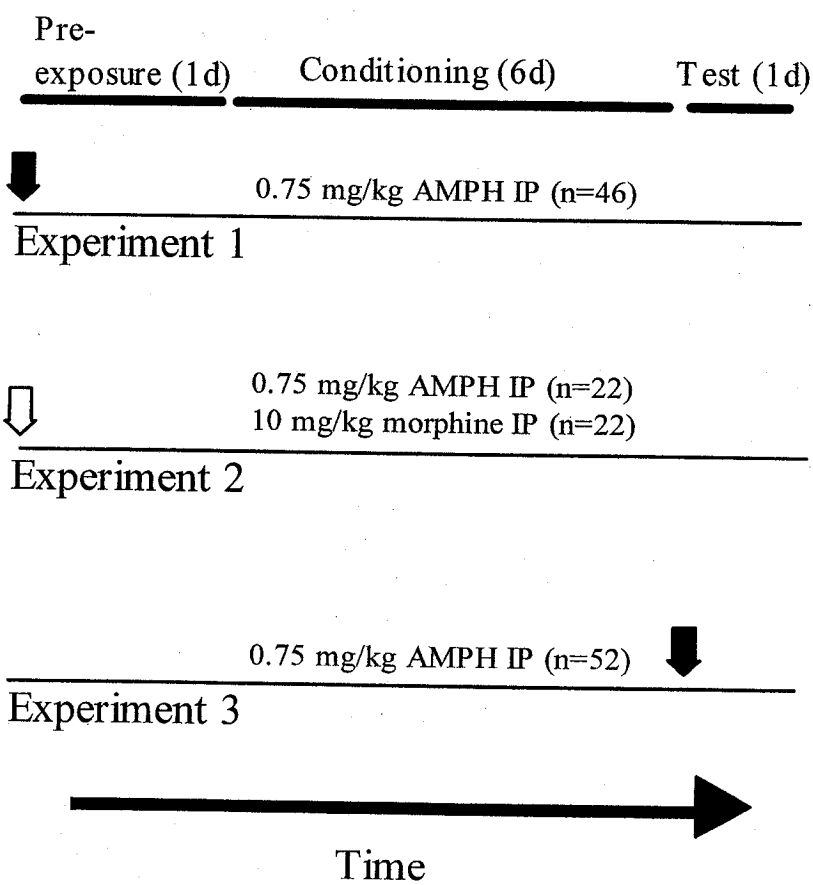


Figure 2. Histological changes associated with infusion of vehicle (*A*) or 6-OHDA (*B*) into the medial shell region of the NAcc. Representative 20 μm Nissl-stained sections are shown ~0.1 mm caudal to the site of injection (10.2 mm anterior to interaural zero). 6-OHDA infusion resulted in disruption of normal tissue morphology local to the infusion site (*B*, black arrow). Much less disruption of normal tissue morphology occurred in rats infused with vehicle. Scale bars, 50 μm. Anterior commissure is indicated by white arrows.

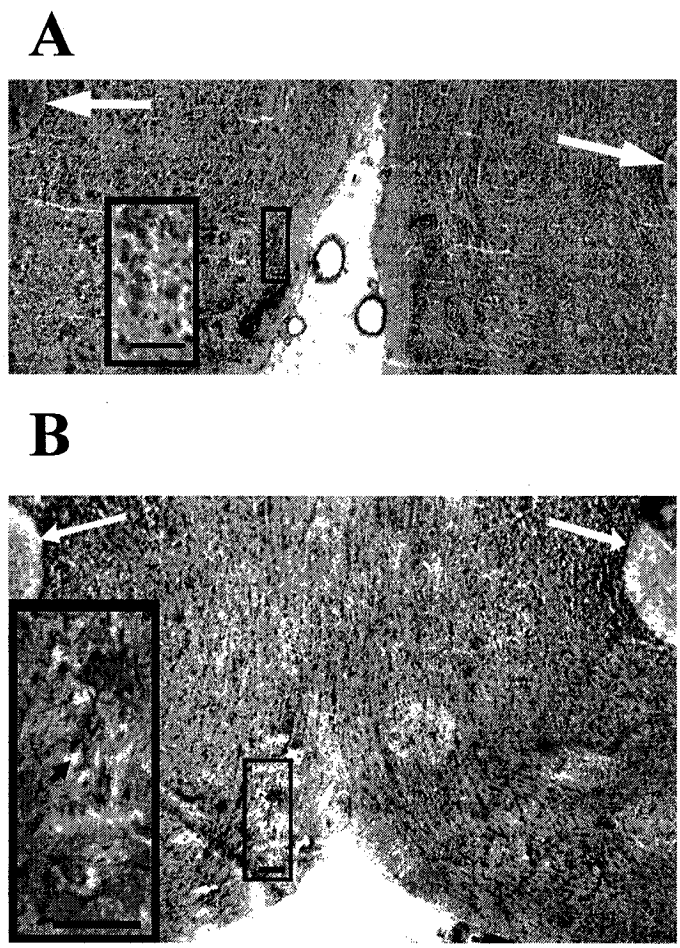


Figure 3. Autoradiographic images of [^{125}I]RTI-55 binding to DAT in animals from core-lesioned, medial shell-lesioned, and sham-operated groups (experiment 3). Because binding was similar between groups that received vehicle in core and medial shell, the latter group has been omitted. Numbers designate distance anterior to interaural zero (in millimeters). Radioligand binding was obtained at a nonsaturating concentration of radioligand and is expressed as attomol per milligram of tissue. Arrows refer to the core subregion. Arrowheads (pointing upward) refer to the medial shell subregion. In most rats, core 6-OHDA lesions were less anatomically selective than shown here (see Fig. 5).

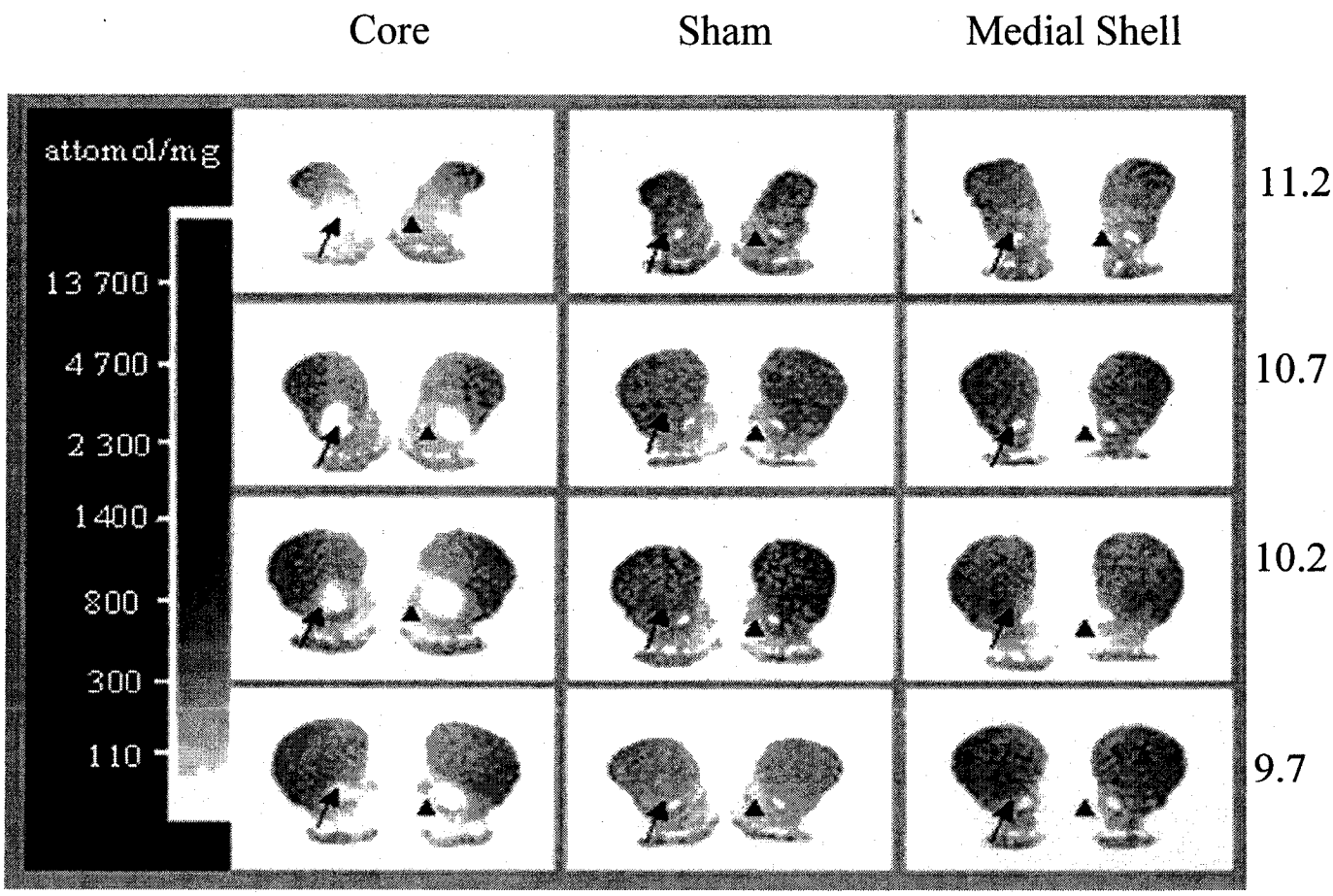


Figure 4. Locations of sampled [^{125}I]RTI-55 binding in nucleus accumbens core, medial shell, ventral shell, ventral caudate-putamen, and olfactory tubercle. Each rat was sampled at four anterior-posterior levels. Numbers are distances (in millimeters) anterior to interaural zero. Sampling areas were circles of 0.3 mm diameter. Three samples per side per structure were taken at each level, except for ventral shell, where one sample per side was taken at level 11.2 and two per side at all other levels. Adapted from Paxinos and Watson (1997).

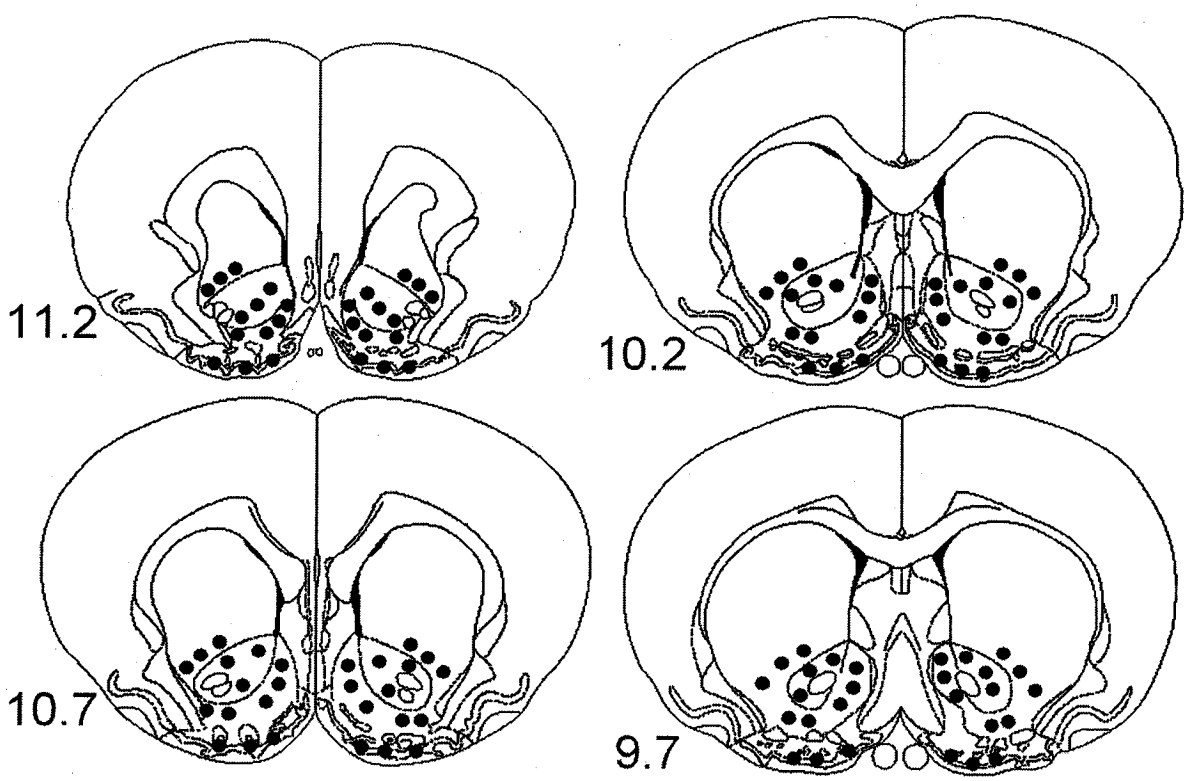


Figure 5. Relationship of DAT labeling in nucleus accumbens core versus medial shell. Data are pooled from experiments 1 and 3 ($n = 98$ rats). DAT labeling was performed by [^{125}I]RTI-55 autoradiography and expressed as a percentage of the mean value of the core-vehicle group for core 6-OHDA animals, or the shell-vehicle group for the shell 6-OHDA group. Correlational analysis revealed a weak but significant relationship between core and medial shell binding ($r = 0.30$; $p < 0.005$). CV, Core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion.

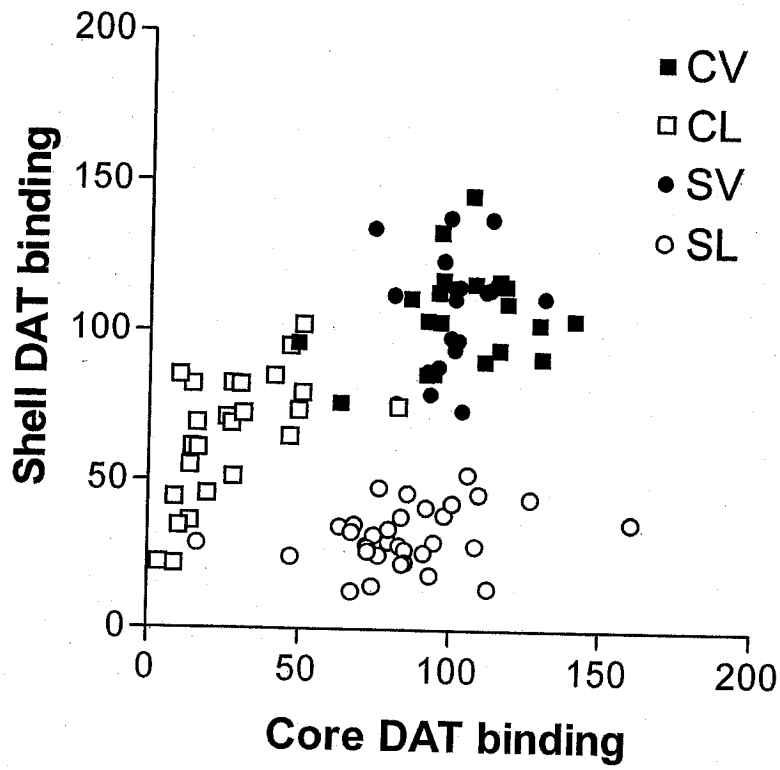
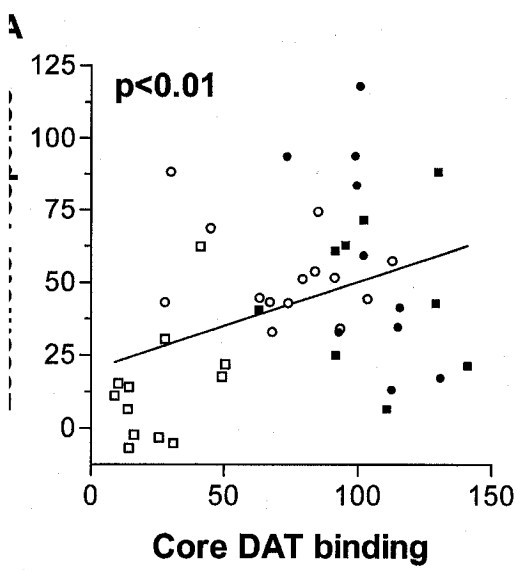
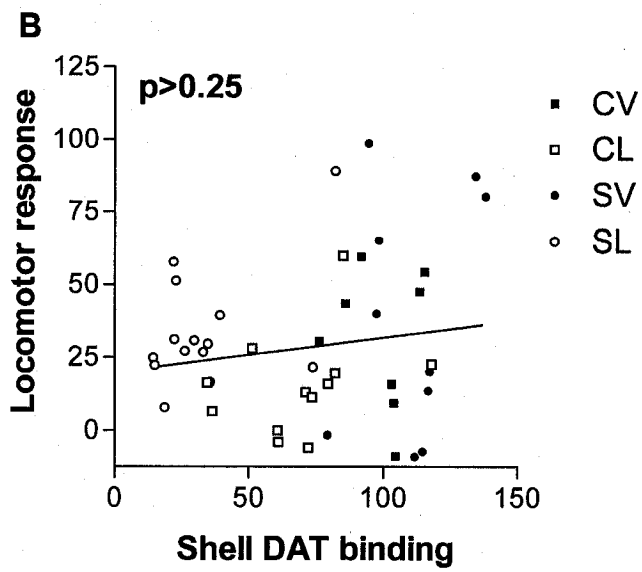
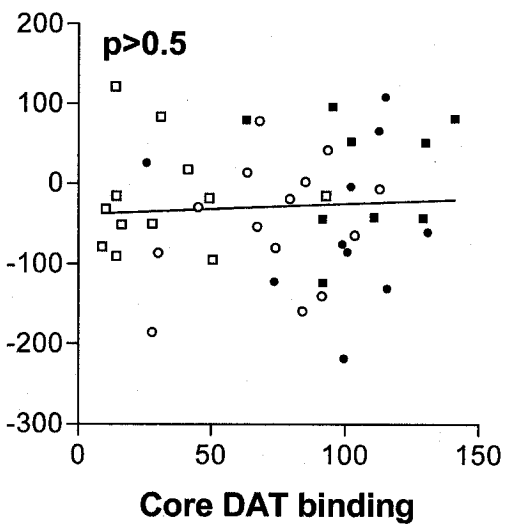


Figure 6. Effect of bilateral 6-OHDA infusion into either NAcc core or medial shell on AMPH-induced locomotor response and CPP (experiment 1). Rats (n = 10–14 per group) were allowed 7–11 d recovery after stereotaxic surgery before conditioning with AMPH (0.75 mg/kg, i.p.). Locomotor responses are expressed for each rat as the difference between the mean distance moved (in meters) during conditioning sessions with AMPH versus with saline. CPP magnitude is the difference between time spent on the drug-paired and saline-paired textures during the 600 sec test. DAT labeling in core or medial shell is expressed as percentage DAT binding of sham-lesioned groups. Saline locomotor scores, in meters, were 134 ± 7 in the core vehicle group, 152 ± 11 in the core 6-OHDA group, 154 ± 10 in the shell vehicle group, and 153 ± 11 in the shell 6-OHDA group. Locomotor responses (AMPH-saline) correlated significantly with DAT binding in NAcc core but not in NAcc medial shell. Conversely, CPP magnitude correlated significantly with DAT binding in medial shell but not core. To visualize the association of each drug response to core or medial shell [^{125}I]RTI-55 labeling, the predicted contribution of the irrelevant brain structure was subtracted from the y-axis variables using the calculated multiple linear regression equation. Significant linear associations (shown by p values) are evident in A and D. CV, Core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion.


A
p<0.01
125
100
75
50
25
0
0
50
100
150
Core DAT binding


B
p>0.25
Locomotor response
125
100
75
50
25
0
0
50
100
150
Shell DAT binding
CV
CL
SV
SL


p>0.5
200
100
0
-100
-200
-300
0
50
100
150
Core DAT binding

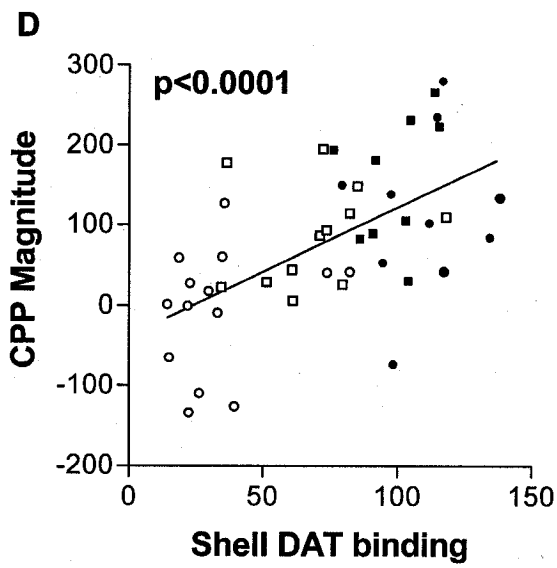

D
p<0.0001
CPP Magnitude
300
200
100
0
-100
-200
0
50
100
150
Shell DAT binding

Figure 7. Effect of 6-OHDA lesions of NAcc medial shell on morphine and AMPH CPP (experiment 2). Stereotaxic surgery was performed 7–11 d before the first conditioning day. CPP magnitudes (mean ± SEM) for morphine (10 mg/kg, i.p.) or AMPH (0.75 mg/kg, i.p.) were calculated as the difference between the time spent on the drug-paired and saline-paired sides (n = 10–12 rats per group). Because the data were not normally distributed, Mann–Whitney U tests were applied to predetermined comparisons. NS, Nonsignificant; $^{*}p < 0.02$ versus corresponding sham-lesioned group (unprotected tests).

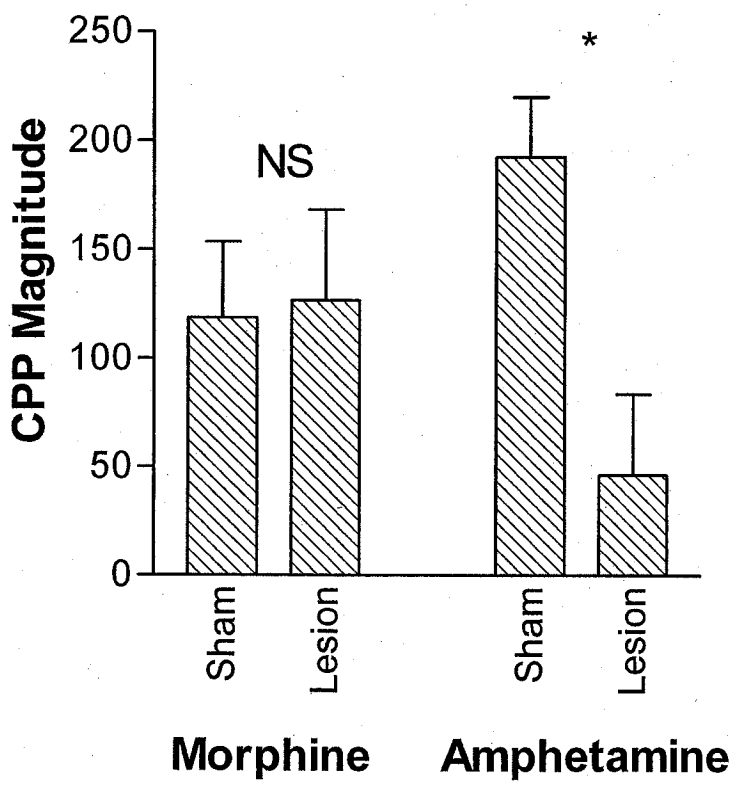


Figure 8. Effect of NAcc core and medial shell lesions on the expression of AMPH CPP (experiment3). Rats (n = 10–19 per group) received bilateral infusion of either 6-OHDA or vehicle into either NAcc core or medial shell after conditioning with AMPH and before CPP testing. Degree of DAT depletion in core or medial shell is expressed as percentage DAT binding of control. To visualize the association of each drug response to core or medial shell [^{125}I]RTI-55 labeling, the predicted contribution of the irrelevant brain structure was subtracted from the y-axis variables using the calculated multiple linear regression equation. CPP magnitude correlated significantly with DAT binding in NAcc medial shell (*B*) but not with DAT binding in NAcc core (*A*). CV, Core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion.

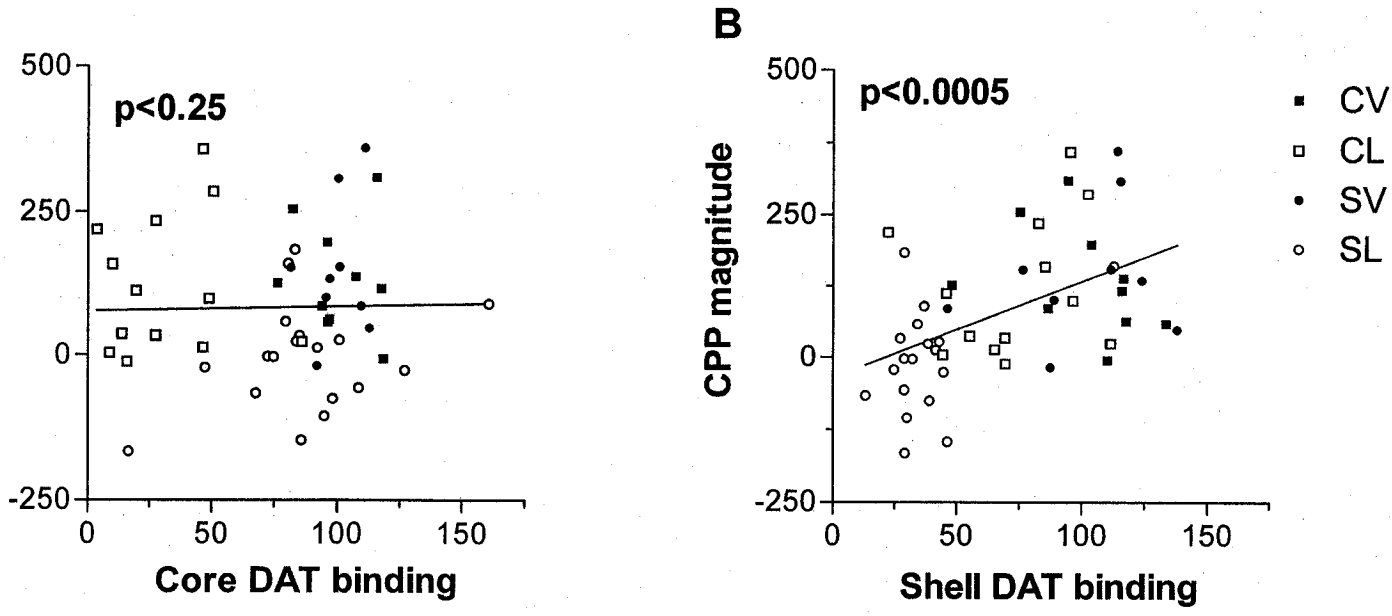


Table 1. Absolute values of DAT and SERT binding in core, medial shell, ventral shell, ventral caudate-putamen (ventral CP), and olfactory tubercle (OT) in experiments 1 and 3

	Core (vehicle)	**Core (6-OHDA)**	**Shell (vehicle)**	**Shell (6-OHDA)**
Experiment 1				
n	10	12	10	14
DAT				
Core	265±20	87±9	261±30	209±20
Medial shell	183±7	128±13	178±18	70±20
Ventral shell	170±8	129±17	158±15	166±16
Ventral CP	152±9	95±11	129±11	137±7
OT	259±16	177±18	282±24	243±16
SERT				
Core	117±12	107±14	117±8	113±7
Medial shell	145±14	149±14	143±6	137±10
Ventral shell	151±17	167±16	136±14	147±15
Ventral CP	152±17	165±16	141±17	143±12
OT	219±9	230±11	242±13	232±9
Experiment 3				
n	10	13	10	19
DAT				
Core	927±43	288±60	941±32	816±61
Medial shell	566±45	411±41	596±56	225±26
Ventral shell	774±38	493±64	787±40	761±40
Ventral CP	641±31	404±35	684±19	660±30
OT	733±36	524±45	743±53	635±36
SERT				
Core	297±10	269±16	310±16	318±14
Medial shell	450±15	441±16	450±23	446±14
Ventral shell	476±27	486±23	497±19	504±20
Ventral CP	300±13	267±10	305±12	321±12
OT	637±25	624±35	637±24	664±26

Values are mean ±SEM [^{125}I]RTI-55 binding to DAT or SERT (expressed as attomol per milligram of tissue), obtained at a subsaturating concentration of radioligand.

Table 2. Absolute values of DAT and SERT binding in core, medial shell, ventral shell, ventral caudate-putamen (ventral CP), and olfactory tubercle (OT) in experiment 2

	Morphine		**Amphetamine**	
	Shell (vehicle)	**Shell (6-OHDA)**	**Shell (vehicle)**	**Shell (6-OHDA)**
Experiment 2				
n	10	12	10	12
DAT				
Core	755±22	715±20	755±22	715±20
Medial shell	473±17	166±21	473±17	166±21
Ventral shell	429±12	382±14	429±12	382±14
Ventral CP	535±18	525±9	535±18	525±9
OT	670±18	612±32	670±18	612±32
SERT				
Core	414±14	408±14	414±14	408±14
Medial shell	517±14	530±19	517±14	530±19
Ventral shell	481±43	534±50	481±43	534±50
Ventral CP	327±31	343±29	327±31	343±29
OT	737±24	688±18	737±24	688±18

Values are mean ± SEM[^{125}I]RTI-55 binding to DAT or SERT (expressed as attomol per milligram of tissue), obtained at a subsaturating concentration of radioligand.

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Intervening Section 1

The previous chapter suggested that DA transmission in the core mediated the locomotor stimulant of amphetamine, while medial shell DA innervation mediated amphetamine CPP. Lesion effects on CPP appear not to be result from a general memory deficit, since medial shell lesions did not disrupt morphine CPP. As such, the findings provided the first evidence for a dissociation between the effects of DA transmission in the accumbens core vs. medial shell in psychostimulant-induced locomotor activity and CPP.

Since an *unconditioned* measure was used for locomotor activity, and a *conditioned* measure for reward, these results were equally consistent with a segregation between the unconditioned and conditioned effects of amphetamine. To examine this issue, we therefore examined the effects of core and medial shell lesions on amphetamine-conditioned locomotor activity. If medial shell lesions reduced conditioned locomotion, this would suggest that a segregation of conditioned vs. unconditioned drug reward would be a more appropriate interpretation of the data. The results supported out initial hypothesis, suggesting that locomotor stimulation and reward processing are indeed segregated within the nucleus accumbens.

CHAPTER 4: 6-hydroxydopamine lesions of nucleus accumbens core, but not medial shell, abolish amphetamine-induced conditioned activity

Laurie H. L. Sellings and Paul B. S. Clarke

Synapse, **59**: 374-377

Abstract: Environmental cues associated with drug experiences appear to play a critical role in drug dependence. We have previously reported that dopamine-depleting lesions of the nucleus accumbens medial shell inhibit amphetamine-conditioned place preference. Here, we examined the effects of analogous lesions on amphetamine-conditioned locomotor activity. Bilateral core, but not medial shell, lesions attenuated unconditioned locomotion and abolished the conditioned locomotor response. Taken with our previous results, these findings confirm a role for accumbens core in amphetamine-induced locomotor activity and suggest that the role of medial shell dopamine transmission in conditioned place preference is related to reward processing rather than conditioning in general.

Rats receiving repeated amphetamine (AMPH) administration in a distinct environment subsequently exhibit hyperlocomotion in that environment in the absence of drug (Gold et al., 1988; Mazurski and Beninger, 1991). The nucleus accumbens appears critical, as 6-hydroxydopamine (6-OHDA) lesions prevented acquisition and expression of AMPH-induced conditioned locomotion (Gold et al., 1988). Recently, we provided 6-OHDA lesion evidence suggesting that conditioned place preference for AMPH was associated with medial shell dopamine (DA) transmission, and that unconditioned locomotor activity was associated with core DA transmission (Sellings and Clarke, 2003). However, reward was assessed using a conditioned measure, whereas the measure of locomotor activation was unconditioned. As such, it is unclear whether the functional segregation we observed represents dissociation between reward and locomotion, or between the

conditioned and unconditioned effects of AMPH.

The aim of the current study was therefore to examine the effects of 6-OHDA lesions of medial shell vs. core on conditioned locomotion. An association between medial shell DA transmission and conditioned locomotion, if observed, would suggest that this subregion may play a general role in mediating conditioned drug effects. Core lesions were expected to reduce the unconditioned locomotor response to AMPH (Sellings and Clarke, 2003), but it was not clear whether this would also prevent the emergence of a conditioned locomotor response (see Discussion). Two experiments were performed. The first established the occurrence of conditioned locomotion in our conditioned place preference apparatus. The second experiment tested behavioural effects of intra-accumbens 6-OHDA.

Subjects were 37 Long-Evans rats (Charles River, St-Constant, QC; 270-310 g at time of surgery). Food and water were available ad libitum except during training. All experiments were approved by the McGill Faculty of Medicine Animal Care Committee in accordance with Canadian Council on Animal Care guidelines.

The testing apparatus was as previously described for conditioned place preference (Sellings and Clarke, 2003). Briefly, test cages comprising four vertical walls forming a rectangle (58 cm L x 29 cm W x 53 cm H) were placed on linoleum flooring covered with a thin layer of sawdust. Two removable square floor tiles (mesh or bar) were inserted into each cage to provide tactile cues during conditioning sessions. Behavioural

experimentation took place over 12 days. After an initial pre-exposure to the test box without tactile cues (Day 1, 20 min), each rat received five AMPH injections (0.75 mg/kg as sulphate salt) on either bar or mesh, and five vehicle injections on the other texture over ten consecutive days (Day 2-11, 45 min/session). On test day, locomotor activity was examined (in a drug-free state) in two separate sessions, one on bar and one on mesh (Day 12, 10 min each). Locomotor activity during conditioning trials and the two test sessions was monitored by a commercial tracking system (EthoVision v3.0, Noldus IT). The order of drug presentation, drug-texture pairing, and order of cue presentation on test day (mesh vs. bar) were counterbalanced. To minimize visual cues, the testing room was lit with a Kodak GBX-2 filter. In Experiment 1, unoperated rats served as experimental subjects. In Experiment 2, rats received bilateral infusions of 6-OHDA or vehicle (0.9% saline plus 0.3 mg/ml sodium metabisulfite) 7-9 days prior to conditioning. This was given via a 30 gauge stainless steel cannula aimed at either core or medial shell, as previously described (Sellings and Clarke, 2003). The extent of the 6-OHDA lesion was quantified by autoradiographic labelling of the DA transporter (DAT) using the radioligand [^{125}I]RTI-55 (2200 Ci/mmol). To assess non-specific damage, serotonin transporter (SERT) autoradiography using [^{125}I]RTI-55 with DAT occluded, as well as cresyl violet staining for Nissl substance (as previously described; Sellings and Clarke, 2003) was used.

A commercial software program (Systat v10.2, SPSS Inc.) was used for data analyses. Unconditioned locomotion was calculated as the difference of locomotor counts between AMPH and saline conditioning sessions. Conditioned locomotion was calculated as the

difference between activity (distance moved in metres) on the AMPH and vehicle paired textures on test day. Group differences were examined by one-way ANOVA, followed by Dunnett's test. Multiple linear regression analysis was used to test for associations between DAT binding in core vs. medial shell and unconditioned or conditioned locomotion. The two sham lesioned groups (core and medial shell) were pooled, as initial examination revealed no significant differences between these two groups. A p value of <0.05 (two-tailed) was considered significant.

In Experiment 1, rats (n=8) expressed significant conditioned locomotion (paired t-test with Bonferroni correction, $p<0.005$; Figure 1A). The distance moved on the AMPH-paired texture was 36.7±2.8 m; on the saline-paired texture, it was 33.0±2.8 m. The magnitude of the conditioned locomotion was not dependent on the floor texture paired with drug (bar vs. mesh, Student's t-test, $p>0.5$).

In Experiment 2, no significant group differences existed for saline activity ($F(2,26)=1.65$, $p>0.2$; Figure 1B) or unconditioned locomotor activation ($F(2,26)=1.45$, $p>0.25$; Figure 1C). However, multiple linear regression analysis revealed a significant association between core, but not medial shell, DAT binding and locomotor activation (core: $p<0.05$, $r=0.37$; Figure 1D; medial shell: $p>0.25$, $r=0.06$; Figure 1E). For conditioned locomotion, only the core-lesioned group differed significantly from sham animals (Dunnett's test, $p<0.02$; Figure 1F). On test day, the distance moved on the AMPH-paired texture was 42.1±3.5 m (sham), 36.5±2.0 m (core) and 37.7±2.9 m (medial shell); on the saline-paired texture, it was 34.2±3.4 m (sham), 36.8±3.6 m (core) and

32.4±2.5 m (medial shell). Additionally, the magnitude of the conditioned locomotion associated positively with core and not medial shell DAT binding (core: $p<0.05$, $r=0.57$; medial shell: $p>0.5$, $r=0.32$; Figures 1G and H). Residual DAT binding, expressed as a percent of combined sham groups, is given in Table 1. Nissl staining revealed minimal nonspecific damage, and residual SERT binding was minimally affected by core and medial shell lesions (92-107% of control).

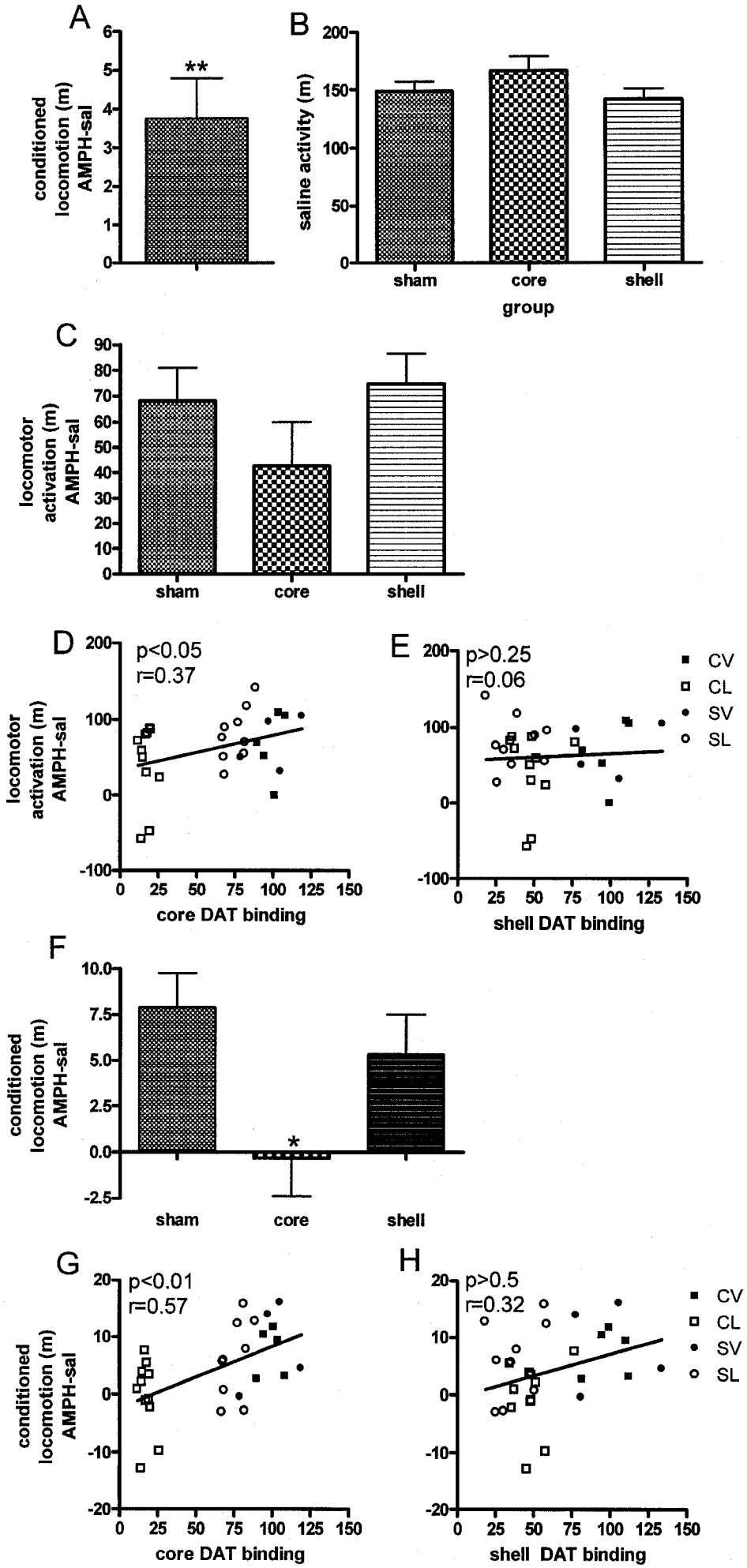
In the present study, DA-depleting lesions of the accumbens medial shell did not inhibit the conditioned locomotor response to AMPH. This finding contrasts with our previous observation that the same kind of lesion inhibited AMPH-conditioned place preference (Sellings and Clarke, 2003). It is important to note that the two studies were designed to be highly comparable. For example, the testing apparatus was identical and the dose of AMPH was the same. Additionally, [^{125}I]RTI-55 binding in medial shell lesioned animals was reduced to the same extent (62%) in both studies. Taken together, the two studies suggest that medial shell DA transmission plays a role in conditioned reward rather than a more general role in conditioning.

Our findings confirm an association between DAT binding in accumbens core and the unconditioned locomotor stimulant effect of AMPH (Sellings and Clarke, 2003). Although the 6-OHDA infusions quite possibly destroyed noradrenaline as well as DA terminals, the critical lesion site (core) receives little noradrenergic input (Berridge et al., 1997; Delfs et al., 1998). As such, the observed effects of core 6-OHDA infusion are most likely attributable to disruption of DAergic transmission.

The conditioned locomotor response was abolished by core 6-OHDA lesions. This is not a trivial result, since several DAergic manipulations (i.e. several DA receptor antagonists, reserpine) have been reported to block the unconditioned locomotor response to AMPH while preserving the conditioned locomotor response in subsequent drug-free tests (Martin-Iverson and McManus, 1990; DiLullo and Martin-Iverson, 1991; DiLullo and Martin-Iverson, 1992a; DiLullo and Martin-Iverson, 1992b). In contrast, other DAergic manipulations given during conditioning have been found to prevent subsequent conditioned locomotion (Beninger and Hahn, 1983; Mazurski and Beninger, 1991; DiLullo and Martin-Iverson, 1992b). Our core lesions only partially inhibited unconditioned locomotion, but they blocked the conditioned response. This result suggests that core lesions affected the acquisition and/or expression of AMPH-conditioned locomotion. Consistent with a role in expression, 6-OHDA lesions of the entire accumbens blocked conditioned locomotion when given before *or after* conditioning (Gold et al., 1988).

The present results add to existing evidence suggesting that unconditioned and conditioned locomotion are controlled via different mechanisms (Beninger and Hahn, 1983; Poncelet et al., 1987; Mazurski and Beninger, 1991; Sutton et al., 2000). They also imply that different AMPH conditioned behaviours may be mediated by DA transmission in separate ventral striatal subregions, with the medial shell underlying conditioned effects of reward, and the core subregion locomotor activation. The extent to which our findings would generalize to conditioning with discrete cues (Hotsenpiller et al., 2002) or natural rewards (Jones and Robbins, 1992) remains to be determined.

Figure 1 - Both conditioned and unconditioned locomotor activity are decreased by 6-OHDA lesions of the core. In Experiment 1, rats (n=8) received multiple pairings of amphetamine with a distinct floor texture, and subsequently exhibited conditioned locomotion (**$p<0.005$, paired t-test, Figure 1A). In Experiment 2, rats received 6-OHDA or vehicle into accumbens medial shell or core (n=8-10 per group). They were then conditioned with amphetamine and tested drug-free. During conditioning, no significant group differences were observed in saline activity (Figure 1B) or in the unconditioned locomotor response to amphetamine (Figure 1C). However, the extent of the core depletion associated significantly with the unconditioned locomotor response (Figure 1D); this was not the case for medial shell lesions (Figure 1E). The conditioned locomotor response was blocked in core-lesioned rats (*$p<0.02$, Dunnett's test, Figure 1F). The magnitude of the conditioned locomotion was significantly associated with core, but not medial shell DAT binding (Figures 1G and H). The apparent, but highly non significant, association between medial shell DAT and the magnitude of the conditioned locomotion reflects covariance between core and medial shell DAT binding ($r=0.57$, $p<0.01$).Shell refers to medial shell. CV, core vehicle; CL, core 6-OHDA; SV, medial shell vehicle; SL, medial shell 6-OHDA.


A
conditioned locomotion (m) AMPH-sal
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B
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CV
CL
SV
SL
shell DAT binding

Table 1. Reductions in DAT binding seen in core and medial shell lesioned groups.

Group	*Core*	*mSh*	*vSh*	*OT*	*vCP*
Sham	100±4	100±6	100±5	100±5	100±3
Core	18±1	49±4	40±5	45±2	48±5
Medial shell	76±3	38±5	88±5	72±4	96±4

Figures are mean ± SEM, and are calculated as a percent of sham-operated control.

Abbreviations are as follows: mSh, medial shell; vSh, ventral shell; OT, olfactory tubercle; vCP, ventral caudate-putamen.

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Intervening Section 2

Taken together, the results of Chapters 3 and 4 suggested that core DA transmission mediated amphetamine-stimulated locomotor activity, and medial shell DA transmission was responsible for reward processing. We therefore set out to examine whether a similar segregation exists for other psychomotor stimulant drugs.

The first drug examined was cocaine. Abuse of this drug has been reported by both the intranasal and intravenous routes. The results were suggestive of an important role for not only medial shell, but also the medial olfactory tubercle in i.v. cocaine CPP. Additionally, core DA transmission appeared to mediate cocaine-stimulated locomotor activity after both intravenous and intraperitoneal administration, at several doses of cocaine. Taken together, these results suggested that a segregation of locomotor stimulation and reward processing, as seen for amphetamine, exists for intravenous but not intraperitoneal cocaine.

CHAPTER 5: Evidence for multiple sites within rat ventral striatum mediating cocaine conditioned place preference and locomotor activation

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J. Pharmacol. Exp. Ther., **317:** 1178-1187

Abstract

Considerable evidence suggests psychostimulants can exert rewarding and locomotor stimulating effects via increased dopamine transmission in the ventral striatum. However, the relative contributions of ventral striatal subregions to each of these effects have been little investigated. The present study examined the contribution of different ventral striatal sites to the rewarding and locomotor activating effects of cocaine. Initially, the effects of bilateral 6-hydroxydopamine lesions of the nucleus accumbens core or medial shell on cocaine-induced locomotor stimulation (0.5-1.5 mg/kg i.v. or 5-20 mg/kg i.p.) and conditioned place preference (0.5 mg/kg i.v. or 10 mg/kg i.p.) were examined. A subsequent study investigated the effects of olfactory tubercle *vs.* medial shell lesions on cocaine conditioned place preference and locomotor activity (0.5 mg/kg i.v.). Dopaminergic lesion extent was quantified by radioligand binding to the dopamine transporter. Multiple linear regression was used to identify associations between behavioral effects and residual dopamine innervation in ventral striatal subregions. On this basis, the accumbens core was associated with locomotor stimulant effects of i.v. and i.p. cocaine. In contrast, the medial shell was associated with the rewarding effect of i.v. cocaine, but not of i.p. cocaine. Finally, the olfactory tubercle was identified as an additional site contributing to conditioned place preference produced by i.v. cocaine. Overall, these findings provide additional evidence that the locomotor stimulant and rewarding effects of systemically-administered psychomotor stimulant drugs are segregated within the ventral striatum.

Introduction

The nucleus accumbens (NAcc) plays an important role in the rewarding and locomotor stimulant effects of systemically-administered amphetamine and cocaine (Koob et al., 1998; Wise, 2004). It is anatomically and neurochemically heterogeneous, with a prominent medioventral shell and dorsolateral core (Zahm and Brog, 1992). Recent behavioral studies, largely relying on intracranial microinjections of dopaminergic agonists, have provided evidence for functional compartmentalization within this structure, although certain details are controversial. Thus, the medial shell subregion has been implicated in reward processes (Di Chiara et al., 2004 ; Ikemoto and Wise, 2004), whereas locomotor stimulation has been elicited from core and/or shell injection sites (Boye et al., 2001; Ikemoto, 2002; Sellings and Clarke, 2003 and references therein).

The technique of intracranial drug microinjection, despite its obvious utility, is limited by the fact that local drug concentrations are usually unknown and may not be comparable with those obtained after systemic administration. Using an alternate approach, we recently evaluated the respective roles of accumbens core and shell in amphetamine-induced locomotion and conditioned place preference (CPP) by combining systemic amphetamine challenge with prior 6-hydroxydopamine (6-OHDA) lesions of either structure (Sellings and Clarke, 2003). In this study, DAergic depletion in core and medial shell reduced amphetamine-induced locomotor stimulation and CPP, respectively.

The present study sought to extend these findings to cocaine. Intra-NAcc infusion of cocaine produces both locomotor stimulation and rewarding effects (Ikemoto, 2002; Ikemoto, 2003; Ikemoto and Witkin, 2003; Rodd-Henricks et al., 2003). However, the interpretation of such findings is complicated by possible sympathomimetic and anesthetic actions within the target tissue (Ikemoto, 2003; Ikemoto and Witkin, 2003). Even after systemic injection, the precise route of administration can be critical. In particular, cocaine is reported to produce DA (dopamine)-dependent or DA-independent rewarding effects, depending on whether it is delivered intravenously or intraperitoneally (Spyraki et al., 1987). In the present study, these two systemic routes of administration were compared.

The less-studied olfactory tubercle (OT) may also play a role in psychomotor stimulant-mediated locomotor activation and reward. This is suggested by studies employing intracranial administration in rats. Thus, direct intra-OT infusions of DA agonists including amphetamine and cocaine produced a marked and prompt locomotor activation (Pijnenburg et al., 1976; Cools, 1986; Ikemoto, 2002), and both these drugs were avidly self-administered at OT sites (Ikemoto, 2003; Ikemoto et al., 2005). Interestingly, intra-OT drug infusions elicited stronger locomotor and reinforcing effects than intra-NAcc infusions (Cools, 1986; Ikemoto, 2003; Ikemoto et al., 2005). Despite these positive findings, we previously tested the impact of profound 6-OHDA lesions of OT on the locomotor stimulant and rewarding (CPP) effects of *systemic* amphetamine challenge, and concluded that DAergic transmission in the OT does not contribute significantly to either behavioral effect (Clarke et al., 1988; Clarke et al., 1990). Hence, at present, it is

an open question whether the OT contributes significantly to the locomotor stimulant and rewarding effects of any systemically-administered psychostimulant.

The overall goal of the present study was therefore to localize the ventral striatal actions of *systemically-administered* cocaine. The first experiment investigated whether the locomotor stimulant effects of i.v. and i.p. cocaine are diminished by DA denervation in the accumbens core or medial shell. The next two experiments determined whether the stimulant and rewarding effects of cocaine could be dissociated by selective 6-OHDA lesions of either structure, as previously seen with amphetamine (Sellings and Clarke, 2003). The final experiment tested for OT involvement in cocaine reward and locomotor activation, again after systemic drug challenge.

Methods

Experimental design. The design of all four experiments is summarized in Table 1.

Subjects. Subjects were male Long–Evans rats (Charles River, St. Constant, Quebec) weighing 250–325 g at time of surgery. Rats were housed individually (Experiment 1) or in groups of three (Experiments 2 - 4) in clear Plexiglas cages in a temperature- and humidity-controlled animal colony, lit from 7 A.M. to 7 P.M. Food and water were available ad libitum except during behavioral testing. All experiments were approved by the McGill Faculty of Medicine Animal Care Committee in accordance with Canadian Council on Animal Care guidelines.

Stereotaxic infusion of 6-OHDA. Surgery was performed 7-10 days prior to the start of behavioral testing. Rats were anesthetized with ketamine HCl (90 mg/kg, i.p.) and xylazine HCl (16 mg/kg, i.p.) prior to placement in a stereotaxic apparatus (Kopf, Tujunga, CA) with the incisor bar set at -3.9 mm. Depending on the experiment (see Table 1), rats received bilateral infusions of either 6-OHDA or vehicle into either NAcc core, medial shell, or anteromedial olfactory tubercle (amOT). Infusions were made via a 30 gauge stainless steel cannula attached by polyethylene tubing to a 10 µl Hamilton syringe driven by a model 5000 Micro Injection Unit (Kopf) (core or medial shell) or via two separate 10 µl Hamilton syringes driven by a multi-channel syringe pump (amOT; MD-1001, BioAnalytical Systems Inc., West Layette, IN). For greater accuracy, coordinates for all three target subregions were derived from the mean of two coordinate systems. Thus, anterior-posterior coordinates were +10.3 mm from interaural zero and +1.3 mm from bregma for both core and shell, and +10.7 mm from interaural zero and +1.7 mm from bregma for amOT. Lateral coordinates were ±0.6 mm (shell), ±2.4 mm (core) and ±0.8 mm (amOT). Ventral coordinates for shell (three injections) were +2.0, +2.4, and +2.8 mm from interaural zero and -8.0, -7.6, and -7.2 mm from bregma. Ventral coordinates for core were +2.7 mm from interaural zero and -7.3 mm from bregma. For amOT, ventral coordinates were +1.1 mm and –8.9 mm respectively from interaural zero and bregma. All coordinates are based on the atlas of Paxinos and Watson (1997). 6-OHDA or vehicle was infused on each side in a volume of 0.1 µl (core), as three infusions of 0.06 µl (medial shell), or 0.2 µl (amOT) on each side. For core and medial shell, 6-OHDA was infused at a rate of 0.1 µl /min; for amOT, the rate of infusion was 0.1 µl/10 min. The concentration of 6-OHDA used was 80 µg/µl (core) or 48 µg/µl

(shell). For amOT, a volume of 0.2 µl of either vehicle or 6-OHDA (40 µg/µl free base) was infused bilaterally over 20 minutes. The different doses of 6-OHDA, infusion volumes and infusion times used at each lesion site were chosen based on pilot studies, and represented the best compromise between efficacy (DA depletion) and anatomical selectivity. For all three lesion sites, the cannula remained at the final infusion site for 5 min.

Intravenous catheterization. During 6-OHDA lesion surgery, rats were implanted with chronic indwelling silastic catheters (0.51 mm I.D. and 0.94 mm O.D., Fisher Scientific, Montreal, Quebec) in the left jugular vein. Tubing was secured to the vein by surgical silk sutures, led subcutaneously to the skull surface, and was then fitted onto a 22 gauge cannula attached to a plastic connector (Model number C313G-5UP, Plastics One, Roanoke, VA). The cannula/connector was fixed to the animal's skull with small stainless steel screws (Lomir, Notre-Dame-de-L'Ile Perrot, Quebec) and dental cement (Stoelting, Wood Dale, IL). To keep catheters patent, 0.1-0.15 ml heparinized 0.9% saline was administered at the end of surgery, on the first day of behavioral testing, and every 2-3 days thereafter.

Locomotor activity testing (Experiment 1). Horizontal locomotor activity was tested in the CPP apparatus (see below for description). Rats were first given one pre-exposure session (20 minutes) in the absence of drug. Each rat then received eight tests on consecutive days with cocaine given i.v. (0, 0.5, 1 or 1.5 mg/kg) or i.p. (0, 5, 10, 20

mg/kg) in a randomized order. Each test session lasted 30 min, starting immediately after injection. Test cages contained one bar and one mesh tile (see below).

Conditioned place preference and locomotor activity testing (Experiments 2, 3 and 4). The apparatus and general procedure were as previously described (Sellings and Clarke, 2003). Briefly, the procedure consisted of three phases: pre-exposure (one day), conditioning (six days) and test (one day). All phases were carried out in a one-compartment box (58 cm x 29 cm x 53 cm) with walls made of white plastic-coated particle board. In the pre-exposure phase, Beta-Chip sawdust bedding covered the floor of the cage. In the conditioning phase, two square tactile tiles of either bar or mesh texture were placed in the bottom of the cage, on top of the bedding. During this phase, the video tracking software (EthoVision v 3.0, Noldus Information Technology, Leesburg, VA) measured locomotor activity, expressed as horizontal distance moved (in meters). During the test phase, one bar and one mesh tile were placed on the bottom of the cage. The time spent on bar or mesh texture was measured by EthoVision software. All three phases were carried out under darkroom lighting using a Kodak GBX-2 safelight filter (Vistek, Toronto, Ontario, Canada), to minimize visual cues. Animals do not spontaneously prefer either texture (unpublished observations), and all experiments were as fully counterbalanced as possible with respect to drug-texture pairing and order of drug pairing (drug-saline or saline-drug) within each surgery group. For all experiments, pre-exposure sessions lasted 20 minutes, and the test session 10 minutes. Conditioning trial duration for i.v. COC was 15 minutes; for i.p. COC, 25 minutes.

For i.v. infusion (Experiments 1, 2 and 4), a fluid swivel was fixed above the center of each cage. Each swivel was connected to on one end a 1 ml syringe, and on the other end to a brass connector (Produits MSM, Laval, Quebec) and protective spring (Heiplex, Montreal) via Tygon tubing of 0.51 mm diameter. The cannula fixed to the skull of the rat was attached to the Tygon tubing, and the brass connector fastened to the plastic connector, to secure the tubing to the cannula, hence allowing administration of drug immediately after placement in the CPP cage. Drug was infused over 25-30 s. Cocaine administered i.p. was injected immediately prior to placement in the CPP cage.

Tissue Preparation. Tissue was prepared for autoradiography and Nissl-staining (cresyl violet) as previously described (Sellings and Clarke, 2003). Briefly, rats were sacrificed 3 to 5 hours following CPP testing, by decapitation under sodium pentobarbital (65 mg/kg, i.p.) anesthesia. Brains were removed, frozen in 2-methylbutane at -50°C for 30 sec, and stored at -40°C.

Coronal sections (20 μm) were taken on a cryostat at several rostrocaudal levels through the ventral striatum. In Experiments 1, 2, and 3, sections were examined at 11.2, 10.7, 10.2, and 9.7 mm anterior to interaural zero; 9.2 and 8.7 mm were also examined in Experiment 4 (Paxinos and Watson 1997). Four adjacent sections were collected for autoradiography and one for Nissl staining with cresyl violet. Sections were thaw mounted onto gelatin-subbed slides, air dried at room temperature for 20–30 min, and stored with desiccant at -40°C.

Quantitative autoradiography. The extent and chemical selectivity of the 6-OHDA lesion was quantified by autoradiographic labeling of the DA transporter (DAT) and the 5-HT transporter (SERT) (Sellings and Clarke, 2003), using a nonsaturating concentration of 3β-(4-iodophenyl)tropan-2-β-carboxylic acid methyl ester ([^{125}I]-RTI-55; 2200 Ci/mmol; NEN-Mandel, Guelph, Ontario).

Sections were thawed at room temperature for 10 min and then placed in a staining dish containing an aqueous buffer solution of 120 mM NaCl, 0.1 M sucrose, 10 mM sodium phosphate buffer, and 10 pM [^{125}I]-RTI-55, with the pH adjusted to 7.4. In the DAT autoradiographic assay, 50 nM citalopram hydrobromide was used to occlude SERT; nonspecific binding was determined by addition of 10 μM 1-(2-[bis(4-flurorphenyl)methoxy]ethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 12909). For SERT autoradiography, 1 μM 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 12935) was added to occlude DAT; nonspecific binding was determined by addition of 50 nM citalopram HBr (Sellings and Clarke, 2003). Slides were incubated at room temperature for 2 hr and then washed three times in cold buffer solution (once for 1 min, twice for 20 min) and for 1-2 sec in distilled and deionized water. They were then blow dried and placed in X-ray film cassettes. Kodak BioMax MS film (Amersham Biosciences, Baie d'Urfé, Québec) was exposed to slides for 48 hr (DAT) or 120 hr (SERT) with [^{125}I] autoradiographic standards (Amersham Biosciences). After development of film, DAT and SERT binding were

quantified using an MCID M4 imaging system (Imaging Research, St. Catherines, Ontario).

Histological examination. Tissue was stained with cresyl violet to assess nonspecific damage, as previously described (Sellings and Clarke, 2003), and examined under a light microscope (40–200X magnification).

Drugs. Drug sources were as follows: cocaine HCl (gift of National Institute on Drug Abuse, Bethesda, MD); citalopram HBr (gift from H. Lundbeck A/S); dipyrone (Vetoquinol, Quebec, Quebec); ketamine HCl (Vetalar, Vetrepharm, London, Ontario); xylazine HCl (Anased, Novopharm, Toronto, Ontario); GBR 12909 (NIMH Chemical Synthesis and Drug Supply Program), and GBR 12935•2HCl (Sigma-Aldrich, Oakville, Ontario). Unless otherwise stated, all other chemicals were obtained from Fisher Scientific (Montreal, Quebec).

Cocaine HCl was dissolved in sterile 0.9% saline and injected at 1 ml/kg (i.v. or i.p.). 6-OHDA HBr was dissolved in sterile 0.9% saline containing 0.3 mg/ml sodium metabisulfite (Sigma-Aldrich) as an antioxidant and protected from light. Vehicle solutions, as well as 6-OHDA to be infused into medial shell or amOT, were neutralized to pH 7.3 ± 0.1 with NaOH (to reduce non-specific damage; see Results). Doses of all drugs except 6-OHDA HBr are expressed as the salt. 6-OHDA HBr doses are expressed as free base.

Data analysis. A commercial software program (Systat v10.2, SPSS Inc., Chicago, IL) was used for all data analyses. In all experiments, locomotor responses to cocaine were calculated as the difference of locomotor counts between drug and saline conditioning sessions. In CPP experiments, saline locomotor scores were calculated as the mean activity over all three conditioning sessions with saline, and are expressed as mean ± SEM. After initial data inspection, sham groups were combined within each experiment. Group differences were analyzed by 1-way ANOVA. CPP magnitude was calculated as the difference between time spent on the drug-paired and vehicle-paired sides during the 10-minute test session. Experiments 2 and 4 were each carried out in different batches, due to space constraints in the animal facility; after initial data inspection, the results within each experiment were pooled. The existence of a significant CPP magnitude or locomotor stimulant effect was determined by one-sample Student's t-test with Bonferroni correction for multiple comparisons. The relationship between behavioral measures *vs.* [^{125}I]-RTI-55 labeling was analyzed by multiple linear regression. A p value of less than 0.05 (two-tailed) was considered significant. Group data are expressed as mean ± SEM throughout.

Results

Neurochemical and anatomical selectivity. To assess nonspecific tissue damage, sections were Nissl-stained with cresyl violet. As previously reported (Sellings and Clarke, 2003), only minimal cell loss was evident at the site of infusion for all vehicle groups (Fig. 1A) and for the group infused with 6-OHDA in the core subregion (not

shown). Among rats lesioned in medial shell or amOT, most rats (~60%) also showed a minimal degree of cell loss, ~30% of rats possessed a small region of decreased cell density at the infusion site (Fig. 1B), and ~10% of rats showed more pronounced non-selective damage (Fig. 1C). This larger region of non-specific damage did not extend more than 0.3 mm from the site of infusion and was almost always found at only one anterior-posterior level.

Sampling locations for DAT and SERT binding density are indicated in Fig. 2. [^{125}I]-RTI-55 autoradiographs of DAT binding are shown in Fig. 3. Residual DAT binding as a percent of combined sham groups is given in Tables 2 (Experiments 1-3) and 3 (Experiment 4). Radioligand binding to SERT in tissue from lesioned animals was minimally changed by all lesion parameters in all experiments (Tables 2 and 3).

In all Experiments, rats were allowed 7-10 days recovery post-surgery before the start of behavioral testing.

The magnitude of core, but not medial shell, DA denervation predicted locomotor responses to i.p. and i.v. cocaine. The effects of 6-OHDA lesions of core vs. medial shell on cocaine-induced locomotion were tested most extensively in Experiment 1. Locomotor responses to i.p. and i.v. cocaine are shown in Fig. 4A and 4B (absolute values) and Fig. 4C and 4D (saline-subtracted values). Saline test scores did not differ significantly between the three surgery groups (Fig. 4A and 4B). The locomotor stimulant effects of cocaine were blunted only in the core-lesioned group. Multiple linear

regression analysis revealed significant positive associations between core DAT binding and the locomotor stimulant response for both administration routes used and at all doses except for 0.5 mg/kg i.v. (range $p < 0.001$ - $p < 0.05$). Significant *negative* associations were observed between medial shell DAT binding and the locomotor stimulant response at several cocaine doses (1 mg/kg i.v., 5 and 10 mg/kg i.p.; $p < 0.05 - p < 0.005$).

The effects of core and medial shell 6-OHDA lesions on cocaine-induced locomotion were also tested in two CPP experiments (i.e. Experiments 2 and 3). Locomotor data were obtained from the three drug and saline conditioning sessions. Experiment 2 examined the locomotor stimulant response to cocaine (0.5 mg/kg i.v.). Here, saline locomotor scores did not differ significantly between groups and were as follows: 52 ± 2 m (sham), 54 ± 2 m (core 6-OHDA), and 55 ± 2 m (medial shell 6-OHDA). A significant locomotor stimulant effect was observed in sham-lesioned and medial shell-lesioned animals, but not in the core-lesioned subjects (Fig. 5A). Multiple linear regression analysis revealed a positive trend between the locomotor response and DAT binding in the core ($p = 0.086$, Fig. 5B) but not medial shell (Fig. 5C).

The locomotor stimulant response to *intraperitoneal* cocaine (10 mg/kg) was also attenuated after core 6-OHDA lesions (Experiment 3, Fig. 6A). No significant group differences were seen for saline locomotor activity. Saline scores were 85 ± 6 (sham), 88 ± 5 (core 6-OHDA), and 87 ± 5 (medial shell 6-OHDA). Multiple linear regression analysis (Fig. 6B and 6C) revealed a positive association between the locomotor response and core DA innervation only ($p < 0.05$).

NAcc medial shell lesions inhibited CPP for i.v. cocaine. In Experiment 2, only the combined sham group and the core-lesioned group exhibited significant CPP (Fig. 5D). Relationships between the CPP magnitude and core *vs.* medial shell DAT binding are shown in Figs. 5E and 5F respectively. The CPP magnitude produced by i.v. cocaine was positively related to medial shell DAT binding ($p < 0.005$, Fig. 5F) with a negative trend in the accumbens core ($p = 0.062$, Fig. 5E).

Conditioned place preference for i.p. cocaine was unaffected by lesions of core or medial shell. In Experiment 3, a significant CPP to i.p. cocaine occurred in the sham-lesioned group, with a similar trend in the two lesion groups (Fig. 6D). No significant relationship was observed between the CPP magnitude and core or medial shell DAT binding (Figs. 6E and 6F).

CPP magnitude for i.v. cocaine was related to OT residual DAT binding. It was recently reported that amOT more robustly supports intracranial self-infusion of cocaine than does medial shell (see Discussion). Therefore, we first re-examined the data from Experiment 2 (i.v. cocaine), to determine if amOT DAT binding may have contributed significantly to the CPP magnitude. However, amOT binding was reduced only slightly in this experiment (by 28% in the core- and 11% in the shell-lesioned group). We therefore addressed the question of amOT involvement by directly comparing the effects of 6-OHDA lesions of the medial shell *vs.* amOT on i.v. cocaine CPP (Experiment 4).

Infusions of 6-OHDA into either amOT or medial shell depleted DAT binding locally, and also tended to produce a smaller and variable depletion in the other structure (Fig. 7). Initial analysis revealed a high degree of co-linearity existing in DAT binding levels between different OT subregions. Accordingly, these values were averaged, and subsequent analyses were carried out using OT rather than amOT values.

Only sham-lesioned animals exhibited significant CPP (Fig. 8A). Multiple linear regression analysis was performed with CPP magnitude as the dependent variable, using residual DAT binding in core, medial shell, ventral shell, ventral caudate putamen and OT as simultaneous predictors. Only OT was retained as a significant predictor ($p < 0.01$, Fig. 8C). Linear regression analysis of CPP magnitude with medial shell as the sole predictor revealed a positive association that bordered on significance ($p = 0.056$). Linear regression analysis of the locomotor stimulant effect revealed that DAT binding in neither medial shell nor OT predicted the degree of locomotor stimulation ($p > 0.5$ for both, data not shown).

Discussion

Novel findings. To our knowledge, the present study is the first to examine the role of ventral striatal subregions in CPP induced by systemically administered cocaine. Cocaine-induced locomotion was related to core DA innervation at several doses of both i.v. and i.p. cocaine. CPP results, in contrast, were more complex. Intravenous cocaine CPP appeared dependent on DA innervation in both OT and medial shell, whereas i.p. cocaine CPP was unaffected by medial shell lesions.

Methodological considerations. The present series of experiments revealed associations between residual DA innervation in various ventral striatal structures and cocaine-induced locomotion or CPP. It is doubtful that these relationships represent segregation between conditioned and unconditioned drug effects rather than between reward and locomotion, as core but not medial shell 6-OHDA lesions abolished amphetamine-induced *conditioned* locomotion (Sellings and Clarke, 2006).

In the present study, quantitative autoradiographic analysis was performed by taking a large number of samples within each structure (e.g. 24 each for medial shell and core). Within each targeted structure, the extent of DAT depletion appeared rather uniform (see Fig. 3 and Sellings and Clarke, 2003), and visual inspection revealed no evidence for smaller sites of preferential depletion. Nevertheless, we cannot rule out the possibility that our behavioral effects resulted from damage to functionally important "hot spots" within the targeted structures.

It is unlikely that non-specific damage caused these lesion effects, since only minimal changes were observed in SERT binding levels, and Nissl staining revealed only slight non-specific damage in a subset of medial shell and medial OT lesioned animals (Fig. 1). However, 6-OHDA infusion almost certainly depleted noradrenaline as well as DA. Preservation of noradrenergic terminals by using systemic desipramine proved impossible, since in pilot studies the routinely used dose of 25 mg/kg (Kelly and Iversen, 1976) caused significant mortality (>25%). Nevertheless, for several reasons, it is unlikely that the observed lesion effects were due to loss of noradrenergic terminals.

First, neither noradrenergic agonists nor antagonists when injected into ventral striatum affected locomotion (Pijnenburg et al., 1975; Pijnenburg et al., 1976). Second, noradrenergic denervation of ventral striatum does not alter locomotor stimulant responses to cocaine and amphetamine (Roberts et al., 1975; Kelly and Iversen, 1976). Third, noradrenergic afferents to NAcc largely avoid the core (Delfs et al., 1998), where lesion effects on locomotor stimulation occurred. Fourth, stimulation of noradrenergic transmission did not produce CPP (Martin-Iverson et al, 1985; Subhan et al, 2000). Fifth, neither α nor β adrenergic receptor antagonists affected the rewarding effects of i.v. cocaine as reflected by self-administration behavior (Johanson and Fischman 1989). Sixth, the disruptive effects of 6-OHDA lesions on cocaine self-administration appear unrelated to noradrenaline depletion (Roberts et al, 1977; Roberts et al, 1980). Lastly, self-administration of cocaine directly into the amOT was blocked by co-infusion of a D1 or D2 DA receptor antagonist (Ikemoto, 2003). On this basis, it seems reasonable to conclude that our 6-OHDA lesions produced their behavioral effects via local depletion of DA.

The accumbens core and locomotor activation. There is currently no consensus on the role of core *vs.* shell in psychostimulant-induced locomotion (Boye et al., 2001; Ikemoto, 2002 and references therein). In particular, studies employing intra-accumbens microinjection of direct or indirect DAergic agonists have implicated core, shell, or both structures, depending on the drug. For example, amphetamine acted with similar potency at either injection site, whereas cocaine stimulated locomotor activity most strongly after injection into medial OT and medial shell (Ikemoto, 2002). Importantly, locomotor

responses from accumbens core injections of cocaine may have been weakened by local anesthesia (Ikemoto and Witkin, 2003).

The present experiments show that the locomotor stimulant effects of systemically administered cocaine are associated with DAergic neurotransmission in core rather than medial shell. This result generalized to several doses of the drug and to both i.p. and i.v. routes of administration. These findings accord with observations using systemic amphetamine (Boye et al., 2001; Sellings and Clarke, 2003 and references therein) and methylphenidate (Sellings et al., submitted). Taken together, they suggest a general mechanism by which systemically administered psychostimulants produce activating effects. Whether core DA transmission directly mediates the locomotor stimulant action of these drugs, or plays an indirect enabling role, remains a question for the future.

Differences between i.p. and i.v. cocaine CPP. In the present study, i.v. cocaine produced CPP that appears dependent on DA transmission in both medial shell and OT. In contrast, i.p. cocaine CPP did not appear dependent on accumbens DA transmission. This finding is consistent with reports suggesting that i.v. cocaine produces DA-dependent CPP, and i.p. cocaine DA-independent CPP (Morency and Beninger 1986; Spyraki et al., 1987). Although neuroadaptation may account for the lack of lesion effect on i.p. cocaine CPP, this appears unlikely considering that similar medial shell lesions reduced CPP both for i.v. cocaine and for amphetamine (Sellings and Clarke 2003). Our results do not rule out other forms of accumbens involvement; indeed glutamatergic and serotonergic manipulations within this structure affect i.p. cocaine CPP (Kaddis et al, 1995; Harris et al, 2001).

Since cocaine produces CPP more potently after i.v. than i.p. administration (Spyraki et al., 1987; O'Dell et al., 1996), care was taken in the present study to select submaximal i.p. and i.v. doses of cocaine approximately matched in terms of CPP magnitude. Hence, it is likely that the differential sensitivity to DA depletion reflected route of administration and not dose.

The neurochemical basis of this differential susceptibility cannot readily be related to changes in extracellular DA. The i.v. dose used (0.5 mg/kg) has been reported to increase dialysate DA levels in the medial shell but not the core (Pontieri et al., 1995), whereas the i.p. dose (10 mg/kg) robustly increased DA levels in both subregions (Cadoni et al., 2000). Another reported difference between i.v. and i.p. cocaine administration is that only the former caused significant increases in glucose metabolism in NAcc and OT (Porrino, 1993); in the latter study, the use of a wide range of doses suggests strongly that route of administration was the critical factor. The basis for route-dependent effects on cerebral glucose utilization, and the possible relation to cocaine reward, remain to be elucidated.

Cocaine CPP: dependence on both medial shell and OT. Although there is a rich literature linking the NAcc to drug reward, possible OT involvement has been largely unexamined (Clarke et al., 1990; Kornetsky et al., 1991; Ikemoto, 2003; Ikemoto, 2005;

Ikemoto and Donahue, 2005). The present results suggest both medial shell and OT play important roles in mediating i.v. cocaine reward.

Self-administration of cocaine directly into the ventral striatum appears strongly site-dependent; responding was vigorous for infusions into amOT, marginal in medial shell, and negligible within accumbens core (Rodd-Henricks et al., 2002; Ikemoto, 2003). In addition, only cocaine infusion at amOT sites produced CPP at the doses tested (Ikemoto, 2003). However, the behavioral effects of focal cocaine infusion into the NAcc (shell or core) may be masked by local anesthesia (Ikemoto and Witkin, 2003). Nevertheless, DA antagonist microinjection experiments suggest that it is medial shell rather than core that mediates the reinforcing effects of self-administered i.v. cocaine (Bari and Pierce, 2005).

In Experiment 2, lesions of the medial shell reduced i.v. cocaine CPP independently of accumbens core; in this experiment, DA denervation in the OT was minimal. When 6-OHDA infusions of medial shell and OT were directly compared (Experiment 4), only OT DA innervation significantly predicted i.v. cocaine CPP. These results may indicate that the OT is a stronger mediator of cocaine reward, as concluded from findings based on intracranial cocaine infusion (Ikemoto, 2003). It is unlikely that these lesion effects represent disruptions of memory or learning, as medial shell lesions did not affect CPP induced by morphine (Sellings and Clarke, 2003) or i.p. cocaine (present study), and extensive 6-OHDA lesions of OT did not disrupt amphetamine CPP (Clarke et al., 1990).

Several factors could determine the relative contributions of OT *vs.* medial shell to psychostimulant CPP. First, the nature of the CPP paradigm used may be a factor. Our CPP procedure is based on tactile cues; other types of stimuli may engage other ventral striatal subregions. Another factor of potential importance is the drug in question. Our results suggest that i.v. cocaine CPP engages OT mechanisms. This does not appear to be the case for i.p. amphetamine CPP (Clarke et al., 1990).

Conclusions. The increase in locomotor activity observed after psychostimulant administration appears related to increased DA transmission in NAcc core. In contrast, CPP appears more complex, likely depending on drug and route of administration. The present study suggests that DA transmission in both medial shell and OT is important for i.v. cocaine CPP. Our findings build on recent evidence suggesting that distinct ventral striatal subregions participate in different aspects of drug reward (Sellings and Clarke, 2003; Ikemoto, 2003; Ikemoto and Donahue, 2005; Pecina and Berridge, 2005). Whether these structures act in concert or independently remains a question for further study (van Dongen et al., 2005).

Figure 1. Representative photomicrographs of Nissl staining in sham-lesioned (A) and amOT-lesioned (B, C) animals adjacent to the infusion site. In some (30%) of amOT lesioned rats, a small region of reduced cell density was observed compared to sham-lesioned rats (black arrow, Panel B). Larger regions of decreased cell density were seen in a subset (~10%) of lesioned animals (black arrow, Panel C). Scale bar 100 μm. Abbreviations: ac, anterior commissure; Tu, medial olfactory tubercle.

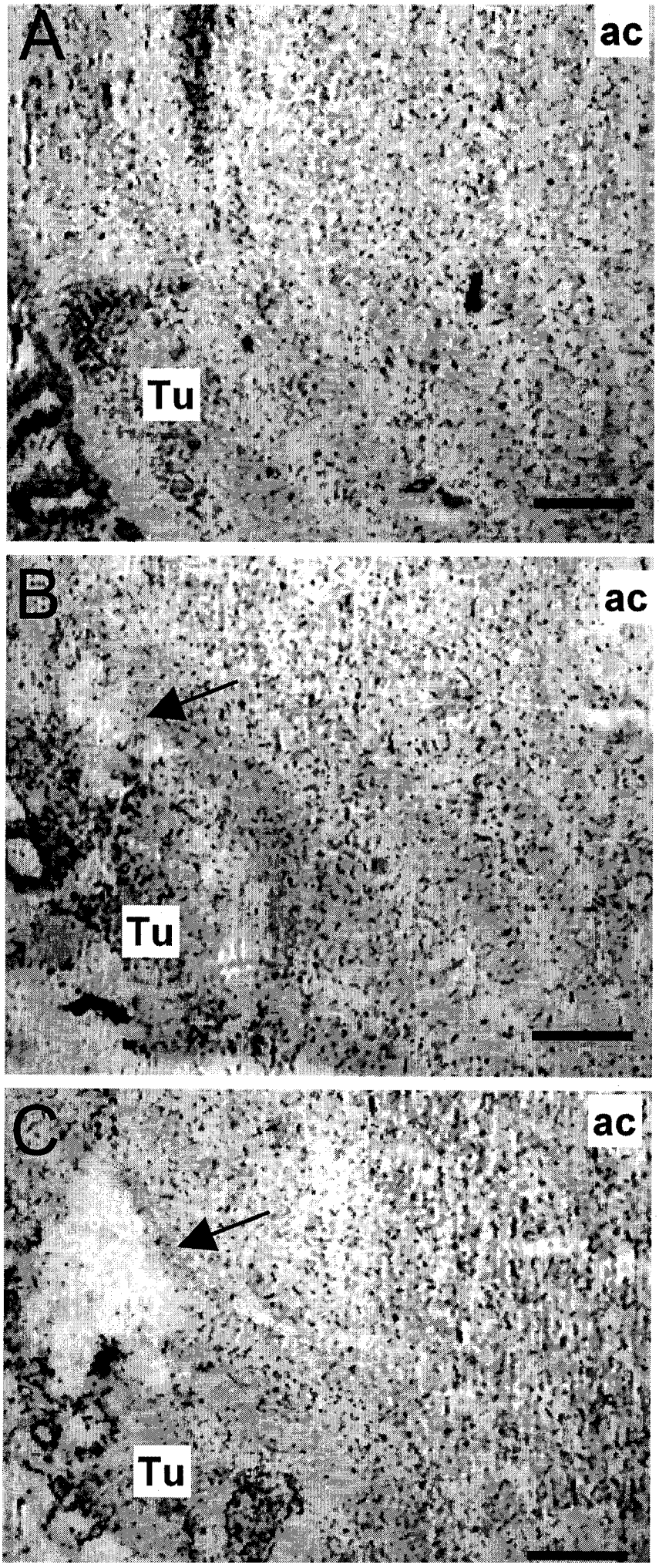
A
ac
Tu
B
ac
Tu
C
ac
Tu

Figure 2. (A) Locations of sampled [^{125}I] RTI-55 binding in core, medial shell, ventral shell, olfactory tubercle and ventral caudate putamen. Each rat was sampled at four anterior-posterior levels. Numbers are distances (in millimetres) anterior to interaural zero. Sampling areas were circles of 0.3 mm diameter. (B) Sampling regions for olfactory tubercle subregions (anteromedial (amOT), anterolateral (alOT) and posterior (pOT)) in Experiment 4, and in post-hoc analyses of Experiment 2. At levels 11.2, 10.7 and 10.2, both amOT and alOT were sampled. At levels 9.7, 9.2 and 8.7, only pOT was sampled. Figures adapted from Paxinos and Watson (1997).

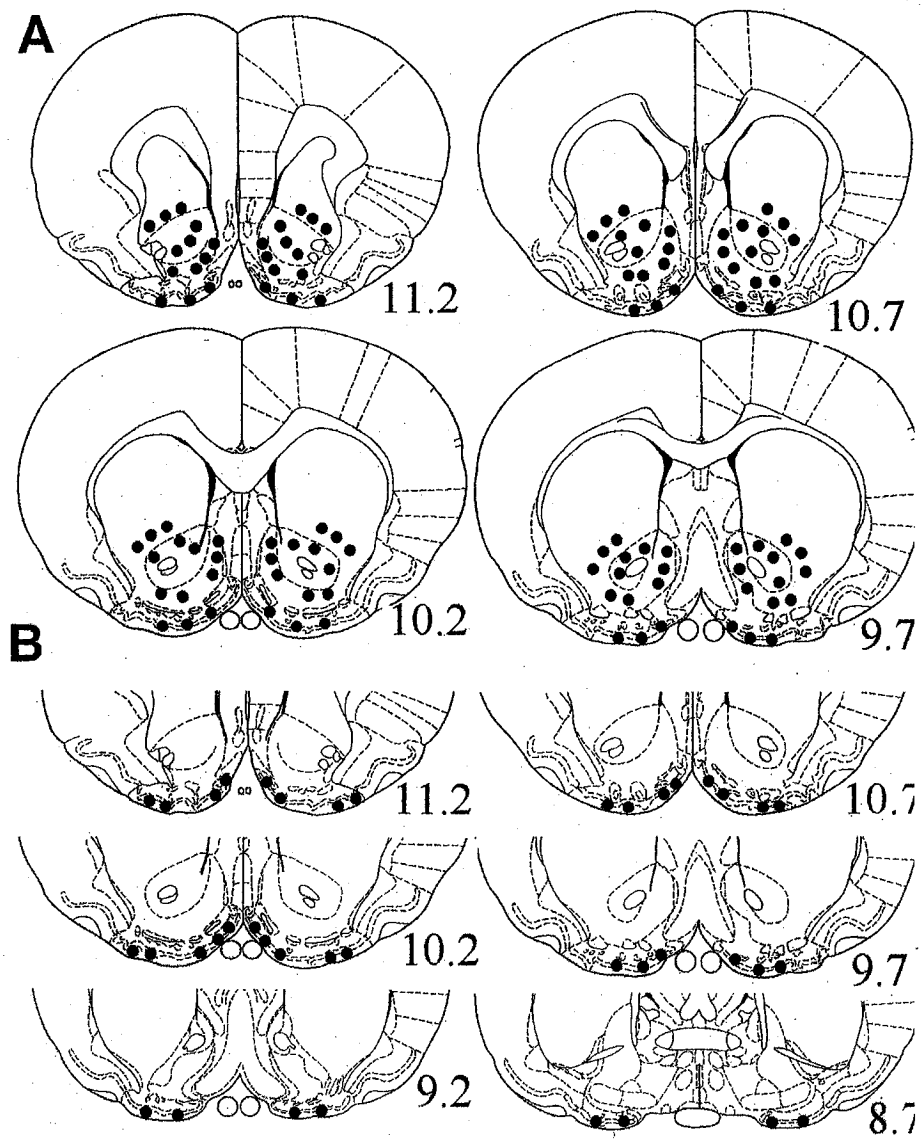

Figure 3. Representative autoradiographic images of [^{125}I] RTI-55 binding to DAT in animals from medial shell-lesioned, anteromedial olfactory tubercle-lesioned and sham-operated groups in Experiment 4. Since binding was similar between groups that received vehicle in medial shell and medial olfactory tubercle, the latter group has been omitted. Numbers designate distance anterior to interaural zero (in millimetres). Radioligand binding was obtained at a nonsaturating concentration of radioligand. Arrows refer to the medial shell. Arrowheads (pointing upward) refer to the anteromedial olfactory tubercle.

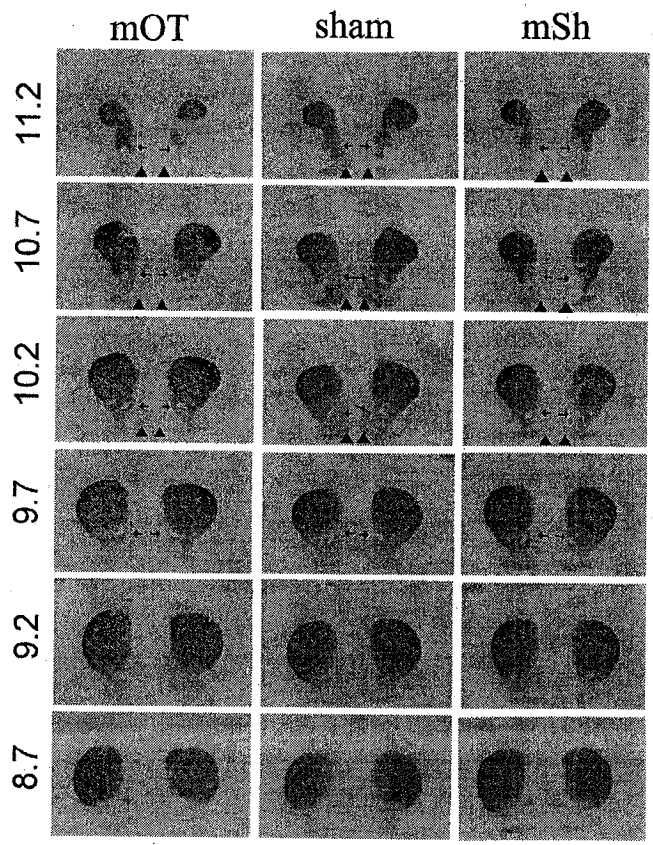

Figure 4. Effect of 6-OHDA lesions of NAcc medial shell or core on locomotor responses to a range of i.p. and i.v. cocaine doses (Experiment 3). Each rat (n = 5-10 per group) was tested with i.v. (0-1.5 mg/kg) and i.p. (0-20 mg/kg) cocaine in a repeated measures design. Absolute locomotor activity at all doses of i.p. and i.v. cocaine are shown in panels A and B respectively. The stimulant effect of cocaine (i.e. cocaine-saline difference score) is illustrated in panels C and D. Locomotor response correlated positively and significantly with DAT binding in core at all doses except 0.5 mg/kg i.v. Shell refers to medial shell.

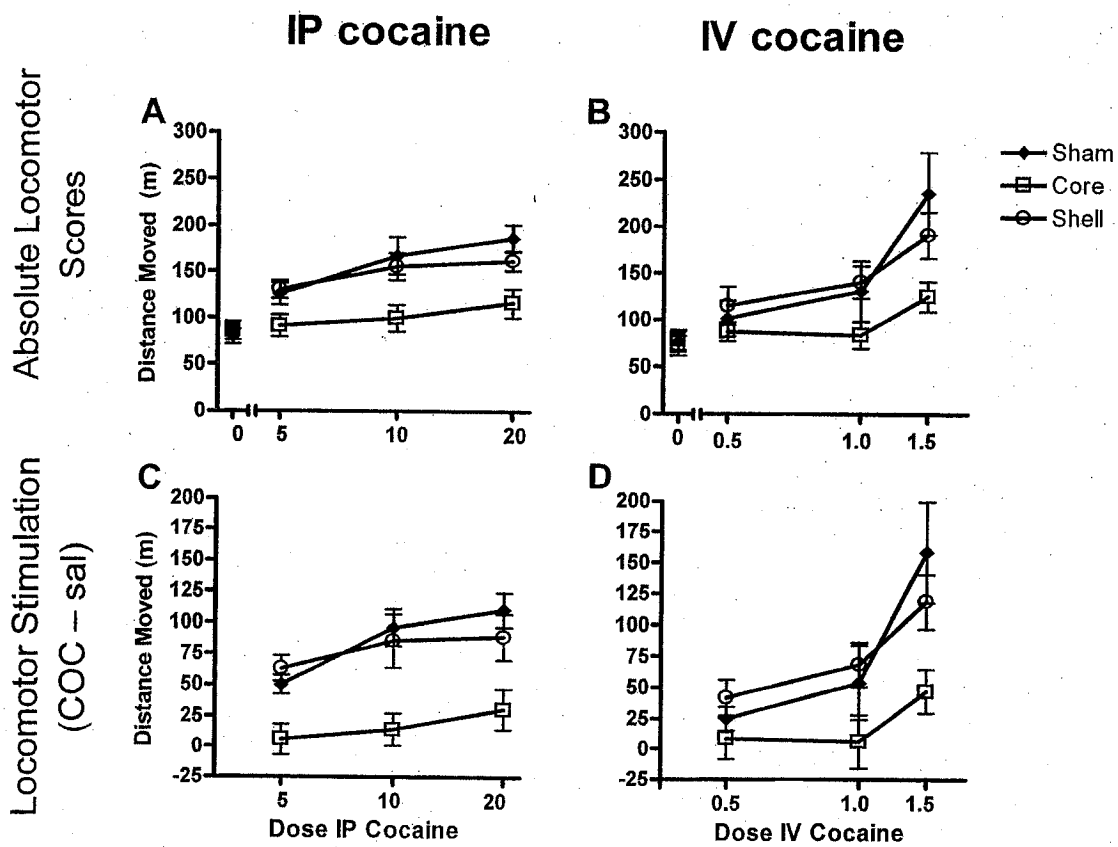

Figure 5. Effect of bilateral 6-OHDA infusion into either NAcc core or medial shell on locomotor response and CPP to i.v. cocaine (Experiment 2). Rats were allowed 7-10 d recovery after jugular catheter implantation and stereotaxic surgery prior to conditioning with i.v. cocaine (0.5 mg/kg). Locomotor responses (panels A-C) are expressed as the difference between the mean distance moved (m) during conditioning sessions with i.v. cocaine *vs.* saline. CPP magnitude (Panels D-F) is expressed as the difference between time spent on the drug-paired and saline-paired floor textures on test day (in s, 600 s test). DAT labeling in core or medial shell is expressed as a percent of combined sham-lesioned groups. Both sham and shell-lesioned groups exhibit significant locomotor stimulation (Panel A). Locomotor response tended to correlate positively with DAT binding in core (Panel B). Both sham and core-lesioned groups exhibit significant CPP (Panel D). CPP magnitude correlated positively and significantly with DAT binding in medial shell (Panel F), and tended to correlate negatively with DAT binding in core (Panel E). CV, Core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion. Shell refers to medial shell.

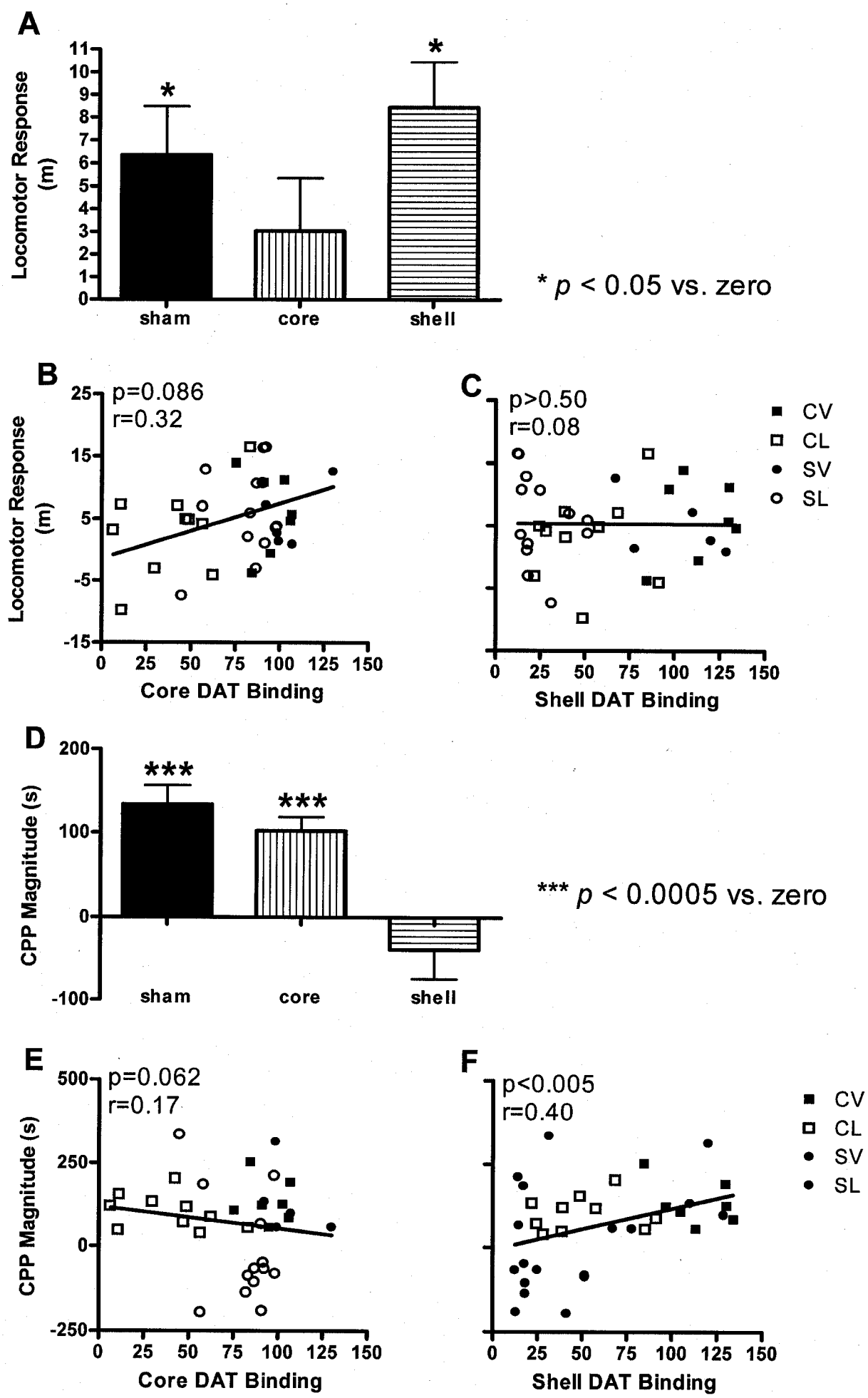
A
Locomotor Response (m)
sham
core
shell
* p < 0.05 vs. zero
B
p=0.086
r=0.32
Locomotor Response (m)
Core DAT Binding
C
p>0.50
r=0.08
CV
CL
SV
SL
Shell DAT Binding
D
CPP Magnitude (s)
***
sham
core
shell
*** p < 0.0005 vs. zero
E
p=0.062
r=0.17
CPP Magnitude (s)
Core DAT Binding
F
p<0.005
r=0.40
CV
CL
SV
SL
Shell DAT Binding

Figure 6. Effect of bilateral 6-OHDA infusion into either NAcc core or medial shell on locomotor response and CPP to intraperitoneal (i.p.) cocaine (Experiment 3). Rats were conditioned with i.p. cocaine (10 mg/kg). Data are presented as in Figure 5. All groups exhibit significant locomotor stimulation (Panel A), but that of core-lesioned animals was smaller than that of the sham- and shell-lesioned groups ($p < 0.05$). Only sham rats exhibited significant CPP, but core and shell-lesioned animals also tended to exhibit CPP (Panel D). Locomotor response correlated positively and significantly with DAT binding in core (Panel B). No other behavioral responses correlated with DAT labeling in either structure (Panels C, E and F). CV, Core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion. Shell refers to medial shell.

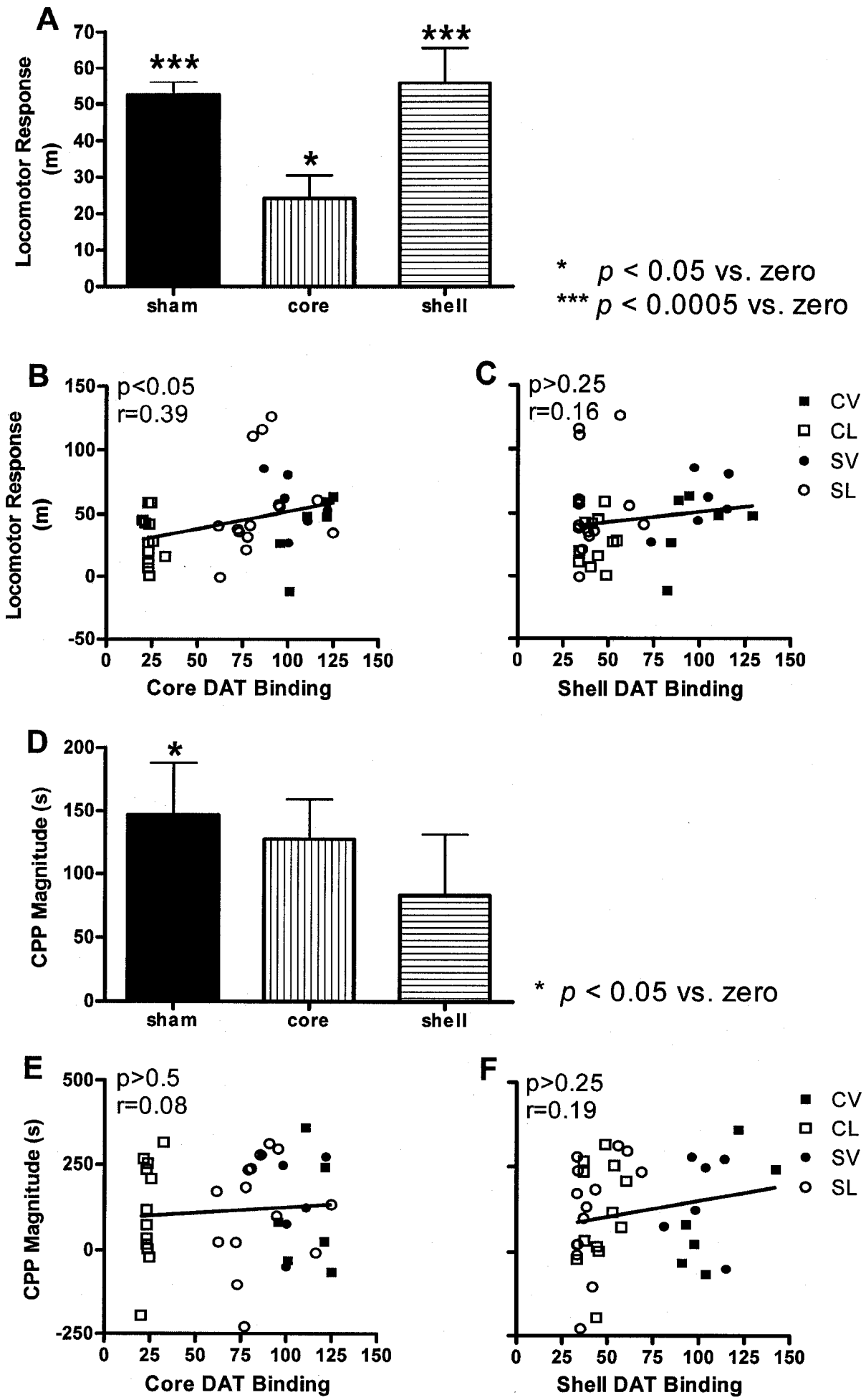
A
Locomotor Response (m)
70
60
50
40
30
20
10
0
***
*
***
sham
core
shell
* p < 0.05 vs. zero
*** p < 0.0005 vs. zero
B
p<0.05
r=0.39
Locomotor Response (m)
150
100
50
0
-50
0 25 50 75 100 125 150
Core DAT Binding
C
p>0.25
r=0.16
CV
CL
SV
SL
0 25 50 75 100 125 150
Shell DAT Binding
D
CPP Magnitude (s)
200
150
100
50
0
*
sham
core
shell
* p < 0.05 vs. zero
E
p>0.5
r=0.08
CPP Magnitude (s)
500
250
0
-250
0 25 50 75 100 125 150
Core DAT Binding
F
p>0.25
r=0.19
CV
CL
SV
SL
0 25 50 75 100 125 150
Shell DAT Binding

Figure 7. Relationship of DAT labeling in nucleus accumbens medial shell *vs.* olfactory tubercle in Experiment 4 (n=46 rats). [^{125}I]RTI-55 autoradiography for DAT was used to assess residual DA innervation (see Materials and Methods), and expressed as a percentage of the mean value of the sum of medial shell-vehicle and olfactory tubercle-vehicle groups. Correlational analysis revealed a significant relationship between medial shell and olfactory tubercle binding (r=0.39, $p < 0.01$). OTV, olfactory tubercle vehicle; OTL, olfactory tubercle lesioned; SV, medial shell vehicle; SL, medial shell lesion.

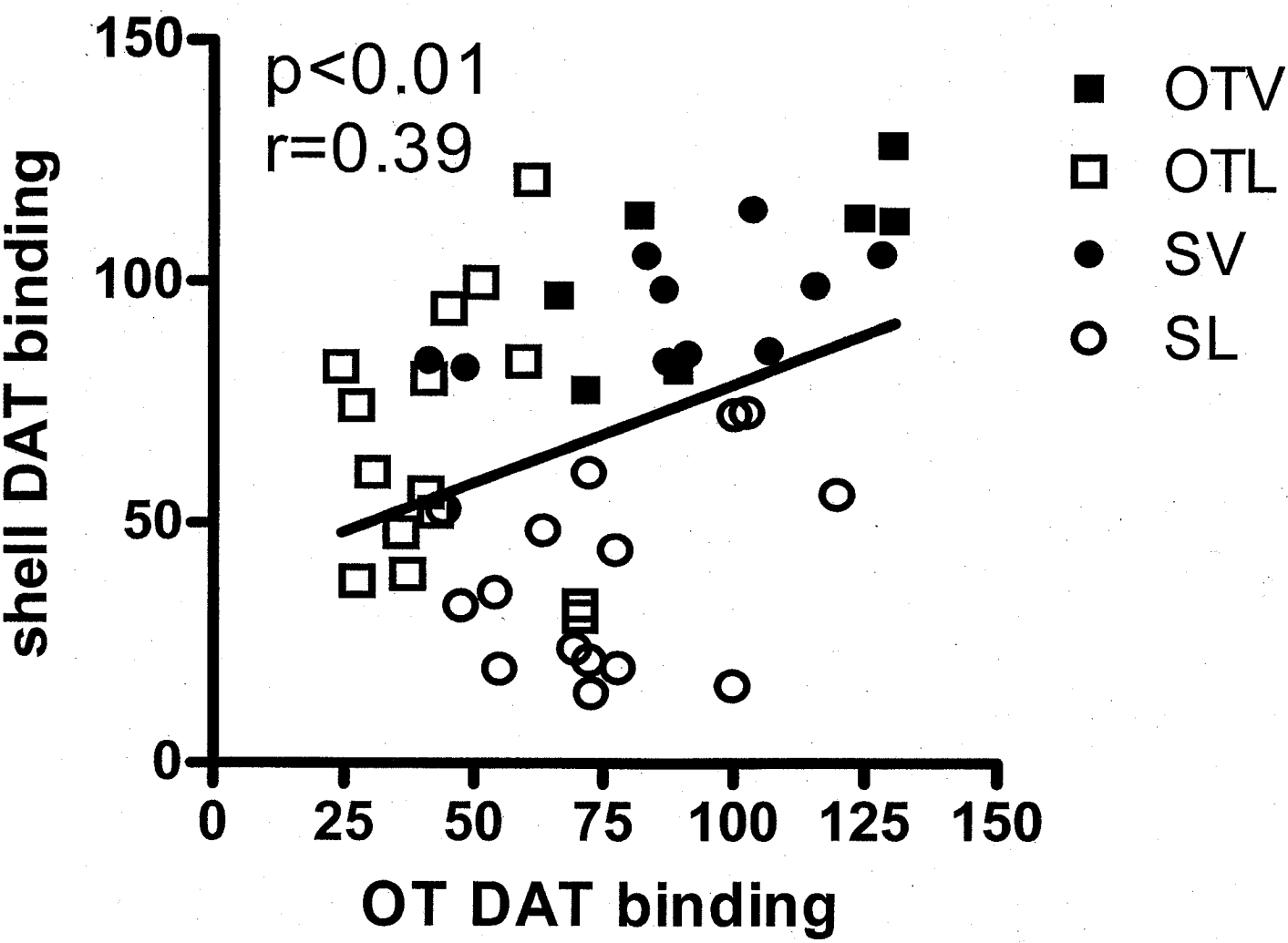

Figure 8. Effect of 6-OHDA lesions of olfactory tubercle and medial shell on i.v. cocaine CPP (Experiment 4). CPP magnitude was calculated as the difference between the time spent on the drug-paired and saline-paired sides. CPP magnitude correlated positively and significantly with DAT binding in olfactory tubercle (Panel C), but not with DAT binding in medial shell (Panel B). OTV, olfactory tubercle vehicle; OTL, olfactory tubercle lesioned; SV, medial shell vehicle; SL, medial shell lesion. Shell refers to medial shell.

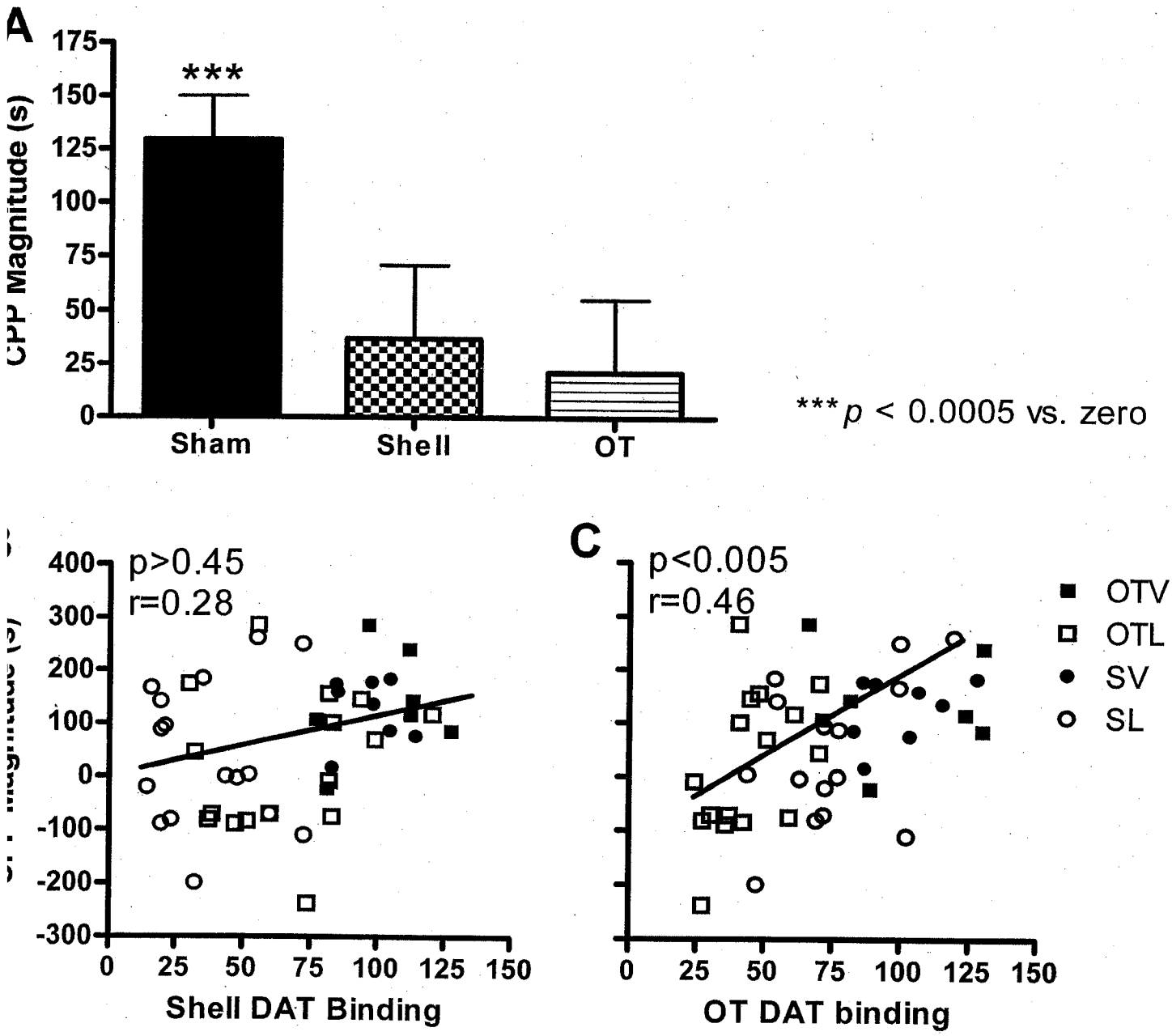

Table 1. Experimental parameters for Experiments 1-4. Sham groups represent a combination of rats infused with vehicle in core and shell (Experiments 1-3) or shell and amOT (Experiment 4).

Experiment	Lesion site[a]	Dose (mg/kg)	Route	Behavior[b]	n[c]
1	Core or shell	0.5-1.5 i.v., 5-20 i.p.	i.v.,, i.p.	LMA	11
2	Core or shell	0.5	i.v.	CPP, LMA	10-14
3	Core or shell	10	i.p.	CPP, LMA	12-14
4	amOT or shell	0.5	i.v.	CPP	15-16

[a] Shell refers to medial shell.

[b] CPP, conditioned place preference; LMA, locomotor activity.

[c] n is number of rats per surgery group (core, medial shell or anteromedial olfactory tubercle, and the combined sham-operated groups).

Table 2. Residual DAT and SERT binding in rats lesioned in core or medial shell in ventral striatal subregions (Experiments 1, 2 and 3). mSh, medial shell; vSh, ventral shell; OT, olfactory tubercle; vCP, ventral caudate putamen. Values given are mean ± SEM as a percent of combined sham group.

Experiment	**1**			**2**			**3**		
6-OHDA site	Sham	Core	mSh	Sham	Core	mSh	Sham	Core	mSh
DAT									
Core	100±4	40±8	85±5	100±3	25±1	86±5	100±7	20±3	95±5
mSh	100±11	60±9	31±7	100±5	47±3	42±3	100±8	48±4	36±6
vSh	100±5	63±12	76±4	100±7	45±3	92±7	100±6	35±6	98±8
OT	100±4	74±11	80±5	100±5	47±5	76±6	100±12	46±4	80±4
vCP	100±4	66±8	92±4	100±5	45±2	102±7	100±10	50±7	107±6
SERT									
Core	100±9	97±7	91±5	100±5	83±4	93±4	100±5	108±6	97±3
mSh	100±6	100±2	93±5	100±2	100±4	97±5	94±3	105±6	95±7
vSh	100±5	102±6	100±5	100±2	90±4	99±4	100±6	118±8	108±3
OT	100±6	110±6	100±5	100±3	100±5	101±5	100±6	116±5	108±4
vCP	100±9	99±6	98±5	100±4	90±5	107±3	100±3	88±3	93±3

Table 3. DAT and SERT binding in medial shell- or anteromedial olfactory tubercle (amOT)- lesioned rats in imaged ventral striatal subregions (Experiment 4). Values are expressed as mean ± SEM. mSh, medial shell; vSh, ventral shell; amOT, anteromedial olfactory tubercle; alOT, anterolateral olfactory tubercle; pOT, posterior olfactory tubercle; vCP, ventral caudate putamen.

6-OHDA site	Sham	Medial shell	amOT
DAT			
Core	100±5	87±6	89±5
mSh	100±4	40±5	66±7
vSh	100±5	77±7	75±3
amOT	100±8	57±7	34±8
alOT	100±9	70±6	55±6
pOT	100±10	85±6	51±6
vCP	100±4	100±5	95±4
SERT			
Core	100±14	110±4	97±9
mSh	100±11	100±4	90±8
vSh	100±15	109±6	97±9
amOT	100±13	101±5	92±10
alOT	100±14	109±4	105±12
pOT	100±13	108±6	95±12
vCP	100±15	123±7	110±10

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Intervening Section 3

In the case of cocaine, it appears as if route of administration is a critical determinant of the neural substrates of drug reward. More specifically, intravenous cocaine CPP was reduced by 6-OHDA lesions of the medial ventral striatum (either medial shell or medial OT), whereas intraperitoneal cocaine CPP was unaffected by medial shell lesions.

In the next chapter, I turned my attention to a third psychomotor stimulant, methylphenidate. Despite its wide-spread use, no causal link had been established between its rewarding effects and DA. In this study, it was first shown that both methylphenidate-stimulated locomotor activity and CPP were dose-dependently reduced by systemic administration of the dopanime receptor antagonist cis-flupenthixol. Hence, anatomical localization of these effects within the ventral striatum was examined. As with other drugs, locomotor stimulation appeared dependent on core DA transmission. In the case of CPP, mOT but not medial shell lesions effectively reduced methylphenidate CPP.

CHAPTER 6: Characterization of rewarding and locomotor stimulant effects of intravenously-administered methylphenidate in rats

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Neuroscience (2006) 141: 1457-1468.

Abstract: In general, psychostimulants are thought to exert rewarding and locomotor stimulating effects via increased dopamine transmission in the ventral striatum. However, little is known about the mechanisms underlying the effects of the stimulant drug methylphenidate. The present study examined the putative role of dopaminergic transmission in intravenous methylphenidate reward as measured by conditioned place preference. Rats were shown to exhibit conditioned place preference for intravenous methylphenidate (5 mg/kg, not 2 mg/kg). Administration of the dopamine receptor antagonist cis-flupenthixol (0.1-0.8 mg/kg i.p.), either during conditioning or on test day, dose-dependently attenuated the magnitude of the conditioned place preference. Finally, we examined the effects of bilateral 6-hydroxydopamine lesions of nucleus accumbens core, medial shell or anteromedial olfactory tubercle on the rewarding and locomotor stimulant effects of methylphenidate. Residual dopamine innervation, as assessed by [^{125}I]-RTI-55 binding to the dopamine transporter, revealed a significant association between core dopamine innervation and the locomotor stimulant effect of methylphenidate. However, neither core nor medial shell dopamine innervation was related to conditioned place preference magnitude. Instead, conditioned place preference magnitude was associated with dopamine innervation in the anteromedial olfactory tubercle. These results establish a role for dopaminergic transmission in both intravenous methylphenidate conditioned place preference and locomotor stimulation. As well, they suggest that different ventral striatal subregions mediate the rewarding (anteromedial olfactory tubercle) and locomotor stimulant (accumbens core) effects of methylphenidate.

Keywords: dopamine, nucleus accumbens core, nucleus accumbens medial shell,

olfactory tubercle, conditioned place preference, cis-flupenthixol

Abbreviations: 6-OHDA, 6-hydroxydopamine; amOT, anteromedial olfactory tubercle; alOT, anterolateral olfactory tubercle; CPP, conditioned place preference; DA, dopamine, DAergic, dopaminergic; DAT, dopamine transporter; ; i.p., intraperitoneal; i.v., intravenous; mSh, medial shell; NAcc, nucleus accumbens; OT, olfactory tubercle; pOT, posterior olfactory tubercle; s.c., subcutaneous; SERT, serotonin transporter; vCP, ventral caudate putamen; vSh, ventral shell

Considerable evidence indicates that the rewarding and behavioural activating effects of cocaine and amphetamine occur via increased dopaminergic (DAergic) transmission in the ventral striatum (Koob et al., 1998; Everitt and Wolf, 2002; Wise, 2004). Much less is known in this regard about other psychostimulant drugs such as methylphenidate. Like cocaine, methylphenidate blocks the dopamine transporter (DAT) and increases interstitial dopamine (DA) levels in the nucleus accumbens (NAcc) in rats (Gerasimov et al., 2000). In addition, PET studies employing [^{11}C]raclopride binding have suggested that intravenous methylphenidate can also increase DA transmission in the human striatum (Volkow et al., 2004). Based on this evidence and by analogy with other psychostimulants, Volkow et al. (2004) have proposed that the euphoric and/or reinforcing effects of methylphenidate are dependent on striatal DA transmission. However, to our knowledge, no causal link between increased DA transmission and methylphenidate reward has been established.

Rewarding effects of methylphenidate occur not only in humans but have also been shown in animals; the drug is self-administered intravenously in several mammalian species including non-human primates as a replacement for other stimulant drugs (Bergman et al., 1989; Kollins et al., 2001), and it also induces conditioned place preference (CPP) in rats (Martin-Iverson et al., 1985; Mithani et al., 1986; Meririnne et al., 2001). The pharmacology of methylphenidate self-administration remains to be explored, but evidence to date suggests that methylphenidate CPP can occur independently of brain DA. In particular, CPP acquisition is inhibited only at very high doses of DA antagonists (Martin-Iverson et al., 1985; Mithani et al., 1986; Meririnne et al., 2001).

In previously published CPP studies, methylphenidate was given by intraperitoneal injection. Studies with cocaine have shown that route of administration can critically determine abuse liability and can also determine whether CPP occurs via a DAergic or non-DAergic mechanism (Spyraki et al., 1982; Spyraki et al., 1987; Nomikos and Spyraki, 1988). Abuse liability of methylphenidate in humans is presumably also route dependent. Although oral methylphenidate exhibits minimal abuse liability (Swanson and Volkow, 2003), intranasal abuse is common (Barrett et al., 2005) and there are several reports of intravenous use (Parran and Jasinski, 1991; Barrett et al., 2005). In light of this, the rewarding effects of intravenous methylphenidate warrant separate examination.

Recent rodent studies using amphetamine and cocaine suggest that rewarding and locomotor stimulant drug effects can be anatomically dissociated within the ventral striatum. To date, reward processes have been most clearly linked to the medial portion of the NAcc shell (Di Chiara et al., 2004). For example, direct and indirect dopaminergic agonists are self-administered by rats directly into this subregion but not into NAcc core (Ikemoto and Wise, 2004). However, recent studies have implicated the (antero)medial olfactory tubercle as potentially more important than the medial shell in both cocaine and amphetamine reward (Ikemoto, 2003; Ikemoto et al., 2005; Sellings et al., 2006). In contrast, locomotor stimulation has been reported in rats after focal infusion into core and/or shell sites, depending not only on the study (see Boye et al., 2001; Sellings and Clarke, 2003) but also on the drug in question (Ikemoto, 2002). Using an alternate approach, we recently combined systemic amphetamine challenge with prior 6-OHDA lesions of NAcc core or medial shell (Sellings and Clarke, 2003). In this study, DAergic depletion in core and medial shell reduced amphetamine-induced locomotor stimulation and CPP, respectively.

The aims of the present study were threefold. First, we set out to establish whether rats would form a CPP for intravenous methylphenidate. Second, we tested if systemic dopamine receptor blockade would affect either the acquisition or the expression of methylphenidate CPP. The final aim was to determine if the rewarding and locomotor stimulant effects of intravenous methylphenidate could be dissociated by anatomically-selective 6-OHDA lesions of ventral striatal subregions, including NAcc core, medial

shell, and medial olfactory tubercle as previously seen with cocaine (Sellings et al., 2006).

Experimental procedures

Subjects

Subjects were 111 male Long–Evans rats (Charles River, St. Constant, Quebec) weighing 270–340 g at time of surgery. Rats were housed individually (Experiments 1 and 4) or in groups of three (Experiments 2, 3 and 5) in clear Plexiglas cages in a temperature- and humidity-controlled animal colony, lit from 7 A.M. to 7 P.M. Food and water were available *ad libitum* except during behavioural testing. All experiments were approved by the McGill Faculty of Medicine Animal Care Committee in accordance with Canadian Council on Animal Care guidelines.

Intravenous catheterization

Rats were implanted with chronic indwelling silastic catheters (0.51 mm I.D. and 0.94 mm O.D., Fisher Scientific, Montreal, Quebec) in the left jugular vein under ketamine (80 mg/kg) and xylazine (16 mg/kg) anaesthesia. Tubing was secured to the vein by surgical silk sutures, led subcutaneously to the skull surface, and was then fitted onto a 22 gauge cannula attached to a plastic connector (Model number C313G-5UP, Plastics One, Roanoke, VA). The cannula/connector was fixed to the animal's skull with small stainless steel screws (Lomir, Notre-Dame-de-L'Ile Perrot, Quebec) and dental cement (Stoelting, Wood Dale, IL). To keep catheters patent, 0.1-0.15 ml heparinized 0.9% saline was administered at the end of surgery, on the first day of behavioural testing, and

every 2-3 days thereafter. Animals were allowed 7-10 days recovery from surgery before starting CPP testing.

Stereotaxic infusion of 6-OHDA

In Experiment 4, at the same time as intravenous catheterization surgery, rats were placed in a stereotaxic apparatus (Kopf, Tujunga, CA) with the incisor bar set at -3.9 mm. Bilateral infusions of either 6-OHDA (lesioned groups) or vehicle (sham-lesioned groups) were made into either NAcc core or medial shell, or anteromedial olfactory tubercle (amOT). Infusions into all three lesion sites were made via a 30 gauge stainless steel cannula attached by polyethylene tubing to a 10 µl Hamilton syringe. For core and medial shell, syringes were driven by a model 5000 Micro Injection Unit (Kopf). For amOT, syringes were driven by a syringe pump. For greater accuracy, coordinates for all three target subregions were derived from the mean of bregma and intraural coordinate systems. Thus, anterior-posterior coordinates were +10.2 mm from interaural zero and +1.2 mm from bregma for both core and shell; in amOT, they were +10.7 mm and +1.7 mm from interaural zero and bregma respectively. Lateral coordinates were ±0.6 mm (shell), ±2.4 mm (core) or ±0.8 mm (amOT). Ventral coordinates for shell (three injections) were +2.0, +2.4, and +2.8 mm from interaural zero and -8.0, -7.6, and -7.2 mm from bregma. Ventral coordinates for core were +2.7 mm from interaural zero and -7.3 mm from bregma; for amOT, they were +1.1 mm from interaural zero and -8.9 mm from bregma. All coordinates are based on the atlas of (Paxinos and Watson, 1997). 6-OHDA or vehicle was infused on each side in a volume of 0.2 µl (amOT), 0.1 µl (core), or as three infusions of 0.05 µl (medial shell). The rate of infusion was 0.1 µl/min for

core and medial shell, and 0.1 µl/10 min for amOT. The concentration of 6-OHDA used was 80 µg/µl (core), 48 µg/µl (medial shell) or 40 µg/µl (amOT). The cannula remained at the final infusion site for 5 min. Animals were allowed 7–10 d recovery prior to the start of conditioning.

Conditioned place preference and locomotor activity testing

The apparatus and general procedure were as previously described (Sellings and Clarke, 2003). Briefly, the procedure consisted of three phases: pre-exposure (one day), conditioning (six days) and test (one day). All phases were carried out in a one-compartment box (58 cm x 29 cm x 53 cm) with walls made of white plastic-coated particle board. In the one-day pre-exposure phase, rats received intravenous saline infusions immediate prior to placement in the CPP cage. Beta-Chip sawdust bedding covered the floor of the cage. The conditioning phase lasted six consecutive days, with one session of 15 min occurring each day. In all, there were three sessions with drug and three sessions with saline administration, occurring on alternating days. Two square tactile tiles of either bar or mesh texture were placed in the bottom of the cage, and paired with drug or saline administration. During this phase, the video tracking software (EthoVision v 3.0, Noldus Information Technology, Leesburg, VA) measured locomotor activity, expressed as horizontal distance moved (in metres). During the test phase, one bar and one mesh tile were placed on the bottom of the cage. The time spent on bar or mesh texture was measured by EthoVision software. All three phases were carried out under darkroom lighting using a Kodak GBX-2 safelight filter (Vistek, Toronto, Ontario, Canada), to minimize visual cues. Animals do not spontaneously prefer either texture (unpublished observations), and all experiments were as fully counterbalanced as

possible with respect to drug-texture pairing and order of drug pairing (drug-saline or saline-drug) within each surgery group. For all experiments, pre-exposure sessions lasted 20 minutes, conditioning sessions for 15 minutes, and the test session 10 minutes. To facilitate intravenous infusion immediately after placement in the test cage, a fluid swivel was fixed above the centre of each cage. Each swivel was connected to on one end to a 1 ml syringe, and on the other end to a brass connector (Produits MSM, Laval, Quebec) and protective spring (Heiplex, Montreal) via Tygon tubing of 0.51 mm diameter. The cannula fixed to the skull of the rat was attached to the Tygon tubing, and the brass connector fastened to the plastic connector, to secure the tubing to the cannula. Drug was infused over 25-30 s at a volume of 1 ml/kg.

Experimental design

Experiment 1. Rats (n=17) were conditioned at one of two doses of intravenous methylphenidate (2 mg/kg, n=8 and 5 mg/kg, n=9) and subsequently tested for CPP.

Experiment 2. Rats (n=25) were administered one of four doses of cis-flupenthixol (0 mg/kg (n=5), 0.1 mg/kg (n=7), 0.3 mg/kg (n=8) or 0.8 mg/kg (n=5)) s.c., 30 minutes prior to each of the six conditioning sessions (drug: 5 mg/kg methylphenidate, i.v.), and subsequently tested for CPP.

Experiment 3. Rats (n=28) were conditioned with 5 mg/kg i.v. methylphenidate. On test day, rats received one of four doses of cis-flupenthixol (0 mg/kg (n=6), 0.1 mg/kg (n=7), 0.3 mg/kg (n=8) or 0.8 mg/kg (n=7)) s.c., 30 minutes prior to placement in the cage.

Experiment 4. Rats (n=25) sustaining vehicle (sham-lesioned; n=6) or 6-OHDA infusion into core (core-lesioned; n=9) or medial shell (shell-lesioned; n=10) were subsequently conditioned with 5 mg/kg intravenous methylphenidate after recovering from surgery as described above.

Experiment 5. Rats (n=17) sustaining anteromedial OT vehicle (sham; n=6) or 6-OHDA (lesion; n=11) infusions were subsequently conditioned with 5 mg/kg intravenous methylphenidate after recovering from surgery.

Tissue Preparation

Tissue was prepared for autoradiography and Nissl-staining (cresyl violet) as previously described (Sellings and Clarke, 2003). Briefly, rats were sacrificed 3 to 5 hours following CPP testing, by decapitation under sodium pentobarbital (20 mg/kg, i.v.) anaesthesia. Rats not anesthetized within 10 s of injection were excluded from statistical analysis. Brains were removed, frozen in 2-methylbutane at -50°C for 30 sec, and stored at -40°C. Coronal sections (20 μm) were taken on a cryostat at four rostrocaudal levels (11.2, 10.7, 10.2 and 9.7 mm anterior to interaural zero) through the ventral striatum. At each level, four adjacent sections were collected for autoradiography and one for Nissl staining with cresyl violet. Sections were thaw mounted onto gelatin-subbed slides, air dried at room temperature for 20–30 min, and stored with desiccant at -40°C.

Quantitative [^{125}I]-RTI-55 autoradiography

The extent of the 6-OHDA lesion was quantified by autoradiographic labelling of DAT (Sellings and Clarke, 2003), using a nonsaturating concentration of [^{125}I]RTI-55 (2200

Ci/mmol; NEN-Mandel, Guelph, Ontario). This radioligand allows visualization of either DAT or 5-HT transporter (SERT) binding. To visualize DAT binding, SERT was occluded using the serotonin selective reuptake inhibitor citalopram HBr (50 nM). Analogously, to visualize SERT binding, DAT was occluded using the DAT reuptake inhibitor GBR 12935•2HCl (1 μM). Sections were thawed at room temperature for 10 min and then placed in a staining dish containing an aqueous buffer solution of 120 mM NaCl, 0.1 M sucrose, 10 mM sodium phosphate buffer, and 10 pM [^{125}I]RTI-55, with the pH adjusted to 7.4. Nonspecific binding was determined by addition of 10 μM GBR 12909 and 50 nM citalopram HBr in the DAT and SERT autoradiographic assays, respectively. Slides were incubated at room temperature for 2 hr and then washed three times in cold buffer solution (once for 1 min, twice for 20 min) and for 1-2 sec in distilled and deionised water. They were then blow dried and placed in X-ray film cassettes. Kodak BioMax MS film (Amersham Biosciences, Baie d'Urfé, Québec) was exposed to slides for 48 hr (DAT) or 120 hr (SERT) with [^{125}I] autoradiographic standards (Amersham Biosciences). After development of film, DAT and SERT binding was quantified using an MCID M4 imaging system (Imaging Research, St. Catherines, Ontario). The mean DAT binding was first calculated at each anteroposterior level and these mean values were then averaged across levels.

Histological examination

Tissue was stained with cresyl violet to assess nonspecific damage, as previously described (Sellings and Clarke, 2003).

Drugs

Drug sources were as follows: methylphenidate SO_4 (gift of National Institute on Drug Abuse, Bethesda, MD); cis-flupenthixol (Sigma-Aldrich, Oakville, Ontario); citalopram HBr (gift from H. Lundbeck A/S); ketamine HCl (Vetalar, Vetrepharm, London, Ontario); xylazine HCl (Anased, Novopharm, Toronto, Ontario); GBR 12909 (NIMH Chemical Synthesis and Drug Supply Program), and GBR 12935•2HCl (Sigma-Aldrich, Oakville, Ontario). Unless otherwise stated, all other chemicals were obtained from Fisher Scientific (Montreal, Quebec). Both methylphenidate SO_4 and cis-flupenthixol were dissolved in sterile 0.9% saline and injected at 1 ml/kg. Methylphenidate was administered intravenously immediately after placement in CPP boxes. Cis-flupenthixol was administered i.p. 30 minutes prior to all conditioning sessions (Experiment 2) or the CPP test (Experiment 3). 6-OHDA HBr was dissolved in sterile 0.9% saline containing 0.3 mg/ml sodium metabisulfite (Sigma-Aldrich) as an antioxidant and protected from light. Vehicle solutions, as well as 6-OHDA to be infused into medial shell, were neutralized to pH 7.3 ± 0.1 with NaOH (to reduce non-specific damage; see Results). Doses of all drugs except 6-OHDA HBr are expressed as the salt. 6-OHDA HBr doses are expressed as the free base.

Data analysis

A commercial software program (Systat v10.2, SPSS Inc., Chicago, IL) was used for all data analyses. CPP magnitude was calculated as the difference between times spent on the drug-paired and vehicle-paired sides during the 10-minute test session. Locomotor responses to methylphenidate were calculated as the difference of locomotor counts between drug and saline conditioning sessions. Saline scores were calculated as the mean

activity over all three conditioning sessions with saline. For Experiment 2, group differences for both CPP magnitude and locomotor stimulation were analysed by ANOVA followed by Dunnett's test. For Experiment 3, since data were not normally distributed, Kruskal-Wallis ANOVA followed by multiple Mann-Whitney U tests with Bonferroni correction were used to compare CPP magnitude after cis-flupenthixol treatment to the control (dose = 0) group. In addition, the existence of a significant CPP magnitude and locomotor stimulant effect was determined by the Wilcoxon test between times spent on the drug-paired vs. saline-paired texture, with Bonferroni correction for multiple comparisons. For Experiment 4, group differences were analysed by ANOVA. To determine whether rats experienced locomotor sensitization, three factors were used: LESION (i.e. 6-OHDA *vs.* vehicle infusion [sham]), AREA (i.e. core *vs.* medial shell) and SESSION (i.e. difference scores [methylphenidate-saline] over successive pairs of conditioning sessions). In Experiment 5, group differences were examined by Student's t-test. For both Experiments 4 and 5, the relationship between behavioural measures *vs.* [^{125}I]-RTI-55 labelling was analyzed by multiple linear regression. A p value of less than 0.05 (two-tailed) was considered significant. Group data are expressed as mean ± SEM throughout. Outliers, as defined by the statistical program, were removed prior to statistical analysis. Additionally, in Experiment 4, CPP data from four rats were missing due to an equipment malfunction during testing.

Results

Experiment 1: Rats express a conditioned place preference for intravenous methylphenidate

The occurrence of intravenous methylphenidate CPP was initially established in Experiment 1. Here, rats were conditioned with either 2 mg/kg methylphenidate (n=8) or 5 mg/kg methylphenidate (n=9). CPP magnitude was calculated as the difference between times spent on the drug-paired and vehicle-paired sides during the 10-minute test session. Rats formed a significant preference for the floor texture paired with the higher dose only ($p<0.005$, Figure 1A). Rats conditioned with 2 mg/kg spent 274 ± 18 s on the saline-paired texture and 326 ± 18 s on the methylphenidate-paired texture. For rats conditioned with 5 mg/kg, 195 ± 18 s were spent on the saline paired texture, and 405 ± 18 s on the methylphenidate-paired texture. Additionally, rats formed CPP regardless of which texture was the conditioned stimulus; CPP magnitude did not differ significantly between rats conditioned with bar vs. those conditioned with mesh texture (calculated as the difference between times spent on the drug-paired and vehicle-paired textures: $p>0.5$, Figure 1B). Locomotor activity measured during the conditioning phase was significantly stimulated by both doses of methylphenidate ($p<0.005$ for both, Figure 1C).

Experiment 2: The acquisition of a conditioned place preference for intravenous methylphenidate is dose-dependently attenuated by cis-flupenthixol given during conditioning

The effect of systemic dopamine receptor blockade on the acquisition of intravenous methylphenidate CPP was investigated in Experiment 2. Here, rats received 0, 0.1, 0.3 or 0.8 mg/kg cis-flupenthixol i.p. 30 minutes prior to each conditioning session. Rats were conditioned with 5 mg/kg methylphenidate. Only rats receiving vehicle or 0.1 mg/kg cis-flupenthixol exhibited significant conditioned place preference ($p<0.01$ for both; one-

sample t-test with Bonferroni correction, Figure 2A). Only the 0.3 mg/kg group differed significantly from control (Dunnett's test $p<0.05$; Figure 2A), with a similar trend in the 0.8 mg/kg group ($p=0.087$; Figure 2A). Locomotor activity after saline administration was significantly inhibited by the 0.8 mg/kg dose (Dunnett's test $p<0.005$, Figure 2B). In view of this, the locomotor stimulant effect of methylphenidate was not examined by a difference score (i.e. drug-saline). Activity in methylphenidate sessions was also reduced by the 0.8 mg/kg dose (Dunnett's test: $p<0.05$, Figure 2B).

Experiment 3: Conditioned place preference expression for intravenous methylphenidate is dose-dependently attenuated by cis-flupenthixol administration on test day

The effect of systemic dopamine receptor blockade on the expression of intravenous methylphenidate CPP was investigated in Experiment 3. Here, rats were conditioned with 5 mg/kg methylphenidate and subsequently received 0, 0.1, 0.3 or 0.8 mg/kg cis-flupenthixol i.p. 30 minutes prior to CPP testing. Only rats receiving vehicle or 0.1 mg/kg cis-flupenthixol exhibited significant conditioned place preference ($p<0.05$ for both; Wilcoxon test with Bonferroni correction; Figure 2C). Only the 0.3 mg/kg group differed significantly from control (Mann-Whitney U with Bonferroni correction $p<0.05$; Figure 2D). At the highest antagonist dose (0.8 mg/kg), CPP magnitude was highly variable and hard to interpret, since the animals were not only less active ($p<0.05$; Mann-Whitney with Bonferroni correction; Figure 2D), but also tended to "camp" in a small area of the test box; indeed, several rats spent the entire ten-minute test session on one side of the test box.

Neurochemical and anatomical selectivity after 6-OHDA lesion (Experiments 4 and 5)

To assess nonspecific tissue damage, sections were Nissl-stained with cresyl violet. As previously reported (Sellings et al., 2006), only minimal cell loss was evident at the site of infusion for all sham-lesioned groups and for the group infused with 6-OHDA in the core subregion. Tissue from rats infused with 6-OHDA in medial shell or mOT exhibited a region of decreased cell density compared to control. This region of nonspecific damage did not extend more than 0.3 mm from the site of infusion. Sampling locations for DAT and SERT binding density, RTI-55 autoradiographs of DAT and SERT binding are shown in Figure 3. For brevity, only one hemisphere is shown; lesions were bilateral and imaging was performed on both hemispheres. Residual DAT and SERT binding as a percent of combined sham groups are given in Tables 1 and 2. Radioligand binding to SERT in tissue from lesioned animals was minimally changed in 6-OHDA vs. sham-lesioned rats. Radioligand binding is not changed after vehicle infusion (i.e. sham lesion) vs. intact tissue, as rats receiving unilateral vehicle infusions show no changes in DAT or SERT binding on the intact vs. sham lesioned side (unpublished observations).

Experiment 4: Effects of 6-OHDA lesions of NAcc core vs. medial shell on intravenous methylphenidate conditioned place preference and locomotor activity

Here, rats received intracerebral infusion of 6-OHDA aimed at either accumbens core or medial shell 7-10 days prior to the start of conditioning.

The locomotor response to intravenous methylphenidate was attenuated by core, but not medial shell lesions

In Experiment 4, rats did not exhibit significant locomotor sensitization (SESSION: $F(2,42)=3.50$, $p>0.05$; Figure 4A). However, to avoid any potential confounding factor of lesion effects on locomotor sensitization, the locomotor difference score (methylphenidate-saline) from the first drug and saline conditioning session were used, so that locomotor scores were examined from only the first drug exposure. Saline test locomotor activity did not differ significantly between surgery groups (LESION x AREA: $F(1,21)=0.03$, $p>0.50$) and were as follows: 58 ± 7 (sham), 54 ± 5 (core 6-OHDA) and 47 ± 1 (medial shell 6-OHDA). All groups exhibited significant locomotor stimulation ($p<0.05$ to $p<0.005$, one-sample t-test with Bonferroni correction; Figure 4B), but this response was smaller in the core-lesioned group compared to the shams ($p<0.05$ for core-lesioned vs. sham-lesioned group, Dunnett's test; Figure 4B). A significant positive association was observed between the locomotor response to intravenous methylphenidate and core DAT binding ($p<0.005$, $r=0.60$, Figure 4C). No relationship was apparent for medial shell (Figure 4D).

CPP magnitude for intravenous methylphenidate related significantly to neither core nor medial shell residual DAT binding

In Experiment 4, both the sham- and medial shell-lesioned groups exhibited a significant CPP, with a similar trend in the core-lesioned group ($p=0.06$; Figure 4E). Multiple regression analysis revealed no significant relationships between CPP magnitude and DAT binding in core (Figure 4F) or medial shell (Figure 4G). In view of this negative

result, we used stepwise multiple linear regression analysis as a post hoc exploratory tool in order to assess a possible contribution of anteromedial olfactory tubercle. Three predictive variables were included in the model: DAT binding in core, medial shell, and anteromedial olfactory tubercle. The iterative model showed that medial shell and anteromedial olfactory tubercle in combination significantly predicted CPP magnitude, but the contribution of neither structure on its own was significant (medial olfactory tubercle positive association, $p=0.055$, medial shell negative association, $p=0.11$).

Experiment 5 - Effects of 6-OHDA lesions of anteromedial olfactory tubercle on intravenous methylphenidate conditioned place preference

In light of recent results suggesting that the entirety of the medial ventral striatum is important in psychostimulant induced reward (see Discussion), the effects of 6-OHDA lesions of the anteromedial olfactory tubercle on i.v. methylphenidate CPP were examined. In Experiment 5, only sham-lesioned animals exhibited significant CPP ($p<0.001$; one-sample t-test with Bonferroni correction; Figure 5A). CPP magnitude differed significantly between sham- and anteromedial OT-lesioned animals ($p<0.005$; Student's t-test; Figure 5A). Additionally, linear regression analysis showed a significant association between the degree of DAT depletion in anteromedial OT and CPP magnitude ($p<0.02$; Figure 5B). Neither activity after saline administration nor methylphenidate-induced locomotor stimulation were significantly altered by the lesion ($p>0.05$ and $p>0.50$ respectively).

Discussion

Novel findings

Previous reports have shown that methylphenidate can sustain intravenous self-administration in several species, including non-human primates, rats and dogs (Kollins et al., 2001; Volkow and Swanson, 2003), and can also produce a CPP when given intraperitoneally (Martin-Iverson et al., 1985; Mithani et al., 1986; Meririnne et al., 2001). Here, we extend these findings to show that methylphenidate can also produce a CPP when given intravenously. Both acquisition and expression of this CPP were dose-dependently reduced by systemic administration of the D1/D2 receptor antagonist cis-flupenthixol. In contrast, CPP following methylphenidate appeared to be unaffected by DA denervation of either core or medial shell prior to conditioning. Instead, DA denervation in anteromedial olfactory tubercle significantly reduced i.v. methylphenidate CPP. The unconditioned locomotor stimulant effect of methylphenidate was dose-dependently reduced by cis-flupenthixol administration and was associated with DA innervation of accumbens core. These results suggest that both the locomotor stimulant and rewarding effects of intravenously administered methylphenidate are dopamine dependent, and that these effects are segregated within the ventral striatum.

Methodological considerations

Given the size, shape and proximity of brain regions lesioned in this study, substantial depletion of one structure was virtually impossible without affecting other nearby structures to some degree. The multiple linear regression analyses used in the current study circumvented this problem in part by considering the degree to which a given structure (core or medial shell) was depleted in each individual animal. One drawback of

this method is that it does not allow for the possibility that lesions may have caused non-uniform DAT depletions; indeed, this was the case in a subset of lesioned animals. However, since there were no consistencies as to which portion of any target structure was spared (rostrocaudally, mediolaterally or dorsoventrally), the current method of sampling appears to faithfully represent DAT depletion in ventral striatal structures. This being said, we cannot rule out the possibility that our behavioral effects resulted from damage to functionally important "hot spots" within the targeted structures.

The statistical approach adopted here is essentially correlational; however, when the present results are integrated with previous findings, causal inferences can be made with some confidence. To infer a causal link, it is important to first exclude the possibility that the lesion effects on behaviour may have resulted from non-specific damage. This appears unlikely for the following reasons. First, 6-OHDA tends to destroy catecholaminergic neurons quite selectively (Jonsson, 1983) and accordingly, our lesions produced little if any change in 5-HT transporter binding levels. Second, Nissl staining revealed only slight non-specific damage in 6-OHDA lesioned animals compared to sham-operated controls; the area of non-specific damage was confined to a small region directly adjacent to the infusion site.

Methylphenidate most likely increased noradrenergic as well as dopaminergic transmission in our experiments (Kuczenski and Segal, 1997), and our 6-OHDA infusions almost certainly destroyed noradrenergic as well as DA terminals. We specifically avoided using desipramine pretreatment to protect noradrenergic afferents

(e.g. Kelly and Iversen, 1976) since we have observed mortality rates of >25% resulting from the commonly used dose (25 mg/kg i.p.) in this strain of rat (unpublished observations). However, loss of noradrenergic afferents is unlikely to account for our lesion effects on either behavioural measure, for the reasons given below.

In terms of psychomotor stimulant-induced locomotion, pharmacological and lesion manipulations of ventral striatal noradrenaline appear to have little or no effect in rats (Pijnenburg et al., 1975; Roberts et al., 1975). Moreover, in the present study, changes in locomotion were associated with lesions in the accumbens core, a subregion which is largely devoid of noradrenergic afferents (Berridge et al., 1997; Delfs et al., 1998).

Reward functions are more clearly associated with medial accumbens shell and anteromedial olfactory tubercle (see below). Although these subregions receive significant noradrenergic input (Versteeg et al., 1976; Berridge et al., 1997), several observations indicate that noradrenergic denervation probably did not significantly influence the magnitude of methylphenidate-induced CPP. First, stimulation of noradrenergic transmission does not appear to produce a CPP (Martin-Iverson et al., 1985; Subhan et al., 2000). Second, neither α nor β adrenergic receptor antagonists affect the rewarding effects of intravenous cocaine, as reflected by self-administration behaviour (Johanson and Fischman, 1989). Finally, the disruptive effects of ventral striatal 6-OHDA lesions on cocaine self-administration appear unrelated to noradrenaline depletion (Roberts et al., 1977; Roberts et al., 1980).

Ventral striatal dopamine and methylphenidate reward

Previous DA antagonist studies focused on the acquisition of intraperitoneal methylphenidate CPP, and yielded only equivocal evidence for blockade (Martin-Iverson et al., 1985). In the present study, cis-flupenthixol was given either during conditioning, or on the test day, and in both cases intravenous methylphenidate CPP was blocked. At the lower effective dose (0.3 mg/kg), cis-flupenthixol would be expected to act principally on DA receptors, with only a weak antagonist effect at 5-HT2 receptors (Matsubara et al., 1993). The inhibition of methylphenidate CPP was probably not due to a disruption of memory recall, since high doses of D1 or D2 antagonists did not inhibit expression of CPP for intraperitoneal cocaine (Cervo and Samanin, 1995).

Dopaminergic transmission in the nucleus accumbens is considered pivotal to psychomotor stimulant reward (Koob et al., 1998; Everitt and Wolf, 2002; Di Chiara et al., 2004; Wise, 2004), and we previously reported a strong association between DA innervation of medial shell and amphetamine CPP (Sellings and Clarke, 2003). However, in the present study, methylphenidate CPP was altered by focal catecholamine depletion in neither accumbens medial shell nor core. One potential explanation of these negative findings is that our lesions were not substantial enough to produce a detectable behavioural deficit, particularly since compensatory neuroadaptations may have occurred in the 7-10 day interval between 6-OHDA infusion and behavioural testing. However, this explanation is unlikely for two reasons. First, core lesions were behaviourally significant, insofar as core-lesioned animals showed a reduced locomotor response to methylphenidate. Second, our medial shell depletions were of similar magnitude to those

in a previous study where significant reductions in CPP magnitude were observed after a similar delay between lesion and CPP training (64% *vs.* 62%; Sellings and Clarke, 2003).

The main finding in the present study was the reduction in CPP magnitude observed after 6-OHDA infusions into amOT. These infusions produced a DAergic depletion of 74% in the target area, with a smaller depletion (36%) in the adjacent anterolateral OT. Importantly, DAT binding in the medial shell was virtually unchanged. This finding suggests that i.v. methylphenidate CPP depends critically on DA transmission in OT, probably in its anteromedial portion. This accords with recent evidence suggesting that the anteromedial olfactory tubercle plays a role in psychostimulant reward, and most likely does not represent a memory deficit, as a 6-OHDA lesion of the OT did not impair amphetamine CPP (Clarke et al., 1990). In particular, both cocaine and amphetamine are avidly self administered into anteromedial OT (Ikemoto, 2003; Ikemoto et al., 2005), and 6-OHDA lesions of the OT appear to reduce CPP for i.v. cocaine (Sellings et al., 2006).

The nucleus accumbens core and locomotor activation

There is currently no consensus on the role of core *vs.* shell in psychostimulant-induced locomotor activation (Boye et al., 2001; Ikemoto, 2002 and references therein). Studies employing intra-accumbens microinjection of direct or indirect DAergic agonists have implicated core, shell, or both structures. After focal administration, the relative importance of core *vs.* shell appears to depend on the drug in question. For example, in a recent study (Ikemoto, 2002), amphetamine acted with similar potency at either injection site, whereas cocaine stimulated locomotor activity more strongly after injection into

medial shell. In contrast to published findings based on intracranial infusion, the locomotor stimulant effect of *systemically-administered* amphetamine and cocaine appears dependent on DA transmission in accumbens core rather than shell (Weiner et al., 1996; Boye et al., 2001; Sellings and Clarke, 2003; Sellings et al., 2006). The present results extend this conclusion to methylphenidate, although a contribution from ventral caudate-putamen cannot be ruled out (Campbell et al., 1997). No previous studies have, to our knowledge, examined the relative contributions of medial shell and core to methylphenidate-induced locomotion. Whether core DA transmission directly mediates the locomotor stimulant action of psychomotor stimulants drugs, or instead plays an indirect enabling role, remains a question for the future.

Conclusions

The present study suggests that the rewarding properties of intravenous methylphenidate are dependent on dopamine transmission, as also suggested by human imaging studies. More specifically, the anatomical site appears to be the anteromedial olfactory tubercle, and not the medial shell. In contrast, a role for intravenous methylphenidate-induced locomotor activity was attributable to accumbens core. These results extend our previous findings with amphetamine and cocaine, and strengthen the hypothesis that psychomotor stimulants exert their stimulant and rewarding effects via increased DA release in functionally segregated territories within ventral striatum. The possibility that drug reward is mediated by small subregions within ventral striatum has several implications, not least for human PET studies where spatial resolution may be a limiting factor.

Figure 1 – Establishment of intravenous methylphenidate conditioned place preference in intact rats. Rats (n=8-9 per group) were tested in a conditioned place preference procedure after three vehicle exposures and three intravenous methylphenidate exposures of either 2 mg/kg or 5 mg/kg. (A) Rats receiving three pairings with 5 mg/kg showed a significant place preference (**p<0.005, one-sample t-test with Bonferroni correction) whereas those receiving 2 mg/kg did not (p>0.30). (B) Rats conditioned to either texture; rats receiving 5 mg/kg methylphenidate expressed a significant conditioned place preference regardless of whether the drug was paired with bar or mesh tiles. (C) Rats exhibit significant locomotor stimulation at both the 2 mg/kg and 5 mg/kg group (**p<0.005, ***p<0.0005, one-sample t-test with Bonferroni correction).

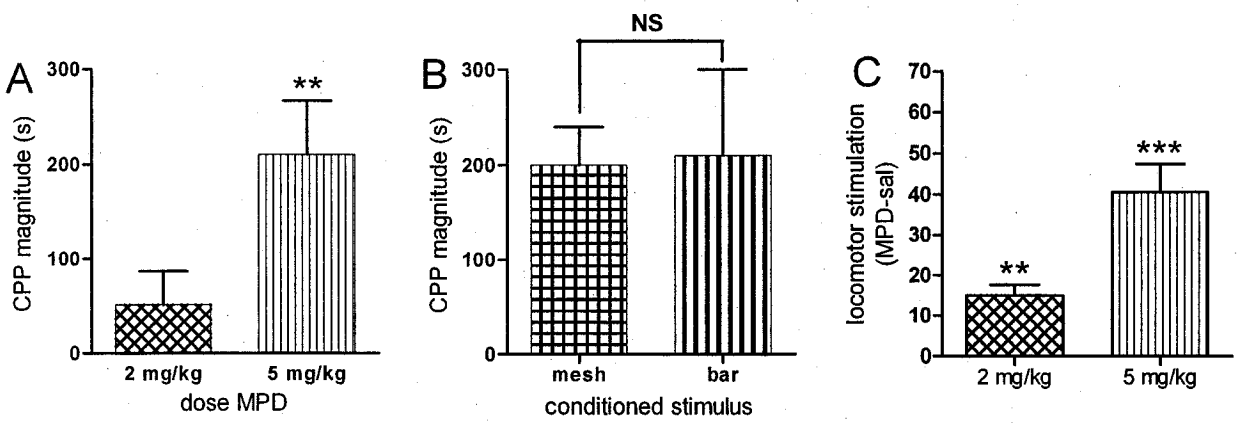
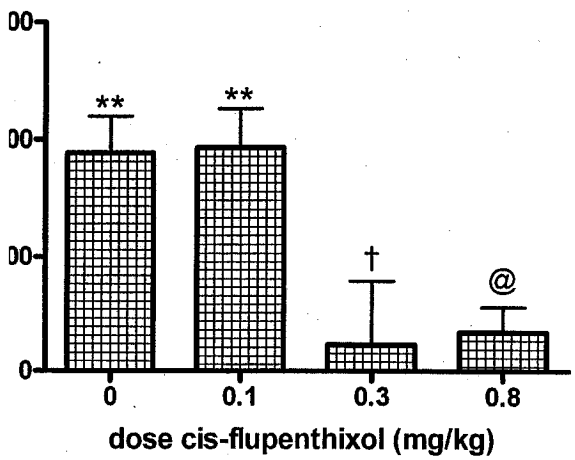
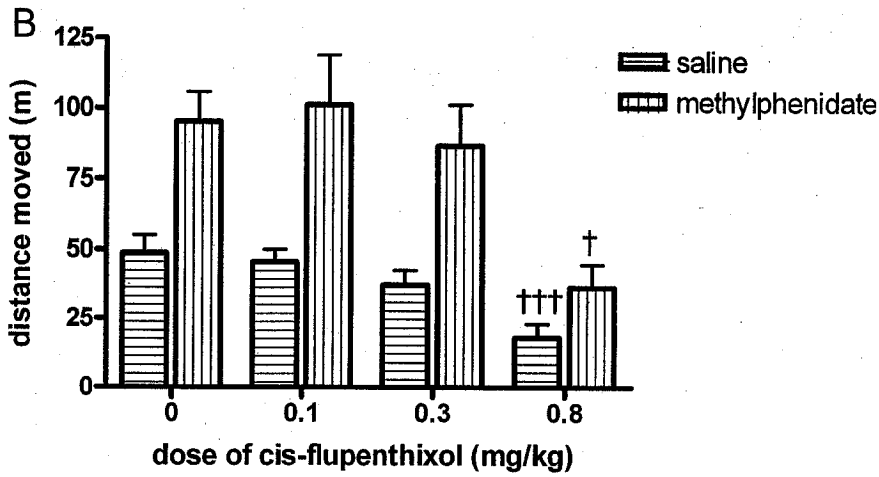


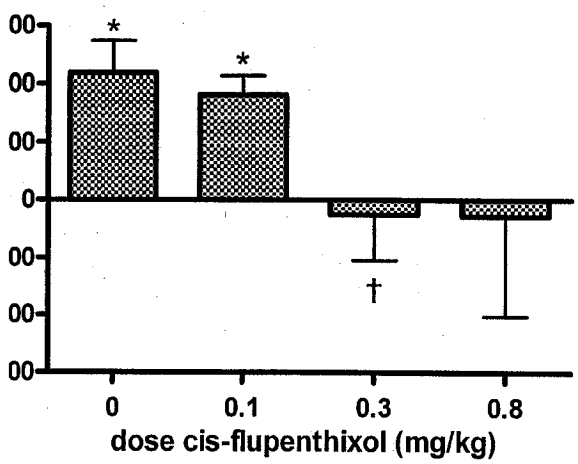
Figure 2 – Effect of cis-flupenthixol on the acquisition and expression of intravenous methylphenidate conditioned place preference. In all experiments, rats were trained in the conditioned place preference paradigm with 5 mg/kg methylphenidate. During conditioning (Figures 2A and 2B), or on test day (Figures 2C and 2D), rats received either 0, 0.1, 0.3 or 0.8 mg/kg cis-flupenthixol (n=5-8 per group). When given during conditioning, only rats receiving vehicle or 0.1 mg/kg cis-flupenthixol exhibited significant CPP (** $p<0.01$, one-sample t-tests with Bonferroni correction, Figure 2A). Cis-flupenthixol dose-dependently reduced CPP acquisition for intravenous methylphenidate (†$p<0.05$, @ $p=0.087$, Dunnett's test, Figure 2A). Cis-flupenthixol treatment also reduced locomotor activity in saline sessions (††† $p<0.005$, Dunnett's test, Figure 2B) and in methylphenidate sessions (†$p<0.05$, Dunnett's test, Figure 2B). When given on test day, only rats receiving 0 or 0.1 mg/kg cis-flupenthixol exhibited significant CPP (*$p<0.05$, one-sample t-test with Bonferroni correction, Figure 2C). Significant reduction of CPP expression was observed after treatment with 0.3 mg/kg cis-flupenthixol (†$p<0.05$, Mann-Whitney U test with Bonferroni correction, Figure 2C). Only rats receiving the highest dose (0.8 mg/kg) exhibited significant hypoactivity on test day (* $p<0.05$, Mann-Whitney U test with Bonferroni correction, Figure 2D).

cis-flupenthixol during conditioning





cis-flupenthixol during CPP test



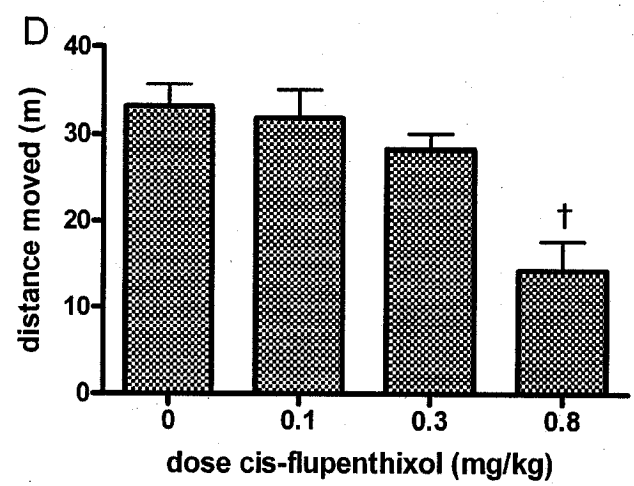
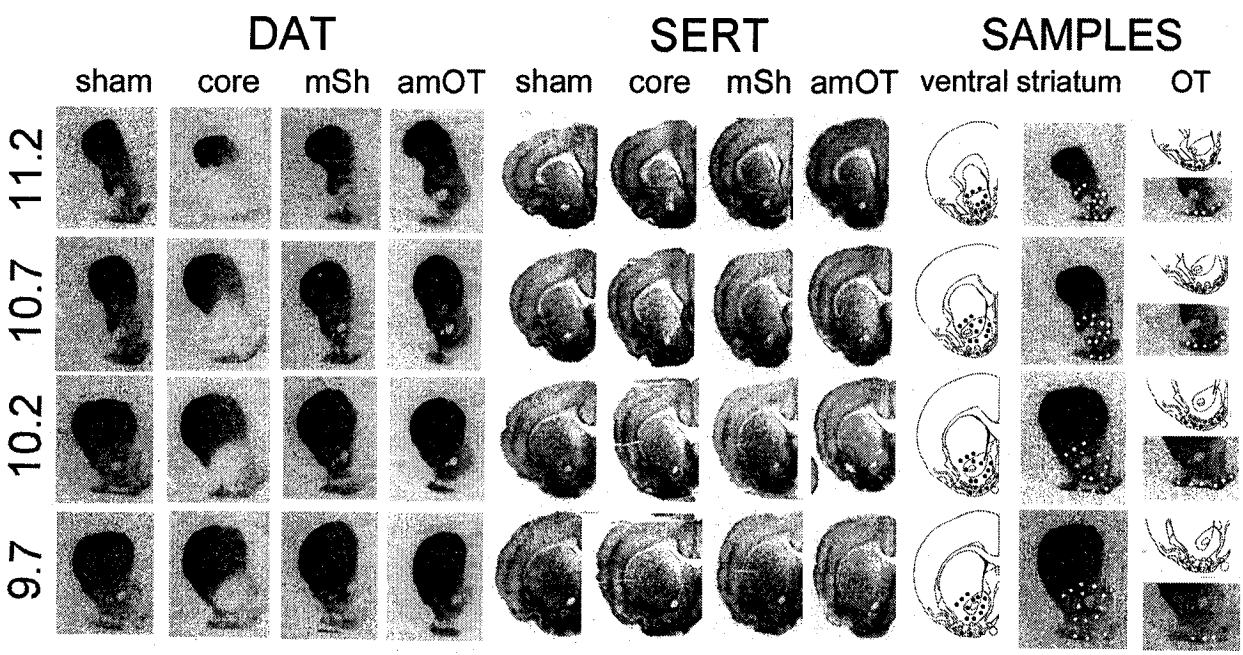


Figure 3 – Autoradiographs for DAT and SERT binding. Figure 3 shows DAT and SERT autoradiographs for sham, core, medial shell and anteromedial olfactory tubercle lesioned rats at four rostrocaudal levels. Excess cold conditions for both DAT and SERT binding were negligible. Areas sampled to determine depletions are shown at right (i.e. nucleus accumbens core, medial shell, ventral shell, olfactory tubercle and ventral caudate-putamen). Depletions in DAT and SERT binding were calculated as a percentage of sham lesioned rats, by taking the mean of all sampled areas (shown at right) at a particular level for all four rostrocaudal levels, and ultimately taking a mean of these four numbers. Sampled areas for core, medial shell, ventral shell and ventral caudate putamen were identical for both Experiments 4 and 5, and are depicted both in cartoon form and superimposed onto DAT autoradiographic images from a sham-lesioned rat. Olfactory tubercle was analyzed as a homogenous structure for Experiment 4 (as seen under "ventral striatal subregions"), but as three heterogeneous subregions in Experiment 5 (based on Ikemoto, 2003; OT subregions). These three subdivisions were anteromedial olfactory tubercle, anterolateral olfactory tubercle and posterior olfactory tubercle. The former two were examined at levels 11.2, 10.7 and 10.2. The latter (posterior olfactory tubercle) was examined at levels 9.7, 9.2 and 8.7 (only level 9.7 shown, see Sellings et al., 2006 for details).


DAT
SERT
SAMPLES
sham
core
mSh
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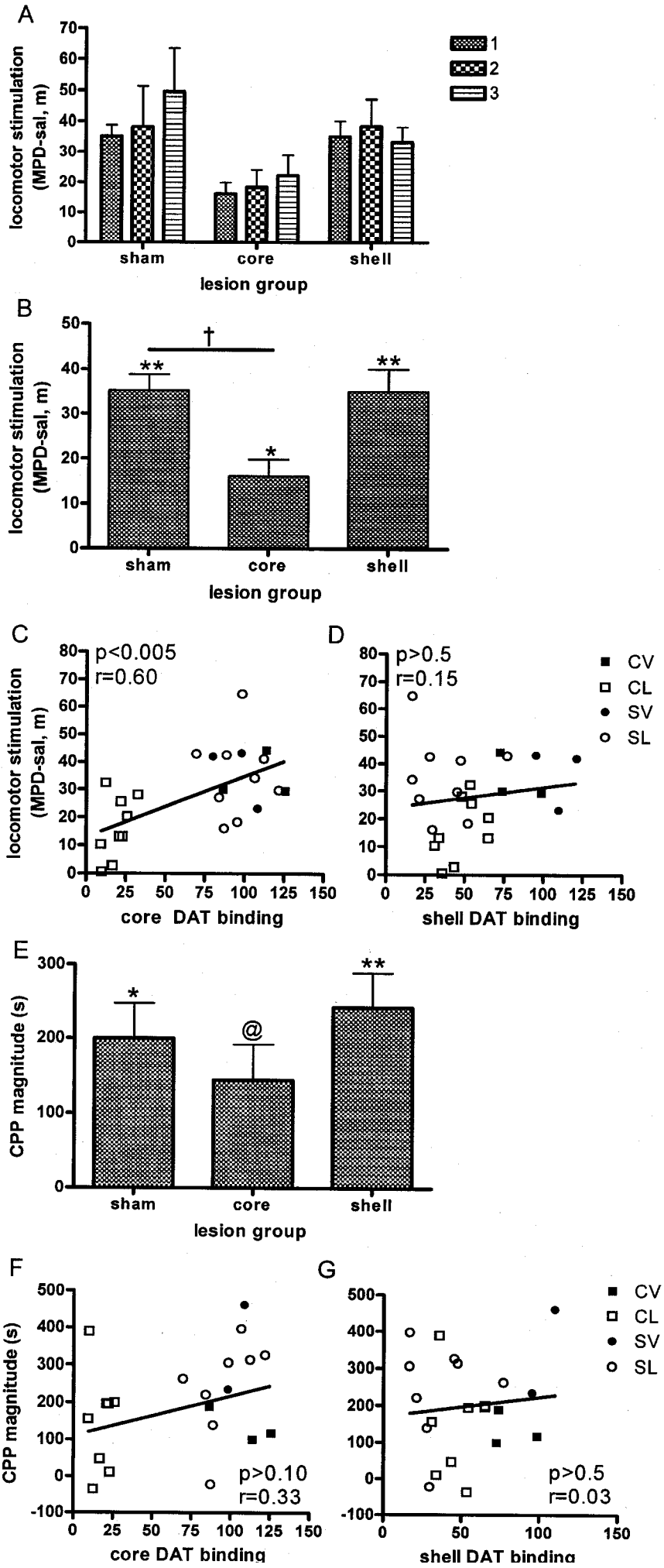
sham
core
mSh
amOT
ventral striatum
OT
11.2
10.7
10.2
9.7

Figure 4 – Effect of 6-OHDA lesions of accumbens core or medial shell on intravenous methylphenidate-induced locomotion and CPP. Rats (n = 6-10 per group) received bilateral 6-OHDA or vehicle infusions (i.e. sham-lesion) into accumbens core or medial shell, and were subsequently conditioned with methylphenidate (5 mg/kg). Locomotor scores (methylphenidate-saline) for all three conditioning pairs (i.e. three pairs of methylphenidate and saline exposures during the CPP conditioning phase; indicated as 1, 2 and 3) are shown in Panel A. Rats did not exhibit significant behavioural (locomotor) sensitization. However, all groups exhibited locomotor stimulation. Locomotor stimulation data for only the first conditioning pair (i.e. the difference between the first drug and saline exposures) was further examined. The stimulant response was smaller in the core lesioned group († $p<0.05$; Dunnett's test; Panel B) but still significant. Locomotor response correlated positively and significantly with DAT binding in core (Panel C), but not in medial shell (Panel D). Sham and medial shell-lesioned groups exhibited a significant CPP, with a similar trend in the core-lesioned group (@ $p=0.06$, *$p<0.05$, **$p<0.005$, one sample t-test with Bonferroni correction, Panel E). CPP magnitude did not correlate significantly with either core or medial shell DAT binding (Panels F and G). DAT binding is expressed as percent of sham-lesioned rats of the target structure being examined. Abbreviations are as follows: CV, core vehicle (sham); CL, core lesion; SV, medial shell vehicle (sham); SL, medial shell lesion. Shell refers to medial shell. N per group: 9 (core lesion), 10 (medial shell lesion), 6 (combined sham groups).


A
locomotor stimulation (MPD-sal, m)
1
2
3
sham
core
shell
lesion group
B
†
**
*
**
locomotor stimulation (MPD-sal, m)
sham
core
shell
lesion group
C
p<0.005
r=0.60
locomotor stimulation (MPD-sal, m)
core DAT binding
D
p>0.5
r=0.15
CV
CL
SV
SL
shell DAT binding
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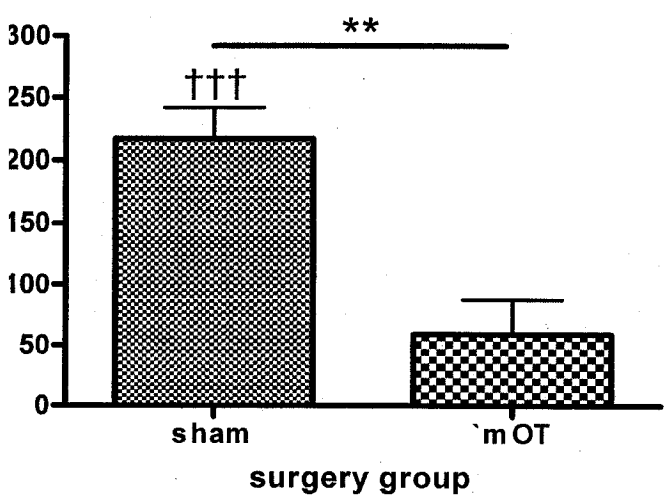
**
CPP magnitude (s)
sham
core
shell
lesion group
F
CPP magnitude (s)
p>0.10
r=0.33
core DAT binding
G
CV
CL
SV
SL
p>0.5
r=0.03
shell DAT binding

Figure 5 – Effect of 6-OHDA lesions of anteromedial olfactory tubercle on intravenous methylphenidate-induced CPP. Rats (n = 6-11 per group) received bilateral 6-OHDA or vehicle infusions (i.e. sham lesion) into accumbens core or medial shell, and were subsequently conditioned with methylphenidate (5 mg/kg). Only sham-lesioned rats exhibited a significant CPP, which differed significantly from the CPP magnitude of anteromedial OT-lesioned rats (††† $p<0.0001$, one-sample t-test with Bonferroni correction; **$p<0.005$, Student's t-test; Panel A). CPP magnitude correlated significantly with anteromedial OT DAT binding ($p<0.02$; Panel B). DAT binding is expressed as percent of sham-lesioned rats of the target structure being examined. Abbreviations are as follows: amOTV, anteromedial olfactory tubercle sham; amOTL, anteromedial olfactory tubercle lesion.



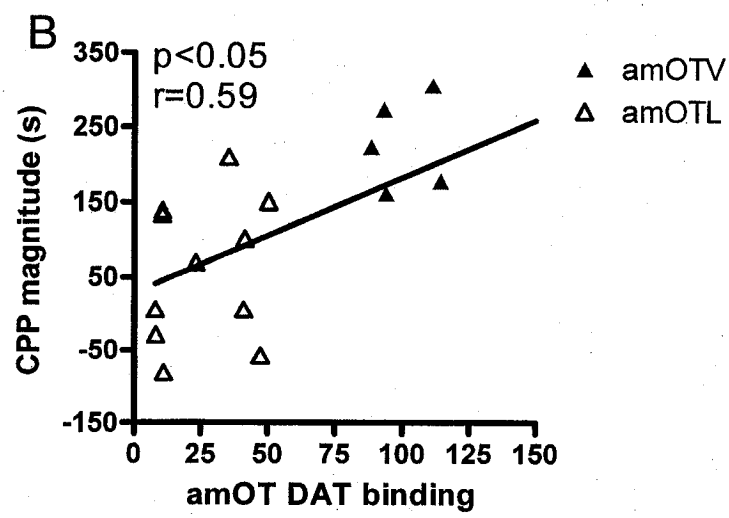


Table 1. Reductions in DAT and SERT binding seen in core and medial shell lesioned groups (Experiment 4)

	DAT			SERT		
Group	Sham	Core	mSh	Sham	Core	mSh
Core	100±7	20±3****	95±5	100±5	108±6	97±3
mSh	100±8	48±4††	36±6***	100±4	97±5	94±3
vSh	100±6	35±6††	98±8	100±6	118±8	108±3
OT	100±12	46±4***	80±4	100±6	116±5	108±4
vCp	100±10	50±7**	107±6	100±3	88±3	93±3

Figures presented are mean ± SEM, and are calculated as a percent of sham-operated control. Abbreviations are as follows: mSh, medial shell; vSh, ventral shell; OT, olfactory tubercle; vCP, ventral caudate-putamen. **p<0.005; ††p<0.001; ***p<0.0005; ****p<0.00005 vs. sham-lesioned control

Table 2. Reductions in DAT and SERT binding seen in anteromedial olfactory tubercle lesioned rats (Experiment 5).

	DAT		SERT	
Group	Sham	amOT	Sham	amOT
Core	100±5	96±4	100±10	97±7
mSh	100±5	95±6	100±9	98±10
vSh	100±7	85±4	100±8	97±12
amOT	100±17	26±5††	100±2	106±10
alOT	100±10	64±7	100±3	107±14
pOT	100±7	78±5	100±6	113±12
vCP	100±7	90±3	100±5	97±7

Figures presented are mean ± SEM, and are calculated as a percent of sham-operated control. Abbreviations are as follows: mSh, medial shell; vSh, ventral shell; amOT, anteromedial olfactory tubercle; alOT, anterolateral olfactory tubercle; pOT, posterior olfactory tubercle; vCP, ventral caudate-putamen. ††$p<0.001$ vs. sham-lesioned control.

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Intervening Section 4

The fourth and final drug examined was nicotine, the major psychoactive component of tobacco smoke. The effects of medial shell and core lesions on CPP were first examined. As previously observed for amphetamine and intravenous cocaine, medial shell lesions reduced CPP. Interestingly, core lesions increased nicotine CPP. This appeared to result from reduced nicotine aversion, since core, but not medial shell lesions abolished nicotine CTA.

CHAPTER 7: Rewarding and aversive effects of nicotine are segregated within the nucleus accumbens

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Submitted to *Journal of Neuroscience,* April 2006

Abstract

Forebrain dopamine plays a critical role in motivated behavior. According to the classic view, mesolimbic dopamine selectively mediates behavior motivated by rewards. However, this has been challenged in favor of a wider role encompassing aversively motivated behavior. This controversy is particularly striking in the case of nicotine, with opposing claims that either the rewarding or aversive effect of nicotine is critically dependent on mesolimbic dopamine transmission. In the present study, the effects of 6-hydroxydopamine lesions of nucleus accumbens core vs. medial shell on intravenous nicotine conditioned place preference and conditioned taste aversion were examined in adult rats. Dopamine denervation in medial shell was associated with decreased nicotine conditioned place preference. Conversely, denervation in core was associated with an *increase* in conditioned place preference. In addition, core but not medial shell dopamine denervation abolished conditioned taste aversion for nicotine. We conclude that core and medial shell dopamine innervation exert segregated effects on rewarding and aversive effects of nicotine. More generally, our findings indicate that DA transmission may be enabling opposing motivational processes within functionally distinct domains of the accumbens.

A rich literature indicates that forebrain dopamine (DA) is intimately related to reward processes. For example, strong evidence suggests that cocaine and amphetamine produce their rewarding effects by increasing mesolimbic DA transmission (Di Chiara et al., 2004; Wise, 2004). Several lines of evidence suggest that nicotine also exerts its rewarding effects by increasing dopaminergic (DAergic) transmission in the nucleus accumbens (Di Chiara et al., 2004; Lecca et al., 2006). Indeed, both nicotine conditioned place preference (CPP) (Spina et al., 2006) and intravenous self administration (Corrigall et al., 1992) are inhibited by disruption of mesolimbic DAergic transmission (Di Chiara et al., 2004). In light of these findings, considerable effort has been expended in identifying the nicotinic receptor subtypes that modulate DAergic transmission (Picciotto et al., 1998; Champtiaux et al., 2003; Wonnacott et al., 2005) , partly in order to try to develop better smoking cessation aids.

The psychobiological role of DA may not, however, be restricted to reward-relevant processes (Horvitz, 2000; Ungless, 2004). Not only do aversive events elicit mesolimbic DA release (Horvitz, 2000), but pharmacological studies have suggested that both drug-conditioned place preference and aversion can be DA-dependent (Acquas et al., 1989; Shippenberg et al., 1993). In addition, both direct and indirect DA agonists produce conditioned taste aversion (CTA) that is blocked by systemic DA antagonist treatment (Asin and Montana, 1989; Huang and Hsiao, 2002). Recently, the aversive effects of nicotine were attributed to mesolimbic DA transmission (Laviolette et al., 2002). In particular, systemic or intra-NAcc administration of the DA antagonist (α-flupenthixol) reduced the *aversive* rather than the rewarding effects of nicotine (Laviolette et al., 2002;

Laviolette and van der Kooy, 2003). On the basis of these results, it was concluded that mesolimbic dopamine transmission may encode the aversive effects of nicotine.

Although rewarding and aversive processes are to some extent anatomically dissociable within the nucleus accumbens (Reynolds and Berridge, 2002; Pecina and Berridge, 2005), the idea that DA in distinct accumbal compartments may underlie reward vs. aversion has been little explored. In this respect, several reports suggest that medial rather than lateral accumbal sites are associated with reward processes. In particular, dopaminergic agonists were self-administered directly into the medial shell but not into the core (Ikemoto et al., 1997; Ikemoto et al., 2005), and injections of amphetamine into shell preferentially induced 50 kHz ultrasonic vocalizations (Thompson et al., 2006). Recently, we found that 6-OHDA lesions of the medial shell reduced CPP for amphetamine and i.v. cocaine (Sellings and Clarke, 2003; Sellings et al., 2006). In addition, the results obtained with i.v. cocaine were suggestive ($p=0.06$) of an inhibitory influence of core DA transmission on CPP (Sellings et al., 2006).

Accordingly, the present study investigated the hypothesis that the acute rewarding and the acute aversive effects of nicotine are both dependent on DA transmission, but in different accumbens subregions. To this end, we examined the effects of focal 6-hydroxydopamine lesions on CPP for intravenous nicotine. As a more direct test of aversion, nicotine CTA was examined in the same way.

Materials and Methods

Subjects. Subjects were Long-Evans rats, weighing 260-310 g at time of surgery (Experiments 1, 2, and 4-8) or start of behavioral testing (Experiment 3). Rats were housed in groups of 2 or 3 in a temperature and humidity controlled environment, in a 12h/12h light-dark cycle (lights on at 07h00).

Surgery

1 – Intravenous catheterization. Rats were implanted with chronic indwelling silastic catheters (0.51 mm I.D. and 0.94 mm O.D., Fisher Scientific, Montreal, Quebec) in the left jugular vein under ketamine (80 mg/kg) and xylazine (16 mg/kg) anesthesia. Tubing was secured to the vein by surgical silk sutures, led subcutaneously to the skull surface, and was then fitted onto a 22 gauge cannula attached to a plastic connector (Model number C313G-5UP, Plastics One, Roanoke, VA). The cannula/connector was fixed to the animal's skull with small stainless steel screws (Lomir, Notre-Dame-de-L'Ile Perrot, Quebec) and dental cement (Stoelting, Wood Dale, IL). To keep catheters patent, 0.1-0.15 ml heparinized 0.9% saline was administered at the end of surgery, on the first day of behavioral testing, and every 2-3 days thereafter.

2 – 6-hydroxydopamine infusion. All procedures are identical to those previously published (Sellings and Clarke, 2003) with two modifications. First, atropine methyl nitrate pretreatment (0.05 mg/kg s.c.) was omitted. Second, in Experiments 6 and 8 of the present work, DMI was added to the 6-hydroxydopamine vehicle (1mM), and given systemically (15 mg/kg i.p.) 20 minutes prior to ketamine/xylazine anesthetic to all rats. This dual route approach (i.e. systemic plus intracerebral) was taken principally to avoid

the high (>25%) mortality rates observed after administration of the commonly used systemic dose of 25 mg/kg i.p. to this particular strain of rats (Long-Evans; supplier Charles River; our unpublished observations). In DMI-treated rats, surgical anesthesia was achieved at a much lower dose (50%) of ketamine and xylazine.

Behavioral testing. Animals were allowed 7-10 days recovery from surgery before starting CPP or CTA testing.

Conditioned place preference. The apparatus and general procedure were as previously described (Sellings and Clarke, 2003). Briefly, the procedure consisted of three phases: pre-exposure (one day), conditioning (six days) and test (one day). All phases were carried out in a one-compartment box (58 cm x 29 cm x 53 cm) with walls made of white plastic-coated particle board, under darkroom lighting using a Kodak GBX-2 safelight filter (Vistek, Toronto, Ontario, Canada), to minimize visual cues. In the pre-exposure phase, Beta-Chip sawdust bedding covered the floor of the cage. In the conditioning phase, two square tactile tiles of either bar or mesh texture, each covering half the area of the bottom of the test cage, were placed on top of the bedding (tile dimensions: 28.5 cm x 28.5 cm x 5.5 cm). During the test phase, one bar and one mesh tile were placed on the bottom of the cage, such that half the floor of the test cage had bar texture, and the other half had mesh texture. The time spent on bar or mesh texture was measured by Noldus EthoVision software. Animals do not spontaneously prefer either texture (LHLS and PBSC, unpublished observations), and as such our procedure can be considered to be "balanced". All experiments were as fully counterbalanced as possible with respect to

drug-texture pairing and order of drug pairing (drug-saline or saline-drug) within each surgery group. For all experiments, pre-exposure sessions lasted 20 minutes, the conditioning trial duration 15 minutes, and the test session 10 minutes. A fluid swivel was fixed above the center of each cage. Each swivel was connected to on one end a 1 ml syringe, and on the other end to a brass connector (Produits MSM, Laval, Quebec) and protective spring (Heiplex, Montreal) via Tygon tubing of 0.5 mm diameter. The cannula fixed to the skull of the rat was attached to the Tygon tubing, and the brass connector fastened to the plastic connector, to secure the tubing to the cannula, hence allowing administration of drug immediately after placement in the CPP cage. Drug was infused over 5s.

Conditioned taste aversion. The present procedure is adapted from a previously published protocol (Laviolette et al., 2002). Testing took place over 16 consecutive days. The procedure consisted of four phases – initial water restriction, conditioning, secondary water restriction, and testing. During the initial water restriction phase (5d), rats were allowed one hour per day access to water. Body weight and the volume of water consumed by each animal were measured each day. During the conditioning phase (Days 6-13), rats received four nicotine and four saline injections, on alternating days, in a counterbalanced fashion within groups. Rats had 15 minutes access to one of two novel flavors (unsweetened cherry or grape Kool-Aid). Immediately after access to the novel flavor was terminated, rats received an intravenous injection of either nicotine or saline, infused over 5 s. Two hours post Kool-Aid, rats had access to water for 15 minutes. Volume of Kool-Aid and water consumed, as well as animal weights, were recorded.

Rats lost about 10% of their free feeding weight by the fourth water deprivation day. Then, they steadily gained weight. By the fifth conditioning day, body weights were not significantly different from the day before water restriction commenced. This pattern was unaffected by nicotine dose (day x dose interaction: $F(45,435) = 1.11$, $p > 0.25$). The conditioning phase was followed by water restriction (Day 14), in which rats had one hour access to water. No Kool-Aid was presented on this day. The final phase comprised two tests (Days 15 and 16), in which rats were water-deprived and given 20 minutes of access to two bottles. One contained cherry, and the other grape, Kool-Aid. The volume of each flavor consumed was recorded. The side position of the flavors was changed between test days, to account for any side preference rats may have developed.

Experimental Design

Experiment 1. Rats were conditioned in the CPP paradigm at one of three doses of intravenous nicotine (5, 15 or 50 μg/kg; n=4-6/group) and subsequently tested for CPP.

Experiment 2. To confirm that rats expressed CPP at 15 μg/kg nicotine, a new group of rats (n=6) was conditioned at this dose of nicotine and tested for CPP.

Experiment 3. Rats were conditioned in the CPP paradigm at one of three doses of subcutaneous nicotine (100, 300 or 600 μg/kg; n=8/group) and subsequently tested for CPP.

Experiment 4. Rats were conditioned in the CTA paradigm at one of four doses of intravenous nicotine (0, 5, 15 or 50 μg/kg; n=7-10/group), and subsequently tested in a two-bottle choice paradigm between the nicotine- and the saline-paired flavor.

Experiment 5. Rats sustaining vehicle or 6-OHDA infusion into core or medial shell (n=5-6/group) were subsequently conditioned in the CPP paradigm with 15 μg/kg intravenous nicotine after recovering from surgery.

Experiment 6. This was identical in design to Experiment 5, except that at surgery, all rats were treated with the noradrenergic transporter (NET) blocker desipramine both systemically (15 mg/kg i.p.), and co-infused in the 6-OHDA solution (1 mM). The number of subjects varied between 6 and 20 per group.

Experiment 7. Rats sustaining vehicle, or 6-OHDA infusion into core or medial shell (n=6-9/group) were subsequently conditioned in the CTA paradigm with 50 μg/kg intravenous nicotine after recovering from surgery as described above.

Experiment 8. This was identical in design to Experiment 7, except that all rats were treated the NET blocker desipramine both systemically (15 mg/kg i.p.), and co-infused in the 6-OHDA solution (1 mM). The number of subjects varied between 10 and 12 per group.

Quantitative autoradiography for the DA, NE and 5-HT transporters. Tissue preparation, DAT and SERT autoradiography, and quantification of DAT and SERT binding are as previously described (Sellings and Clarke, 2003). The procedure for [^{125}I] nisoxetine autoradiography to visualize norepinephrine transporters was as follows. Sections were thawed at room temperature for 10 min and then placed in a staining dish containing an aqueous buffer solution of 50 mM tris, 300 mM NaCl, 5 mM KCl, 50 nM citalopram, 100 nM GBR 12909, and 10 pM [^{125}I]-nisoxetine (2200 Ci/mmol; gift of Mei-Ping Kung, University of Pennsylvania, nonsaturating concentration), with the pH

adjusted to 7.4. Nonspecific binding was determined by addition of 1 μM desipramine. Slides were incubated at 4°C for 2 hr and then washed three times for five minutes each in cold buffer solution, and finally for 1-2 sec in distilled and deionized water. They were then blow dried and placed in X-ray film cassettes. Kodak BioMax MS film (Amersham Biosciences, Baie d'Urfé, Québec) was exposed to slides for 14 days with [^{125}I] autoradiographic standards (Amersham Biosciences). Radiolabeling of DAT, SERT and NET was quantified using an MCID M4 imaging system (Imaging Research, St. Catherines, Ontario).

Histological examination. Tissue was stained with cresyl violet to assess nonspecific damage, as previously described (Sellings and Clarke, 2003), and examined under a light microscope.

Drugs. (-)Nicotine bitartrate salt (Sigma) was dissolved in sterile physiological saline neutralized to pH=7.3±0.1, and administered 1 ml/kg body weight; doses represent free base. Desipramine HCl (Sigma-RBI) administered prior to surgery was also dissolved in sterile physiological saline administered at 2 ml/kg body weight (dose as salt).

Statistical analysis. All analyses were performed using a commercially available program (SYSTAT v. 10). Outliers, as defined by the statistical program, were excluded prior to analysis. CPP magnitude was calculated as the difference between times spent on nicotine-paired and saline-paired sides on test day. CTA magnitude was defined as the difference between saline- and nicotine- paired fluids consumed in test sessions.

Significance was set at $p<0.05$ (two-tailed) throughout. Group values are expressed as mean ± SEM.

In Experiments 1 and 2, the presence or absence of a significant CPP was determined by a Wilcoxon signed rank test (i.e. time on nicotine- vs. saline-paired sides), since data were not normally distributed. In Experiment 3, multiple one-sample t-tests with Bonferroni correction were used for this purpose. In Experiment 4 (CTA), the amount of fluid drunk during conditioning sessions was initially analyzed by a repeated measures ANOVA, with three factors: DOSE (i.e. conditioning dose of nicotine), DRUG (i.e. saline or nicotine) and DAY (i.e. conditioning day with either nicotine or saline). The effect of nicotine DOSE on CTA magnitude was analyzed by one-way ANOVA. For rats receiving saline paired with both fluids, to test if these control rats spontaneously preferred either flavor, a one-sample t-test was performed on the cherry vs. grape fluid consumed on test day. Dunnett's test was performed post-hoc, to determine which doses differed significantly from control. In Experiments 5 and 6, group differences in CPP magnitude were analyzed by one-way ANOVA and a post-hoc Tukey's test. The presence of a CPP within individual groups of animals was tested by multiple one-sample Bonferroni t-tests. Multiple linear regression analysis was performed with CORE and MEDIAL SHELL DAT binding as factors, and CPP magnitude as the dependent variable. Pooled data from Experiments 5 + 6 were examined in the same fashion. In Experiments 7 and 8, CTA magnitude data were clearly not normally distributed, and group differences were assessed by multiple Mann-Whitney tests with Bonferroni correction. The presence of a CTA within individual groups of animals was tested by multiple

Wilcoxon signed-rank tests with Bonferroni correction. Multiple linear regression analyses were performed on CORE vs. MEDIAL SHELL DAT binding as factors, and CTA magnitude as the dependent variable. Pooled data from Experiments 7 + 8 were analyzed in an analogous fashion. One rat that was recorded as being lesioned in core in Experiment 7, but exhibiting core DAT binding at 94% of sham-lesioned rats, was removed prior to analysis.

Results

Rats can express conditioned place preference for intravenous nicotine

We first determined whether rats form a CPP for intravenous nicotine in a "balanced" CPP procedure. Rats were implanted with an intravenous jugular catheter and conditioned at one of three doses of intravenous nicotine: 5, 15 or 50 μg/kg (Experiment 1). Only the group of rats receiving 15 μg/kg showed a significant CPP magnitude, defined as the difference between time spent on the nicotine-paired and saline-paired textures on test day (Wilcoxon test, uncorrected, $p<0.05$; Fig. 1a). To confirm this result, CPP testing was repeated at a dose of 15 μg/kg with different rats (n=6 rats, Experiment 2). As before, rats spent significantly more time on the nicotine-paired side than on the saline-paired side (Wilcoxon test, $p<0.05$; Fig. 1b). Hence, rats formed CPP to 15 μg/kg nicotine (i.v.).

Rats do not show conditioned place preference for subcutaneous nicotine in a balanced paradigm

The previous experiment provides the first evidence for nicotine CPP using a balanced design. We next examined whether subcutaneous nicotine doses would also be effective (Experiment 3). Rats (n=8/group) were first treated with 400 μg/kg nicotine s.c. for two days to render them tolerant to the drug's locomotor depressant effect. Two days later, they received one of three doses of nicotine: 100, 300 or 600 μg/kg. None of these doses produced CPP (one-sample t-tests; t(7): 0.69-1.01; $p>0.35$ for all; Fig. 1c). Thus, the CPP observed with intravenous nicotine appears to be a function of route of administration.

Non-lesioned rats exhibit conditioned taste aversion to intravenous nicotine

The expression of a conditioned taste aversion to intravenous nicotine was established in Experiment 4. After a period of water restriction (5 d, 1 h/d access), rats (n=7-10/group) received one of four doses (0, 5, 15 or 50 μg/kg) of intravenous nicotine after presentation of one bottle containing a novel flavor. The amount of novel flavor consumed prior to nicotine vs. saline infusion during conditioning did not differ significantly at any dose (DRUG x SESSION x DOSE interaction: $F(9,84)=0.65$, $p>0.7$, Fig. 2a). Additionally, there was no overall effect of dose on the mean total fluid consumed during the two-bottle choice test ($F(3,27)=0.89$, $p>0.45$), and no single dose differed significantly from the 0 μg/kg group (Dunnett's test: $p>0.5$ for all; Fig. 2b).

CTA magnitude was calculated as the mean of the difference between the volume of saline- and nicotine-paired flavors consumed on both test days. As expected, control rats receiving saline injections paired with both novel flavors (i.e. no nicotine infusions) did

not significantly prefer either novel flavor ($t(9)=0.71$, $p>0.5$, one-sample t-test, Fig. 2c). CTA magnitude was significantly dose-dependent (One-way ANOVA; $F(3,29)=4.40$; $p<0.02$); only the 50 μg/kg group differed significantly from the 0 μg/kg group (Dunnett's test; $p<0.005$; Fig. 2c).

Anatomical and neurochemical lesion selectivity

In Experiments 5-8, the effects of core vs. medial shell lesions on intravenous nicotine CPP and CTA was examined. Autoradiographic binding to the dopamine transporter (DAT) and serotonin transporter (SERT) for Experiments 5-8 are shown in Table 1. To determine the degree of preservation of norepinephrine (NE) terminals after DMI pretreatment, autoradiographic labeling of the noradrenergic transporter (NET) was performed in Experiments 6 and 8. NET binding in the core subregion was negligible (Table 1). NET binding was not significantly affected in lesioned rats receiving DMI pretreatment. This suggests that DMI pretreatment preserved NE, but not DA terminals (Table 1). Infusions of 6-OHDA did not significantly affect SERT binding in any sampled subregion (Table 1), and Nissl staining revealed minimal non-specific damage, as previously reported (Sellings et al., 2006).

Conditioned place preference is reduced by medial shell lesions, and increased by core lesions

The effect of 6-OHDA lesions of the nucleus accumbens core vs. medial shell on nicotine CPP were examined in Experiment 5. A significant CPP was observed in sham- and core-lesioned rats (n=5-6 /group; $p<0.05$ [sham], $p<0.05$ [core]; one-sample t-test with

Bonferroni correction) but not in medial shell-lesioned rats (Fig. 3a). Multiple linear regression analysis revealed a significant *negative* association between core DAT binding and the magnitude of the CPP ($p<0.005$; Fig. 3b). Conversely, a significant positive association was observed between medial shell DAT binding and CPP magnitude ($p<0.05$; Fig. 3c).

Since intracerebral administration of 6-OHDA destroys both DA and NE innervation, the above experiment was repeated using desipramine pretreatment, designed to protect against NE toxicity (Experiment 6; n=6-20/group). Here, only core-lesioned animals exhibited significant CPP ($p<0.01$; one-sample t-test with Bonferroni correction; Fig. 3d). A significant group difference was observed between core- and medial shell-lesioned rats ($p<0.005$; Tukey's test). As in the previous experiment, CPP magnitude was negatively associated with core DAT binding ($p<0.05$; Fig. 3e) and positively associated with medial shell DAT binding ($p<0.05$; Fig. 3f).

After initial data inspection, the results of Experiments 5 and 6 were pooled for further analysis. Overall, only core-lesioned animals exhibited significant CPP ($p<0.02$; one-sample t-test with Bonferroni correction; Fig. 3g). CPP magnitude in shell-lesioned animals differed significantly from sham-operated and core-lesioned ($p<0.05$ and $p<0.005$, respectively; Tukey's test; Fig. 3g) rats. A significant negative association was observed between core DAT binding and CPP magnitude ($p<0.0005$; Fig. 3h), and a

significant, positive association between medial shell DAT binding and CPP magnitude ($p<0.01$; Fig. 3i).

Intravenous nicotine conditioned taste aversion is not observed in core lesioned animals

In Experiment 7, rats (n=6-9/group) sustaining intra-core or intra-medial shell infusion of 6-OHDA (or vehicle) were examined for acquisition of a conditioned taste aversion to 50 μg/kg i.v. nicotine. Only core-lesioned animals differed significantly from sham-lesioned rats in the magnitude of the CTA ($p<0.05$; Mann-Whitney test with Bonferroni correction; Fig. 4a). Additionally, only sham-lesioned animals displayed significant CTA ($p<0.05$; Wilcoxon test with Bonferroni correction; Fig. 4a). The magnitude of the CTA was significantly and negatively related to core DAT binding ($p<0.0001$; Fig. 4b), and to medial shell DAT binding ($p<0.05$; Fig. 4c).

Experiment 8 differed from Experiment 7 in that rats were pretreated with DMI to protect NE terminals. As in the previous experiment, only sham-lesioned animals exhibited significant CTA (n=10-12/group; $p<0.01$; Wilcoxon test with Bonferroni correction; Fig. 4d); significant group differences existed between core- and sham-lesioned animals ($p<0.002$; Mann-Whitney test with Bonferroni correction; Fig. 4d). As before, multiple linear regression analysis revealed a significant negative association between core DAT binding and the magnitude of the taste aversion ($p<0.05$; Fig. 4e). However, in this experiment, no significant association was observed between medial shell DAT binding and CTA magnitude ($p>0.7$; Fig. 4f).

When the data from Experiments 7 and 8 were pooled, both sham- and medial shell-lesioned rats expressed a significant CTA ($p<0.0002$ and $p<0.02$ respectively; Wilcoxon test with Bonferroni correction; Fig. 4g). CTA magnitude differed significantly between core- and medial shell-lesioned animals, and between both lesion groups and sham-lesioned animals ($p<0.0001$ [sham vs. core], $p<0.05$ [sham vs. medial shell, $p<0.005$ [core vs. medial shell]; Mann-Whitney test with Bonferroni correction, Fig. 4g). Core, but not medial shell, DAT binding was significantly associated with CTA magnitude (Core: $p<0.00001$; medial shell: $p>0.25$; Figs. 4h and 4i).

Discussion

The present study implicates accumbal DA transmission in both rewarding and aversive effects of nicotine, and demonstrates that these effects are anatomically dissociable. As discussed below, these findings may help to resolve conflicting evidence regarding the role of DA in nicotine reward vs. aversion (Corrigall et al., 1992; Rose and Corrigall, 1997; Laviolette and van der Kooy, 2004)

Methodological issues

The present study represents the first published report of CPP following intravenous nicotine administration. This route of administration was chosen to more closely model the pharmacokinetics of nicotine after inhalation of tobacco smoke. The effective dose

(15 μg/kg) is at the low end of the range self-administered in animals (Rose and Corrigall, 1997) and represents the dose of nicotine typically self-administered by smokers after one cigarette (Benowitz and Jacob, 1984). However, nicotine CPP was variable and not always statistically significant. Nevertheless, intravenous nicotine may be more effective than other systemic routes of administration, as evinced by our negative results with subcutaneous nicotine. The latter result is consistent with previous studies using subcutaneous nicotine in an "unbiased" test (Le Foll and Goldberg, 2005b). An additional factor that may account for the presence of i.v. nicotine CPP is the nature of our CPP paradigm. Since visual cues are absent throughout the procedure, conditioned approach behavior is unlikely to account for CPP. Hence, the paradigm appears to provide a purer measure of conditioned reward than other CPP procedures.

Although 6-OHDA infusions do not discriminate between NE and DA, similar results were obtained both in the absence and presence of the NET blocker DMI. Hence, reduced DA transmission probably underlies lesion-induced behavioral alterations in nicotine CPP and CTA. As previously observed (Sellings and Clarke, 2003), core and medial shell lesions were not anatomically specific; this variability was exploited through the use of multiple linear regression.

Nicotine reward and the nucleus accumbens medial shell

Nicotine CPP was observed to be dependent on medial shell DA innervation. Although CPP magnitude depends in part on the learned association between drug and sensory

cues, it is unlikely that medial shell lesions impaired learning, since similar lesions do not reduce CPP produced by i.p. morphine or cocaine (Sellings and Clarke, 2003; Sellings et al., 2006). The finding that medial shell lesions reduce nicotine CPP accords with a recent report that intra-shell infusion of a D1 receptor antagonist dose-dependently reduced nicotine CPP (Spina et al., 2006). More generally, these findings mirror evidence that psychostimulants produce CPP by increasing dopamine transmission in the medial shell (Di Chiara et al., 2004), although a role for the adjacent olfactory tubercle in nicotine CPP cannot be excluded (Ikemoto, 2003; Ikemoto et al., 2005; Sellings et al., 2006).

Nicotine CPP and the nucleus accumbens core

In the present study, DA-depleting lesions of the core increased nicotine CPP magnitude. This is unlikely to have resulted from global memory improvement, since analogous core lesions did not increase CPP associated with amphetamine or i.p. cocaine in the same apparatus (Sellings and Clarke, 2003; Sellings et al., 2006). CPP magnitude is potentially sensitive to test day activity, in that rats on the test day will tend to locomote more on the drug paired side if they develop a conditioned locomotor response to the drug. This in turn could affect CPP magnitude. We have previously observed that core lesions decreased amphetamine-conditioned locomotion (Sellings and Clarke, 2006). However, core lesions did not affect amphetamine CPP (Sellings and Clarke, 2003), suggesting that any lesion effect on conditioned locomotion in the present study would not have appreciably affected nicotine CPP. Based on these considerations, our CPP findings

suggest that DA transmission in accumbens core may be signaling or enabling the acute aversive properties of intravenously-administered nicotine.

Nicotine CTA and the nucleus accumbens core

CTA provides a sensitive measure of the aversive effects of drugs, including nicotine (Shoaib and Stolerman, 1995; Di Chiara et al., 2004). Two recent reports suggest that nicotine CTA may be DA dependent. In the first, CTA following systemic nicotine administration was abolished by systemic DA receptor blockade (Di Chiara et al., 2004). In the second, CTA produced by intra-VTA nicotine was blocked by intra-NAcc α-flupenthixol (Laviolette et al., 2002).

In the present study, DA transmission in the core and not the medial shell subregion was consistently associated with intravenous nicotine CTA. It is unlikely that the disruption of CTA by core lesions was due to an amnesic effect, as a role for accumbens shell but not core has been established in the learning aspects of CTA (Fenu et al., 2001). However, a deficit in learning may explain the slight decrease in CTA magnitude observed in medial shell-lesioned rats (Fig. 4). The present CTA results, taken with the above CPP findings, provide convergent evidence that DA transmission in accumbens core mediates nicotine aversion.

Segregated motivational effects of nicotine

It is well established that nicotine exerts both rewarding and aversive motivational effects. However, the aversive properties of nicotine appear more robust than with other abused drugs, as evinced by several lines of convergent evidence. First, animals will self-administer nicotine only under restricted conditions (Le Foll and Goldberg, 2005a). Second, intravenous nicotine is weakly self-administered unless drug delivery is paired with environmental cues (Donny et al., 2003; Le Foll and Goldberg, 2005a), and even when such cues are presented, rats reliably choose cocaine over nicotine infusions in a two-lever choice paradigm (Manzardo et al., 2002). Third, studies in squirrel monkeys have shown that a given dose of intravenous nicotine may be self-administered or can serve as a punisher, depending on the experimental conditions (Spealman, 1983). Fourth, whereas cocaine and many other drugs of abuse produce a reliable CPP in rats, systemic nicotine has been reported to elicit either a CPP (Fudala et al., 1985; Iwamoto, 1990; Shoaib et al., 1994; Le Foll and Goldberg, 2005b; Spina et al., 2006), a conditioned place aversion (Jorenby et al., 1990; Laviolette et al., 2002), or neither (Clarke and Fibiger, 1987; Calcagnetti and Schechter, 1994). Finally, aversive effects of systemic or intra-accumbens nicotine are also evident in the conditioned taste aversion paradigm (Shoaib and Stolerman, 1995; Laviolette et al., 2002). Taken together, these findings suggest that the aversive effects of nicotine may frequently mask its rewarding effects.

The findings of the present study suggest that accumbens DA mediates or enables two anatomically segregated effects of nicotine: a rewarding effect via the medial shell and an aversive effect via the core. The notion that DA can play an aversive role in the motivational effects of nicotine has been suggested by studies using the DA receptor

antagonist α-flupenthixol (Laviolette et al., 2002). However, α-flupenthixol also has significant affinity for 5-HT2 receptors (Matsubara et al., 1993). The current study used a 5-HT sparing neurotoxin, suggesting that DA does play a critical role. Opposing roles of core and medial shell DA would go some way towards reconciling the controversy surrounding the role of DA in nicotine's motivational effects (Rose and Corrigall, 1997; Laviolette and van der Kooy, 2004).

Anatomical division of dopamine functions

A core/shell functional segregation is consistent with a recently proposed division of the ventral striatum based on a medial-lateral or medioventral-dorsolateral organization (Voorn et al., 2004; Ikemoto et al., 2005). Since our medial shell and core lesions reduced DA innervation over similar rostrocaudal extents, a rostrocaudal gradient of positive to negative motivated behaviour (Reynolds and Berridge, 2002; Pecina and Berridge, 2005) is unlikely to be responsible for our findings.

The present observations appear relevant to the ongoing controversy surrounding the role of DA in signaling rewarding vs. aversive or salient events. Considerable evidence suggests that DA plays a role in reward processing or reward prediction (Berridge and Robinson, 2003; Wise, 2004; Schultz, 2005). DA also appears to subserve a general role in salience and attentional switching (Redgrave et al., 1999; Ungless, 2004). The relationship between DA and aversion is less clear. Several reports using single-cell recording suggest that DA cells do not fire in response to mildly aversive events

(Mirenowicz and Schultz, 1996), and that DA cell firing may be reduced in response to aversive stimuli (Ungless et al., 2004). In the accumbens, however, stressful stimuli (e.g. footshock, restraint) increase DA transmission preferentially in the shell (Deutch and Cameron, 1992; Kalivas and Duffy, 1995). It is unclear whether these powerful stressors accurately model the aversive effects of drugs such as nicotine; indeed, available evidence suggests that less obviously stressful stimuli (i.e. quinine and saturated saline solution) increase extracellular DA in the core but not the shell (Bassareo et al., 2002), suggesting that core DA release can be aversive.

In response to the public health threat imposed by tobacco smoking, the brain mechanisms that underlie nicotine dependence have been widely investigated. However, no consensus mechanism has emerged (Laviolette and van der Kooy, 2004; Dani and Harris, 2005). The present findings implicate the core in the aversive effects of nicotine, and the medial shell in the rewarding effects of nicotine. More generally, the present findings indicate that DA transmission may enable multiple, opposing motivational processes within the nucleus accumbens.

Table 1. DAT, SERT and NET binding. Values are percent of sham-operated control, and are expressed as mean ± SEM. und, undetectable; nd, not determined.

	DAT			SERT			NET		
	sham	**core**	**mSh**	**sham**	**core**	**mSh**	**sham**	**core**	**mSh**
EXPERIMENT 5									
Core	100±9	29±15	87±12	100±5	93±4	105±8	nd	nd	nd
medial shell	100±3	57±17	41±6	100±7	106±9	107±11	nd	nd	nd
ventral shell	100±15	50±24	107±14	100±4	107±9	101±10	nd	nd	nd
OT	100±14	64±26	101±8	100±6	101±8	103±7	nd	nd	nd
ventral CP	100±10	48±18	100±12	100±4	87±5	109±10	nd	nd	nd
EXPERIMENT 6									
Core	100±5	27±4	81±6	100±6	99±12	119±18	und	und	und
medial shell	100±6	67±7	37±2	100±6	98±13	109±16	100±20	86±41	87±23
ventral shell	100±6	55±8	81±10	100±7	106±15	108±12	100±26	99±46	111±37
OT	100±4	56±6	100±6	100±6	100±11	116±17	100±24	116±50	90±27
ventral CP	100±7	64±8	93±10	100±7	106±17	107±11	100±32	73±64	58±47
EXPERIMENT 7									
Core	100±2	25±7	100±5	100±5	90±3	102±6	nd	nd	nd
medial shell	100±3	62±9	41±5	100±3	101±5	105±8	nd	nd	nd
ventral shell	100±4	50±10	86±6	100±5	94±4	112±11	nd	nd	nd
OT	100±7	65±8	76±13	100±11	92±9	96±6	nd	nd	nd
ventral CP	100±1	47±5	107±5	100±4	96±5	103±7	nd	nd	nd
EXPERIMENT 8									
Core	100±4	14±3	81±5	100±2	100±5	92±3	und	und	und
medial shell	100±4	54±4	42±6	100±3	110±5	94±4	100±29	126±33	161±44
ventral shell	100±4	47±8	60±5	100±3	107±5	91±2	100±26	94±31	130±32
OT	100±3	51±5	64±5	100±4	102±5	91±3	100±14	76±19	87±21
ventral CP	100±4	47±8	89±4	100±2	101±4	96±3	100±15	82±24	115±24

Figure 1. Rats expressed conditioned place preference for intravenous but not subcutaneous nicotine. Rats appeared to express place preference for an intermediate dose of intravenous nicotine (*p<0.05, uncorrected Wilcoxon test, panel a). A separate group of rats was subsequently tested at this dose, and expressed a significant CPP magnitude (*p<0.05, panel b). Rats did not express CPP for subcutaneous nicotine at any dose tested (panel c).

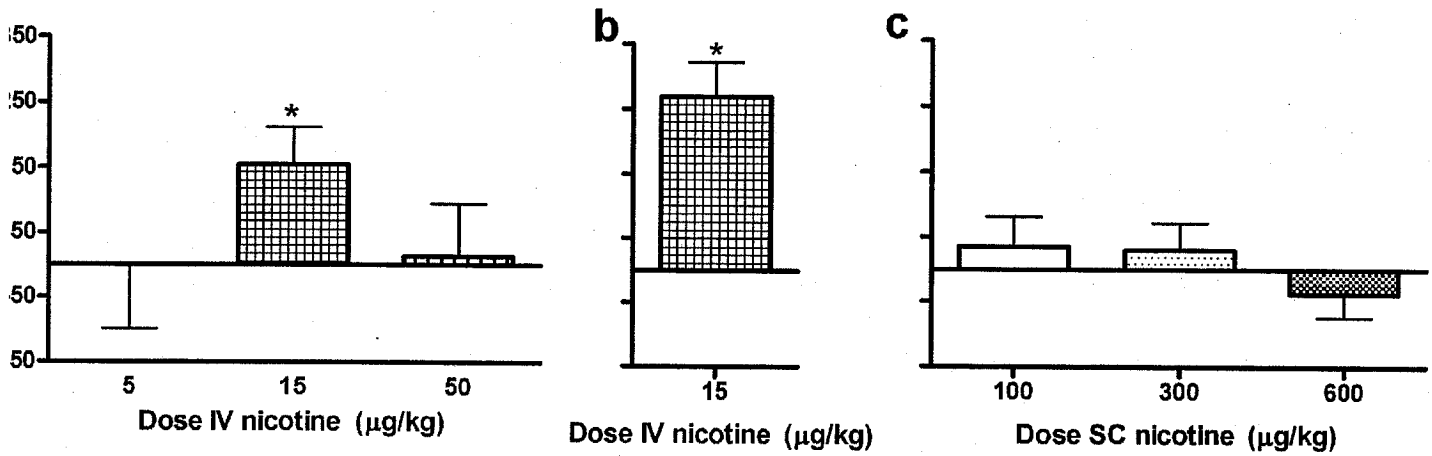


Figure 2. Rats expressed conditioned taste aversion for intravenous nicotine. The volume of novel flavor consumed did not differ significantly between nicotine and saline conditioning sessions at any nicotine dose given (panel a). Likewise, the total volume of fluid consumed (i.e. nicotine-conditioned *plus* saline-conditioned) on test day did not vary with dose of nicotine (panel b). CTA magnitude (panel c) was defined as the volume of saline-paired flavor consumed, minus the volume of nicotine-paired flavor consumed. Rats conditioned with 50 μg/kg nicotine drank significantly less nicotine-paired flavor than control rats receiving saline (**p<0.005, panel c).

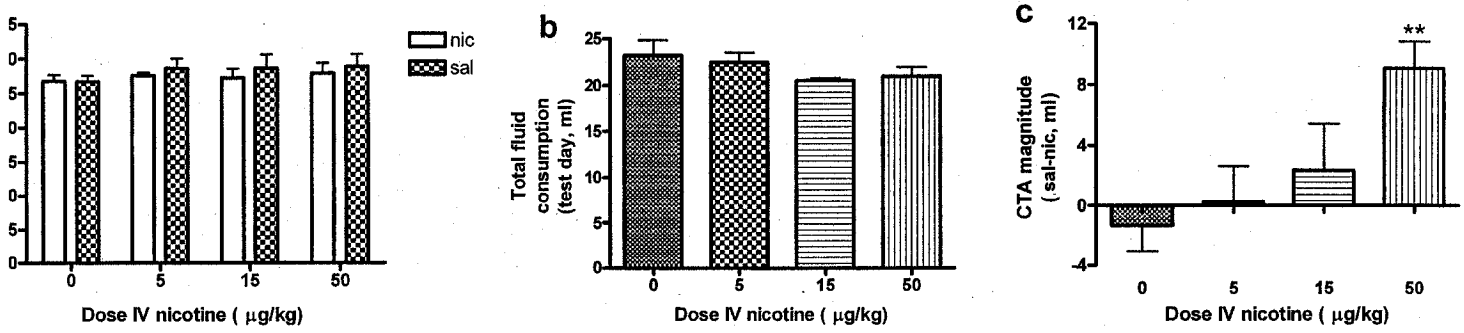


Figure 3. Core lesions increased, and medial shell lesions decreased, conditioned place preference for intravenous nicotine. In animals not pretreated with DMI prior to surgery, both sham- and core-lesioned animals exhibited significant CPP (†$p<0.05$, panel a). CPP magnitude differed significantly between core- and medial shell-lesioned animals (*$p<0.05$, Tukey's test, panel A). A negative linear association was observed between core DAT binding and CPP magnitude ($p<0.005$, panel b), and a positive association between medial shell DAT binding and CPP magnitude ($p<0.05$, panel c). In rats receiving DMI prior to surgery, only core-lesioned animals exhibited significant CPP (†$p<0.05$, panel D). Additionally, core- and medial shell-lesioned animals differed significantly in their CPP magnitude (*$p<0.005$, panel d). As with rats not receiving pre-surgical DMI, a negative association was observed between core DAT binding and CPP magnitude, and a positive association between medial shell DAT binding and CPP magnitude ($p<0.05$ for both, panels e and f respectively). After combining data from both experiments, only core-lesioned animals exhibited significant CPP (†$p<0.05$, panel g). Additionally, CPP magnitude differed significantly between medial shell- and both sham- (*$p<0.05$) and core- (**$p<0.005$) lesioned rats. A significant, negative association was observed between core DAT binding and CPP magnitude (panel h), and a significant, positive association between medial shell DAT binding and CPP magnitude (panel i). To better visualize the association between core vs. medial shell and CPP magnitude in panels h and i, the contribution of the irrelevant structure (i.e. medial shell or core) as calculated from the multiple regression equation has been subtracted to obtain an adjusted score.

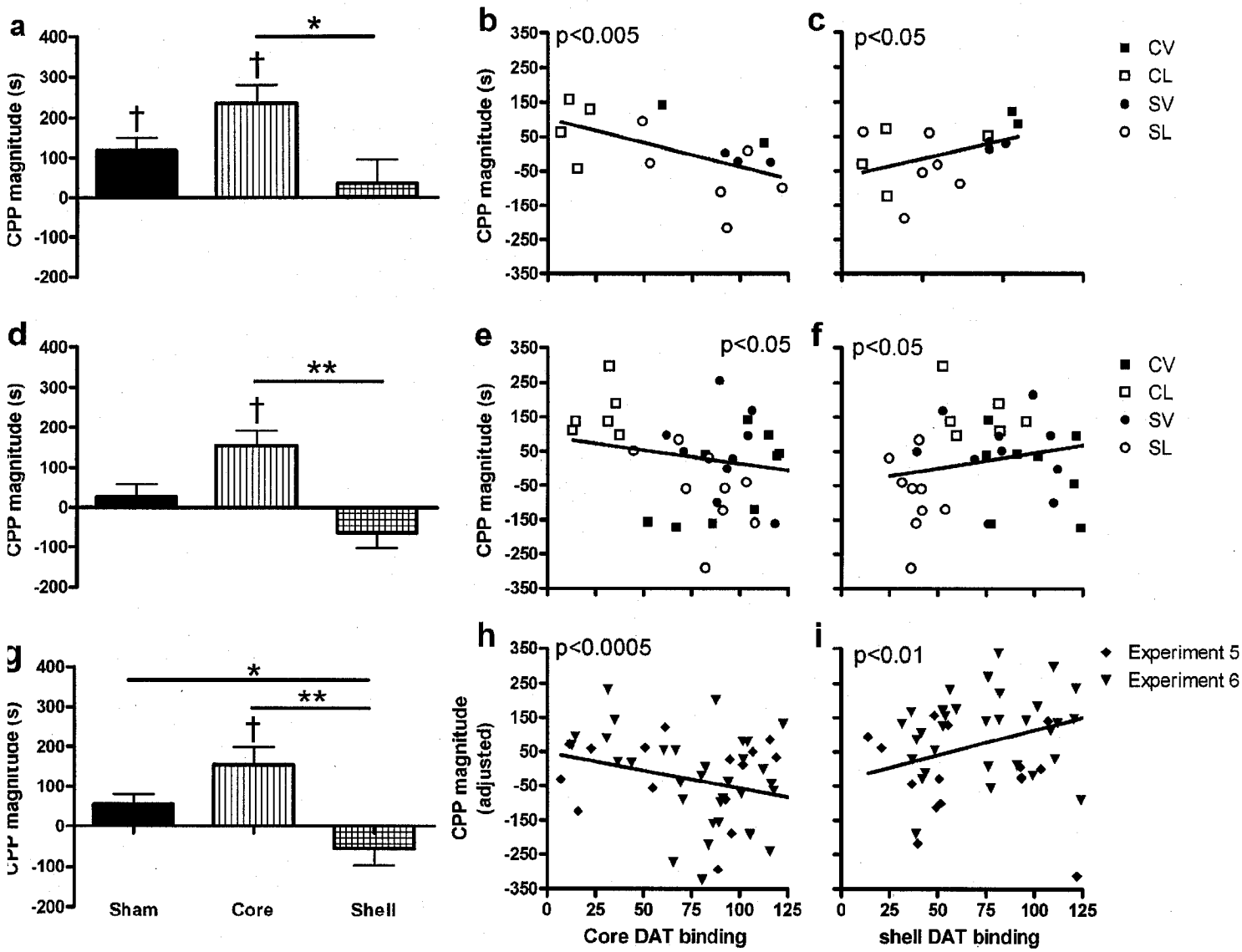
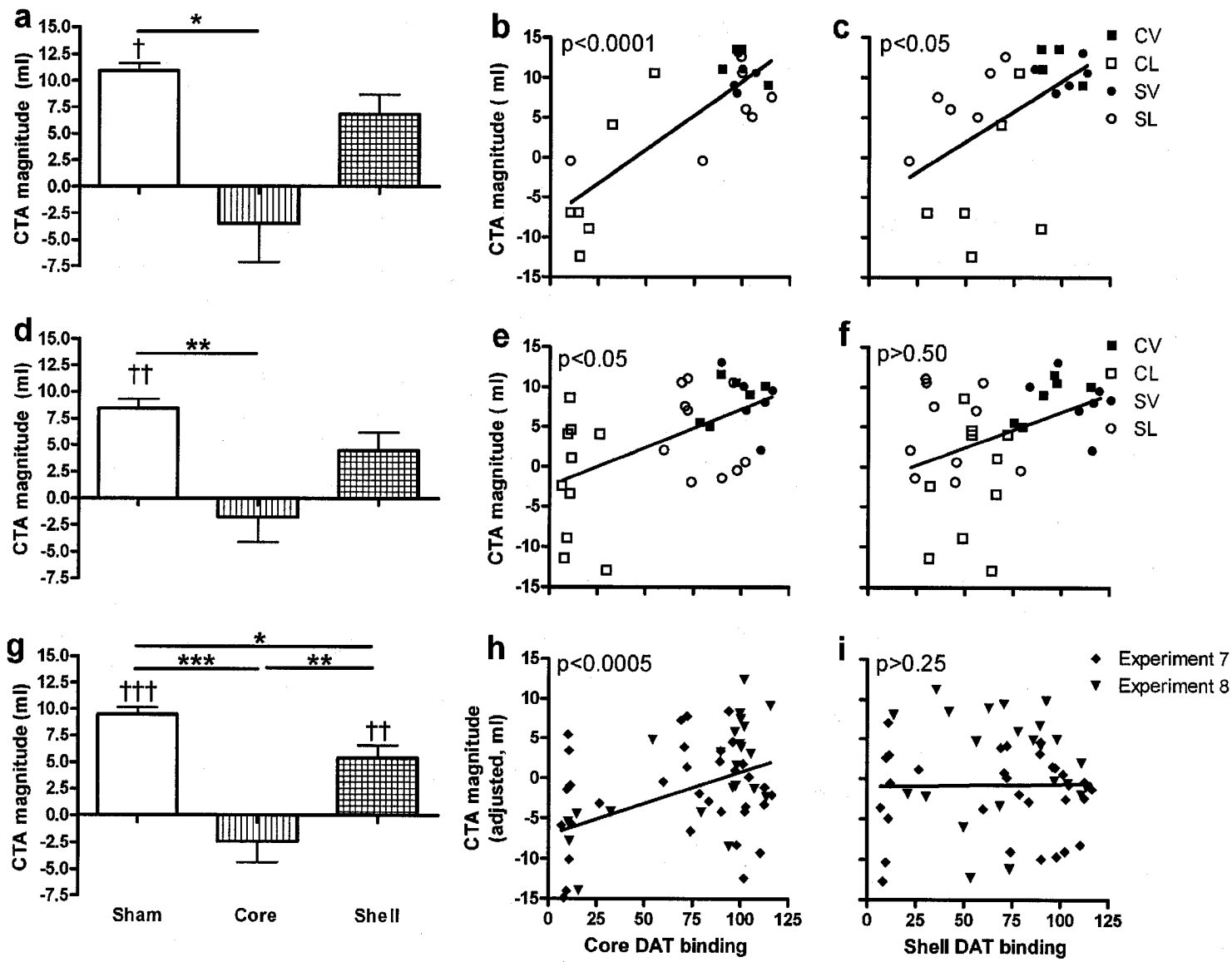

a
b
c
d
e
f
g
h
i
CPP magnitude (s)
CPP magnitude (adjusted)
p<0.005
p<0.05
p<0.0005
p<0.01
CV
CL
SV
SL
Experiment 5
Experiment 6
Sham
Core
Shell
Core DAT binding
shell DAT binding

Figure 4. Core lesions abolished conditioned taste aversion for intravenous nicotine. CTA magnitude, shown on the y-axis, was calculated as in Fig. 2. In the absence of DMI pretreatment before surgery (panels a-c), only sham-lesioned animals exhibited significant conditioned taste aversion (†$p<0.05$, Wilcoxon test; panel a). Additionally, only core-lesioned animals differed from sham-lesioned animals in the magnitude of the CTA (*$p<0.05$; panel a). Significant negative linear associations were observed between CTA magnitude and core DA innervation ($p<0.00005$, panel b) and medial shell DA innervation ($p<0.05$, panel c). In animals pretreated with DMI before surgery, again, only sham-lesioned rats exhibited significant CTA (††$p<0.005$, panel d). Core, but not medial shell DA innervation related significantly to the magnitude of the CTA ($p<0.05$ vs. $p>0.50$, panels e and f). When data from both experiments were combined, both sham- and medial shell-lesioned rats exhibited significant CTA (††$p<0.005$, †††$p<0.0005$, panel g). Both core- and medial shell-lesioned rats differed significantly from sham-lesioned rats in terms of CTA magnitude (*$p<0.05$, *** $p<0.0005$, panel g). A significant association was observed between core DAT binding and CTA magnitude (panel h), but not between medial shell DAT binding and CTA magnitude (panel i). To better visualize the association between core vs. medial shell and CTA magnitude, in panels h and i, the contribution of the irrelevant structure (i.e. medial shell or core) as calculated from the multiple regression equation has been subtracted to obtain an adjusted score.


a
b
c
d
e
f
g
h
i
CTA magnitude (ml)
CTA magnitude (ml)
CTA magnitude (adjusted, ml)
p<0.0001
p<0.05
p>0.50
p<0.0005
p>0.25
CV
CL
SV
SL
Experiment 7
Experiment 8
Sham
Core
Shell
Core DAT binding
Shell DAT binding

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CHAPTER 8: General Discussion

Laurie H. L. Sellings

8.1 Summary

This thesis examined the effects of catecholamine-depleting lesions of discrete ventral striatal subregions on drug-induced behaviours relating to behavioural arousal and reward vs. aversive processing. Psychostimulant-induced locomotor activity was reduced by 6-OHDA lesions of the accumbens core (Chapters 3-6). Effects on reward processing, however, were more complex (Table 1). The segregation of locomotor stimulation and conditioned place preference between core and medial ventral striatum (i.e. medial shell or medial olfactory tubercle) appears to reflect a segregation of reward and locomotion rather than conditioned and unconditioned drug effects, since core but not shell lesions abolished amphetamine-conditioned locomotor activity (Chapter 4). Finally, the core subregion may also underlie the aversive effects of nicotine, since lesions of the core abolished a CTA for nicotine (Chapter 7).

Taken together, these results suggest that medial ventral striatum underlies the rewarding effects and the core subregion the stimulant effects, of psychomotor stimulants. Additionally, the core may subserve a role in the aversive effects of nicotine.

8.2 Methodological limitations

The 6-OHDA lesion model was chosen in this thesis, since 6-OHDA can selectively lesion catecholaminergic terminals (see Introduction and Chapter 2). One drawback of the 6-OHDA lesion technique is the occurrence of behaviourally relevant compensatory neuroadaptations. For example, rats exhibited a similar degree of hypomotility after low-dose apomorphine challenge as did sham-lesioned controls at 24 weeks, but not at 4

Table 1. Effects of 6-OHDA lesions of discrete ventral striatal subregions on locomotor stimulation, conditioned place preference, conditioned activity and conditioned taste aversion. Significant positive association: +. Significant negative association: -. Symbols followed by a question mark indicate a non-significant statistical trend.

Lesion site		**Core**	**Medial shell**	**Anteromedial olfactory tubercle**
Behaviour	Drug			
Locomotor stimulation	Amphetamine (i.p.)	+		
	Cocaine (i.v.)	+		
	Cocaine (i.p.)	+		
	Methylphenidate (i.v.)	+		
Conditioned place preference	Amphetamine (i.p.)		+	
	Cocaine (i.v.)	-?	+	++
	Cocaine (i.p.)			
	Methylphenidate (i.v.)			+
	Nicotine (i.v.)	-	+	
	Morphine (i.p.)			
Conditioned activity	Amphetamine (i.p.)	+		
Conditioned taste aversion	Nicotine (i.v.)	+		

weeks following-6-OHDA infusion into the nucleus accumbens, (Vos et al., 1999). Additionally, the hypomotility observed one week after accumbal 6-OHDA infusion disappeared 3-4 weeks after the lesion (Wolterink et al., 1990). The types and extent of functional recovery that occurs during behavioural testing in the studies performed in this thesis (i.e. 1-3 weeks post-lesion) have not been characterized. However, since all three lesion sites were shown to be behaviourally effective in different behavioural tests, it is unlikely that neuroadaptations significantly rescued function within this time frame.

Anatomical selectivity proved difficult to achieve, especially in the case of accumbens core lesions. Thus, the experiments presented in this thesis cannot rule out the possibility that observed behavioural effects required some DA denervation in structures adjacent to the target structure. Further refinement of the lesion technique will be required to investigate this possibility. Another potential problem lies in the possibility that depletion of NE innervation contributed significantly to the observed behavioural effects. This seems unlikely for many reasons, as discussed below.

In the case of locomotor activation, although disruption of noradrenergic transmission can inhibit amphetamine-induced locomotion (Ogren et al., 1983; Archer et al., 1986; Dickinson et al., 1988; Darracq et al., 1998; Harro et al., 2000; Drouin et al., 2002a; Drouin et al., 2002b), the medial prefrontal cortex has been identified as the probable site of action (Blanc et al., 1994; Darracq et al., 1998). In contrast, noradrenergic transmission in the NAcc does not contribute directly to locomotor stimulation (Pijnenburg et al., 1975; Roberts et al., 1975; Kelly and Iversen, 1976; Joyce et al., 1983).

Additionally, NE afferents largely avoid the core (Berridge et al., 1997; Delfs et al., 1998), which was the subregion most clearly associated with the locomotor stimulant effect of the psychomotor stimulants examined in this thesis.

In the case of reward processing, noradrenergic transmission appears unrelated to both CPP and IVSA, for the following reasons. First, stimulation of noradrenergic transmission does not produce a CPP (Martin-Iverson et al., 1985; Subhan et al., 2000). Second, neither α nor β adrenergic receptor antagonists affected i.v. cocaine self-administration behaviour (Johanson and Fischman, 1989). Third, the disruptive effects of 6-OHDA lesions on cocaine self-administration persist even when NE terminals are protected with DMI (Roberts et al., 1980). Fourth, self-administration of cocaine directly into the amOT was blocked by co-infusion of a D1 or D2 DA receptor antagonist (Ikemoto, 2003). Finally, in the case of nicotine, the effects of 6-OHDA lesions on noradrenergic transmission appear unrelated to lesion effects on CPP and CTA. More specifically, similar lesion effects were observed in rats pre-treated with DMI prior to 6-OHDA infusion, and those not receiving DMI (Chapter 7).

Transporter autoradiography was used to quantify DA, 5-HT or NE innervation. Although this measure provided an indirect measure of DA innervation, there is evidence suggesting that DAT binding density accurately reflects DA innervation after 6-OHDA lesion (Joyce, 1991a; Joyce, 1991b). Similarly, SERT binding can be used as a marker for 5-HT innervation, since 5-HT afferents to the accumbens express SERT, and >90% of accumbal SERT is expressed on 5-HTergic axons and axon terminals (Pickel and Chan,

1999). Although the distribution of NET in the accumbens has not been thoroughly characterized, noradrenergic afferents from the nucleus tractus solitarius, and to a lesser extent from the locus coeruleus, are known to innervate the medial accumbens (Gaspar et al., 1985; Delfs et al., 1998). Importantly, locus coeruleus noradrenergic neurons express both NET mRNA and protein (Zhu et al., 2002), and nucleus tractus solitarius NE content is reduced by 6-OHDA infusion (Itoh et al., 1992), suggesting that these neurons also express NET. Since NET autoradiography revealed negligible binding in core, the region of ventral striatum virtually devoid of noradrenergic innervation (Berridge et al., 1997; Delfs et al., 1998), NET binding appears to represent an acceptable marker of ventral striatal NE innervation.

An additional point of discussion is how the experiments presented represent *addiction.* Addiction in humans is characterized by compulsive drug seeking and drug taking behaviour that can relapse after protracted abstinence. Intense drug craving, and the reinstatement of drug seeking behaviour can be precipitated by exposure to drug-related environmental cues, stress, or a small dose of the drug (see Introduction). In this thesis, rats' affinity for drug-associated cues was examined extensively, by CPP, CLMA and CTA. As such, these experiments examined how the association of environmental cues with the drug experience may be encoded by NAcc DA transmission. However, these studies do not fully model addiction in the human sense, for two main reasons. First, acute drug administration was used – rats received drug on between three and five occasions. Second, drug was experimenter delivered, as opposed to being self-administered. Although self-administration paradigms may come closer to modelling

addiction, drug access is still typically limited, and as such, it usually represents an acute model.

Two recent reports have somewhat convincingly modelled "addictive" behaviours in a chronic cocaine self-administration paradigm in rats (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Vanderschuren et al., 2005). In the first such study, it was demonstrated that the presence of an aversive CS did not prevent drug administration in rats self-administering cocaine on a prolonged schedule (Vanderschuren and Everitt, 2004). In the second study (Deroche-Gamonet et al., 2004), rats were scored on three addiction-like criteria – namely persistence of drug seeking (measured by resistance to extinction in the absence of drug), resistance to punishment (self-administration despite drug being delivered with a punishing footshock) and motivation to self-administer (breaking point on a progressive ratio). Approximately 17% of rats were in the top third for all three addiction-like criteria examined in the study, a number that these authors point out corresponds with the percentage of human cocaine users who progress to cocaine addiction (Anthony et al., 2006). Unfortunately, no comparisons were made between the rat population exhibiting all three addiction-like behaviours and addiction resistant populations at the molecular, cellular, or systems level. Such differences certainly merit further investigation, as they may provide insight into determinants of whether an individual is a part of an addiction susceptible or non-susceptible population.

8.3 Future directions – how do discrete 6-OHDA lesions of ventral striatal subregions affect behaviours that are altered in psychiatric disorders?

Changes in DA transmission in the ventral striatum are by no means observed only after exposure to natural rewards and stressors, or in response to drug administration or exposure to drug-associated stimuli. Alteration of DA transmission has been observed in several psychiatric disorders in humans (Cropley et al., 2006). Additionally, several of these psychiatric disorders are co-morbid with substance abuse (Green, 2005), suggesting that a link between the two may exist. This link may be ventral striatal DA transmission. Animal evidence for mesolimbic DA transmission as a critical component of specific behavioural deficits observed in several psychiatric disorders is examined below, with special reference to core vs. shell contributions.

8.3.1 Sensorimotor gating

Deficits in sensorimotor gating as measured by the prepulse inhibition of the acoustic startle reflex (PPI) are observed in a number of psychiatric disorders, including schizophrenia and obsessive-compulsive disorder (Swerdlow et al., 1993). PPI is the decreased startle observed after a loud and sudden acoustic tone if it is preceded by a weak auditory "prepulse". DA neurotransmission in the accumbens appears to be critical in sensorimotor gating, as evinced by the disruption in PPI observed after intra-accumbens infusion of exogenous DA (Swerdlow et al., 1990), and the restoration of PPI by 6-OHDA lesions of the ventral striatum in rats treated with systemic amphetamine (Swerdlow et al., 1990).

Taken as a whole, the core subregion appears to play a more significant role than the shell in mediating PPI, for the following reasons. First, inactivation of core but not shell

by infusion of the $GABA_A$ receptor agonist muscimol disrupted PPI (Pothuizen et al., 2005a). Second, lesions of the core *enhanced* dizocilpine-induced PPI disruption (Pothuizen et al., 2006b). Third, NMDA infusion in core disrupted PPI (Reijmers et al., 1995). Fourth, infusions of the DA D2/D3 receptor agonist quinpirole into the core more effectively disrupted PPI than did intra-shell infusions (Wan et al., 1994). Fifth, systemic haloperidol administration rescued PPI only after intra-core infusions (Wan and Swerdlow, 1996). In summary, although the core appears more functionally important than the shell, the precise role of *dopamine* in the core vs. shell warrants further investigation. The mini-lesion approach developed in this thesis could be informative in this respect, to examine the effect of DA denervation in core vs. shell on PPI.

8.3.2 Latent inhibition and attentional switching

Latent inhibition refers to the delayed acquisition of a CS-US association resulting from prior presentation of the CS. Changes in the persistence of latent inhibition, which can be considered as deficits in attentional switching, are observed in schizophrenic patients (Weiner, 2003). Such deficits are reversed by neuroleptic treatment, suggesting they may be DA-dependent in humans (Weiner, 2003). This appears to be the case in rodents, as reducing DAergic transmission in the accumbens by pharmacological blockade or 6-OHDA infusion made latent inhibition abnormally persistent, while intra-NAcc amphetamine in combination with systemic amphetamine attenuated latent inhibition (Gray et al., 1997).

The core and the shell seem to exert opposing effects on latent inhibition. More specifically, whereas electrolytic or excitotoxic lesions of the shell disrupted latent inhibition in a conditioned avoidance paradigm (Tai et al., 1995; Weiner et al., 1996; Gal et al., 2005), electrolytic lesions of the entire accumbens made latent inhibition abnormally persistent (Gal et al., 2005). This suggests that neurotransmission in the shell is important in learning that a potential CS has no motivational importance, and that the core underlies learning that a CS previously considered unimportant is in fact predictive of a motivationally relevant outcome. This may relate to DA release in core vs. shell, since pre-exposure to the CS+ prevented subsequent conditioned DA release in the shell, but not in the core (Murphy et al., 2000). Again, the mini-lesion approach developed in this thesis has potential to be informative in this respect.

8.3.3 Impulsive choice

One aspect of impulsivity can be examined in animals by investigating responses to delays in reward receipt. For example, an animal that preferentially responds for a small, immediate reward rather than for a large, delayed reward has made an impulsive choice. Impulsive responding is one of the hallmarks of several human psychiatric disorders, including attention deficit hyperactivity disorder (King et al., 2003), personality disorders, bipolar disorder, and addiction (Moeller et al., 2001; Bornovalova et al., 2005).

Impulsive responding for rewards is thought to be dependent on ventral striatal transmission, and is probably DA-dependent, for the following reasons. First, both systemic and intra-accumbens amphetamine increase impulsive responses in rats (Cole

and Robbins, 1987). Second, 6-OHDA lesions of the ventral striatum prevent systemic AMPH-induced increases in impulsive responding (Cole and Robbins, 1989). Third, 6-OHDA lesions of the NAcc reduced the impulsive responding resulting from systemic administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (Winstanley et al., 2005). The latter finding also indicates that 5-HT-DA interactions in the accumbens are critical in impulsive responding (Harrison et al., 1997; Koskinen and Sirvio, 2001).

Manipulations of the NAcc core rather than the shell affect choice selection (Cardinal et al., 2004). For example, excitotoxic lesions of the core, but not of the shell, increased responding for small but immediate vs. large but delayed rewards (Cardinal et al., 2001; Pothuizen et al., 2005b). A similar deficit is also observed when choosing between intermittent and continuous reinforcement. More specifically, in rats sustaining excitotoxic core lesions, continuously reinforced rewards were consistently chosen, even when intermittent reinforcement provided substantially larger food reward (Cardinal and Howes, 2005). The input from the prefrontal cortex to the core appears to be essential in choice selection, as rats sustaining disconnection lesions of the core and prefrontal cortex exhibited impulsive responding (Christakou et al., 2004). Although these results clearly implicate neurotransmission in the core rather than the shell in choice selection, how DAergic transmission in either subregion may influence choice selection warrants further investigation, and again, the 6-OHDA mini-lesions employed in this thesis may shed light on this question.

8.4 Mesolimbic dopamine – reward signal or enabler of reward-related behaviour?

Changes in DA transmission are clearly associated with drug delivery and self-administration. Several influential hypotheses have proposed specific roles for DA signalling in motivated behaviour (e.g. Wise, 1978; Wise, 1987; Wise and Rompre, 1989; Schultz, 1998; Wise, 2004; Ikemoto and Wise, 2004; Schultz, 2005). Indeed, much evidence supports a role for DA in reward and reinforcement (see Introduction). The term reward, however, signifies several separate psychological processes. In a recent review, Berridge and Robinson (2003) outlined different aspects of reward-related processing. Three major categories of reward processing were outlined – namely the learning of relationships between stimuli, the experience of hedonic consequences and the motivation to receive reward. These three categories of reward processing shall be considered below.

8.4.1 Reward-related learning

The first category to be considered shall be the learning of relationships between environmental cues and the drug experience. Since several conditioned drug measures (i.e. CPP, CTA and CLMA) were examined in this thesis, this type of reward processing is of particular relevance in reference to the experiments presented here. In support of a role for DA cell firing specifically in *reward* learning, a series of elegant studies using single-cell recording in the conscious monkey have suggested that DA cells fire in response to rewarding stimuli (Mirenowicz and Schultz, 1996; Schultz, 1998; Schultz, 2005). More specifically, presumed DA cells fire in response to the initial reward presentation, but this response quickly habituates. Instead, cues predictive of future reward receipt elicit increased DA cell firing, and the omission of expected reward is

accompanied by a *decrease* in DA cell firing (Schultz, 1998; Schultz, 2005). Conversely, DA cell firing was not observed in response to a mildly aversive stimulus (Mirenowicz and Schultz, 1996), which was interpreted as support of a reward-selective function for DA cell firing. Taken together, the results of these studies led this group to propose that DA cell firing is important in reward prediction. Since extracellular DA levels in the rat nucleus accumbens have been observed to increase in response to conditioned stimuli predicting the receipt of food (Bassareo and Di Chiara, 1999), sucrose (Datla et al., 2002) or cocaine (Ito et al., 2000), increased DA cell firing associated with reward prediction may translate into increased extracellular DA levels. However, extracellular DA levels in the accumbens have also been observed to increase in response to salient or aversive stimuli (e.g. Young et al., 1998; Horvitz, 2000).

Reward prediction can be examined in the CPP paradigm. Here, cues paired with the drug experience come to serve as predictors of reward receipt. Studies examining CPP in this thesis are consistent with a role for DA transmission in the ventral striatum in reward prediction, since 6-OHDA lesions of the medial shell or medial OT reduced CPP for several psychomotor stimulant drugs. However, investigations of nicotine CPP and CTA, in which 6-OHDA lesions of the core both increased CPP magnitude and abolished CTA, suggest that DA transmission encompasses a broader role than just reward prediction.

Aberrant learning has been proposed to underlie addictive behaviours. Supporting this idea, processes important in learning such as long term potentiation and long term depression have been observed in the NAcc after repeated drug administration (reviewed

in Berke and Hyman, 2000; Hyman and Malenka, 2001; Hyman et al., 2006). One such proposal posits that aberrant stimulus-response habit learning may represent a stepping stone on the route to compulsive drug-taking behaviour (Berke and Hyman, 2000; Everitt and Robbins, 2005), and DA transmission in the dorsal striatum appears to support habit learning. In particular, it was recently shown that infusion of the DA receptor antagonist cis-flupenthixol into the dorsal striatum dose-dependently reduced responding for a cocaine-associated cue, but only after habitual responding had been established (Vanderschuren et al., 2005). On the basis of such results, this group has hypothesized that responding for drug receipt becomes an aberrant stimulus-response habit – that is, drug seeking is no a longer goal-directed behaviour, but becomes an automated response (Everitt and Robbins, 2005). This is an attractive theory that can incorporate much of the experimental animal literature examining drug-seeking behaviour. However, it is unclear if automated habit responding can fully incorporate the flexibility of responding required in human drug-seeking behaviour; more specifically, human drug-seeking requires flexible behaviour in order to obtain drug in a variety of situations, not just a simple operant response as in animal models (Robinson and Berridge, 2003). Whereas this concern does not rule out a role for aberrant learning per se in the development of compulsive drug seeking behaviour, it does suggest that aberrantly strong stimulus-response learning cannot fully account for addiction.

8.4.2 Hedonic responses to reward presentation

The second "reward" category is the hedonic or affective component, which has also been referred to as 'liking' the reward (e.g. Robinson and Berridge, 2003). One approach

that has been taken to study this phenomenon in the rat is taste reactivity. In this paradigm, the rat is examined after the consumption of a substance (e.g. sucrose, quinine solution) and the number of positive hedonic reactions (e.g. tongue protrusions, paw licking) vs. aversive reactions (e.g. head shakes, chin rubs) is scored. DAergic mechanisms within the accumbens shell do not appear necessary for the expression of positive 'liking' reactions to sucrose reward; such studies have implicated opioid transmission rather than DA transmission in the accumbens in the positive 'liking' reactions to sweet tastes (Pecina and Berridge, 2000; Wyvell and Berridge, 2000; Pecina and Berridge, 2005). However, it remains unknown if liking of drug reward is dependent on DAergic signalling. Additionally, the taste reactivity test is not useful for examination of primary drug reward. Indeed, examination of taste reactivity in response to tastes conditioned to reward-relevant drugs often leads to an avoidance of that flavour, as in conditioned taste aversion.

One potential measure of affect that may be useful to examine drug liking is the 50 kHz ultrasonic vocalization (see Introduction). Although it is not universally accepted that such an index represents affect, it has been proposed that rats can communicate both 'positive' and 'negative' affect by ultrasonic vocalization (Knutson et al., 2002). 'Positive' hedonic state calls are produced by intra-shell amphetamine infusion, suggesting that positive affect induced by drugs may indeed be dependent on DA transmission (Thompson et al., 2006). This would accord with other studies suggestive of a role for shell DA transmission in several aspects of reward-related processing (Di Chiara et al., 2004). Further characterization of 50 kHz ultrasonic vocalizations as an

affective measure will be useful to resolve the question of DA involvement in affective state induced by drugs vs. natural rewards.

8.4.3 Motivation for reward receipt

The third and final category is the motivational aspect of reward. In contrast to the hedonic aspect of reward, which is an affective measure, motivation examines the desire or drive to obtain the reward. This is not a trivial distinction. Indeed, in a recent human study, liking and wanting of alcoholic beverages were shown to be dissociable (Hobbs et al., 2005). More specifically, administration of a priming alcoholic beverage increased the motivation to consume alcohol without affecting self-reports of liking, whereas addition of an unpleasant flavour to the alcohol reduced liking, but not consumption (Hobbs et al., 2005). Animal studies have suggested that NAcc DA may mediate the attribution of incentive salience – that is, the motivation or drive to obtain a reward (Robinson and Berridge, 2003). It is proposed that sensitized DA release after repeated drug use attributes excessive incentive salience to drug-associated cues, and would ultimately cause a state of "incentive sensitization", contributing to compulsive drug-seeking behaviour (Robinson and Berridge, 2003). The results observed in the present thesis are consistent with the notion that NAcc DA transmission is important in the association of drug-related cues with the drug experience. However, whether the persistence of such associations – that is, the ability of cues to precipitate drug-seeking and drug-taking behaviour after protracted abstinence - is dependent on DA release remains a question for the future. All things considered, it is clear that the DA hypothesis

must be refined to consider which aspects of reward (Berridge and Robinson, 2003) depend critically on NAcc DA transmission and which do not.

8.4.4 Dopamine – beyond a reward-specific function?

Although NAcc DA transmission is clearly associated with psychostimulant reward, hypotheses indicating that NAcc DA is reward-specific suffer two important criticisms. The first is that extracellular DA levels are altered in the ventral striatum in response to salient, stressful and aversive stimuli (Horvitz, 2000; Ungless, 2004). As such, a selective role for DA in reward processing is unlikely. It has been proposed that DA release accompanying such stimuli may simply represent an opponent process (Ungless, 2004). Briefly, opponent process theory posits that a stimulus activates two, opposing responses. The first is of rapid onset and offset, and the second – the opponent process - serves to counter the first. In the case of aversive stimuli, it has been proposed that the increase in extracellular DA levels represents an opponent process to an initial decrease in DA cell firing induced by the aversive stimulus (Ungless, 2004; Ungless et al., 2004). However, such an explanation does not exclude a role for DA in signalling aversive stimuli. It simply means that the role DA is playing in encoding aversive stimuli is different from that of rewarding stimuli. Accordingly, Ikemoto and Panksepp (1999) proposed that NAcc DA transmission enables sensorimotor integration that facilitates flexible approach responses. Such a general role for NAcc DA in motivated behaviour would encompass both positive and negatively motivated behaviour, as well as behavioural activation.

A second criticism of the idea that DA transmission is reward-selective is that manipulations of NAcc DA transmission alter the locomotor stimulant effect of a wide array of psychoactive drugs, and it is unclear how this unconditioned response directly represents reward processing. Although it has been proposed that drug reward and reinforcement are critically dependent on behavioural activation, the two effects are indeed segregable (Robledo et al., 1993; Burns et al., 1993; Sellings and Clarke, 2003; Ventura et al., 2003; Sellings and Clarke, 2006; Sellings et al., 2006a; Sellings et al., 2006b).

8.5 Of mice *and* men?

Although it is interesting to consider what DA transmission encodes in animals, it is important to consider how such theories may apply to the human case.

8.5.1 Does the core-shell divide exist in humans?

Compared to the rodent, little is known about potential subdivisions of the ventral striatum in the human. A limited number of studies have examined post mortem human brain tissue using histochemical markers that are differentially distributed between core and shell in the rat. A table of comparisons between known human markers and the equivalent in the rodent is shown in Table 2. In short, histochemical marker distributions typically support the existence of a dorsolateral/medioventral division in the human ventral striatum that resembles the core/shell divide in the rat. However, it is not known if these compartments in the human are accompanied by hodological and functional differences. Unfortunately, the spatial resolution of brain imaging techniques

Table 2. Distribution of histochemical markers between rat core and shell, and human core-like and shell-like areas

Species		**Rat**	**Human**
Marker	*Structure*		
Dopamine D3 receptor	Core	Low	Low
	Shell	High	High
μ opioid receptor	Core	Low	Low
	Shell	Intermediate-high	Intermediate-high
κ opioid receptor	Core	Low-moderate	Low
	Shell	High	High
Calbindin D-28k	Core	High	High
	Shell	Low	Low
Calretinin	Core	Low	Low
	Shell	High, especially caudal shell	High
Choline acetyltransferase	Core	Low	Low-intermediate
	Shell	High in caudal shell	Low-intermediate
Acetylcholinesterase	Core	Low	Low-intermediate
	Shell	High	Low-intermediate
Substance P	Core	Low	Low
	Shell	Intermediate-high	High

References: Zaborszky et al., 1985; Tempel and Zukin, 1987; Meredith et al., 1989; Zahm and Brog, 1992; Levesque et al., 1992; Voorn et al., 1994; Jongen-Relo et al., 1994; Voorn et al., 1996; Meredith et al., 1996; Bubser et al., 2000; Prensa et al., 2003.

is not such that differences in DA transmission between the core-like and shell-like areas of human ventral striatum can be determined.

8.5.2 Does DA signalling encode drug cues and experiences in humans?

8.5.2.1 DA and cue-induced craving

DA transmission may play a role in selective attention; such a role could influence the attention attributed to drug-related stimuli (Franken et al., 2005). Indeed, pharmacological treatments that act on DA receptors have been shown to influence cue-induced craving in humans. For example, administration of the indirect DA agonist amphetamine increased cue-induced cigarette craving in both abstinent and non-abstinent smokers (Alsene et al., 2005). Although the DA receptor antagonist haloperidol did not attenuate cue-induced craving in abstinent smokers, this study had a relatively small sample size (Mahler and De Wit, 2005). Conversely, the atypical antipsychotic olanzepine successfully reduced cue-elicited craving in abstinent smokers in a double blind study (Hutchison et al., 2004), suggesting that DA may signal cue-elicited craving. Whether this extends to other drugs is unclear. More specifically, in the case of cocaine, risperidone reduced cue-induced cocaine craving in an open label study (Smelson et al., 1997), but was ineffective in preventing cue-induced craving for cocaine in a double-blind, placebo-controlled study (Smelson et al., 2004). In further support of a role for DA transmission in cue-induced craving, feeding non-dependent cocaine users a diet lacking amino acid precursors of DA synthesis successfully reduced cue-induced cocaine craving (Leyton et al., 2005). It is possible that the properly controlled risperidone study lacked power due to a small sample size. In summary, it appears that DA transmission in

humans may be causally related to cue-induced drug craving. However, more properly controlled and large-scale studies are needed to define a role for DA signalling in such a process.

The proposal that DA may encode selective attention in humans (Franken et al., 2005) is consistent with rodent studies suggesting that DA release accompanies exposure to a wide array of salient stimuli (Horvitz, 2000). Additionally, the incentive sensitization theory (Robinson and Berridge, 2003) accords with the idea that excessive attention may be paid to drug-associated cues in humans, although evidence for drug sensitization in humans is lacking.

8.5.2.2 DA and subjective drug measures

Several PET imaging studies have shown an association between DA transmission and drug-liking or euphoria affect in humans. For example, a reduction in DA D2 receptor binding potential in the extended striatum (measured using the PET ligand [^{11}C] raclopride), an indirect measure of DA release, was associated with subjective reports of drug liking for both methylphenidate (Volkow et al., 1999, 2002) and nicotine (Barrett et al., 2004). Additionally, the euphoria experienced after intravenously-administered amphetamine correlated significantly with the same measure in *ventral* striatum (Drevets et al., 2001), with one study suggesting lateralization to the left ventral striatum (Oswald et al., 2005). However, studies examining the correlation between mood and raclopride binding potential after oral amphetamine administration failed to show such a relationship (Leyton et al., 2002; Riccardi et al., 2006), despite a clear relationship

between DA release and drug wanting (Leyton et al., 2002). In the case of oral amphetamine, administration of the DA receptor antagonist pimozide prior to drug receipt had no effect on drug liking (Brauer and De Wit, 1996; Brauer and De Wit, 1997). It is possible that subjective liking for intravenous, but not for oral amphetamine is mediated by DA transmission. Indeed, pre-clinical studies in laboratory animals have demonstrated that route of administration is a critical determinant of whether or not cocaine CPP is DA-dependent (Spyraki et al., 1987; Sellings et al., 2006b). In other words, the route of administration may be a critical factor in determining whether a drug-induced effect is dependent on or independent of DA transmission. It is unclear if DA transmission mediates or merely accompanies the experience of positive affect associated with drug taking. Additionally, such a correlation between increased DA transmission and positive affect or euphoria has not been universally observed. Whether increased striatal DA transmission mediates, enables or merely accompanies drug-induced positive affect in humans remains a subject for further investigation. In summary, the above evidence linking striatal DA release and drug-induced affect in humans is at best correlative.

8.6 Concluding statements

The studies in this thesis examined the relative contribution of DA transmission in the core, medial shell and medial olfactory tubercle to behavioural attributes of amphetamine, cocaine, methylphenidate and nicotine. Although locomotor stimulation was associated with DA transmission in the core for all drugs tested (Chapters 3-6), the pattern of results for reward was more complex (Chapters 3, 5-7; see Table 8-1).

Additionally, 6-OHDA lesions of the core appeared to reduce the *aversive* effects of intravenous nicotine (Chapter 7). Taken as a whole, these studies suggest that DA transmission in small, adjacent brain structures can encode different aspects of motivated behaviour. Such complexities must be considered in the development of more effective therapeutics in drug cessation. It is clear that *in vitro* screening assays commonly employed in pharmaceutical research cannot mimic such complexities, and such reductionist approaches to drug discovery may account in part for the lack of efficacious drugs in the battle against drug addiction.

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APPENDICES

APPENDIX A – Copyright waivers and permissions

APPENDIX B: Radioactivity permits and animal use protocols and permits

APPENDIX C: Copies of published articles

Behavioral/Systems/Cognitive

Segregation of Amphetamine Reward and Locomotor Stimulation between Nucleus Accumbens Medial Shell and Core

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Convergent evidence suggests that amphetamine (AMPH) exerts its rewarding and locomotor stimulating effects via release of dopamine in the nucleus accumbens. However, there is no consensus as to the relative contributions of core and medial shell subregions to these effects. Moreover, the literature is based primarily on intracranial administration, which cannot fully mimic the drug distribution achieved by systemic administration. In the present study, the effects of bilateral 6-hydroxydopamine lesions of the accumbens core or medial shell on rewarding and locomotor stimulating effects of systemically administered amphetamine (0.75 mg/kg, i.p.) were examined in a conditioned place preference (CPP) procedure relying solely on tactile cues (floor texture). Residual dopamine innervation was quantified by [^{125}I]-RTI-55 binding to the dopamine transporter. When lesions were performed before the conditioning phase, AMPH-induced locomotor stimulation and CPP magnitude were positively correlated with residual dopamine transporter binding in core and medial shell, respectively. Medial shell lesions did not affect morphine CPP, arguing that a sensory or mnemonic deficit was not responsible for the lesion-induced reduction in AMPH CPP. Medial shell lesions performed between the conditioning phase and the test day reduced the expression of amphetamine CPP. These results suggest that after systemic amphetamine administration, rewarding and locomotor stimulating effects of the drug are anatomically dissociated within the nucleus accumbens: the medial shell contributes to rewarding effects, whereas the core contributes to behavioral activation.

Key words: nucleus accumbens core; nucleus accumbens medial shell; amphetamine; 6-hydroxydopamine; locomotion; reward; conditioned place preference; morphine

Introduction

Convergent evidence suggests that the rewarding and behavioral activating effects of psychomotor stimulant drugs are initiated by increased dopaminergic transmission in the nucleus accumbens (NAcc). Evidence is perhaps strongest for the prototypic psychomotor stimulant, amphetamine (AMPH). For example, the locomotor stimulant effect of systemic AMPH is mimicked by intra-accumbens infusion of AMPH or dopamine (DA) (Pijnenburg et al., 1976; Campbell et al., 1997) and is inhibited by intra-accumbens administration of DA antagonists (Pijnenburg et al., 1975; Roberts et al., 1975; Phillips et al., 1994) or 6-hydroxydopamine (6-OHDA) (Kelly et al., 1975; Joyce et al., 1983; Clarke et al., 1988). Similarly, the rewarding effects of AMPH are either mimicked or inhibited by the same types of manipulations (Yokel and Wise, 1976; Lyness et al., 1979; Spyraki et al., 1982; Carr and White, 1991; Phillips et al., 1994; Izzo et al., 2001).

The NAcc is a heterogeneous structure, as evinced by immunohistochemical staining and neuronal projection patterns (Zahm and Brog, 1992). The major subdivisions are a medioventral shell and a dorsolateral core. These subregions are functionally distinct (Maldonado-Irizarry and Kelley, 1995; Weiner et al., 1996; Kelley et al., 1997; Parkinson et al., 1999; Boye et al., 2001; Ikemoto, 2002), but their precise roles in reward and locomotor activation are uncertain. For example, the locomotor stimulant effect of AMPH has been attributed to an action in the core (Weiner et al., 1996; West et al., 1999; Boye et al., 2001) or in medial shell (Heidbreder and Feldon, 1998; Parkinson et al., 1999) or in both structures (Pierce and Kalivas, 1995; Ikemoto, 2002). In contrast, certain dopaminergic drugs have been shown to maintain responding when infused into (medial) shell but not core (Carlezon and Wise, 1996; Ikemoto et al., 1997). Intra-shell self-administration of AMPH has also been reported (Hoebel et al., 1983; Chevrette et al., 2002), but in these studies intra-core infusions were not examined.

A feature of almost all the behavioral studies using AMPH was that the drug was given directly into the NAcc; after intracranial administration, drug distribution and local concentration differ markedly from that achieved after systemic administration. Recently, we combined systemic AMPH administration with

Received March 19, 2003; revised April 23, 2003; accepted May 15, 2003.

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Institutes of Health Research. L.H.L.S holds an NSERC Postgraduate Award, and P.B.S.C. is a Chercheur National of the Fonds de la Recherche en Santé du Québec. We thank Dr. Paul Vezina for advice on conditioned tactile preference, Lindsey McQuade and Robert Biskin for help assembling the test apparatus, Rebecca J. Grant for discussions regarding lesion parameters, and Dr. Sandra M. Boye for comments on this manuscript and help producing figures.

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6-OHDA lesions and found that locomotor stimulation was blunted by dopaminergic denervation of core and not medial shell (Boye et al., 2001).

The present study aimed to establish the relative involvement of NAcc core and medial shell subregions in systemic AMPH-induced behavioral activation and reward. Rats that had sustained 6-OHDA lesions of NAcc core or medial shell were assessed for AMPH-induced locomotor activation and conditioned place preference (CPP). To assess the possibility that decreased CPP indicated a deficit not in reward but in learning, memory, or sensory function, morphine CPP was also tested.

Materials and Methods

Subjects

Subjects were 142 male Long–Evans rats (Charles River, St. Constant, Quebec) weighing 270–310 gm at time of surgery. Rats were housed in groups of three in clear Plexiglas cages in a temperature- and humidity-controlled animal colony that was lit from 7 A.M. to 7 P.M. Food and water were available *ad libitum* except during behavioral testing. All experiments were approved by the McGill Faculty of Medicine Animal Care Committee in accordance with Canadian Council on Animal Care guidelines.

Stereotaxic infusion of 6-OHDA

Rats were anesthetized with ketamine HCl (90 mg/kg, i.p.) and xylazine HCl (16 mg/kg, i.p.) 15 min after pretreatment with atropine methyl nitrate (0.05 mg/kg, s.c.). The rat was placed in a stereotaxic apparatus (Kopf, Tujunga, CA) with the incisor bar set at −3.9 mm. Rats received bilateral infusions of either 6-OHDA or vehicle into either NAcc core or medial shell. Infusions were made via a 30 gauge stainless steel cannula attached by polyethylene tubing to a 10 μl Hamilton syringe driven by a model 5000 Micro Injection Unit (Kopf). For greater accuracy, coordinates for both the core and the medial shell were derived from the mean of two coordinate systems. Thus, anterior-posterior coordinates were +10.3 mm from interaural zero and +1.3 mm from bregma for both core and shell. Lateral coordinates were ±0.6 mm (shell) and ±2.4 mm (core). Ventral coordinates for shell (three injections) were +2.0, +2.4, and +2.8 mm from interaural zero and −8.0, −7.6, and −7.2 mm from bregma. Ventral coordinates for core were +2.9 mm from interaural zero and −7.1 mm from bregma. All coordinates are based on the atlas of Paxinos and Watson (1997). 6-OHDA or vehicle was infused on each side in a volume of 0.1 μl (core) or as three infusions of 0.06 μl each (shell) at a rate of 0.1 μl/min. The concentration of 6-OHDA used was 80 μg/μl (core) or 48 μg/μl (shell). The cannula remained at the final infusion site for 5 min. Dipyrone (100 mg/kg, s.c.) provided analgesia after surgery. Animals were allowed 7–11 d recovery before conditioning (experiments 1 and 3) or testing (experiment 2). Four animals died after surgery in experiment 3.

Conditioned place preference testing

General procedure. The method was modified from that of Vezina and Stewart (1987). Eight CPP cages [58.1 cm (length) × 28.8 cm (width) × 53.0 cm (height)] were used, each comprising four outer walls made of white plastic-coated particle board (Melamine) and an open top. Cages sat on linoleum flooring covered with a thin layer of Beta Chip bedding. There was no wall dividing the cage into two compartments. Two removable square floor tiles [28.5 cm (length) × 28.5 cm (width) × 5.5 cm (height)] were inserted into each cage; these served as tactile cues. Floor tiles were of two types: mesh and bar. These two textures were provided, respectively, by a stainless steel grid with squares of 1 × 1 cm and by 12 stainless steel bars of 1.2 cm diameter separated by 1.5 cm edge to edge. Both floor types were mounted on square Melamine frames. All behavioral testing was performed in a room lit with a Kodak GBX-2 safelight filter (Vistek, Toronto, Ontario, Canada) providing far-red illumination (wavelength >650 nm) to minimize visual cues. The location and movements of rats during behavioral testing were monitored by a closed circuit television video camera (Panasonic) linked to a commercial tracking system (EthoVision v3.0, Noldus Information Technology, Leesburg, VA).

Behavioral testing took place over 8 consecutive days and consisted of three phases: preexposure, conditioning, and testing. During all three phases, animals were habituated to the test room in home cages for 15 min before placement into test CPP cages. The preexposure phase served to habituate each animal to the CPP cage itself. This phase comprised a single 20 min session performed in the absence of floor tiles. The conditioning phase took place on days 2–7. It comprised six daily sessions of 45 min each: three sessions with drug and three sessions with saline administration. Drug and saline were administered on alternating days. After injection, each rat was immediately placed in the middle of a CPP cage. During the conditioning trials, rats had access to the entire cage, which provided a single tactile floor cue (either two mesh tiles or two bar tiles). On the day immediately after the final conditioning trial, a single 10 min test session was given. Here, the CPP cages contained one bar tile and one mesh tile. Animals in a drug-free state were placed in the middle of the cage and given free choice between the half of their cage with the bar texture and that with the mesh texture. Before a new test or conditioning session was started, half of the soiled Beta Chip was removed and replaced with new bedding, and the cage walls and tiles were wiped with 40% ethanol and allowed to dry. Groups of animals were counterbalanced as fully as possible, not only with respect to the texture that was paired with drug but also with respect to the position of that texture within the test cage on test day and the order of drug versus saline administration during conditioning.

On the test day, the time spent on each side of the apparatus was recorded. The location of a rat was defined as its center, as determined by the tracking system. During conditioning trials, locomotor activity was recorded as total horizontal distance moved. All testing was done between 8:30 A.M. and 5:30 P.M. A pilot study in which rats received saline paired with both floor textures showed that rats had no significant preference for either texture on test day (our unpublished observations). Thus the procedure can be considered unbiased.

Experimental procedures. In experiment 1, rats received bilateral infusion of 6-OHDA or vehicle into either core or medial shell 7–11 d before preexposure. Rats were then conditioned with 0.75 mg/kg AMPH intraperitoneally. In experiment 2, rats received bilateral 6-OHDA or vehicle infusions into medial shell. Half of the rats in each surgery group received 0.75 mg/kg AMPH intraperitoneally; the other half were conditioned with 10 mg/kg morphine intraperitoneally. Experiment 3 is similar in design to experiment 1 except that rats underwent stereotaxic infusion surgery after conditioning but before testing (Fig. 1).

Quantitative [^{125}I]RTI-55 autoradiography

The extent of the 6-OHDA lesion was quantified by autoradiographic labeling of the plasmalemmal DA transporter (DAT) using a nonsaturating concentration of [^{125}I]RTI-55 (2200 Ci/mmol; NEN-Mandel, Guelph, Ontario), because it has been shown previously that percentage loss of DAT accurately represents tissue DA loss (Joyce, 1991a,b). [^{125}I]RTI-55 binds selectively to DAT provided the serotonin transporter (SERT) is inhibited (Boja et al., 1992; Coulter et al., 1995). Conversely, SERT can be selectively labeled via occlusion of DAT (Pradhan et al., 2002). The day after CPP testing, rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and decapitated. Brains were removed rapidly and frozen in 2-methylbutane at −50°C for 30 sec and stored at −40°C. Coronal sections (20 μm) were taken on a cryostat at four rostrocaudal levels through the nucleus accumbens: +11.2, +10.7, +10.2, and + 9.7 mm anterior to interaural zero (Paxinos and Watson, 1997). At each level, five adjacent sections were collected: four for autoradiography and one for Nissl staining with cresyl violet. Sections were thaw mounted onto gelatin-subbed slides, air dried at room temperature for 20–30 min, and stored with desiccant at −40°C.

Sections were thawed at room temperature for 10 min and then placed in a staining dish containing an aqueous buffer solution of 120 mM NaCl, 0.1 M sucrose, 10 mM sodium phosphate buffer, and 10 pM [^{125}I]RTI-55. To assay for DAT binding, 50 nM citalopram hydrobromide was used to occlude SERT; nonspecific binding was determined by addition of 10 μM GBR 12909. To measure SERT binding, 1 μM GBR 12935·2HCl was added to occlude DAT; nonspecific binding was determined by addition of 50 nM citalopram HBr (Pradhan et al., 2002). Slides were incubated at room temperature for 2 hr and then washed three times in cold buffer

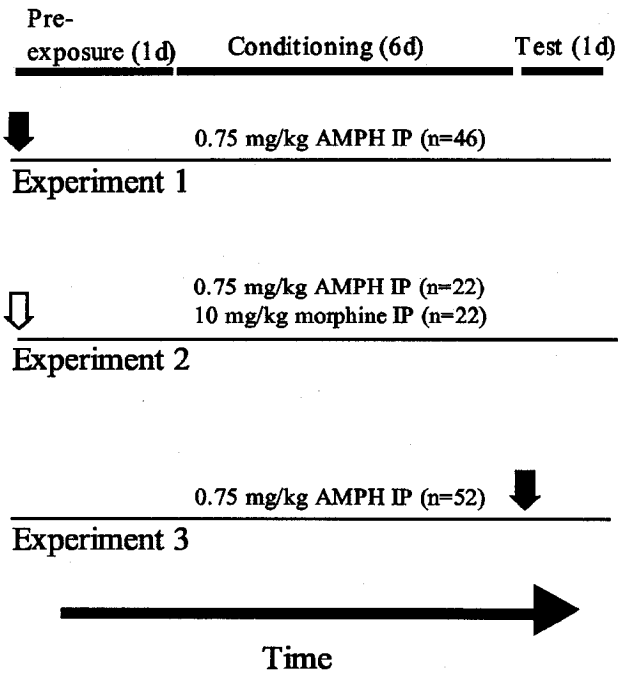

Figure 1. Experimental design of experiments 1, 2 and 3. Vehicle or 6-OHDA infusions were given at the time indicated by the arrows. In experiments 1 and 3, rats received infusions into either core or medial shell, depending on group (filled arrows). In experiment 2, only medial shell was targeted (white arrow). During the conditioning phase, each rat received saline and a drug (AMPH or morphine, dose as indicated) on alternating days (see Materials and Methods). IP, Intraperitoneal.

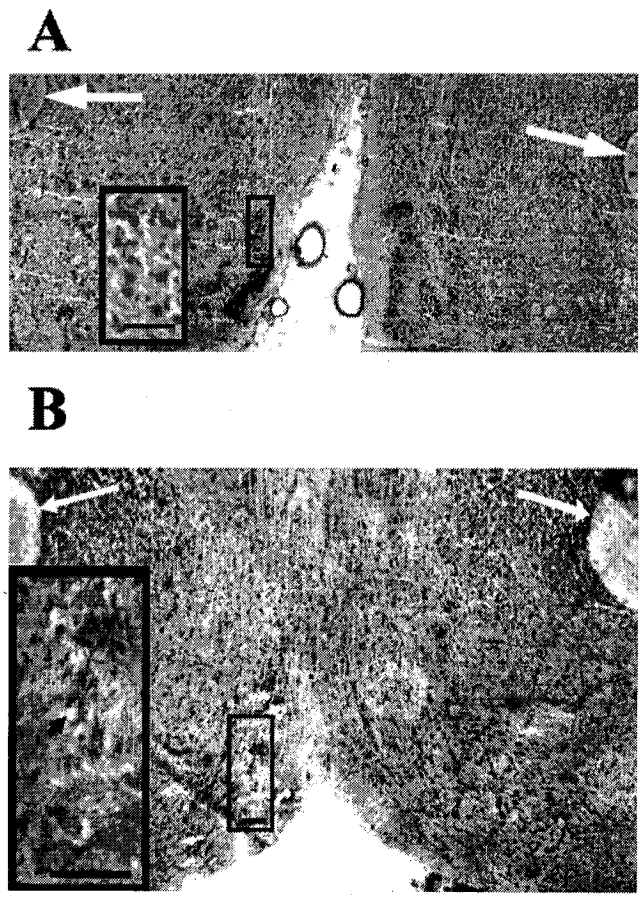

Figure 2. Histological changes associated with infusion of vehicle (*A*) or 6-OHDA (*B*) into the medial shell region of the NAcc. Representative 20 μm Nissl-stained sections are shown ~0.1 mm caudal to the site of injection (10.2 mm anterior to interaural zero). 6-OHDA infusion resulted in disruption of normal tissue morphology local to the infusion site (*B*, black arrow). Much less disruption of normal tissue morphology occurred in rats infused with vehicle. Scale bars, 50 μm. Anterior commissure is indicated by white arrows.

solution (once for 1 min, twice for 20 min) and for 1 sec in distilled and deionized water. They were then blow dried and placed in an x-ray film cassette. Kodak BioMax MS film (Amersham Biosciences, Baie d'Urfé, Québec) was exposed to slides for 48 hr (DAT) or 120 hr (SERT) with [^{125}I] autoradiographic standards (Amersham Biosciences). Films were then processed with Kodak D19 developer and Kodak GBX fixer (Amersham Biosciences). DAT and SERT binding were quantified using an MCID M4 imaging system (Imaging Research, St. Catherines, Ontario).

Histological examination

Tissue was stained with cresyl violet to assess nonspecific damage, as follows. Sections were thawed at room temperature for 10 min and then placed in 0.5% cresyl violet (Sigma-Aldrich, Oakville, Ontario) in distilled water for 20 min. They were rinsed in 95% ethanol twice for 2 min and then in 100% ethanol three times for 15 sec and were dehydrated in xylene three times for 5 min. Slides were coverslipped with Permount and examined under a light microscope (40–200× magnification).

Drugs

Drug sources were as follows: morphine sulfate (gift from Sabex 2002 Inc., Boucherville, Quebec); D-amphetamine sulfate (Bureau of Drug Research, Ottawa, Ontario); citalopram HBr (gift from H. Lundbeck A/S); dipyrone (Vetoquinol, Quebec, Quebec); ketamine HCl (Vetalar, Vetrepharm, London, Ontario); xylazine HCl (Anased, Novopharm, Toronto, Ontario); atropine methyl nitrate, 6-OHDA HBr, GBR 12909, and GBR 12935·2HCl (Sigma-Aldrich, Oakville, Ontario). All other chemicals were obtained from Fisher Scientific (Montreal, Quebec).

Morphine sulfate and D-amphetamine sulfate were dissolved in sterile 0.9% saline and injected at 1 ml/kg. 6-OHDA HBr was dissolved in sterile 0.9% saline containing 0.3 mg/ml sodium metabisulfite (Sigma-Aldrich) as an antioxidant and protected from light. Both 6-OHDA and vehicle solutions were made to pH 7.3 ± 0.1 with NaOH. Doses of all drugs except 6-OHDA HBr are expressed as the salt. 6-OHDA HBr doses are expressed as free base.

Data analysis

A commercial software program (Systat v10.2, SPSS Inc., Chicago, IL) was used for all data analyses. Locomotor response to AMPH was calculated as the difference of locomotor counts between AMPH and saline conditioning sessions; baseline saline scores were calculated as the mean activity over all three conditioning sessions with saline on test day. CPP magnitude was calculated as the difference between time spent on the drug-paired and vehicle-paired sides. The relationship between locomotor and reward measures versus [^{125}I]-RTI-55 labeling was analyzed by multiple linear regression (experiments 1 and 3) or Mann–Whitney *U* test (experiment 2). Activity scores (experiment 1) were analyzed by ANOVA. A p value of <0.05 (two-tailed) was considered significant.

Results

Histological and autoradiographic characterization of lesions

Minimal neuronal loss was evident at the site of injection in both vehicle groups and in the core lesioned group in all three experiments. A representative coronal section of the medial shell vehicle-infused group is shown in Figure 2*A*. In the medial shell lesioned group, tissue damage was more extensive but was nevertheless confined to 0.3 mm from the infusion site, sparing most of the structure (Fig. 2*B*).

[^{125}I]RTI-55 autoradiographs of DAT binding are shown in Figure 3 at four anterior-posterior levels. Sampling locations for DAT binding density are indicated in Figure 4. Absolute values

for [^{125}I]RTI-55 binding to DAT and SERT are given in Tables 1 and 2. In all experiments, core lesions were less anatomically selective than shell lesions (Fig. 5). Pooled across experiments, core 6-OHDA animals showed a mean decrease in DAT binding of 68% in core, 29% in medial shell, 30% in ventral shell, 37% in ventral caudate-putamen, and 30% in olfactory tubercle (OT). In contrast, medial shell-infused 6-OHDA reduced DAT binding in medial shell by 62%, but only by 13, 7, 1, and 12% in core, ventral shell, ventral caudate-putamen, and OT, respectively. SERT binding was virtually unchanged (89–111% of control) by the 6-OHDA lesions in all three experiments (Tables 1, 2).

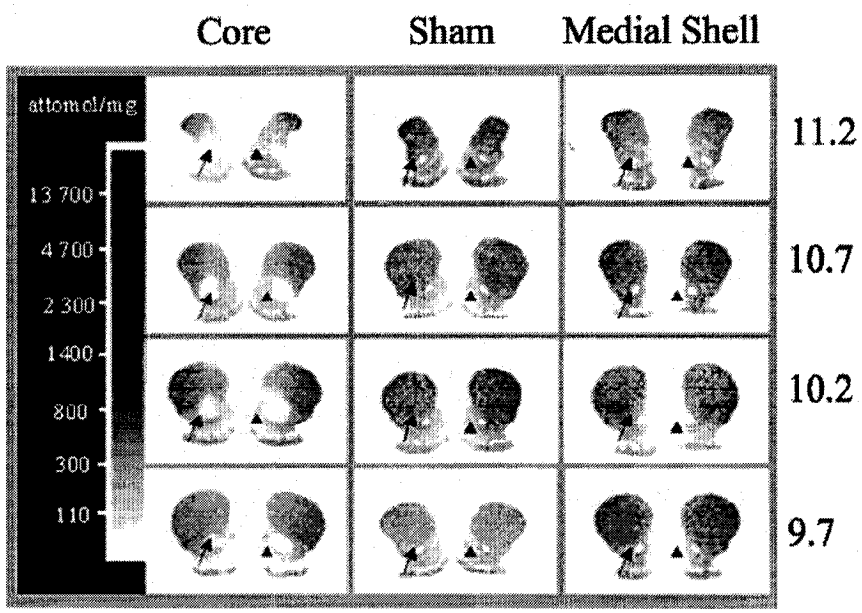

Figure 3. Autoradiographic images of [^{125}I]RTI-55 binding to DAT in animals from core-lesioned, medial shell-lesioned, and sham-operated groups (experiment 3). Because binding was similar between groups that received vehicle in core and medial shell, the latter group has been omitted. Numbers designate distance anterior to interaural zero (in millimeters). Radioligand binding was obtained at a nonsaturating concentration of radioligand and is expressed as attomol per milligram of tissue. Arrows refer to the core subregion. Arrowheads (pointing upward) refer to the medial shell subregion. In most rats, core 6-OHDA lesions were less anatomically selective than shown here (see Fig. 5).

NAcc core and medial shell lesions before conditioning inhibited AMPH-mediated locomotor activation and CPP, respectively

In experiment 1, lesions were performed before drug conditioning. Overall, the AMPH locomotor stimulant effect differed across successive conditioning sessions (SESSION: $F_{(2,84)} = 4.47$, $p < 0.02$; mean ± SEM; AMPH-saline difference score 40 ± 5, 63 ± 6, and 53 ± 8 m). However, locomotor data were pooled across sessions, because an initial three-way ANOVA revealed no significant interactions between SESSION and either AREA or LESION ($F_{(2,84)} < 1.31$, $p > 0.2$). Saline session locomotor scores did not differ significantly between surgery groups (AREA: $F_{(1,42)} = 1.01$, $p > 0.25$; LESION: $F_{(1,42)} = 0.70$, $p > 0.25$; AREA × LESION: $F_{(1,42)} = 0.95$, $p > 0.25$) (Fig. 6, legend). Because lesions were not anatomically specific (Fig. 4), multiple linear regression analysis was performed to assess contributions of core and shell DA innervation to the AMPH-induced locomotor response. Figure 6, *A* and *B*, shows the relationship between locomotor responses to AMPH during conditioning versus DAT binding in core and medial shell. The locomotor stimulant response was significantly correlated with DAT binding in NAcc core ($p < 0.01$) but not NAcc medial shell ($p > 0.25$) (Fig. 6*A*,*B*). Conversely, the magnitude of AMPH CPP was significantly correlated with residual DAT in the medial shell ($p < 0.0001$) but not in the core ($p > 0.5$) (Fig. 6*C*,*D*).

NAcc medial shell lesions did not prevent acquisition of a CPP for morphine

In experiment 2, the effects of preconditioning lesions of medial shell were tested in rats conditioned with either morphine (10 mg/kg, i.p.) or AMPH (0.75 mg/kg, i.p.). As in experiment 1, AMPH CPP magnitude was reduced or abolished by medial shell 6-OHDA infusion (lesion vs sham: Mann–Whitney $U = 90$; $p < 0.02$) (Fig. 7). In contrast, lesioned rats did acquire a morphine CPP, and this was of similar magnitude to that of sham controls (lesion vs sham: Mann–Whitney $U = 63$; $p > 0.5$) (Fig. 7).

Expression of a conditioned place preference for AMPH was abolished by NAcc medial shell, but not NAcc core, lesions

In experiment 3, lesions were performed after conditioning but before testing. Figure 8, *A* and *B*, shows the relationship between DAT binding in NAcc core or medial shell and the CPP magnitude. Two extreme outliers, as defined by the Systat software, were excluded before data analysis. Multiple linear regression analysis showed that CPP magnitude correlated significantly with residual DAT binding in NAcc medial shell ($p < 0.0005$) but not in NAcc core ($p > 0.25$).

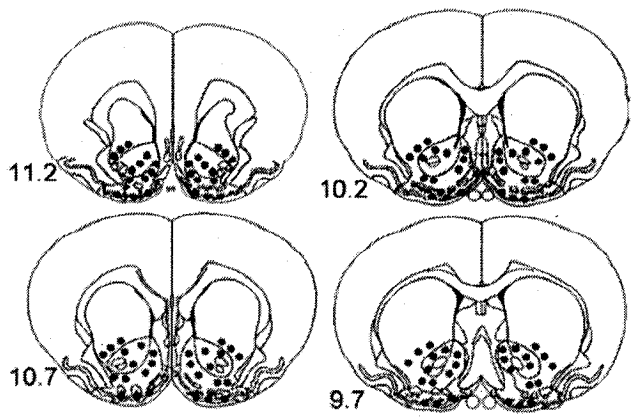

Figure 4. Locations of sampled [^{125}I]RTI-55 binding in nucleus accumbens core, medial shell, ventral shell, ventral caudate-putamen, and olfactory tubercle. Each rat was sampled at four anterior-posterior levels. Numbers are distances (in millimeters) anterior to interaural zero. Sampling areas were circles of 0.3 mm diameter. Three samples per side per structure were taken at each level, except for ventral shell, where one sample per side was taken at level 11.2 and two per side at all other levels. Adapted from Paxinos and Watson (1997).

Discussion

Methodological aspects

Dopaminergic denervation in core or medial shell has rarely been achieved with any anatomical selectivity (Boye et al., 2001). The present study incorporated several methodological improve-

Table 1. Absolute values of DAT and SERT binding in core, medial shell, ventral shell, ventral caudate-putamen (ventral CP), and olfactory tubercle (OT) in experiments 1 and 3

	Core (vehicle)	Core (6-OHDA)	Shell (vehicle)	Shell (6-OHDA)
Experiment 1				
n	10	12	10	14
DAT				
Core	265 ± 20	87 ± 9	261 ± 30	209 ± 20
Medial shell	183 ± 7	128 ± 13	178 ± 18	70 ± 20
Ventral shell	170 ± 8	129 ± 17	158 ± 15	166 ± 16
Ventral CP	152 ± 9	95 ± 11	129 ± 11	137 ± 7
OT	259 ± 16	177 ± 18	282 ± 24	243 ± 16
SERT				
Core	117 ± 12	107 ± 14	117 ± 8	113 ± 7
Medial shell	145 ± 14	149 ± 14	143 ± 6	137 ± 10
Ventral shell	151 ± 17	167 ± 16	136 ± 14	147 ± 15
Ventral CP	152 ± 17	165 ± 16	141 ± 17	143 ± 12
OT	219 ± 9	230 ± 11	242 ± 13	232 ± 9
Experiment 3				
n	10	13	10	19
DAT				
Core	927 ± 43	288 ± 60	941 ± 32	816 ± 61
Medial shell	566 ± 45	411 ± 41	596 ± 56	225 ± 26
Ventral shell	774 ± 38	493 ± 64	787 ± 40	761 ± 40
Ventral CP	641 ± 31	404 ± 35	684 ± 19	660 ± 30
OT	733 ± 36	524 ± 45	743 ± 53	635 ± 36
SERT				
Core	297 ± 10	269 ± 16	310 ± 16	318 ± 14
Medial shell	450 ± 15	441 ± 16	450 ± 23	446 ± 14
Ventral shell	476 ± 27	486 ± 23	497 ± 19	504 ± 20
Ventral CP	300 ± 13	267 ± 10	305 ± 12	321 ± 12
OT	637 ± 25	624 ± 35	637 ± 24	664 ± 26

Values are mean ± SEM [^{125}I]RTI-55 binding to DAT or SERT (expressed as attomol per milligram of tissue), obtained at a subsaturating concentration of radioligand.

Table 2. Absolute values of DAT and SERT binding in core, medial shell, ventral shell, ventral caudate-putamen (ventral CP), and olfactory tubercle (OT) in experiment 2

	Morphine		Amphetamine	
	Shell (vehicle)	Shell (6-OHDA)	Shell (vehicle)	Shell (6-OHDA)
Experiment 2				
n	10	12	10	12
DAT				
Core	755 ± 22	715 ± 20	760 ± 25	674 ± 20
Medial shell	473 ± 17	166 ± 21	407 ± 53	157 ± 17
Ventral shell	429 ± 12	382 ± 14	437 ± 21	364 ± 15
Ventral CP	535 ± 18	525 ± 9	517 ± 21	501 ± 16
OT	670 ± 18	612 ± 32	695 ± 43	618 ± 34
SERT				
Core	414 ± 14	408 ± 14	385 ± 9	382 ± 15
Medial shell	517 ± 14	530 ± 19	510 ± 12	501 ± 23
Ventral shell	481 ± 43	534 ± 50	533 ± 55	474 ± 41
Ventral CP	327 ± 31	343 ± 29	354 ± 40	322 ± 26
OT	737 ± 24	688 ± 18	672 ± 19	672 ± 24

Values are mean ± SEM [^{125}I]RTI-55 binding to DAT or SERT (expressed as attomol per milligram of tissue), obtained at a subsaturating concentration of radioligand.

ments. First, stereotaxic lesion coordinates were improved. Second, multiple infusion sites were used for medial shell lesions. Third, diffusion of 6-OHDA from the infusion site was minimized by administering a high concentration in a small volume. Consequently, core and medial shell DAT binding were largely independent (Pearson $r = 0.30$), which was not the case in our previous study (Pearson $r = 0.84$) (Boye et al., 2001). In addition, nonspecific tissue damage was reduced by neutralizing the 6-OHDA solution before infusion. Thus, despite the unusually high concentration of 6-OHDA used, Nissl staining and SERT autoradiography revealed minimal nonspecific damage.

The present CPP procedure possesses several attractive features. First, latent inhibition can be avoided during the initial preexposure phase by omitting the conditioned stimuli. Second, rats conditioned with saline on both textures showed no significant preference for either texture on test day (our unpublished observations). Hence, our procedure is balanced and avoids the interpretational difficulties inherent in "biased" procedures (Bardo and Bevins, 2000). The current study is the first to show an AMPH CPP using solely tactile cues.

Mechanisms of amphetamine-induced locomotor activation

The present findings suggest that after systemic AMPH administration, locomotor stimulation is dependent on transmission in NAcc core and not medial shell. To date, only three published studies have examined this question using systemic rather than

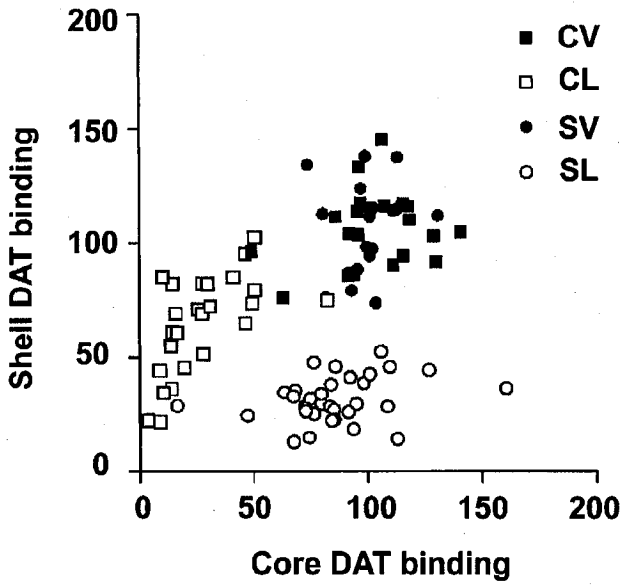

Figure 5. Relationship of DAT labeling in nucleus accumbens core versus medial shell. Data are pooled from experiments 1 and 3 ($n = 98$ rats). DAT labeling was performed by [^{125}I]RTI-55 autoradiography and expressed as a percentage of the mean value of the core-vehicle group for core 6-OHDA animals, or the shell-vehicle group for the shell 6-OHDA group. Correlational analysis revealed a weak but significant relationship between core and medial shell binding ($r = 0.30$; $p < 0.005$). CV, Core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion.

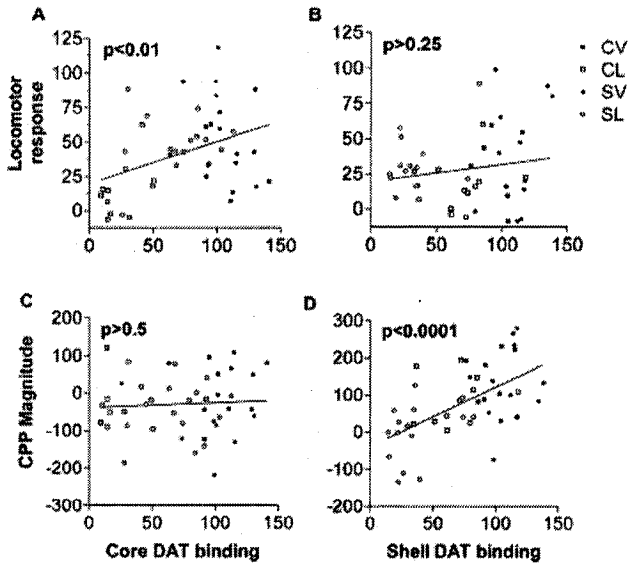

Figure 6. Effect of bilateral 6-OHDA infusion into either NAcc core or medial shell on AMPH-induced locomotor response and CPP (experiment 1). Rats ($n = 10–14$ per group) were allowed 7–11 d recovery after stereotaxic surgery before conditioning with AMPH (0.75 mg/kg, i.p.). Locomotor responses are expressed for each rat as the difference between the mean distance moved (in meters) during conditioning sessions with AMPH versus with saline. CPP magnitude is the difference between time spent on the drug-paired and saline-paired textures during the 600 sec test. DAT labeling in core or medial shell is expressed as percentage DAT binding of sham-lesioned groups. Saline locomotor scores, in meters, were 134 ± 7 in the core vehicle group, 152 ± 11 in the core 6-OHDA group, 154 ± 10 in the shell vehicle group, and 153 ± 11 in the shell 6-OHDA group. Locomotor responses (AMPH-saline) correlated significantly with DAT binding in NAcc core but not in NAcc medial shell. Conversely, CPP magnitude correlated significantly with DAT binding in medial shell but not core. To visualize the association of each drug response to core or medial shell [^{125}I]RTI-55 labeling, the predicted contribution of the irrelevant brain structure was subtracted from the *y*-axis variables using the calculated multiple linear regression equation. Significant linear associations (shown by *p* values) are evident in *A* and *D*. CV, Core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion.

intracranial AMPH (Weiner et al., 1996; Parkinson et al., 1999; Boye et al., 2001). Two of these studies showed that core rather than shell lesions reduced AMPH-induced locomotor activation (Weiner et al., 1996; Boye et al., 2001). In contrast, Parkinson et al. (1999) reported that excitotoxic lesions of the NAcc core enhanced locomotor stimulant responses to systemic AMPH, whereas medial shell lesions had the opposite effect. On this basis, these authors attributed a critical role to the shell; however, in the latter study, shell lesions attenuated AMPH locomotion to only a modest extent, and core lesions increased baseline locomotion, complicating the interpretation of drug effects. On balance, therefore, the available evidence suggests that NAcc core plays an important role in the locomotor stimulant effect of systemically administered AMPH.

In the present study, medial shell DA innervation was not related to AMPH locomotor stimulation. In contrast, we previously observed a significant negative correlation ($p < 0.02$), such that DA denervation in the medial shell was associated with greater locomotor responses (Boye et al., 2001). This discrepancy may reflect differences in lesions coordinates or functional gradients within each NAcc subregion (Essman et al., 1993; Campbell et al., 1997).

Other striatal regions, notably ventromedial striatum (Dickson et al., 1994), OT (Cools, 1986; Ikemoto, 2002), and anteromedial caudate (Fink and Smith, 1979, 1980), have also been implicated in AMPH-induced locomotion. It is doubtful that denervation of ventromedial striatum played a significant role in the present study, because lesions were restricted to the anterior portion, which appears not to contribute to AMPH locomotor activation (Dickson et al., 1994). On the basis of intracranial infusion studies (Cools, 1986; Ikemoto, 2002), the OT has been proposed as a key structure mediating the locomotor stimulant effects of AMPH. In contrast, locomotor stimulation after systemic AMPH administration was unaffected by 6-OHDA lesions of OT, despite substantial loss of tissue DA (Clarke et al., 1988). The anteromedial caudate has been proposed to mediate AMPH-induced locomotion (Fink and Smith, 1979), but this area was probably spared by our lesions. The ventral shell subregion was partially depleted by our core 6-OHDA infusions and, to our knowledge, has not been studied with respect to AMPH locomotion.

Our 6-OHDA infusions almost certainly destroyed noradrenaline (NA) as well as DA terminals in the ventral striatum (Robbins et al., 1983). Disruption of noradrenergic transmission tends to inhibit AMPH-induced locomotion (Ogren et al., 1983; Archer et al., 1986; Dickinson et al., 1988; Blanc et al., 1994; Darracq et al., 1998; Harro et al., 2000; Drouin et al., 2002a,b; Auclair et al., 2002) [but see Ventura et al. (2003)], with the medial prefrontal cortex identified as a potential site of action (Blanc et al., 1994; Darracq et al., 1998). In contrast, noradrenergic transmission in the NAcc appears not to contribute directly to locomotor stimulation (Pijnenburg et al., 1975; Roberts et al., 1975; Kelly and Iversen, 1976; Joyce et al., 1983). Thus, our 6-OHDA lesion effects on AMPH-induced locomotor activation probably reflect decreased DA rather than NA transmission.

Mechanisms of AMPH-induced reward

Considerable evidence suggests that AMPH exerts its rewarding effects via DA release in the NAcc (Di Chiara, 1995; Koob et al.,

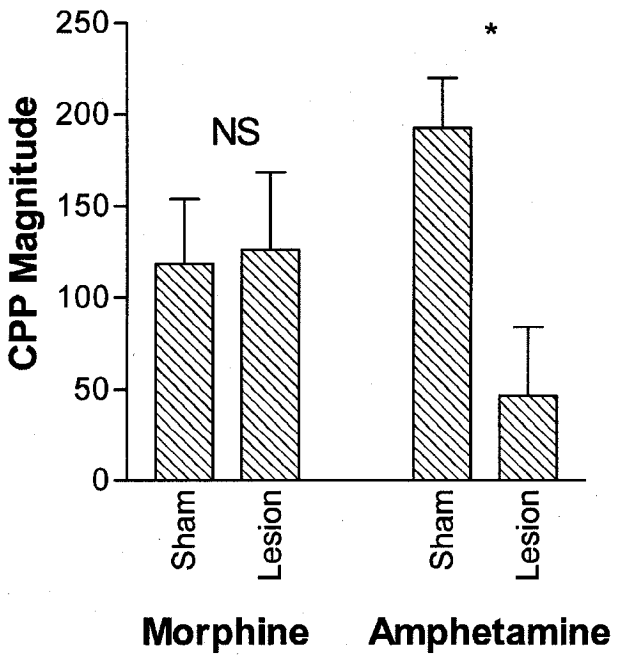

Figure 7. Effect of 6-OHDA lesions of NAcc medial shell on morphine and AMPH CPP (experiment 2). Stereotaxic surgery was performed 7–11 d before the first conditioning day. CPP magnitudes (mean ± SEM) for morphine (10 mg/kg, i.p.) or AMPH (0.75 mg/kg, i.p.) were calculated as the difference between the time spent on the drug-paired and saline-paired sides ($n = 10–12$ rats per group). Because the data were not normally distributed, Mann–Whitney U tests were applied to predetermined comparisons. NS, Nonsignificant; $*p < 0.02$ versus corresponding sham-lesioned group (unprotected tests).

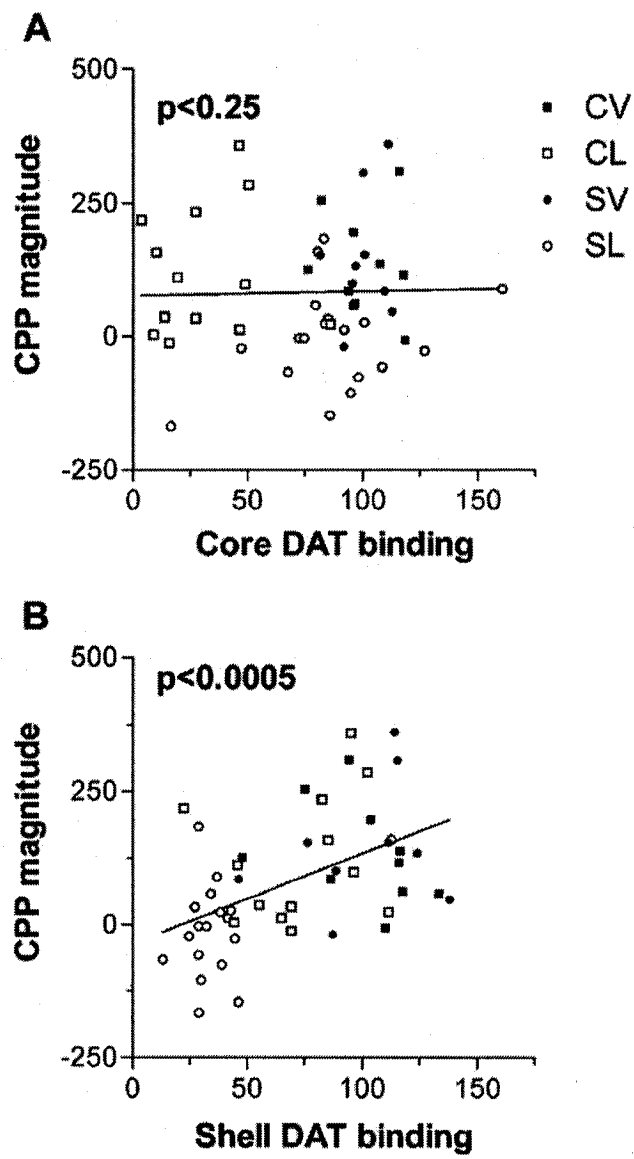

Figure 8. Effect of NAcc core and medial shell lesions on the expression of AMPH CPP (experiment 3). Rats ($n = 10–19$ per group) received bilateral infusion of either 6-OHDA or vehicle into either NAcc core or medial shell after conditioning with AMPH and before CPP testing. Degree of DAT depletion in core or medial shell is expressed as percentage DAT binding of control. To visualize the association of each drug response to core or medial shell [^{125}I]RTI-55 labeling, the predicted contribution of the irrelevant brain structure was subtracted from the y-axis variables using the calculated multiple linear regression equation. CPP magnitude correlated significantly with DAT binding in NAcc medial shell (*B*) but not with DAT binding in NAcc core (*A*). CV, Core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion.

1998), with little if any contribution from NA in this structure (Yokel and Wise, 1975; Roberts et al., 1977). In the present study, medial shell DA denervation was associated with attenuated AMPH CPP. It is unlikely that neighboring structures contributed to this effect, because they were only slightly denervated (Tables 1, 2). Moreover, substantial 6-OHDA lesions of OT did not alter a CPP for systemic AMPH (Clarke et al., 1990). Our findings therefore support a role for NAcc medial shell DA in the rewarding effect of AMPH. This conclusion accords with intracranial self-administration studies using other dopaminergic drugs (Carlezon and Wise, 1996; Ikemoto et al., 1997).

The inhibition of AMPH CPP caused by preconditioning 6-OHDA lesions could reflect impaired acquisition or expression, or both. It is well established that acquisition and expression of CPP are mediated by different dopaminergic mechanisms (Hiroi and White, 1990, 1991a,b; Acquas and Di Chiara, 1994; Bardo et al., 1999). For example, DA antagonist studies show that DA D1 and D2 receptors are required for acquisition, but only DA D1 receptors are required for expression (Hiroi and White, 1991a,b; Acquas and Di Chiara, 1994; Bardo et al., 1999). Because our 6-OHDA lesions presumably impaired transmission at both DA receptor types, both acquisition and expression are likely to be affected.

In the present study, morphine served as a positive control. The finding that morphine CPP was unaffected by medial shell lesions (experiment 2) suggests that lesion-induced reduction of AMPH CPP did not result from impaired sensory, motor, or mnemonic function. The present findings also accord with evidence that morphine CPP occurs via a DA-independent mechanism when drug exposure is minimized (Mackey and van der Kooy, 1985; Bechara and van der Kooy, 1992; Bechara et al., 1992; Nader and van der Kooy, 1997; Laviolette et al., 2002). In contrast, 6-OHDA lesions of the NAcc have been found to reduce opiate CPP in nondependent rats (Spyraki et al., 1983; Shippenberg et al., 1993). Several factors could account for this discrepancy. First, these authors denervated the entire NAcc. It is possible that the NAcc medial shell subregion is neither necessary nor sufficient to mediate opiate reward. Second, although our lesions eliminated AMPH CPP, they may not have decreased DA transmission sufficiently to affect morphine CPP. Third, it is possible that different neural mechanisms underlie morphine CPP depending on whether multiple sensory cues or solely tactile cues are used.

Dissociation of locomotion and reward

The current findings demonstrate a double dissociation in NAcc core versus shell with regard to AMPH-induced locomotor activation and reward. They extend evidence from other behavioral paradigms that also suggest that locomotion and reward are dissociable (Burns et al., 1993; Robledo et al., 1993; Kelley et al., 1997; Ventura et al., 2003). Burns et al. (1993) performed lesions of the ventral subiculum or basolateral amygdala and demonstrated a double dissociation of the locomotor stimulation and conditioned reinforcement produced by intra-NAcc AMPH. However, it is not clear whether the lesion affected reward processes or produced a memory or sensory deficit. Robledo et al. (1993) showed that neurotensin administered into the NAcc core decreased the locomotor stimulant effect of cocaine but did not affect intravenous self-administration of the drug. In this study, cocaine was given intraperitoneally in the locomotor tests, making interpretation difficult. Kelley et al. (1997) found that administration of an NMDA receptor antagonist into NAcc core, but not shell, disrupted the acquisition of food-reinforced responding without affecting spontaneous locomotor activity. However, this study did not examine the effects of psychostimulants. Last, Ventura et al. (2003) demonstrated that in mice, NA-depleting lesions of the medial prefrontal cortex blocked both AMPH-induced NAcc DA release and CPP while preserving the locomotor stimulant response. It would be interesting to determine whether core and medial shell DA release are differentially affected by these lesions and whether this result extends to rats.

In conclusion, the present study provides the first clear anatomical dissociation between the rewarding and locomotor-activating effects of the prototypic psychostimulant drug AMPH in rats. These acute behavioral effects were mapped onto NAcc medial shell and core, respectively. The experimental approach used here should help to further define mechanisms underlying acute and chronic behavioral effects of other drugs of abuse. Finally, the present core/shell dissociation may be relevant to the role of DA in reward anticipation versus consumption (Wise, 2002), incentive salience (Berridge and Robinson, 1998), and other forms of learning (Redgrave et al., 1999; Schultz, 2002).

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SYNAPSE 59:374–377 (2006)

Short Communication

6-Hydroxydopamine Lesions of Nucleus Accumbens Core Abolish Amphetamine-Induced Conditioned Activity

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KEY WORDS accumbens; core; shell; conditioned locomotion; amphetamine; 6-hydroxydopamine; striatum

ABSTRACT Environmental cues associated with drug experiences appear to play a critical role in drug dependence. We have previously reported that dopamine-depleting lesions of the nucleus accumbens medial shell inhibit amphetamine-conditioned place preference. Here, we examined the effects of analogous lesions on amphetamine-conditioned locomotor activity. Bilateral core, but not medial shell, lesions attenuated unconditioned locomotion and abolished the conditioned locomotor response. Taken with our previous results, these findings confirm a role for accumbens core in amphetamine-induced locomotor activity and suggest that the role of medial shell DA transmission in conditioned place preference is related to reward processing rather than conditioning in general. **Synapse 59:374–377, 2006.**

Rats receiving repeated amphetamine (AMPH) administration in a distinct environment subsequently exhibit hyperlocomotion in that environment in the absence of drug (Gold et al., 1988; Mazurski and Beninger, 1991). The nucleus accumbens appears critical, as 6-hydroxydopamine (6-OHDA) lesions prevented acquisition and expression of AMPH-induced conditioned locomotion (Gold et al., 1988). Recently, we provided 6-OHDA lesion evidence, suggesting that conditioned place preference for AMPH was associated with medial shell dopamine (DA) transmission, and that unconditioned locomotor activity was associated with core DA transmission (Sellings and Clarke, 2003). However, reward was assessed using a conditioned measure (conditioned place preference, Bardo and Bevins, 2000), whereas the measure of locomotor activation was unconditioned. As such, it is unclear whether the functional segregation we observed represents a dissociation between reward and locomotion, or between the conditioned and unconditioned effects of AMPH.

The aim of the current study was, therefore, to examine the effects of 6-OHDA lesions of medial shell vs. core on conditioned locomotion. This measure was chosen because the underlying neural processes appear distinct from those mediating CPP and unconditioned locomotor activity (Beninger and Hahn, 1983; Brown and Fibiger, 1993; Hemby et al., 1992; Mazurski and Beninger, 1991; Poncelet et al., 1987; Sutton et al., 2000). An association between medial shell DA transmission and conditioned locomotion, if observed, would suggest that this subregion may play a general role in mediating conditioned drug effects. Core lesions were expected to reduce the unconditioned locomotor response to AMPH (Sellings and Clarke, 2003), but it was not clear whether this would also prevent the emergence of a conditioned locomotor response (see Discussion). Two experiments were performed. The first established the occurrence of conditioned locomotion in our conditioned place preference apparatus. The second experiment tested behavioral effects of intra-accumbens 6-OHDA.

Subjects were 37 Long-Evans rats (Charles River, St-Constant, QC; 270–310 g at time of surgery). Food

Contract grant sponsors: Natural Sciences and Engineering Research Council of Canada (NSERC), Canadian Institutes of Health Research.

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Received 20 September 2005; Accepted 2 December 2005

DOI 10.1002/syn.20247

Published online in Wiley InterScience (www.interscience.wiley.com).

and water were available ad libitum except during training. All experiments were approved by the McGill Faculty of Medicine Animal Care Committee in accordance with Canadian Council on Animal Care guidelines.

The testing apparatus was as previously described for conditioned place preference (Sellings and Clarke, 2003). Briefly, test cages comprising four vertical walls forming a rectangle (58 cm (L) × 29 cm (W) × 53 cm (H)) were placed on linoleum flooring covered with a thin layer of sawdust. Two removable square floor tiles (mesh or bar) were inserted into each cage to provide tactile cues during conditioning sessions. Behavioral experimentation took place over 12 days. After an initial pre-exposure to the test box without tactile cues (day 1, 20 min), each rat received five AMPH injections (0.75 mg/kg as sulfate salt) on either bar or mesh, and five vehicle injections on the other texture over 10 consecutive days (day 2–11, 45 min/session). On test day, locomotor activity was examined (in a drug-free state) in two separate sessions, one on bar and one on mesh (day 12, 10 min each). The two test sessions were separated by 4 h. Locomotor activity during conditioning trials and the two test sessions was monitored by a commercial tracking system (EthoVision v3.0, Noldus IT). The order of drug presentation, drug-texture pairing, and order of cue presentation on test day (mesh vs. bar) were counterbalanced. As such, the conditioned stimulus (CS) was bar or mesh, depending on which texture was paired with the unconditioned conditioned stimulus (UCS) amphetamine. To minimize visual cues, the testing room was lit with a Kodak GBX-2 filter. In experiment 1, unoperated rats served as experimental subjects. In experiment 2, rats received bilateral infusions of 6-OHDA or vehicle (0.9% saline plus 0.3 mg/ml sodium metabisulfite) 7–9 days prior to conditioning. This was given via a stainless steel cannula of 0.30 mm o.d. (30 gauge) aimed at either core or medial shell, as previously described (Sellings and Clarke, 2003). The extent of the 6-OHDA lesion was quantified by autoradiographic labeling of the DA transporter (DAT) using the radioligand [^{125}I]RTI-55 (2200 Ci/mmol). To assess nonspecific damage, serotonin transporter (SERT) autoradiography using [^{125}I]RTI-55 with DAT occluded, as well as cresyl violet staining for Nissl substance (as previously described; Sellings and Clarke, 2003) was used.

A commercial software program (Systat v10.2, SPSS Inc.) was used for data analyses. The unconditioned locomotor response was calculated as the difference of locomotor counts between AMPH and saline conditioning sessions. The conditioned locomotor response was calculated as the difference between activity (distance moved in meters) on the AMPH and vehicle paired textures on test day. Group differences were examined by one-way ANOVA, followed by Dunnett's test. Multiple linear regression analysis was used to test for associations between DAT binding in core vs. medial shell and unconditioned or conditioned locomotion. The two sham lesioned groups (core and medial shell) were pooled, as initial examination revealed no significant differences between these two groups. $P < 0.05$ (two-tailed) was considered significant. Within-group variability is expressed as SEM throughout.

In experiment 1, rats ($n = 8$) expressed significant conditioned locomotion (paired t-test with Bonferroni correction, $P < 0.005$; Fig. 1A). The distance moved on the AMPH-paired texture was 36.7 ± 2.8 m; on the saline-paired texture, it was 33.0 ± 2.8 m. The magnitude of the conditioned locomotion was not dependent on the floor texture paired with drug (bar vs. mesh, Student's t-test, $P > 0.5$).

In experiment 2, no significant group differences existed for saline activity ($F(2,26) = 1.65$, $P > 0.2$; Fig. 1B) or unconditioned locomotor activation ($F(2,26) = 1.45$, $P > 0.25$; Fig. 1C). The mean distance moved during conditioning trials (i.e., unconditioned locomotion) was as follows. During saline sessions (Fig. 1B), it was 148.6 ± 8.5 m (sham), 166.6 ± 12.2 m (core), and 141.7 ± 8.9 m (medial shell); during AMPH sessions, it was 216.8 ± 14.6 m (sham), 209.3 ± 11.1 m (core), and 216.5 ± 9.7 m (medial shell). However, multiple linear regression analysis revealed a significant association between core, but not medial shell, DAT binding and locomotor activation (core: $P < 0.05$, $r = 0.37$; Fig. 1D; medial shell: $P > 0.25$, $r = 0.06$; Fig. 1E). For conditioned locomotion, only the core-lesioned group differed significantly from sham animals (Dunnett's test, $P < 0.02$; Fig. 1F). On test day, the distance moved on the AMPH-paired texture was 42.1 ± 3.5 m (sham), 36.5 ± 2.0 m (core) and 37.7 ± 2.9 m (medial shell); on the saline-paired texture, it was 34.2 ± 3.4 m (sham), 36.8 ± 3.6 m (core) and 32.4 ± 2.5 m (medial shell). Additionally, the magnitude of the conditioned locomotion associated positively with core and not medial shell DAT binding (core: $P < 0.05$, $r = 0.57$; medial shell: $P > 0.5$, $r = 0.32$; Figs. 1G and 1H). Residual DAT binding, expressed as a percent of combined sham groups, is given in Table I. Nissl staining revealed minimal nonspecific damage, and residual SERT binding was minimally affected by core and medial shell lesions (92–107% of control).

In the present study, DA-depleting lesions of the accumbens medial shell did not inhibit the conditioned locomotor response to AMPH. This finding contrasts with our previous observation that the same kind of lesion inhibited AMPH-conditioned place preference (Sellings and Clarke, 2003). Although it cannot be ruled out that a larger medial shell lesion may have resulted in reduced AMPH conditioned locomotion, it is important to note that the two studies were designed to be highly comparable. For example, the testing apparatus was identical and the dose of AMPH was the same. Additionally, [^{125}I]RTI-55 binding in medial

shell lesioned animals was reduced to the same extent (62%) in both studies. Taken together, the two studies suggest that medial shell DA transmission plays a role in conditioned reward rather than a more general role in conditioning.

Our findings confirm an association between DAT binding in accumbens core and the unconditioned locomotor stimulant effect of AMPH (Sellings and Clarke, 2003). Although the 6-OHDA infusions quite possibly destroyed noradrenaline as well as DA terminals, the critical lesion site (core) receives little noradrenergic input (Berridge et al., 1997; Delfs et al., 1998). As such, the observed effects of core 6-OHDA infusion are most likely attributable to disruption of DAergic transmission.

TABLE I. Reductions in DAT binding seen in core and medial shell lesioned groups

Group	Core	mSh	vSh	OT	vCP
Sham	100 ± 4	100 ± 6	100 ± 5	100 ± 5	100 ± 3
Core	18 ± 1	49 ± 4	40 ± 5	45 ± 2	48 ± 5
mSh	76 ± 3	38 ± 5	88 ± 5	72 ± 4	96 ± 4

Values given are mean ± SEM, and are calculated as a percent of sham-operated control. Abbreviations: mSh, medial shell; vSh, ventral shell; OT, olfactory tubercle; vCP, ventral caudate-putamen.

The conditioned locomotor response was abolished by core 6-OHDA lesions. This is not a trivial result, since several DAergic manipulations (i.e., several DA receptor antagonists, reserpine) have been reported to block the unconditioned locomotor response to AMPH while preserving the conditioned locomotor response in subsequent drug-free tests (DiLullo and Martin-Iverson, 1991, 1992a,b; Martin-Iverson and McManus, 1990). In contrast, other DAergic manipulations given during conditioning have been found to prevent subsequent conditioned locomotion (Beninger and Hahn, 1983; DiLullo and Martin-Iverson, 1992b; Mazurski and Beninger, 1991). Our core lesions only partially inhibited unconditioned locomotion, but they blocked the conditioned response. This result suggests that core lesions affected the acquisition and/or expression of AMPH-conditioned locomotion. Consistent with a role in expression, 6-OHDA lesions of the entire accumbens blocked conditioned locomotion when given before *or after* conditioning (Gold et al., 1988).

The present results add to existing evidence suggesting that unconditioned and conditioned locomotion are controlled via different mechanisms (Beninger and Hahn, 1983; Mazurski and Beninger, 1991; Poncelet et al., 1987; Sutton et al., 2000). They also imply that different AMPH conditioned behaviors may be mediated by DA transmission in separate ventral striatal

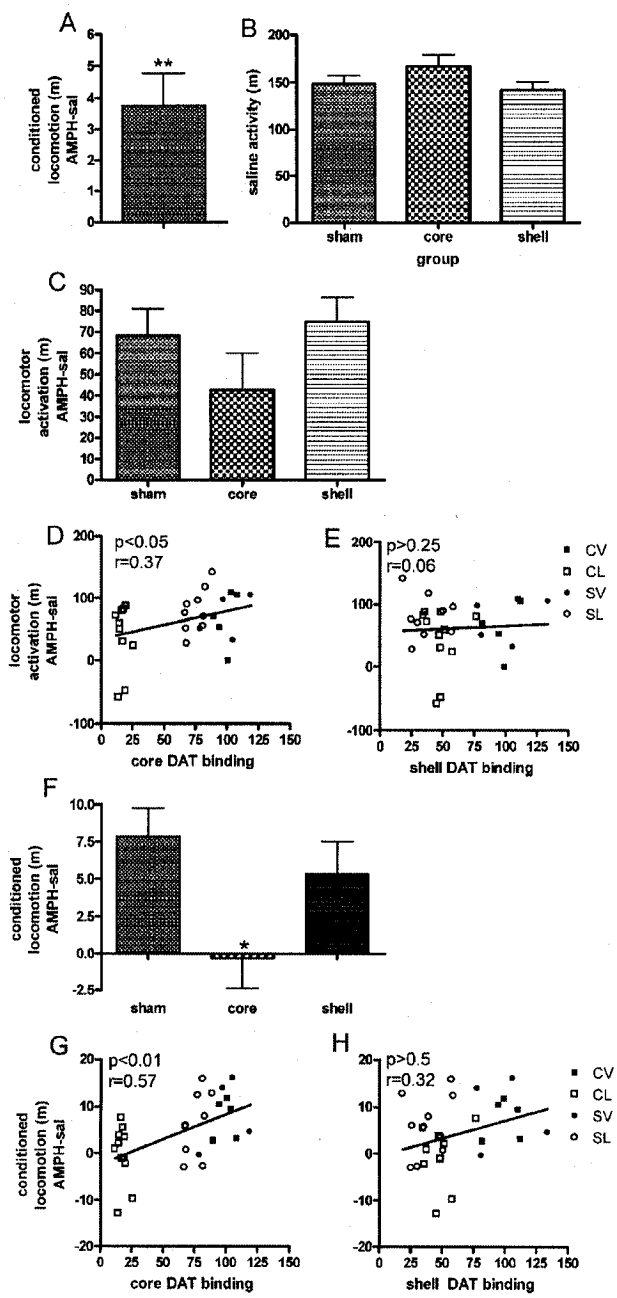

Fig. 1. Both conditioned and unconditioned locomotor activity are decreased by 6-OHDA lesions of the core. In experiment 1, rats ($n = 8$) received multiple pairings of amphetamine with a distinct floor texture, and subsequently exhibited conditioned locomotion (**$P < 0.005$, paired t-test; **A**). In experiment 2, rats received 6-OHDA or vehicle into accumbens medial shell or core ($n = 8$–10 per group). They were then conditioned with amphetamine and tested drug-free. During conditioning, no significant group differences were observed in saline activity (**B**) or in the unconditioned locomotor response to amphetamine (**C**). However, the extent of the core depletion associated significantly with the unconditioned locomotor response (**D**); this was not the case for medial shell lesions (**E**). The conditioned locomotor response was blocked in core-lesioned rats (*$P < 0.02$, Dunnett's test; **F**). The magnitude of the conditioned locomotion was significantly associated with core, but not medial shell DAT binding (**G**, **H**). The apparent, but highly nonsignificant, association between medial shell DAT and the magnitude of the conditioned locomotion reflects a positive correlation existing between core and medial shell DAT binding ($r = 0.57$, $P < 0.01$). Shell refers to medial shell. CV, core vehicle; CL, core 6-OHDA; SV, medial shell vehicle; SL, medial shell 6-OHDA.

subregions, with the medial shell underlying conditioned effects of reward, and the core subregion locomotor activation. The extent to which our findings would generalize to conditioning with discrete cues (Hotsenpiller et al., 2002) or natural rewards (Jones and Robbins, 1992) remains to be determined.

ACKNOWLEDGMENTS

L.H.L.S holds an NSERC Canada Graduate Scholarship.

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0022-3565/06/3173-1178–1187$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

JPET 317:1178–1187, 2006

Vol. 317, No. 3
100339/3113724
Printed in U.S.A.

Evidence for Multiple Sites within Rat Ventral Striatum Mediating Cocaine-Conditioned Place Preference and Locomotor Activation

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Received December 20, 2005; accepted February 27, 2006

ABSTRACT

Considerable evidence suggests that psychostimulants can exert rewarding and locomotor-stimulating effects via increased dopamine transmission in the ventral striatum. However, the relative contributions of ventral striatal subregions to each of these effects have been little investigated. In the present study, we examined the contribution of different ventral striatal sites to the rewarding and locomotor-activating effects of cocaine. Initially, the effects of bilateral 6-hydroxydopamine lesions of the nucleus accumbens core or medial shell on cocaine-induced locomotor stimulation (0.5–1.5 mg/kg i.v. or 5–20 mg/kg i.p.) and conditioned place preference (0.5 mg/kg i.v. or 10 mg/kg i.p.) were examined. In a subsequent study, we investigated the effects of olfactory tubercle versus medial shell lesions on cocaine-conditioned place preference and locomotor activity (0.5 mg/kg i.v.). Dopaminergic lesion extent was quantified by radioligand binding to the dopamine transporter. Multiple linear regression was used to identify associations between behavioral effects and residual dopamine innervation in ventral striatal subregions. On this basis, the accumbens core was associated with the locomotor stimulant effects of i.v. and i.p. cocaine. In contrast, the medial shell was associated with the rewarding effect of i.v. cocaine, but not of i.p. cocaine. Finally, the olfactory tubercle was identified as an additional site contributing to conditioned place preference produced by i.v. cocaine. Overall, these findings provide additional evidence that the locomotor stimulant and rewarding effects of systemically administered psychomotor stimulant drugs are segregated within the ventral striatum.

The nucleus accumbens (NAcc) plays an important role in the rewarding and locomotor stimulant effects of systemically administered amphetamine and cocaine (Koob et al., 1998; Everitt and Wolf, 2002; Wise, 2004). It is anatomically and neurochemically heterogeneous, with a prominent medioventral shell and dorsolateral core (Zahm and Brog, 1992). Recent behavioral studies, largely relying on intracranial microinjections of dopaminergic agonists, have provided evidence for functional compartmentalization within this structure, although certain details are controversial. Thus, the medial shell subregion has been implicated in reward processes (Di Chiara et al., 2004; Ikemoto and Wise, 2004), whereas locomotor stimulation has been elicited from core and/or shell injection sites (Boye et al., 2001; Ikemoto, 2002; Sellings and Clarke, 2003, and references therein).

The technique of intracranial drug microinjection, despite its obvious utility, is limited by the fact that local drug concentrations are usually unknown and may not be comparable with those obtained after systemic administration. Using an alternate approach, we recently evaluated the respective roles of accumbens core and shell in amphetamine-induced locomotion and conditioned place preference (CPP) by combining a systemic amphetamine challenge with prior 6-hydroxydopamine (6-OHDA) lesions of either structure (Sellings and Clarke, 2003). In this study, DAergic depletion in core and medial shell reduced amphetamine-induced locomotor stimulation and CPP, respectively.

In the present study, we sought to extend these findings to cocaine. Intra-NAcc infusion of cocaine produces both locomotor stimulation and rewarding effects (Ikemoto, 2002, 2003; Rodd-Henricks et al., 2002; Ikemoto and Witkin, 2003). However, the interpretation of such findings is complicated by possible sympathomimetic and anesthetic actions within

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Institutes of Health Research. L.H.L.S. holds an NSERC Canada Graduate Scholarship. Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
doi:10.1124/jpet.105.100339.

ABBREVIATIONS: NAcc, nucleus accumbens; CPP, conditioned place preference; 6-OHDA, 6-hydroxydopamine; DAergic, dopaminergic; DA, dopamine; OT, olfactory tubercle; amOT, anteromedial olfactory tubercle; DAT, dopamine transporter; SERT, 5-hydroxytryptamine (serotonin) transporter; RTI-55, 3β-(4-iodophenyl)tropan-2-β-carboxylic acid methyl ester; GBR 12909; 1-{2-[bis(4-fluorophenyl)methoxy]ethyl}-4-(3-phenylpropyl)piperazine dihydrochloride; GBR 12935, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride.

the target tissue (Ikemoto, 2003; Ikemoto and Witkin, 2003). Even after systemic injection, the precise route of administration can be critical. In particular, cocaine is reported to produce dopamine (DA)-dependent or DA-independent rewarding effects, depending on whether it is delivered i.v. or i.p. (Spyraki et al., 1987). In the present study, these two systemic routes of administration were compared.

The less-studied olfactory tubercle (OT) may also play a role in psychomotor stimulant-mediated locomotor activation and reward. This is suggested by studies using intracranial administration in rats. Thus, direct intra-OT infusions of DA agonists including amphetamine and cocaine produced marked and prompt locomotor activation (Pijnenburg et al., 1976; Cools, 1986; Ikemoto, 2002), and both these drugs were avidly self-administered at OT sites (Ikemoto, 2003; Ikemoto et al., 2005). Interestingly, intra-OT drug infusions elicited stronger locomotor and reinforcing effects than intra-NAcc infusions (Cools, 1986; Ikemoto, 2003; Ikemoto et al., 2005). Despite these positive findings, we previously tested the impact of profound 6-OHDA lesions of OT on the locomotor stimulant and rewarding (CPP) effects of *systemic* amphetamine challenge and concluded that DAergic transmission in the OT does not contribute significantly to either behavioral effect (Clarke et al., 1988, 1990). Hence, at present, it is an open question whether the OT contributes significantly to the locomotor stimulant and rewarding effects of any systemically administered psychostimulant.

The overall goal of the present study was, therefore, to localize the ventral striatal actions of *systemically administered* cocaine. The first experiment investigated whether the locomotor stimulant effects of i.v. and i.p. cocaine are diminished by DA denervation in the accumbens core or medial shell. The next two experiments determined whether the stimulant and rewarding effects of cocaine could be dissociated by selective 6-OHDA lesions of either structure, as previously seen with amphetamine (Sellings and Clarke, 2003). The final experiment tested for OT involvement in cocaine reward and locomotor activation, again after systemic drug challenge.

Materials and Methods

Experimental Design. The design of all four experiments is summarized in Table 1.

Subjects. Subjects were male Long-Evans rats (Charles River, St. Constant, QC, Canada) weighing 250 to 325 g at the time of surgery. Rats were housed individually (experiment 1) or in groups of three (experiments 2–4) in clear Plexiglas cages in a temperature- and humidity-controlled animal colony, lit from 7:00 AM to 7:00 PM. Food and water were available ad libitum except during behavioral testing. All experiments were approved by the McGill Faculty of Medicine Animal Care Committee in accordance with Canadian Council on Animal Care guidelines.

Stereotaxic Infusion of 6-OHDA. Surgery was performed 7 to 10 days before the start of behavioral testing. Rats were anesthetized with ketamine HCl (90 mg/kg i.p.) and xylazine HCl (16 mg/kg i.p.) before placement in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the incisor bar set at −3.9 mm. Depending on the experiment (see Table 1), rats received bilateral infusions of either 6-OHDA or vehicle into either NAcc core, medial shell, or anteromedial olfactory tubercle (amOT). Infusions were made via a 30 gauge stainless steel cannula attached by polyethylene tubing to a 10-μl Hamilton syringe driven by a model 5000 Micro Injection Unit (David Kopf Instruments) (core or medial shell) or via two separate 10-μl Hamilton syringes driven by a multichannel syringe pump (amOT; MD-1001, BAS Bioanalytical Systems Inc., West Lafayette, IN). For greater accuracy, coordinates for all three target subregions were derived from the mean of two coordinate systems. Thus, anterior-posterior coordinates were +10.3 mm from interaural zero and +1.3 mm from bregma for both core and shell and +10.7 mm from interaural zero and +1.7 mm from bregma for amOT. Lateral coordinates were ±0.6 mm (shell), ±2.4 mm (core), and ±0.8 mm (amOT). Ventral coordinates for shell (three injections) were +2.0, +2.4, and +2.8 mm from interaural zero and −8.0, −7.6, and −7.2 mm from bregma. Ventral coordinates for core were +2.7 mm from interaural zero and −7.3 mm from bregma. For amOT, ventral coordinates were +1.1 and −8.9 mm, respectively, from interaural zero and bregma. All coordinates are based on the atlas of Paxinos and Watson (1997). 6-OHDA or vehicle was infused on each side in a volume of 0.1 μl (core), as three infusions of 0.06 μl (medial shell) or 0.2 μl (amOT) on each side. For core and medial shell, 6-OHDA was infused at a rate of 0.1 μl/min; for amOT, the rate of infusion was 0.1 μl/10 min. The concentration of 6-OHDA used was 80 μg/μl (core) or 48 μg/μl (shell). For amOT, a volume of 0.2 μl of either vehicle or 6-OHDA (40 μg/μl free base) was infused bilaterally over 20 min. The different doses of 6-OHDA, infusion volumes, and infusion times used at each lesion site were chosen based on pilot studies and represented the best compromise between efficacy (DA depletion) and anatomical selectivity. For all three lesion sites, the cannula remained at the final infusion site for 5 min.

Intravenous Catheterization. During 6-OHDA lesion surgery, rats were implanted with chronic indwelling Silastic catheters (0.51 mm i.d. and 0.94 mm o.d., Fisher Scientific, Montreal, QC, Canada) in the left jugular vein. Tubing was secured to the vein by surgical silk sutures, was led s.c. to the skull surface, and was then fitted onto a 22-gauge cannula attached to a plastic connector (model number C313G-5UP; Plastics One, Roanoke, VA). The cannula/connector was fixed to the animal's skull with small stainless steel screws (Lomir, Notre-Dame-de-L'Ile Perrot, QC, Canada) and dental cement (Stoelting, Wood Dale, IL). To keep catheters patent, 0.1- to 0.15-ml heparinized 0.9% saline was administered at the end of surgery, on the first day of behavioral testing, and every 2 to 3 days thereafter.

Locomotor Activity Testing (Experiment 1). Horizontal locomotor activity was tested in the CPP apparatus (see below for description). Rats were first given one pre-exposure session (20 min) in

TABLE 1
Experimental parameters for experiments 1 to 4
Sham groups represent a combination of rats infused with vehicle in core and shell (experiments 1–3) or shell and amOT (experiment 4).

Experiment	Lesion Site[a]	Dose	Route	Behavior	n^b
		mg/kg			
1	Core or shell	0.5–1.5 i.v., 5–20 i.p.	i.v., i.p.	LMA	11
2	Core or shell	0.5	i.v.	CPP, LMA	10–14
3	Core or shell	10	i.p.	CPP, LMA	12–14
4	amOT or shell	0.5	i.v.	CPP	15–16

LMA, locomotor activity.
[a] Shell refers to medial shell.
[b] n is number of rats per surgery group (core, medial shell or anteromedial olfactory tubercle, and the combined sham-operated groups).

the absence of drug. Each rat then received eight tests on consecutive days with cocaine given i.v. (0, 0.5, 1, or 1.5 mg/kg) or i.p. (0, 5, 10, or 20 mg/kg) in a randomized order. Each test session lasted 30 min, starting immediately after injection. Test cages contained one bar and one mesh tile (see below).

Conditioned Place Preference and Locomotor Activity Testing (Experiments 2, 3, and 4). The apparatus and general procedure were as described previously (Sellings and Clarke, 2003). In brief, the procedure consisted of three phases: pre-exposure (1 day), conditioning (6 days), and test (1 day). All phases were carried out in a one-compartment box (58 cm × 29 cm × 53 cm) with walls made of white plastic-coated particle board. In the pre-exposure phase, Beta-Chip sawdust bedding covered the floor of the cage. In the conditioning phase, two square tactile tiles of either bar or mesh texture were placed in the bottom of the cage, on top of the bedding. During this phase, video tracking software (EthoVision version 3.0; Noldus Information Technology, Leesburg, VA) measured locomotor activity, expressed as horizontal distance moved (in meters). During the test phase, one bar and one mesh tile were placed on the bottom of the cage. The time spent on bar or mesh texture was measured by EthoVision software. All three phases were carried out under dark-room lighting using a Kodak GBX-2 safelight filter (Vistek, Toronto, ON, Canada), to minimize visual cues. Animals do not spontaneously prefer either texture (L. H. L. Sellings and P. B. S. Clarke, unpublished data), and all experiments were as fully counterbalanced as possible with respect to drug-texture pairing and order of drug pairing (drug-saline or saline-drug) within each surgery group. For all experiments, pre-exposure sessions lasted 20 min, and the test session lasted 10 min. Conditioning trial duration for i.v. cocaine was 15 min and for i.p. cocaine was 25 min.

For i.v. infusion (experiments 1, 2, and 4), a fluid swivel was fixed above the center of each cage. Each swivel was connected on one end to a 1-ml syringe and on the other end to a brass connector (Produits MSM, Laval, QC, Canada) and a protective spring (Heiplex, Montreal, QC, Canada) via Tygon tubing of 0.51-mm diameter. The cannula fixed to the skull of the rat was attached to the Tygon tubing, and the brass connector was fastened to the plastic connector to secure the tubing to the cannula, hence allowing administration of drug immediately after placement of the animal in the CPP cage. Drug was infused over 25 to 30 s. Cocaine administered i.p. was injected immediately before placement in the CPP cage.

Tissue Preparation. Tissue was prepared for autoradiography and Nissl staining (Cresyl violet) as described previously (Sellings and Clarke, 2003). In brief, rats were sacrificed 3 to 5 h after CPP testing by decapitation under sodium pentobarbital (65 mg/kg i.p.) anesthesia. Brains were removed, frozen in 2-methylbutane at −50°C for 30 s, and stored at −40°C.

Coronal sections (20 μm) were taken on a cryostat at several rostrocaudal levels through the ventral striatum. In experiments 1, 2, and 3, sections were examined at 11.2, 10.7, 10.2, and 9.7 mm anterior to interaural zero; 9.2 and 8.7 mm were also examined in experiment 4 (Paxinos and Watson, 1997). Four adjacent sections were collected for autoradiography and one for Nissl staining with Cresyl violet. Sections were thaw mounted onto gelatin-subbed slides, air-dried at room temperature for 20 to 30 min, and stored with desiccant at −40°C.

Quantitative Autoradiography. The extent and chemical selectivity of the 6-OHDA lesion was quantified by autoradiographic labeling of the DA transporter (DAT) and the 5-hydroxytryptamine (serotonin) transporter (SERT) (Sellings and Clarke, 2003), using a nonsaturating concentration of [^{125}I]3β-(4-iodophenyl)tropan-2-β-carboxylic acid methyl ester ([^{125}I]RTI-55) (2200 Ci/mmol; NEN-Mandel, Guelph, ON, Canada).

Sections were thawed at room temperature for 10 min and then placed in a staining dish containing an aqueous buffer solution of 120 mM NaCl, 0.1 M sucrose, 10 mM sodium phosphate buffer, and 10 pM [^{125}I]RTI-55, with the pH adjusted to 7.4. In the DAT autoradiographic assay, 50 nM citalopram hydrobromide was used to occlude SERT; nonspecific binding was determined by addition of 10 μM 1-{2-[bis(4-fluorophenyl)methoxy]ethyl}-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 12909). For SERT autoradiography, 1 μM 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 12935) was added to occlude DAT; nonspecific binding was determined by addition of 50 nM citalopram HBr (Sellings and Clarke, 2003). Slides were incubated at room temperature for 2 h and then washed three times in ice-cold buffer solution (once for 1 min and twice for 20 min) and for 1 to 2 s in distilled and deionized water. They were then blow-dried and placed in X-ray film cassettes. Kodak BioMax MS film (Amersham Biosciences, Baie d'Urfé, QC, Canada) was exposed to slides for 48 h (DAT) or 120 h (SERT) with ^{125}I autoradiographic standards (Amersham Biosciences). After development of film, DAT and SERT binding were quantified using an MCID M4 imaging system (Imaging Research, St. Catherines, ON, Canada).

Histological Examination. Tissue was stained with cresyl violet to assess nonspecific damage, as described previously (Sellings and Clarke, 2003) and examined under a light microscope (40–200× magnification).

Drugs. Drug sources were as follows: cocaine HCl (gift of National Institute on Drug Abuse, Bethesda, MD); citalopram HBr (gift from H. Lundbeck A/S, Copenhagen, Denmark); dipyrone (Vétoquinol, Quebec, QC, Canada); ketamine HCl (Vetalar; Vetrepharm, London, ON, Canada); xylazine HCl (Anased; Novopharm, Toronto, ON, Canada); GBR 12909 (National Institute of Mental Health Chemical Synthesis and Drug Supply Program, Bethesda, MD), and GBR 12935·2HCl (Sigma-Aldrich, Oakville, ON, Canada). Unless otherwise stated, all other chemicals were obtained from Fisher Scientific (Montreal, QC, Canada).

Cocaine HCl was dissolved in sterile 0.9% saline and injected at 1 ml/kg (i.v. or i.p.). 6-OHDA HBr was dissolved in sterile 0.9% saline containing 0.3 mg/ml sodium metabisulfite (Sigma-Aldrich) as an antioxidant and protected from light. Vehicle solutions as well as 6-OHDA to be infused into the medial shell or amOT were neutralized to pH 7.3 ± 0.1 with NaOH (to reduce nonspecific damage; see *Results*). Doses of all drugs except 6-OHDA HBr are expressed as the salt. 6-OHDA HBr doses are expressed as free base.

Data Analysis. A commercial software program (Systat v10.2; SPSS Inc., Chicago, IL) was used for all data analyses. In all experiments, locomotor responses to cocaine were calculated as the difference of locomotor counts between drug and saline conditioning sessions. In CPP experiments, saline locomotor scores were calculated as the mean activity over all three conditioning sessions with saline and are expressed as means ± S.E.M. After initial data inspection, sham groups were combined within each experiment. Group differences were analyzed by one-way analysis of variance. CPP magnitude was calculated as the difference between time spent on the drug-paired and vehicle-paired sides during the 10-min test session. Experiments 2 and 4 were each carried out in different batches because of space constraints in the animal facility; after initial data inspection, the results within each experiment were pooled. The existence of a significant CPP magnitude or locomotor stimulant effect was determined by a one-sample Student's t test with the Bonferroni correction for multiple comparisons. The relationship between behavioral measures versus [^{125}I]RTI-55 labeling was analyzed by multiple linear regression. A p value of <0.05 (two-tailed) was considered significant. Group data are expressed as means ± S.E.M. throughout.

Results

Neurochemical and Anatomical Selectivity. To assess nonspecific tissue damage, sections were Nissl-stained with Cresyl violet. As reported previously (Sellings and Clarke, 2003), only minimal cell loss was evident at the site of infusion for all vehicle groups (Fig. 1A) and for the group infused with

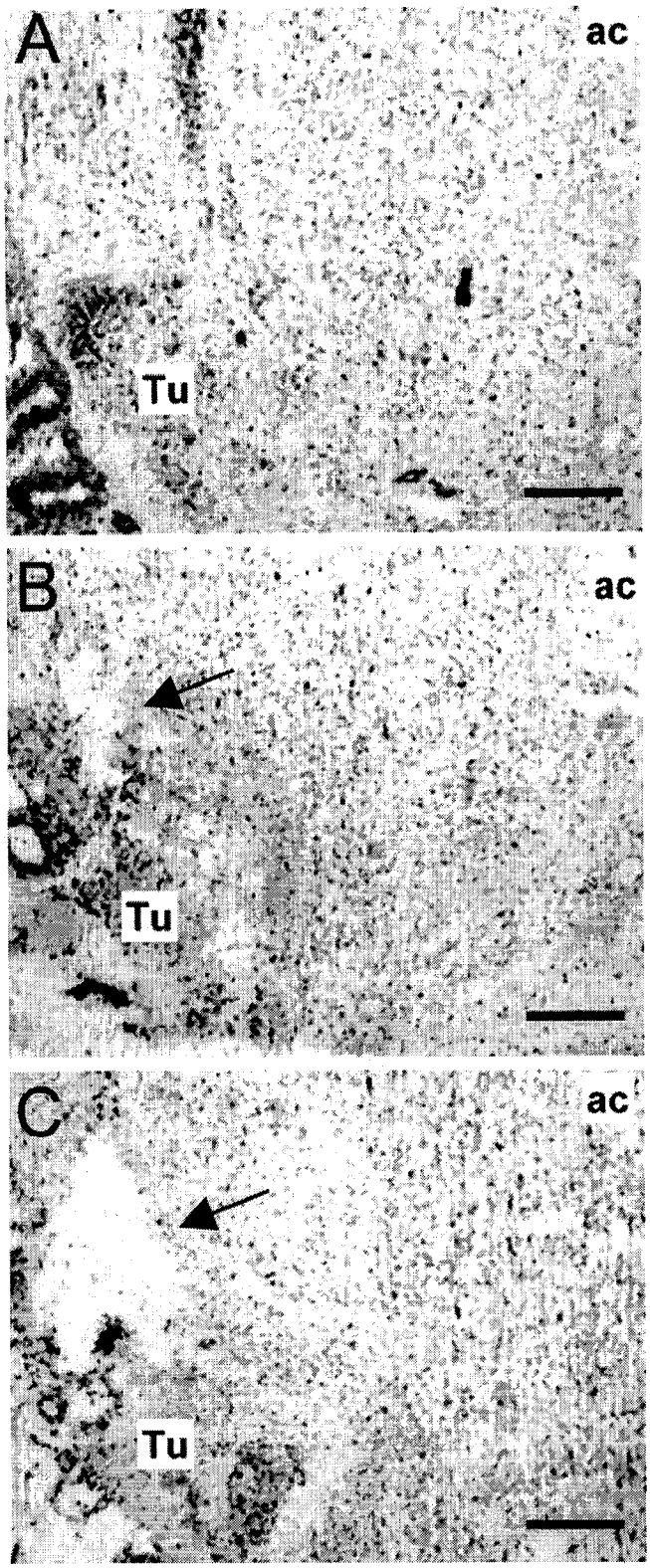

Fig. 1. Representative photomicrographs of Nissl staining in sham-lesioned (A) and amOT-lesioned (B and C) animals adjacent to the infusion site. In some (30%) of amOT-lesioned rats, a small region of reduced cell density was observed compared with sham-lesioned rats (B, black arrow). Larger regions of decreased cell density were seen in a subset (~10%) of lesioned animals (C, black arrow). Scale bar, 100 μm. ac, anterior commissure; Tu, medial olfactory tubercle.

6-OHDA in the core subregion (not shown). Among rats lesioned in the medial shell or amOT, most (~60%) also showed a minimal degree of cell loss, ~30% of rats possessed a small region of decreased cell density at the infusion site (Fig. 1B), and ~10% of rats showed more pronounced nonselective damage (Fig. 1C). This larger region of nonspecific damage did not extend more than 0.3 mm from the site of infusion and was almost always found at only one anterior-posterior level.

Sampling locations for DAT and SERT binding density are indicated in Fig. 2. [^{125}I]RTI-55 autoradiographs of DAT binding are shown in Fig. 3. Residual DAT binding as a percentage of combined sham groups is given in Tables 2 (experiments 1–3) and 3 (experiment 4). Radioligand binding to SERT in tissue from lesioned animals was minimally changed by all lesion parameters in all experiments (Tables 2 and 3). In all experiments, rats were allowed 7 to 10 days recovery postsurgery before the start of behavioral testing.

The Magnitude of Core, but Not Medial Shell, DA Denervation Predicted Locomotor Responses to Intraperitoneal and Intravenous Cocaine. The effects of 6-OHDA lesions of core versus medial shell on cocaine-induced locomotion were tested most extensively in experiment 1. Locomotor responses to i.p. and i.v. cocaine are shown in Fig. 4, A and B (absolute values), and Fig. 4, C and D (saline-subtracted values). Saline test scores did not differ significantly among the three surgery groups (Fig. 4, A and B). The locomotor stimulant effects of cocaine were blunted only in the core-lesioned group. Multiple linear regression analysis revealed significant positive associations between core DAT binding and the locomotor stimulant response for both administration routes used and at all doses except for 0.5 mg/kg i.v. (range $p < 0.001$–$p < 0.05$). Significant *negative* associations were observed between medial shell DAT binding and the locomotor stimulant response at several cocaine doses (1 mg/kg i.v. and 5 and 10 mg/kg i.p.; $p < 0.05$–$p < 0.005$).

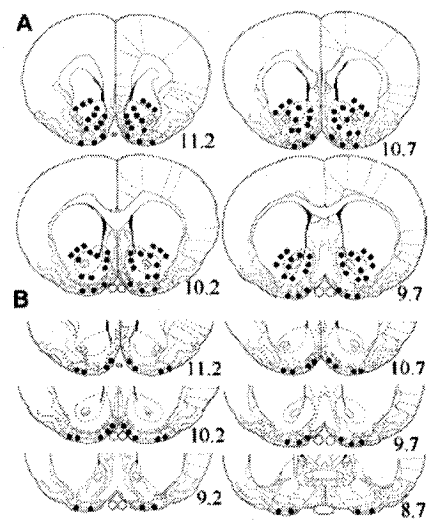

Fig. 2. A, locations of sampled [^{125}I]RTI-55 binding in core, medial shell, ventral shell, olfactory tubercle, and ventral caudate putamen. Each rat was sampled at four anterior-posterior levels. Numbers are distances (in millimeters) anterior to interaural zero. Sampling areas were circles of 0.3 mm diameter. B, sampling regions for olfactory tubercle subregions (amOT, anterolateral OT, and posterior OT) in experiment 4 and in post hoc analyses of experiment 2. At levels 11.2, 10.7, and 10.2, both amOT and anterolateral OT were sampled. At levels 9.7, 9.2, and 8.7, only posterior OT was sampled. Figure adapted from Paxinos and Watson (1997).

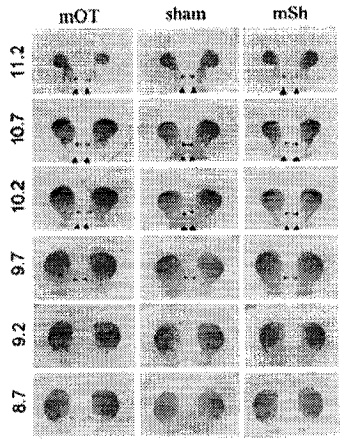

Fig. 3. Representative autoradiographic images of [^{125}I]RTI55 binding to DAT in animals from medial shell-lesioned (mSh), anteromedial olfactory tubercle-lesioned (mOT), and sham-operated groups (sham) in experiment 4. Because binding was similar among groups that received vehicle in the medial shell and medial olfactory tubercle, the latter group has been omitted. Numbers designate distance anterior to interaural zero (in millimeters). Radioligand binding was obtained at a nonsaturating concentration of radioligand. Arrows refer to the medial shell. Arrowheads (pointing upward) refer to the anteromedial olfactory tubercle.

The effects of core and medial shell 6-OHDA lesions on cocaine-induced locomotion were also tested in two CPP experiments (i.e., experiments 2 and 3). Locomotor data were obtained from the three drug and saline conditioning sessions. Experiment 2 examined the locomotor stimulant response to cocaine (0.5 mg/kg i.v.). Here, saline locomotor scores did not differ significantly among groups and were as follows: 52 ± 2 m (sham), 54 ± 2 m (core 6-OHDA), and 55 ± 2 m (medial shell 6-OHDA). A significant locomotor stimulant effect was observed in sham-lesioned and medial shell-lesioned animals, but not in the core-lesioned subjects (Fig. 5A). Multiple linear regression analysis revealed a positive trend between the locomotor response and DAT binding in the core ($p = 0.086$; Fig. 5B) but not medial shell (Fig. 5C).

The locomotor stimulant response to *i.p.* cocaine (10 mg/kg) was also attenuated after core 6-OHDA lesions (experiment 3; Fig. 6A). No significant group differences were seen for saline locomotor activity. Saline scores were 85 ± 6 (sham), 88 ± 5 (core 6-OHDA), and 87 ± 5 (medial shell 6-OHDA). Multiple linear regression analysis (Fig. 6, B and C) revealed a positive association between the locomotor response and core DA innervation only ($p < 0.05$).

TABLE 3

DAT and SERT binding in medial shell- or anteromedial olfactory tubercle (amOT)-lesioned rats in imaged ventral striatal subregions (experiment 4)

Values are expressed as means ± S.E.M.

6-OHDA Site	Sham	Medial Shell	amOT
DAT			
Core	100 ± 5	87 ± 6	89 ± 5
mSh	100 ± 4	40 ± 5	66 ± 7
vSh	100 ± 5	77 ± 7	75 ± 3
amOT	100 ± 8	57 ± 7	34 ± 8
alOT	100 ± 9	70 ± 6	55 ± 6
pOT	100 ± 10	85 ± 67	51 ± 6
vCP	100 ± 4	100 ± 5	95 ± 4
SERT			
Core	100 ± 14	110 ± 4	97 ± 9
mSh	100 ± 11	100 ± 4	90 ± 8
vSh	100 ± 15	109 ± 6	97 ± 9
amOT	100 ± 13	101 ± 5	92 ± 10
alOT	100 ± 14	109 ± 4	105 ± 12
pOT	100 ± 13	108 ± 6	95 ± 12
vCP	100 ± 15	123 ± 7	110 ± 10

mSh, medial shell; vSh, ventral shell; alOT, anterolateral olfactory tubercle; pOT, posterior olfactory tubercle; vCP, ventral caudate putamen.

NAcc Medial Shell Lesions Inhibited CPP for Intravenous Cocaine. In experiment 2, only the combined sham group and the core-lesioned group exhibited significant CPP (Fig. 5D). Relationships between the CPP magnitude and core versus medial shell DAT binding are shown in Fig. 5, E and F, respectively. The CPP magnitude produced by i.v. cocaine was positively related to medial shell DAT binding ($p < 0.005$; Fig. 5F) with a negative trend in the accumbens core ($p = 0.062$; Fig. 5E).

Conditioned Place Preference for Intraperitoneal Cocaine Was Unaffected by Lesions of Core or Medial Shell. In experiment 3, a significant CPP to i.p. cocaine occurred in the sham-lesioned group, with a similar trend in the two lesion groups (Fig. 6D). No significant relationship was observed between the CPP magnitude and core or medial shell DAT binding (Fig. 6, E and F).

CPP Magnitude for Intravenous Cocaine Was Related to OT Residual DAT Binding. It was recently reported that amOT more robustly supports intracranial self-infusion of cocaine than does medial shell (see *Discussion*). Therefore, we first re-examined the data from experiment 2 (i.v. cocaine) to

TABLE 2

Residual DAT and SERT binding in rats lesioned in core or medial shell in ventral striatal subregions (experiments 1, 2, and 3)

Values given are means ± S.E.M. as a percentage of combined sham group.

6-OHDA Site	1			2			3		
	Sham	Core	mSh	Sham	Core	mSh	Sham	Core	mSh
DAT									
Core	100 ± 4	40 ± 8	85 ± 5	100 ± 3	25 ± 1	86 ± 5	100 ± 7	20 ± 3	95 ± 5
mSh	100 ± 11	60 ± 9	31 ± 7	100 ± 5	47 ± 3	42 ± 3	100 ± 8	48 ± 4	36 ± 6
vSh	100 ± 5	63 ± 12	76 ± 4	100 ± 7	45 ± 3	92 ± 7	100 ± 6	35 ± 6	98 ± 8
OT	100 ± 4	74 ± 11	80 ± 5	100 ± 5	47 ± 5	76 ± 6	100 ± 12	46 ± 4	80 ± 4
vCP	100 ± 4	66 ± 8	92 ± 4	100 ± 5	45 ± 2	102 ± 7	100 ± 10	50 ± 7	107 ± 6
SERT									
Core	100 ± 9	97 ± 7	91 ± 5	100 ± 5	83 ± 4	93 ± 4	100 ± 5	108 ± 6	97 ± 3
mSh	100 ± 6	105 ± 6	95 ± 7	100 ± 2	93 ± 5	90 ± 4	100 ± 4	97 ± 5	94 ± 3
vSh	100 ± 5	102 ± 6	100 ± 5	100 ± 2	90 ± 4	99 ± 4	100 ± 6	118 ± 8	108 ± 3
OT	100 ± 6	110 ± 6	100 ± 5	100 ± 3	100 ± 5	101 ± 5	100 ± 6	116 ± 5	108 ± 4
vCP	100 ± 9	99 ± 6	98 ± 5	100 ± 4	90 ± 5	107 ± 3	100 ± 3	88 ± 3	93 ± 3

mSh, medial shell; vSh, ventral shell; vCP, ventral caudate putamen.

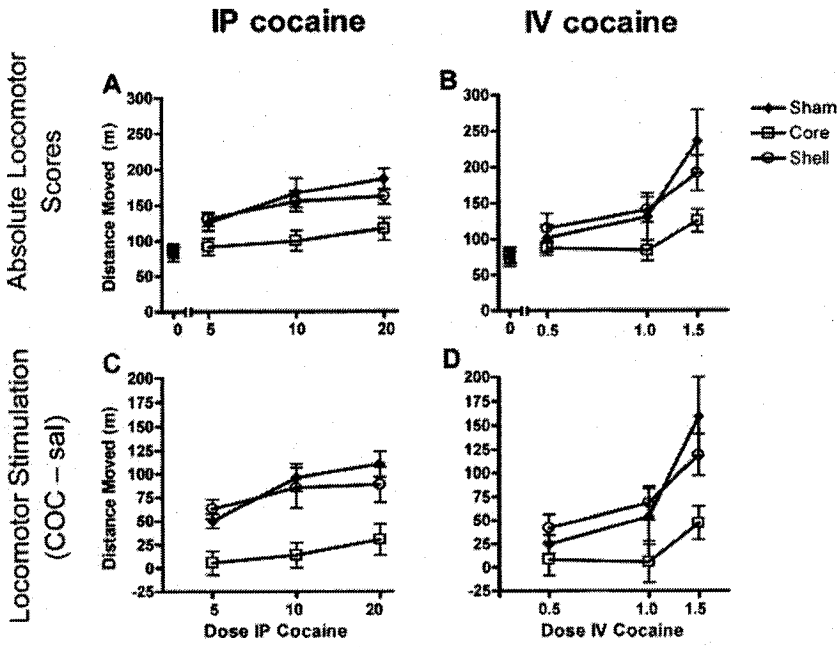

Fig. 4. Effect of 6-OHDA lesions of NAcc medial shell or core on locomotor responses to a range of i.p. and i.v. cocaine doses (experiment 3). Each rat (n = 5–10 per group) was tested with i.v. (0–1.5 mg/kg) and i.p. (0–20 mg/kg) cocaine in a repeated measures design. Absolute locomotor activity at all doses of i.p. and i.v. cocaine are shown in A and B, respectively. The stimulant effect of cocaine (i.e., cocaine-saline difference score) is illustrated in C and D. Locomotor response correlated positively and significantly with DAT binding in the core at all doses except 0.5 mg/kg i.v. Shell refers to medial shell.

determine whether amOT DAT binding may have contributed significantly to the CPP magnitude. However, amOT binding was reduced only slightly in this experiment (by 28% in the core- and 11% in the shell-lesioned group). We therefore addressed the question of amOT involvement by directly comparing the effects of 6-OHDA lesions of the medial shell versus amOT on i.v. cocaine CPP (experiment 4).

Infusions of 6-OHDA into either amOT or medial shell depleted DAT binding locally and also tended to produce a smaller and variable depletion in the other structure (Fig. 7). Initial analysis revealed a high degree of colinearity existing in DAT binding levels between different OT subregions. Accordingly, these values were averaged, and subsequent analyses were carried out using OT rather than amOT values.

Only sham-lesioned animals exhibited significant CPP (Fig. 8A). Multiple linear regression analysis was performed with CPP magnitude as the dependent variable, using residual DAT binding in core, medial shell, ventral shell, ventral caudate putamen, and OT as simultaneous predictors. Only OT was retained as a significant predictor ($p < 0.01$) (Fig. 8C). Linear regression analysis of CPP magnitude with medial shell as the sole predictor revealed a positive association that bordered on significance ($p = 0.056$). Linear regression analysis of the locomotor stimulant effect revealed that DAT binding in neither medial shell nor OT predicted the degree of locomotor stimulation ($p > 0.5$ for both, data not shown).

Discussion

Novel Findings. To our knowledge, the present study is the first to examine the role of ventral striatal subregions in CPP induced by systemically administered cocaine. Cocaine-induced locomotion was related to core DA innervation at several doses of both i.v. and i.p. cocaine. CPP results, in contrast, were more complex. Intravenous cocaine CPP appeared to be dependent on DA innervation in both OT and medial shell, whereas i.p. cocaine CPP was unaffected by medial shell lesions.

Methodological Considerations. The present series of experiments revealed associations between residual DA innervation in various ventral striatal structures and cocaine-induced locomotion or CPP. It is doubtful that these relationships represent segregation between conditioned and unconditioned drug effects rather than between reward and locomotion, as core but not medial shell 6-OHDA lesions abolished amphetamine-induced *conditioned* locomotion (Sellings and Clarke, 2006).

In the present study, quantitative autoradiographic analysis was performed by taking a large number of samples within each structure (e.g., 24 each for medial shell and core). Within each targeted structure, the extent of DAT depletion appeared rather uniform (see Fig. 3 and Sellings and Clarke, 2003), and visual inspection revealed no evidence for smaller sites of preferential depletion. Nevertheless, we cannot rule out the possibility that our behavioral effects resulted from damage to functionally important "hot spots" within the targeted structures.

It is unlikely that nonspecific damage caused these lesion effects, since only minimal changes were observed in SERT binding levels, and Nissl staining revealed only slight nonspecific damage in a subset of medial shell- and medial OT-lesioned animals (Fig. 1). However, 6-OHDA infusion almost certainly depleted noradrenaline as well as DA. Preservation of noradrenergic terminals by using systemic desipramine proved impossible, since in pilot studies the routinely used dose of 25 mg/kg (Kelly and Iversen, 1976) caused significant mortality (>25%). Nevertheless, for several reasons, it is unlikely that the observed lesion effects were due to loss of noradrenergic terminals. First, neither noradrenergic agonists nor antagonists, when injected into ventral striatum, affected locomotion (Pijnenburg et al., 1975, 1976). Second, noradrenergic denervation of ventral striatum does not alter locomotor stimulant responses to cocaine and amphetamine (Roberts et al., 1975; Kelly and Iversen, 1976). Third, noradrenergic afferents to NAcc largely avoid the core (Delfs et al., 1998), where lesion effects on locomotor stimulation oc-

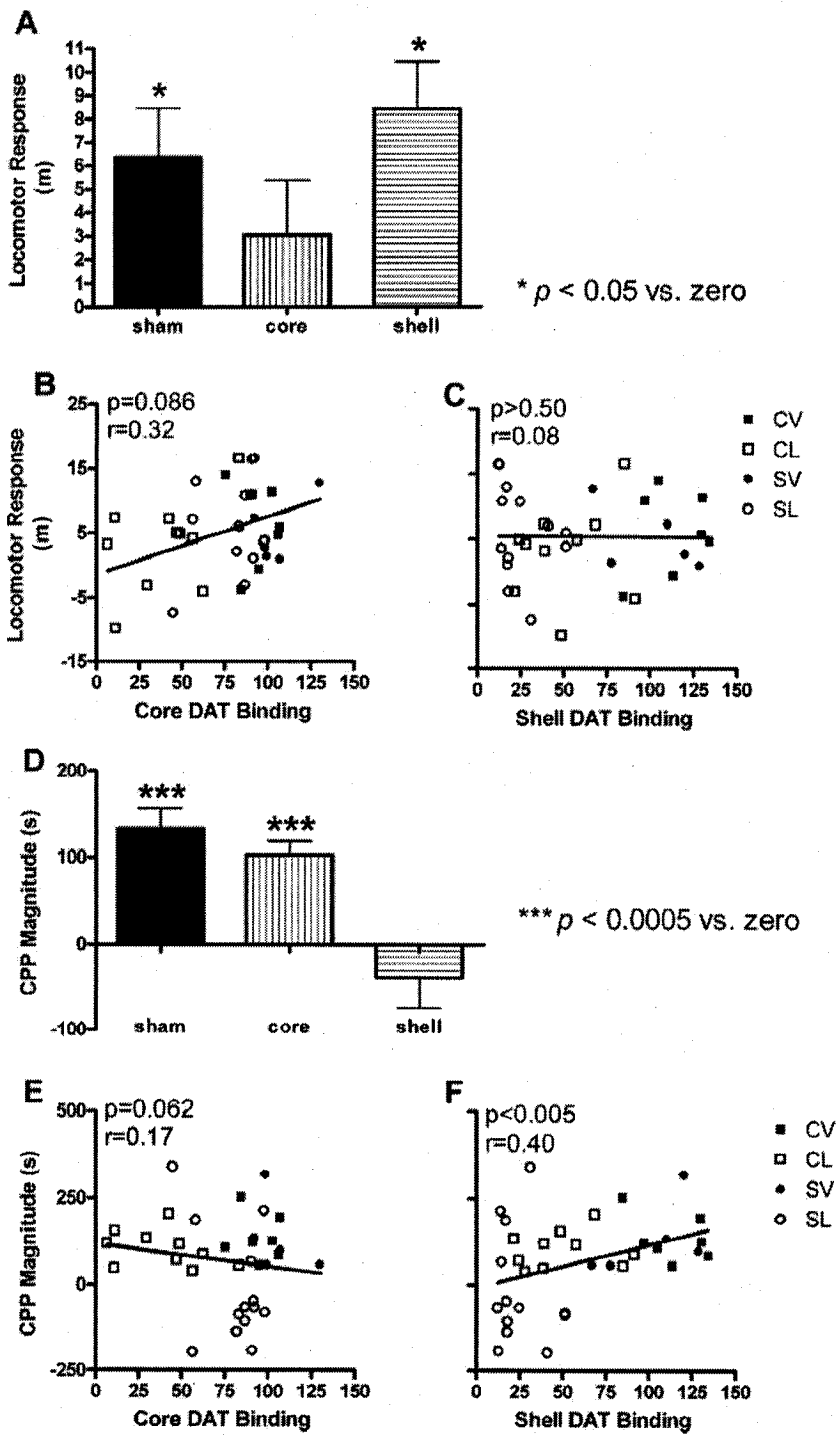

Fig. 5. Effect of bilateral 6-OHDA infusion into either NAcc core or medial shell on locomotor response and CPP to i.v. cocaine (experiment 2). Rats were allowed 7 to 10 days recovery after jugular catheter implantation and stereotaxic surgery before conditioning with i.v. cocaine (0.5 mg/kg). Locomotor responses (A–C) are expressed as the difference between the mean distance moved (m) during conditioning sessions with i.v. cocaine versus saline. CPP magnitude (D–F) is expressed as the difference between time spent on the drug-paired and saline-paired floor textures on test day (in seconds, 600-s test). DAT labeling in core or medial shell is expressed as a percentage of combined sham-lesioned groups. Both sham- and shell-lesioned groups exhibit significant locomotor stimulation (A). Locomotor response tended to correlate positively with DAT binding in core (B). Both sham- and core-lesioned groups exhibit significant CPP (D). CPP magnitude correlated positively and significantly with DAT binding in medial shell (F) and tended to correlate negatively with DAT binding in core (E). CV, core vehicle; CL, core lesioned; SV, medial shell vehicle; SL, medial shell lesion. Shell refers to medial shell.

cured. Fourth, stimulation of noradrenergic transmission did not produce CPP (Martin-Iverson et al., 1985; Subhan et al., 2000). Fifth, neither α- nor β-adrenergic receptor antagonists affected the rewarding effects of i.v. cocaine as reflected by self-administration behavior (Johanson and Fischman, 1989). Sixth, the disruptive effects of 6-OHDA lesions on cocaine self-administration appear to be unrelated to noradrenaline depletion (Roberts et al., 1975, 1977). Lastly, self-administration of cocaine directly into the amOT was blocked by coinfusion of a D1 or D2 DA receptor antagonist (Ikemoto, 2003). On this basis, it seems reasonable to conclude that our 6-OHDA lesions produced their behavioral effects via local depletion of DA.

The Accumbens Core and Locomotor Activation. There is currently no consensus on the role of core versus shell in psychostimulant-induced locomotion (Boye et al., 2001; Ikemoto, 2002, and references therein). In particular, studies with intra-accumbens microinjection of direct or indirect DAergic agonists have implicated core, shell, or both structures, depending on the drug. For example, amphet-

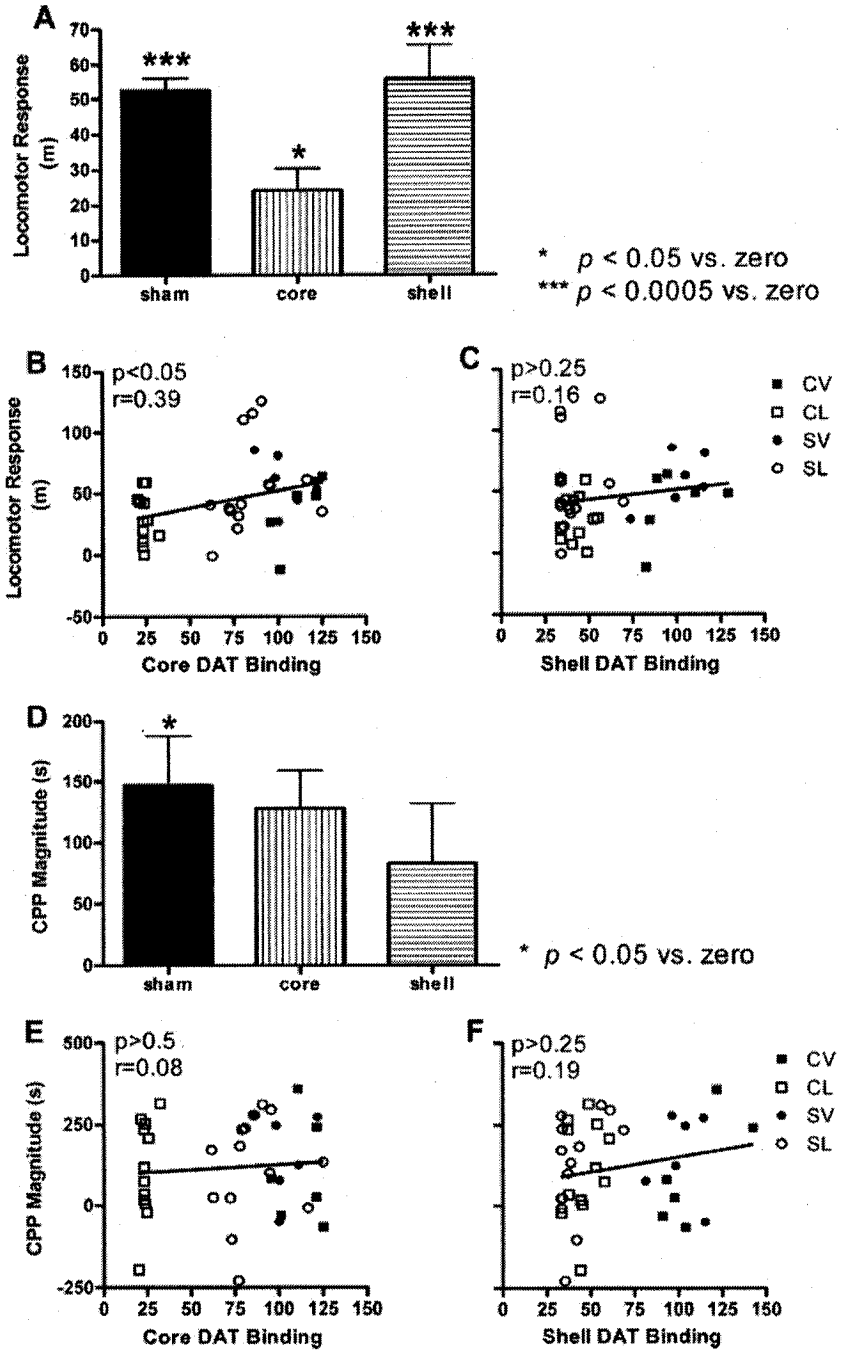

Fig. 6. Effect of bilateral 6-OHDA infusion into either NAcc core or medial shell on locomotor response and CPP to i.p. cocaine (experiment 3). Rats were conditioned with i.p. cocaine (10 mg/kg). Data are presented as in Fig. 3. All groups exhibit significant locomotor stimulation (A), but that of core-lesioned animals was smaller than that of the sham- and shell-lesioned groups ($p < 0.05$). Only sham rats exhibited significant CPP, but core- and shell-lesioned animals also tended to exhibit CPP (D). Locomotor response correlated positively and significantly with DAT binding in core (B). No other behavioral responses correlated with DAT labeling in either structure (C, E, and F). CV, core vehicle; CL, core-lesioned; SV, medial shell vehicle; SL, medial shell lesion. Shell refers to medial shell.

amine acted with similar potency at either injection site, whereas cocaine stimulated locomotor activity most strongly after injection into medial OT and medial shell (Ikemoto, 2002). Importantly, locomotor responses from accumbens core injections of cocaine may have been weakened by local anesthesia (Ikemoto and Witkin, 2003).

The present experiments show that the locomotor stimulant effects of systemically administered cocaine are associated with DAergic neurotransmission in core rather than medial shell. This result generalized to several doses of the drug and to both i.p. and i.v. routes of administration. These findings accord with observations using systemic amphetamine (Boye et al., 2001; Sellings and Clarke, 2003, and references therein) and methylphenidate (L. H. L. Sellings, L. E. McQuade, and P. B. S. Clarke, manuscript submitted for publication). Taken together, they suggest a general mechanism by which systemically administered psychostimulants produce activating effects. Whether core DA transmission directly mediates the locomotor stimulant action of these drugs or plays an indirect enabling role remains a question for the future.

Differences between Intraperitoneal and Intravenous Cocaine CPP. In the present study, i.v. cocaine produced CPP that appears to be dependent on DA transmission

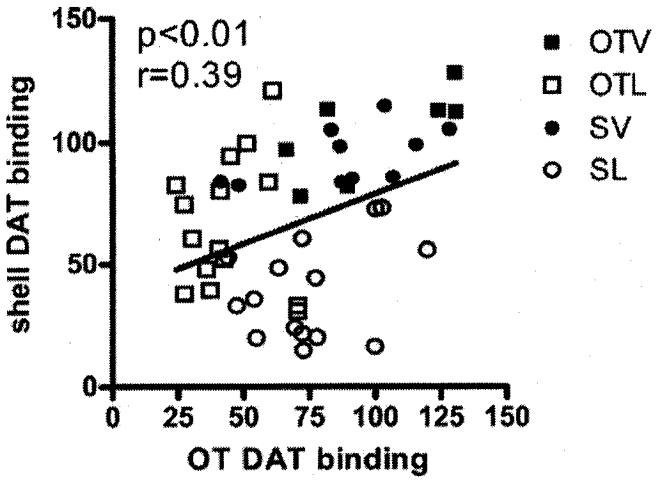

Fig. 7. Relationship of DAT labeling in nucleus accumbens medial shell versus olfactory tubercle in experiment 4 (n = 46 rats). [^{125}I]RTI-55 autoradiography for DAT was used to assess residual DA innervation (see *Materials and Methods*) and expressed as a percentage of the mean value of the sum of medial shell-vehicle and olfactory tubercle-vehicle groups. Correlational analysis revealed a significant relationship between medial shell and olfactory tubercle binding (r = 0.39, p < 0.01). OTV, olfactory tubercle vehicle; OTL, olfactory tubercle-lesioned; SV, medial shell vehicle; SL, medial shell lesion.

in both medial shell and OT. In contrast, i.p. cocaine CPP did not appear to be dependent on accumbens DA transmission. This finding is consistent with reports suggesting that i.v. cocaine produces DA-dependent CPP and i.p. cocaine produces DA-independent CPP (Morency and Beninger, 1986; Spyraki et al., 1987). Although neuroadaptation may account for the lack of lesion effect on i.p. cocaine CPP, this appears unlikely considering the fact that similar medial shell lesions reduced CPP both for i.v. cocaine and for amphetamine (Sellings and Clarke, 2003). Our results do not rule out other forms of accumbens involvement; indeed glutamatergic and serotonergic manipulations within this structure affect i.p. cocaine CPP (Kaddis et al., 1995; Harris et al., 2001).

Because cocaine produces CPP more potently after i.v. than after i.p. administration (Spyraki et al., 1987; O'Dell et al., 1996), care was taken in the present study to select submaximal i.p. and i.v. doses of cocaine approximately matched in terms of CPP magnitude. Hence, it is likely that the differential sensitivity to DA depletion reflected route of administration and not dose.

The neurochemical basis of this differential susceptibility cannot readily be related to changes in extracellular DA. The i.v. dose used (0.5 mg/kg) has been reported to increase dialysate DA levels in the medial shell but not the core (Pontieri et al., 1995), whereas the i.p. dose (10 mg/kg) robustly increased DA levels in both subregions (Cadoni et al., 2000). Another reported difference between i.v. and i.p. cocaine administration is that only the former caused significant increases in glucose metabolism in NAcc and OT (Porrino, 1993); in the latter study, the use of a wide range of doses suggests strongly that route of administration was the critical factor. The basis for route-dependent effects on cerebral glucose utilization and the possible relation to cocaine reward remain to be elucidated.

Cocaine CPP: Dependence on Both Medial Shell and OT. Although there is a rich body of literature linking the NAcc to drug reward, possible OT involvement has been largely unexamined (Clarke et al., 1990; Kornetsky et al., 1991; Ikemoto, 2003; Ikemoto et al., 2005; Ikemoto and Donahue, 2005). The present results suggest that both medial shell and OT play important roles in mediating i.v. cocaine reward.

Self-administration of cocaine directly into the ventral striatum appears strongly site-dependent; responding was vigorous for infusions into amOT, marginal in medial shell, and negligible within accumbens core (Rodd-Henricks et al., 2002; Ikemoto, 2003). In addition, only cocaine infusion at amOT sites produced CPP at the doses tested (Ikemoto, 2003). However, the behavioral effects of focal cocaine infusion into the NAcc (shell

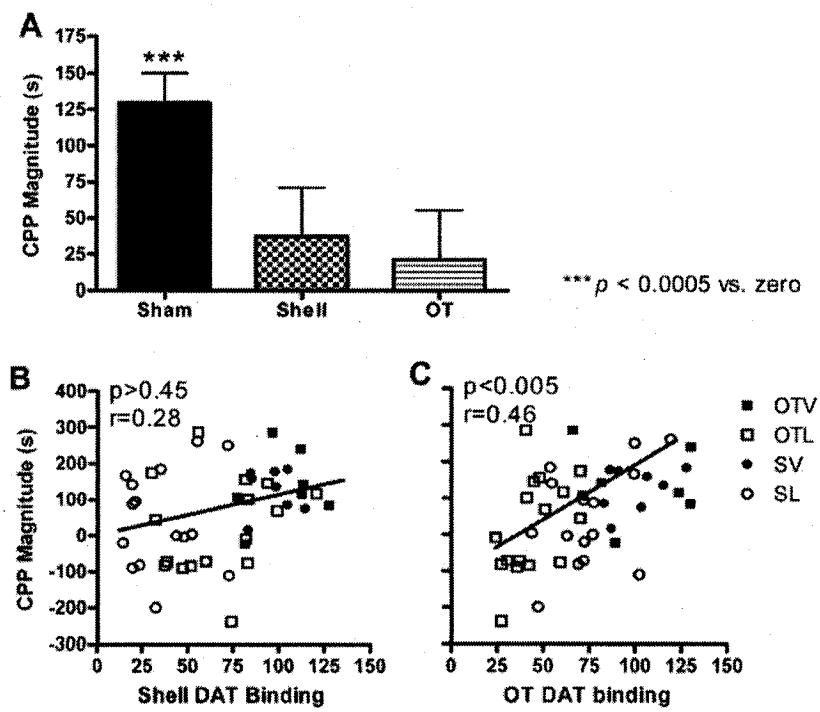

Fig. 8. Effect of 6-OHDA lesions of olfactory tubercle and medial shell on i.v. cocaine CPP (experiment 4). CPP magnitude was calculated as the difference between the time spent on the drug-paired and saline-paired sides. CPP magnitude correlated positively and significantly with DAT binding in olfactory tubercle (C) but not with DAT binding in medial shell (B). OTV, olfactory tubercle vehicle; OTL, olfactory tubercle lesioned; SV, medial shell vehicle; SL, medial shell lesion. Shell refers to medial shell.

or core) may be masked by local anesthesia (Ikemoto and Witkin, 2003). Nevertheless, DA antagonist microinjection experiments suggest that it is the medial shell rather than the core that mediates the reinforcing effects of self-administered i.v. cocaine (Bari and Pierce, 2005).

In experiment 2, lesions of the medial shell reduced i.v. cocaine CPP independently of accumbens core; in this experiment, DA denervation in the OT was minimal. When 6-OHDA infusions of medial shell and OT were directly compared (experiment 4), only OT DA innervation significantly predicted i.v. cocaine CPP. These results may indicate that the OT is a stronger mediator of cocaine reward, as concluded from findings based on intracranial cocaine infusion (Ikemoto, 2003). It is unlikely that these lesion effects represent disruptions of memory or learning, as medial shell lesions did not affect CPP induced by morphine (Sellings and Clarke, 2003) or i.p. cocaine (present study), and extensive 6-OHDA lesions of OT did not disrupt amphetamine CPP (Clarke et al., 1990).

Several factors could determine the relative contributions of OT versus medial shell to psychostimulant CPP. First, the nature of the CPP paradigm used may be a factor. Our CPP procedure is based on tactile cues; other types of stimuli may engage other ventral striatal subregions. Another factor of potential importance is the drug in question. Our results suggest that i.v. cocaine CPP engages OT mechanisms. This does not appear to be the case for i.p. amphetamine CPP (Clarke et al., 1990).

Conclusions

The increase in locomotor activity observed after psychostimulant administration appears to be related to increased DA transmission in the NAcc core. In contrast, CPP appears more complex, probably depending on the drug and route of administration. The present study suggests that DA transmission in both medial shell and OT is important for i.v. cocaine CPP. Our findings build on recent evidence suggesting that distinct ventral striatal subregions participate in different aspects of drug reward (Ikemoto, 2003; Sellings and Clarke, 2003; Ikemoto and Donahue, 2005; Pecina and Berridge, 2005). Whether these structures act in concert or independently remains a question for further study (van Dongen et al., 2005).

Acknowledgments

We thank Robert Sorge for help with intravenous catheterization surgeries, Annie Landau for making photomicrographs, Annie Constantin for optimizing lesion coordinates, and the National Institute on Drug Abuse for providing cocaine.

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Neuroscience 141 (2006) 1457–1468

CHARACTERIZATION OF DOPAMINE-DEPENDENT REWARDING AND LOCOMOTOR STIMULANT EFFECTS OF INTRAVENOUSLY-ADMINISTERED METHYLPHENIDATE IN RATS

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Abstract—In general, psychostimulants are thought to exert rewarding and locomotor stimulating effects via increased dopamine transmission in the ventral striatum. However, little is known about the mechanisms underlying the effects of the stimulant drug methylphenidate. The present study examined the putative role of dopaminergic transmission in i.v. methylphenidate reward as measured by conditioned place preference. Rats were shown to exhibit conditioned place preference for i.v. methylphenidate (5 mg/kg, not 2 mg/kg). Administration of the dopamine receptor antagonist cis-flupenthixol (0.1–0.8 mg/kg i.p.), either during conditioning or on test day, dose-dependently attenuated the magnitude of the conditioned place preference. Finally, we examined the effects of bilateral 6-hydroxydopamine lesions of nucleus accumbens core, medial shell or anteromedial olfactory tubercle on the rewarding and locomotor stimulant effects of methylphenidate. Residual dopamine innervation, as assessed by radioligand binding to the dopamine transporter, revealed a significant association between core dopamine innervation and the locomotor stimulant effect of methylphenidate. However, neither core nor medial shell dopamine innervation was related to conditioned place preference magnitude. Instead, conditioned place preference magnitude was associated with dopamine innervation in the anteromedial olfactory tubercle. These results establish a role for dopaminergic transmission in both i.v. methylphenidate conditioned place preference and locomotor stimulation. As well, they suggest that different ventral striatal subregions mediate the rewarding (anteromedial olfactory tubercle) and locomotor stimulant (accumbens core) effects of methylphenidate.

Key words: dopamine, nucleus accumbens core, nucleus accumbens medial shell, olfactory tubercle, conditioned place preference, cis-flupenthixol.

Considerable evidence indicates that the rewarding and behavioral activating effects of cocaine and amphetamine occur via increased dopaminergic (DAergic) transmission in the ventral striatum (Koob et al., 1998; Everitt and Wolf, 2002; Wise, 2004). Much less is known in this regard about other psychostimulant drugs such as methylphenidate. Like cocaine, methylphenidate blocks the dopamine transporter (DAT) and increases interstitial dopamine (DA) levels in the nucleus accumbens (NAcc) in rats (Gerasimov et al., 2000). In addition, PET studies employing [^{11}C]raclopride binding have suggested that i.v. methylphenidate can also increase DA transmission in the human striatum (Volkow et al., 2004). Based on this evidence and by analogy with other psychostimulants, Volkow et al. (2004) have proposed that the euphoric and/or reinforcing effects of methylphenidate are dependent on striatal DA transmission. However, to our knowledge, no causal link between increased DA transmission and methylphenidate reward has been established.

Rewarding effects of methylphenidate occur not only in humans but have also been shown in animals; the drug is self-administered i.v. in several mammalian species including non-human primates as a replacement for other stimulant drugs (Bergman et al., 1989; Kollins et al., 2001), and it also induces conditioned place preference (CPP) in rats (Martin-Iverson et al., 1985; Mithani et al., 1986; Meririnne et al., 2001). The pharmacology of methylphenidate self-administration remains to be explored, but evidence to date suggests that methylphenidate CPP can occur independently of brain DA. In particular, CPP acquisition is inhibited only at very high doses of DA antagonists (Martin-Iverson et al., 1985; Mithani et al., 1986; Meririnne et al., 2001), and DAT knock-out mice are capable of exhibiting methylphenidate CPP (Sora et al., 1998).

In previously published CPP studies, methylphenidate was given by i.p. injection. Studies with cocaine have shown that route of administration can critically determine abuse liability and can also determine whether CPP occurs via a DAergic or non-DAergic mechanism (Spyraki et al., 1982, 1987; Nomikos and Spyraki, 1988). Abuse liability of methylphenidate in humans is presumably also route dependent. Although oral methylphenidate exhibits minimal abuse liability (Swanson and Volkow, 2003), intranasal abuse is common (Barrett et al., 2005) and there are several reports of i.v. use (Parran and Jasinski, 1991; Barrett et al., 2005). In light of this, the rewarding effects of i.v. methylphenidate warrant separate examination.

Recent rodent studies using amphetamine and cocaine suggest that rewarding and locomotor stimulant drug effects can be anatomically dissociated within the ventral striatum. To date, reward processes have been most clearly linked to the medial portion of the NAcc shell (Di Chiara et al., 2004). For example, direct and indirect

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Abbreviations: amOT, anteromedial olfactory tubercle; CPP, conditioned place preference; DA, dopamine; DAergic, dopaminergic; DAT, dopamine transporter; [^{125}I]-RTI-55, [^{125}I]-3-β-(4-iodophenyl)tropan-2-β-carboxylic acid methyl ester; NAcc, nucleus accumbens; OT, olfactory tubercle; SERT, serotonin transporter; 6-OHDA, 6-hydroxydopamine.

0306-4522/06$30.00+0.00
doi:10.1016/j.neuroscience.2006.04.040

DAergic agonists are self-administered by rats directly into this subregion but not into NAcc core (Ikemoto and Wise, 2004). However, recent studies have implicated the (antero)medial olfactory tubercle (amOT) as potentially more important than the medial shell in both cocaine and amphetamine reward (Ikemoto, 2003; Ikemoto et al., 2005; Sellings et al., 2006). In contrast, locomotor stimulation has been reported in rats after focal infusion of psychostimulants and direct DA agonists into core and/or shell sites, depending on the drug in question (e.g. Ikemoto, 2002). Using an alternate approach, we recently combined systemic amphetamine challenge with prior 6-hydroxydopamine (6-OHDA) lesions of NAcc core or medial shell (Sellings and Clarke, 2003). In this study, DAergic depletion in core and medial shell reduced amphetamine-induced locomotor stimulation and CPP, respectively.

The aims of the present study were three-fold. First, we set out to establish whether rats would form a CPP for i.v. methylphenidate. Second, we tested if systemic DA receptor blockade would affect either the acquisition or the expression of methylphenidate CPP. The final aim was to determine if the rewarding and locomotor stimulant effects of i.v. methylphenidate could be dissociated by anatomically-selective 6-OHDA lesions of ventral striatal subregions, including NAcc core, medial shell, and medial olfactory tubercle (OT) as previously seen with cocaine (Sellings et al., 2006).

EXPERIMENTAL PROCEDURES

Subjects

Subjects were 111 male Long-Evans rats (Charles River, St. Constant, Quebec, Canada) weighing 270–340 g at time of surgery. Rats were housed individually (experiments 1 and 4) or in groups of three (experiments 2, 3 and 5) in clear Plexiglas cages in a temperature- and humidity-controlled animal colony, lit from 7 A.M. to 7 P.M. Food and water were available *ad libitum* except during behavioral testing. All experiments were approved by the McGill Faculty of Medicine Animal Care Committee in accordance with Canadian Council on Animal Care guidelines. Experiments were carried out in accordance with the European Communities Council Directive (86/609/ECC) for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering.

Intravenous catheterization

Rats were implanted with chronic indwelling Silastic catheters (0.51 mm I.D. and 0.94 mm O.D., Fisher Scientific, Montreal, Quebec, Canada) in the left jugular vein under ketamine (80 mg/kg) and xylazine (16 mg/kg) anesthesia. Tubing was secured to the vein by surgical silk sutures, led s.c. to the skull surface, and was then fitted onto a 22 gauge cannula attached to a plastic connector (Model number C313G-5UP, Plastics One, Roanoke, VA, USA). The cannula/connector was fixed to the animal's skull with small stainless steel screws (Lomir, Notre-Dame-de-L'Ile Perrot, Quebec, Canada) and dental cement (Stoelting, Wood Dale, IL, USA). To keep catheters patent, 0.1–0.15 ml heparinized 0.9% saline was administered at the end of surgery, on the first day of behavioral testing, and every 2–3 days thereafter. Animals were allowed 7–10 days recovery from surgery before starting CPP testing.

Stereotaxic infusion of 6-OHDA

In experiment 4, at the same time as i.v. catheterization surgery, rats were placed in a stereotaxic apparatus (Kopf, Tujunga, CA, USA) with the incisor bar set at −3.9 mm. Bilateral infusions of either 6-OHDA (lesioned groups) or vehicle (sham-lesioned groups) were made into either NAcc core or medial shell, or amOT. Infusions into all three lesion sites were made via a 30 gauge stainless steel cannula attached by polyethylene tubing to a 10 μl Hamilton syringe. For core and medial shell, syringes were driven by a model 5000 Micro Injection Unit (Kopf). For amOT, syringes were driven by a syringe pump. For greater accuracy, coordinates for all three target subregions were derived from the mean of bregma and interaural coordinate systems. Thus, anterior–posterior coordinates were +10.2 mm from interaural zero and +1.2 mm from bregma for both core and shell; in amOT, they were +10.7 mm and +1.7 mm from interaural zero and bregma respectively. Lateral coordinates were ±0.6 mm (shell), ±2.4 mm (core) or ±0.8 mm (amOT). Ventral coordinates for shell (three injections) were +2.0, +2.4, and +2.8 mm from interaural zero and −8.0, −7.6, and −7.2 mm from bregma. Ventral coordinates for core were +2.7 mm from interaural zero and −7.3 mm from bregma; for mOT, they were +1.1 mm from interaural zero and −8.9 mm from bregma. These lesion parameters were based on pilot studies, and represented the best compromise between target structure DAT depletion and anatomical selectivity. The core and amOT are roughly spherical, lending themselves to single infusions, whereas the medial shell is vertically elongated and hence best depleted with a ventrodorsal series of infusions. All coordinates are based on the atlas of (Paxinos and Watson, 1997).

6-OHDA or vehicle was infused on each side in a volume of 0.2 μl (amOT), 0.1 μl (core), or as three infusions of 0.05 μl (medial shell). The rate of infusion was 0.1 μl/min for core and medial shell, and 0.1 μl/10 min for amOT. The concentration of 6-OHDA used was 80 μg/μl (core), 48 μg/μl (medial shell) or 40 μg/μl (amOT). The cannula remained at the final infusion site for 5 min. Animals were allowed 7–10 days' recovery prior to the start of conditioning.

CPP and locomotor activity testing

The apparatus and general procedure were as previously described (Sellings and Clarke, 2003). Briefly, the procedure consisted of three phases: pre-exposure (one day), conditioning (six days) and test (one day). All phases were carried out in a one-compartment box (58 cm×29 cm×53 cm) with walls made of white plastic-coated particle board. In the one-day pre-exposure phase, rats received i.v. saline infusions immediate prior to placement in the CPP cage. Beta Chip sawdust bedding (NEPCO, Warrensburg, NY, USA) covered the floor of the cage. The conditioning phase lasted six consecutive days, with one session of 15 min occurring each day. In all, there were three sessions with drug and three sessions with saline administration, occurring on alternating days. Two square tactile tiles of either bar or mesh texture were placed in the bottom of the cage, and paired with drug or saline administration. During this phase, the video tracking software (EthoVision v 3.0, Noldus Information Technology, Leesburg, VA, USA) measured locomotor activity, expressed as horizontal distance moved (in meters). During the test phase, one bar and one mesh tile were placed on the bottom of the cage. The time spent on bar or mesh texture was measured by EthoVision software. All three phases were carried out under darkroom lighting using a Kodak GBX-2 safelight filter (Vistek, Toronto, Ontario, Canada), to minimize visual cues. Animals do not spontaneously prefer either texture (L. H. L. Sellings and P. B. S. Clarke, unpublished observations), and all experiments were as fully counterbalanced as possible with respect to drug–texture pairing and order of drug pairing (drug–saline or saline–drug) within each surgery group. For all experiments, pre-exposure sessions lasted 20 min, conditioning sessions

for 15 min, and the test session 10 min. To facilitate i.v. infusion immediately after placement in the test cage, a fluid swivel was fixed above the center of each cage. Each swivel was connected to on one end to a 1 ml syringe, and on the other end to a brass connector (Produits MSM, Laval, Quebec, Canada) and protective spring (Heiplex, Montreal, Canada) via Tygon tubing of 0.51 mm diameter (Fisher Scientific). The cannula fixed to the skull of the rat was attached to the Tygon tubing, and the brass connector fastened to the plastic connector, to secure the tubing to the cannula. Drug was infused over 25–30 s at a volume of 1 ml/kg.

Experimental design

Experiment 1. Rats (n=17) were conditioned at one of two doses of i.v. methylphenidate (2 mg/kg, n=8 and 5 mg/kg, n=9) and subsequently tested for CPP.

Experiment 2. Rats (n=25) were administered one of four doses of cis-flupenthixol (0 mg/kg (n=5), 0.1 mg/kg (n=7), 0.3 mg/kg (n=8) or 0.8 mg/kg (n=5)) s.c., 30 min prior to each of the six conditioning sessions (drug: 5 mg/kg methylphenidate, i.v.), and subsequently tested for CPP.

Experiment 3. Rats (n=28) were conditioned with 5 mg/kg i.v. methylphenidate. On test day, rats received one of four doses of cis-flupenthixol (0 mg/kg (n=6), 0.1 mg/kg (n=7), 0.3 mg/kg (n=8) or 0.8 mg/kg (n=7)) s.c., 30 min prior to placement in the cage.

Experiment 4. Rats (n=25) sustaining vehicle (sham-lesioned; n=6) or 6-OHDA infusion into core (core-lesioned; n=9) or medial shell (shell-lesioned; n=10) were subsequently conditioned with 5 mg/kg i.v. methylphenidate after recovering from surgery as described above.

Experiment 5. Rats (n=17) sustaining amOT vehicle (sham; n=6) or 6-OHDA (lesion; n=11) infusions were subsequently conditioned with 5 mg/kg i.v. methylphenidate after recovering from surgery.

Tissue preparation

Tissue was prepared for autoradiography and Nissl-staining (Cresyl Violet) as previously described (Sellings and Clarke, 2003). Briefly, rats were killed 3–5 h following CPP testing, by decapitation under sodium pentobarbital (20 mg/kg, i.v.) anesthesia. Rats not anesthetized within 10 s of injection were excluded from statistical analysis. Brains were removed, frozen in 2-methylbutane at −50 °C for 30 s, and stored at −40 °C. Coronal sections (20 μm) were taken on a cryostat at four rostrocaudal levels (11.2, 10.7, 10.2 and 9.7 mm anterior to interaural zero) through the ventral striatum. At each level, four adjacent sections were collected for autoradiography and one for Nissl staining with Cresyl Violet. Sections were thaw mounted onto gelatin-subbed slides, air dried at room temperature for 20–30 min, and stored with desiccant at −40 °C.

Quantitative autoradiography

The extent of the 6-OHDA lesion was quantified by autoradiographic labeling of DAT (Sellings and Clarke, 2003), using a nonsaturating concentration of [^{125}I]-3-β-(4-iodophenyl)tropan-2-β-carboxylic acid methyl ester ([^{125}I]RTI-55; 2200 Ci/mmol; NEN-Mandel, Guelph, Ontario, Canada). This radioligand allows visualization of either DAT or serotonin transporter (SERT) binding. To visualize DAT binding, SERT was occluded using the serotonin selective reuptake inhibitor citalopram HBr (50 nM). Analogously, to visualize SERT binding, DAT was occluded using the DAT reuptake inhibitor GBR 12935·2HCl (1 μM). Sections were thawed at room temperature for 10 min and then placed in a staining dish containing an aqueous buffer solution of 120 mM NaCl, 0.1 M sucrose, 10 mM sodium phosphate buffer, and 10 pM [^{125}I]RTI-55, with the pH adjusted to 7.4. Nonspecific binding was determined by addition of 10 μM GBR 12909 and 50 nM citalopram HBr in the DAT and SERT autoradiographic assays, respectively. Slides were incubated at room temperature for 2 h and then washed three times in cold buffer solution (once for 1 min, twice for 20 min) and for 1–2 s in distilled and deionized water. They were then blow dried and placed in X-ray film cassettes. Kodak BioMax MS film (Amersham Biosciences, Baie d'Urfé, Québec, Canada) was exposed to slides for 48 h (DAT) or 120 h (SERT) with [^{125}I] autoradiographic standards (Amersham Biosciences). After development of film, DAT and SERT binding was quantified using an MCID M4 imaging system (Imaging Research, St. Catherines, Ontario, Canada). The mean DAT binding was first calculated at each anteroposterior level and these mean values were then averaged across levels.

Histological examination

Tissue was stained with Cresyl Violet to assess nonspecific damage, as previously described (Sellings and Clarke, 2003).

Drugs

Drug sources were as follows: methylphenidate SO_4 (gift of National Institute on Drug Abuse, Bethesda, MD, USA); cis-flupenthixol (Sigma-Aldrich, Oakville, Ontario, Canada); citalopram HBr (gift from H. Lundbeck A/S); ketamine HCl (Vetalar, Vetrepharm, London, Ontario, Canada); xylazine HCl (Anased, Novopharm, Toronto, Ontario, Canada); GBR 12909 (NIMH Chemical Synthesis and Drug Supply Program), and GBR 12935·2HCl (Sigma-Aldrich). Unless otherwise stated, all other chemicals were obtained from Fisher Scientific. Both methylphenidate SO_4 and cis-flupenthixol were dissolved in sterile 0.9% saline and injected at 1 ml/kg. Methylphenidate was administered i.v. immediately after placement in CPP boxes. Cis-flupenthixol was administered i.p. 30 min prior to all conditioning sessions (experiment 2) or the CPP test (experiment 3). 6-OHDA HBr was dissolved in sterile 0.9% saline containing 0.3 mg/ml sodium metabisulfite (Sigma-Aldrich) as an antioxidant and protected from light. Vehicle solutions, as well as 6-OHDA to be infused into medial shell, were neutralized to pH 7.3±0.1 with NaOH (to reduce non-specific damage; see Results). Doses of all drugs except 6-OHDA HBr are expressed as the salt. 6-OHDA HBr doses are expressed as the free base.

Data analysis

A commercial software program (Systat v10.2, SPSS Inc., Chicago, IL, USA) was used for all data analyses. CPP magnitude was calculated as the difference between times spent on the drug-paired and vehicle-paired sides during the 10-minute test session. Locomotor responses to methylphenidate were calculated as the difference of locomotor counts between drug and saline conditioning sessions. Saline scores were calculated as the mean activity over all three conditioning sessions with saline. For experiment 2, group differences for both CPP magnitude and saline or methylphenidate-induced locomotor activity were analyzed by ANOVA followed by Dunnett's test. The existence of significant CPP magnitude was determined by one-sample t-tests with Bonferroni correction. For experiment 3, since data were not normally distributed, Kruskal-Wallis ANOVA followed by multiple Mann-Whitney U tests with Bonferroni correction were used to compare CPP magnitude after cis-flupenthixol treatment to the control (dose=0) group. In addition, the existence of a significant CPP magnitude and locomotor stimulant effect was determined by the Wilcoxon test between times spent on the drug-paired vs. saline-paired texture, with Bonferroni correction for multiple comparisons. For experiment 4, group differences were analyzed by

ANOVA. To determine whether rats experienced locomotor sensitization, three factors were used: LESION (i.e. 6-OHDA vs. vehicle infusion [sham]), AREA (i.e. core vs. medial shell) and SESSION (i.e. difference scores [methylphenidate-saline] over successive pairs of conditioning sessions). In experiment 5, group differences were examined by Student's *t*-test. For both experiments 4 and 5, the relationship between behavioral measures vs. [^{125}I]-RTI-55 labeling was analyzed by linear regression (multiple in experiment 4, single in experiment 5). Values given for *r* are derived from single linear regression analyses in both experiments. A *P* value of less than 0.05 (two-tailed) was considered significant. Group data are expressed as mean±S.E.M. throughout. In experiments 4 and 5, analyses on the DAT and SERT autoradiography were done by post hoc Dunnett's test and multiple *t*-tests, respectively, with Bonferroni correction. Outliers, as defined by the statistical program, were removed prior to statistical analysis. Additionally, in experiment 4, CPP data from four rats were missing due to an equipment malfunction during testing.

RESULTS

Experiment 1: rats express a CPP for i.v. methylphenidate

The occurrence of i.v. methylphenidate CPP was initially established in experiment 1. Here, rats were conditioned with either 2 mg/kg methylphenidate (n=8) or 5 mg/kg methylphenidate (n=9). CPP magnitude was calculated as the difference between times spent on the drug-paired and vehicle-paired sides during the 10-minute test session. Rats formed a significant preference for the floor texture paired with the higher dose only (P<0.005, Fig. 1A). Rats conditioned with 2 mg/kg spent 274±18 s on the saline-paired texture and 326±18 s on the methylphenidate-paired texture. For rats conditioned with 5 mg/kg, 195±18 s were spent on the saline paired texture, and 405±18 s on the methylphenidate-paired texture. Additionally, rats formed CPP regardless of which texture was the conditioned stimulus; CPP magnitude did not differ significantly between rats conditioned with bar vs. those conditioned with mesh texture (calculated as the difference between times spent on the drug-paired and vehicle-paired textures: P>0.5, Fig. 1B). Locomotor activity measured during the conditioning phase was significantly stimulated by both doses of methylphenidate (P<0.005 for both, Fig. 1C).

Experiment 2: the acquisition of a CPP for i.v. methylphenidate is dose-dependently attenuated by cis-flupenthixol given during conditioning

The effect of systemic DA receptor blockade on the acquisition of i.v. methylphenidate CPP was investigated in experiment 2. Here, rats received 0, 0.1, 0.3 or 0.8 mg/kg cis-flupenthixol i.p. 30 min prior to each conditioning session. Rats were conditioned with 5 mg/kg methylphenidate. Only rats receiving vehicle or 0.1 mg/kg cis-flupenthixol exhibited significant CPP (P<0.01 for both; one-sample *t*-test with Bonferroni correction, Fig. 2A). Only the 0.3 mg/kg group differed significantly from control (Dunnett's test P<0.05; Fig. 2A), with a similar trend in the 0.8 mg/kg group (P=0.087; Fig. 2A). Locomotor activity after saline administration was significantly inhibited by the 0.8 mg/kg dose (Dunnett's test P<0.005, Fig. 2B). In view of this, the locomotor stimulant effect of methylphenidate was not examined by a difference score (i.e. drug–saline). Activity in methylphenidate sessions was also reduced by the 0.8 mg/kg dose (Dunnett's test: P<0.05, Fig. 2B).

Experiment 3: CPP expression for i.v. methylphenidate is dose-dependently attenuated by cis-flupenthixol administration on test day

The effect of systemic DA receptor blockade on the expression of i.v. methylphenidate CPP was investigated in experiment 3. Here, rats were conditioned with 5 mg/kg methylphenidate and subsequently received 0, 0.1, 0.3 or 0.8 mg/kg cis-flupenthixol i.p. 30 min prior to CPP testing. Only rats receiving vehicle or 0.1 mg/kg cis-flupenthixol exhibited significant CPP (P<0.05 for both; Wilcoxon test with Bonferroni correction; Fig. 2C). Only the 0.3 mg/kg

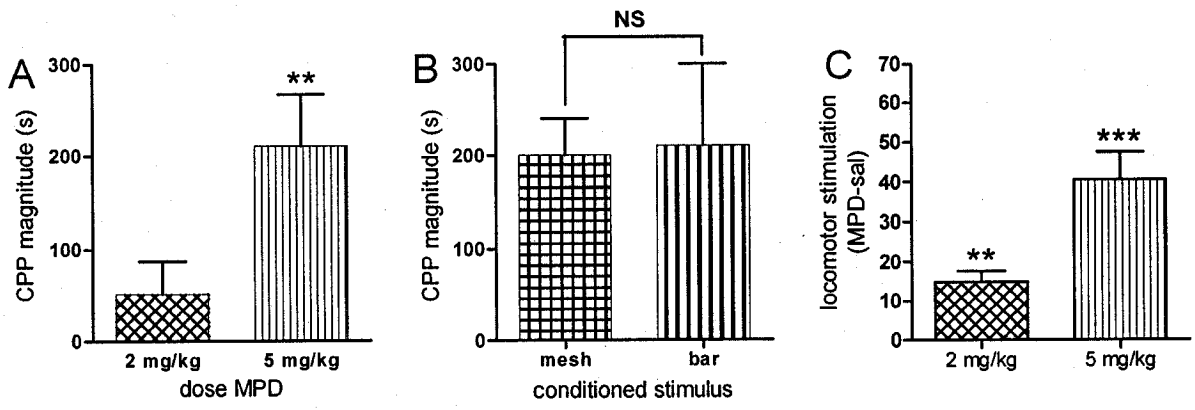

Fig. 1. Establishment of i.v. methylphenidate CPP in intact rats. Rats (n=8–9 per group) were tested in a CPP procedure after three vehicle exposures and three i.v. methylphenidate exposures of either 2 mg/kg or 5 mg/kg. (A) Rats receiving three pairings with 5 mg/kg showed a significant place preference (** P<0.005, one-sample *t*-test with Bonferroni correction) whereas those receiving 2 mg/kg did not (P>0.30). (B) Rats conditioned to either texture; rats receiving 5 mg/kg methylphenidate expressed a significant CPP regardless of whether the drug was paired with bar or mesh tiles. (C) Rats exhibit significant locomotor stimulation at both the 2 mg/kg and 5 mg/kg group (** P<0.005, *** P<0.0005, one-sample *t*-test with Bonferroni correction).

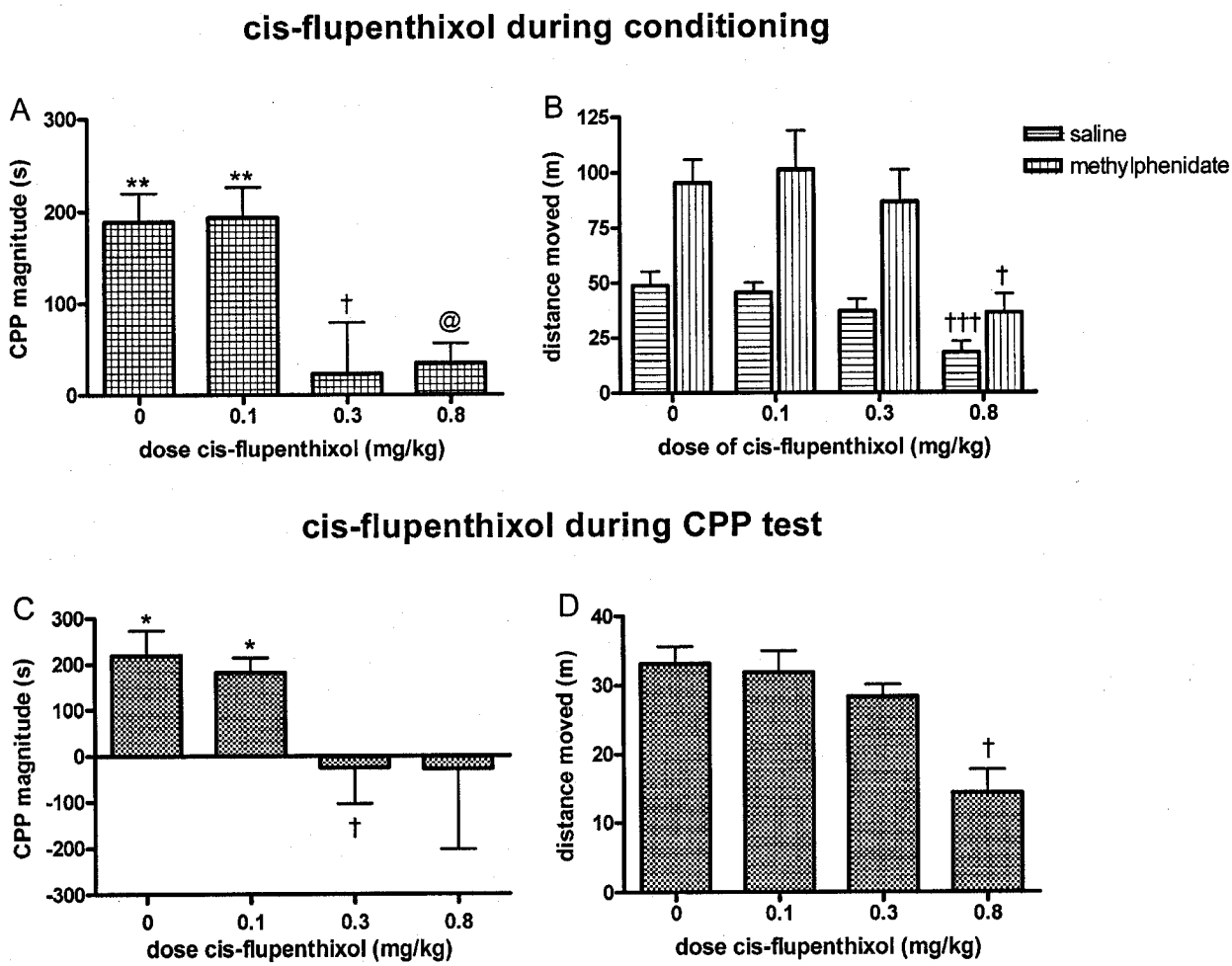

Fig. 2. Effect of cis-flupenthixol on the acquisition and expression of i.v. methylphenidate CPP. In all experiments, rats were trained in the CPP paradigm with 5 mg/kg methylphenidate. During conditioning (A and B), or on test day (C and D), rats received either 0, 0.1, 0.3 or 0.8 mg/kg cis-flupenthixol (n=5–8 per group). When given during conditioning, only rats receiving vehicle or 0.1 mg/kg cis-flupenthixol exhibited significant CPP (** $P<0.01$, one-sample t-tests with Bonferroni correction, A). Cis-flupenthixol dose-dependently reduced CPP acquisition for i.v. methylphenidate († $P<0.05$, @ $P=0.087$, Dunnett's test, A). Cis-flupenthixol treatment also reduced locomotor activity in saline sessions (††† $P<0.005$, Dunnett's test, B) and in methylphenidate sessions († $P<0.05$, Dunnett's test, B). When given on test day, only rats receiving 0 or 0.1 mg/kg cis-flupenthixol exhibited significant CPP (* $P<0.05$, one-sample t-test with Bonferroni correction, C). Significant reduction of CPP expression was observed after treatment with 0.3 mg/kg cis-flupenthixol († $P<0.05$, Mann-Whitney U test with Bonferroni correction, C). Only rats receiving the highest dose (0.8 mg/kg) exhibited significant hypoactivity on test day (* $P<0.05$, Mann-Whitney U test with Bonferroni correction, D).

group differed significantly from control (Mann-Whitney U with Bonferroni correction $P<0.05$; Fig. 2C). At the highest antagonist dose (0.8 mg/kg), CPP magnitude was highly variable and hard to interpret, since the animals were not only less active ($P<0.05$; Mann-Whitney with Bonferroni correction; Fig. 2D), but also tended to "camp" in a small area of the test box; indeed, several rats spent the entire 10-minute test session on one side of the test box.

Neurochemical and anatomical selectivity after 6-OHDA lesion (experiments 4 and 5)

To assess nonspecific tissue damage, sections were Nissl-stained with Cresyl Violet. As previously reported (Sellings et al., 2006), only minimal cell loss was evident at the site of infusion for all sham-lesioned groups and for the group infused with 6-OHDA in the core subregion. Tissue from rats infused with 6-OHDA in medial shell or amOT exhibited a region of decreased cell density compared with control. This region of nonspecific damage did not extend more than 0.3 mm from the site of infusion. Sampling locations for DAT and SERT binding density, RTI-55 autoradiographs of DAT and SERT binding are shown in Fig. 3. For brevity, only one hemisphere is shown; lesions were bilateral and imaging was performed on both hemispheres. Residual DAT and SERT binding as a percent of combined sham groups are given in Tables 1 and 2. Radioligand binding to SERT in tissue from lesioned animals was minimally changed in 6-OHDA vs. sham-lesioned rats. Radioligand binding is not changed after vehicle infusion

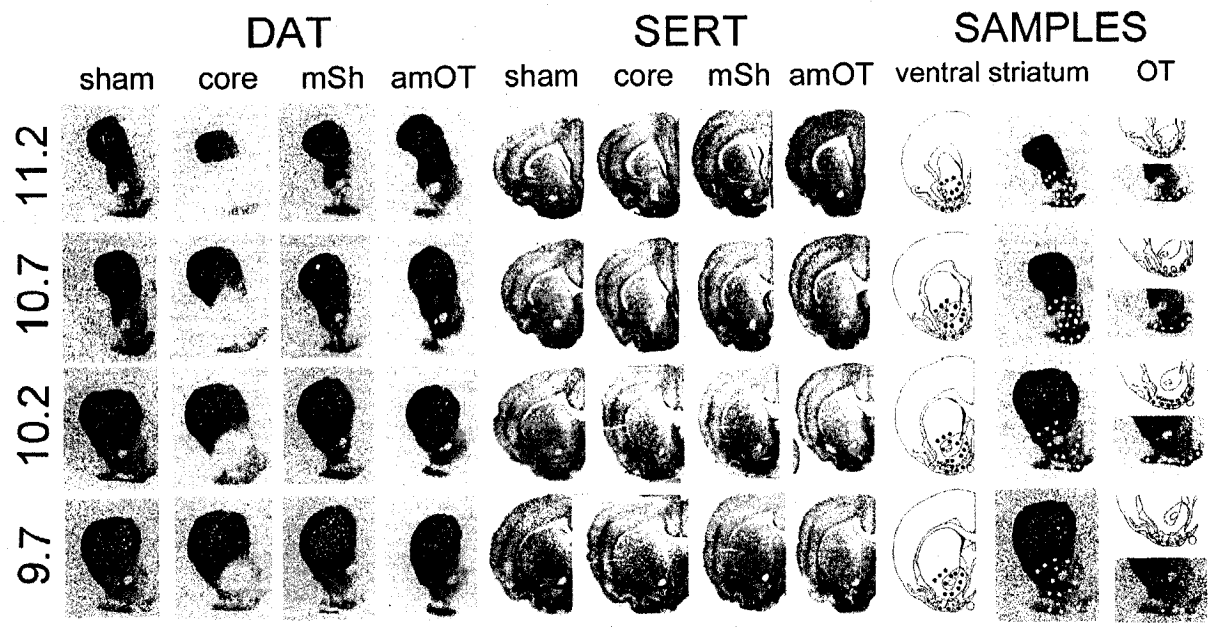

Fig. 3. Autoradiographs for DAT and SERT binding. Fig. 3 shows DAT and SERT autoradiographs for sham-, core-, medial shell- and amOT-lesioned rats at four rostrocaudal levels. Excess cold conditions for both DAT and SERT binding were negligible. Areas sampled to determine depletions are shown at right (i.e. NAcc core, medial shell, ventral shell, OT and ventral caudate-putamen). Depletions in DAT and SERT binding were calculated as a percentage of sham-lesioned rats, by taking the mean of all sampled areas (shown at right) at a particular level for all four rostrocaudal levels, and ultimately taking a mean of these four numbers. Sampled areas for core, medial shell, ventral shell and ventral caudate putamen were identical for both experiments 4 and 5, and are depicted both in cartoon form and superimposed onto DAT autoradiographic images from a sham-lesioned rat. OT was analyzed as a homogenous structure for experiment 4 (as seen under "ventral striatal subregions"), but as three heterogeneous subregions in experiment 5 (based on Ikemoto, 2003; OT subregions). These three subdivisions were amOT, anterolateral OT and posterior OT. The former two were examined at levels 11.2, 10.7 and 10.2. The latter (posterior OT) was examined at levels 9.7, 9.2 and 8.7 (only level 9.7 shown, see Sellings et al., 2006 for details).

(i.e. sham lesion) vs. intact tissue, as rats receiving unilateral vehicle infusions show no changes in DAT or SERT binding on the intact vs. sham lesioned side (L. H. L. Sellings and P. B. S. Clarke, unpublished observations).

Experiment 4: Effects of 6-OHDA lesions of NAcc core vs. medial shell on i.v. methylphenidate CPP and locomotor activity

Here, rats received intracerebral infusion of 6-OHDA aimed at either accumbens core or medial shell 7–10 days prior to the start of conditioning.

The locomotor response to i.v. methylphenidate was attenuated by core, but not medial shell lesions

In experiment 4, rats did not exhibit significant locomotor sensitization (SESSION: $F(2,42)=3.50$, $P>0.05$; Fig. 4A). However, to avoid any potential confounding factor of lesion effects on locomotor sensitization, the locomotor difference score (methylphenidate–saline) from the first drug and saline conditioning session were used, so that locomotor scores were examined from only the first drug exposure. Saline test locomotor activity did not differ significantly be-

Table 1. Reductions in DAT and SERT binding seen in core- and medial shell–lesioned groups (experiment 4)

Surgery group	DAT			SERT		
	Sham	Core	mShell	Sham	Core	mShell
Sampled region						
Core	100±7	20±3****	95±5	100±5	108±6	97±3
mSh	100±8	48±4††	38±6***	100±4	97±5	94±3
vSh	100±6	35±6††	98±8	100±6	118±8	108±3
OT	100±12	46±4***	80±4	100±6	116±5	108±4
vCP	100±10	50±7**	107±6	100±3	88±3	93±3

Columns represent each of the three different surgery groups: sham-lesioned, core and medial shell. DAT and SERT binding were determined in each rat. Rows represent the transporter binding in five ventral striatal subregions, expressed as the mean±SEM percentage of sham-lesioned rats. Abbreviations are as follows: mSh, medial shell; vCP, ventral caudate-putamen; vSh, ventral shell.

** $P<0.005$; †† $P<0.001$; *** $P<0.0005$; **** $P<0.00005$ vs. sham-lesioned control, Dunnett's test for each brain area, Bonferroni-corrected.

Table 2. Reductions in DAT and SERT binding seen in anteromedial OT-lesioned rats (experiment 5)

Surgery group	DAT		SERT	
	Sham	amOT	Sham	amOT
Sampled region				
Core	100±5	96±4	100±10	97±7
mSh	100±5	95±6	100±9	98±10
vSh	100±7	85±4	100±8	97±12
amOT	100±17	26±5††	100±2	106±10
alOT	100±10	64±7	100±3	107±14
pmOT	100±5	82±4	100±8	104±10
plOT	100±6	95±3	100±5	113±9
vCP	100±7	90±3	100±5	97±7

Columns represent each of the two different surgery groups: sham-lesioned and anteromedial OT-lesioned. DAT and SERT binding were determined in each rat. Rows represent the transporter binding in eight ventral striatal subregions, expressed as the mean±SEM SEM percentage of sham-lesioned rats. Abbreviations are as follows: alOT, anterolateral olfactory tubercle; mSh, medial shell; pmOT, posteriormedial olfactory tubercle; plOT, posteriorlateral olfactory tubercle; vCP, ventral caudate-putamen; vSh, ventral shell.

†† $P<0.001$ vs. sham-lesioned control, multiple *t*-tests with Bonferroni correction.

tween surgery groups (LESION×AREA: $F(1,21)=0.03$, $P>0.50$) and were as follows: 58±7 (sham), 54±5 (core 6-OHDA) and 47±1 (medial shell 6-OHDA). All groups exhibited significant locomotor stimulation ($P<0.05$ to $P<0.005$, one-sample *t*-test with Bonferroni correction; Fig. 4B), but this response was smaller in the core-lesioned group compared with the shams ($P<0.05$ for core-lesioned vs. sham-lesioned group, Dunnett's test; Fig. 4B). A significant positive association was observed between the locomotor response to i.v. methylphenidate and core DAT binding ($P<0.005$, $r=0.60$, Fig. 4C). No relationship was apparent for medial shell (Fig. 4D).

CPP magnitude for i.v. methylphenidate related significantly to neither core nor medial shell residual DAT binding

In experiment 4, both the sham- and medial shell-lesioned groups exhibited a significant CPP, with a similar trend in the core-lesioned group ($P=0.06$; Fig. 4E). Multiple regression analysis revealed no significant relationships between CPP magnitude and DAT binding in core (Fig. 4F) or medial shell (Fig. 4G). In view of this negative result, we used stepwise multiple linear regression analysis as a post hoc exploratory tool in order to assess a possible contribution of amOT. Three predictive variables were included in the model: DAT binding in core, medial shell, and amOT. The iterative model showed that medial shell and amOT in combination significantly predicted CPP magnitude, but the contribution of neither structure on its own was significant (amOT positive association, $P=0.055$, medial shell negative association, $P=0.11$).

Experiment 5: effects of 6-OHDA lesions of amOT on i.v. methylphenidate CPP

In light of recent results suggesting that the entirety of the medial ventral striatum is important in psychostimulant induced reward (see Discussion), the effects of 6-OHDA lesions of the amOT on i.v. methylphenidate CPP were examined. In experiment 5, only sham-lesioned animals exhibited significant CPP ($P<0.001$; one-sample *t*-test with Bonferroni correction; Fig. 5A). CPP magnitude differed significantly between sham- and anteromedial OT-lesioned animals ($P<0.005$; Student's *t*-test; Fig. 5A). Additionally, linear regression analysis showed a significant association between the degree of DAT depletion in anteromedial OT and CPP magnitude ($P<0.02$; Fig. 5B). Neither activity after saline administration nor methylphenidate-induced locomotor stimulation was significantly altered by the lesion ($P>0.05$ and $P>0.50$ respectively).

The possibility of contribution of other OT subregions to methylphenidate CPP was examined post hoc using stepwise linear regression analysis. The OT subregions included were amOT, anterolateral olfactory tubercle (alOT), posteriormedial olfactory tubercle (pmOT) and posteriorlateral olfactory tubercle (plOT). The only predictor retained in the final equation was amOT ($P<0.05$).

DISCUSSION

Previous reports have shown that methylphenidate can sustain i.v. self-administration in several species, including non-human primates, rats and dogs (Kollins et al., 2001; Volkow and Swanson, 2003), and can also produce a CPP when given intraperitoneally (Martin-Iverson et al., 1985; Mithani et al., 1986; Meririnne et al., 2001). Here, we extend these findings to show that methylphenidate can also produce a CPP when given i.v. Both acquisition and expression of this CPP were dose-dependently reduced by systemic administration of the D1/D2 receptor antagonist cis-flupenthixol. In contrast, CPP following methylphenidate appeared to be unaffected by DA denervation of either core or medial shell prior to conditioning. Instead, DA denervation in amOT significantly reduced i.v. methylphenidate CPP. The unconditioned locomotor stimulant effect of methylphenidate was dose-dependently reduced by cis-flupenthixol administration and was associated with DA innervation of accumbens core. These results suggest that both the locomotor stimulant and rewarding effects of i.v. administered methylphenidate are DA dependent, and that these effects are segregated within the ventral striatum.

Methodological considerations

Given the size, shape and proximity of brain regions lesioned in this study, substantial depletion of one structure was virtually impossible without affecting other nearby structures to some degree. The multiple linear regression analyses used in the current study circumvented this problem in part by considering the degree to

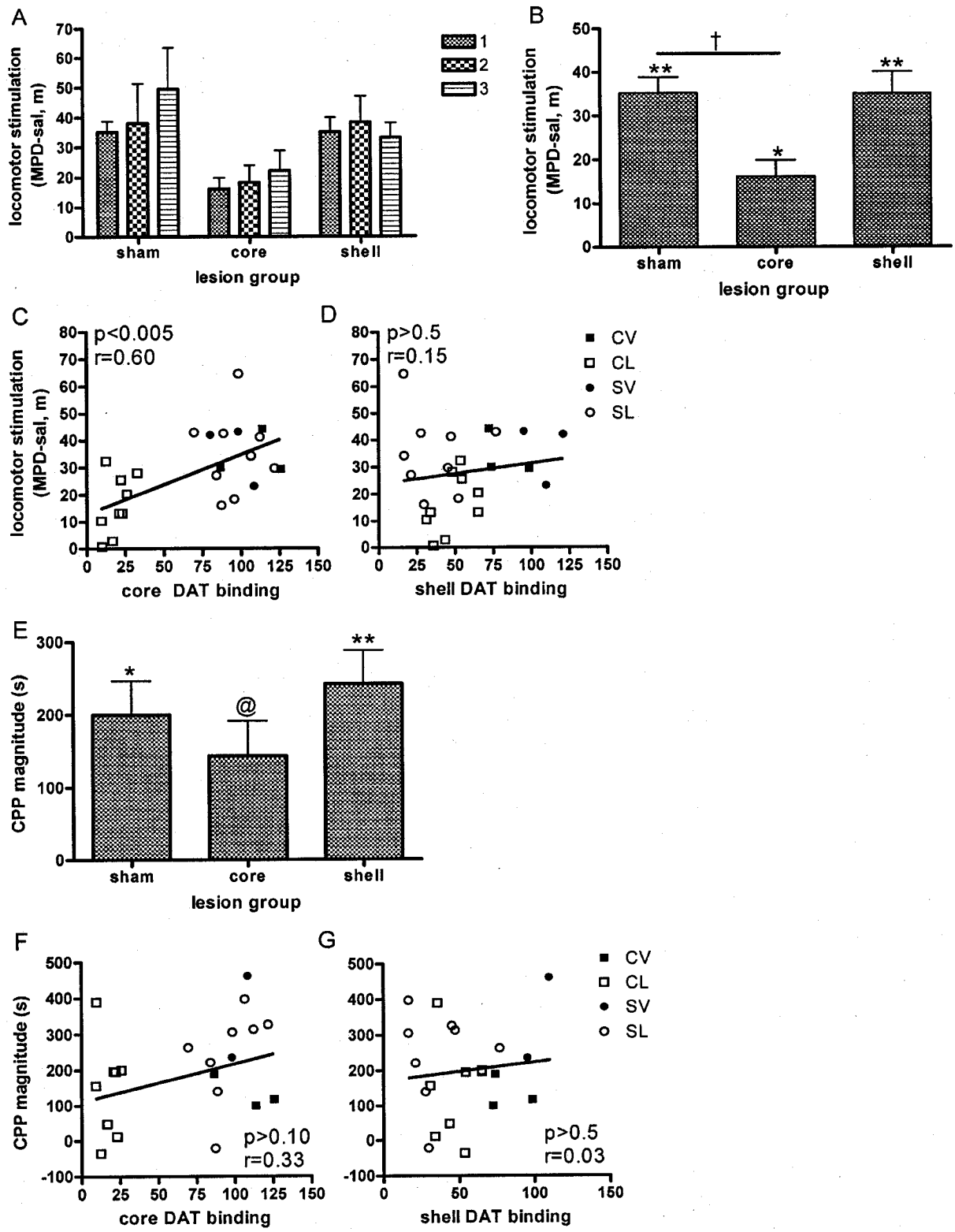

Fig. 4. Effect of 6-OHDA lesions of accumbens core or medial shell on i.v. methylphenidate-induced locomotion and CPP. Rats (n=6–10 per group) received bilateral 6-OHDA or vehicle infusions (i.e. sham-lesion) into accumbens core or medial shell, and were subsequently conditioned with methylphenidate (5 mg/kg). Locomotor scores (methylphenidate–saline) for all three conditioning pairs (i.e. three pairs of methylphenidate and saline exposures during the CPP conditioning phase; indicated as 1, 2 and 3) are shown in panel A. Rats did not exhibit significant behavioral (locomotor)

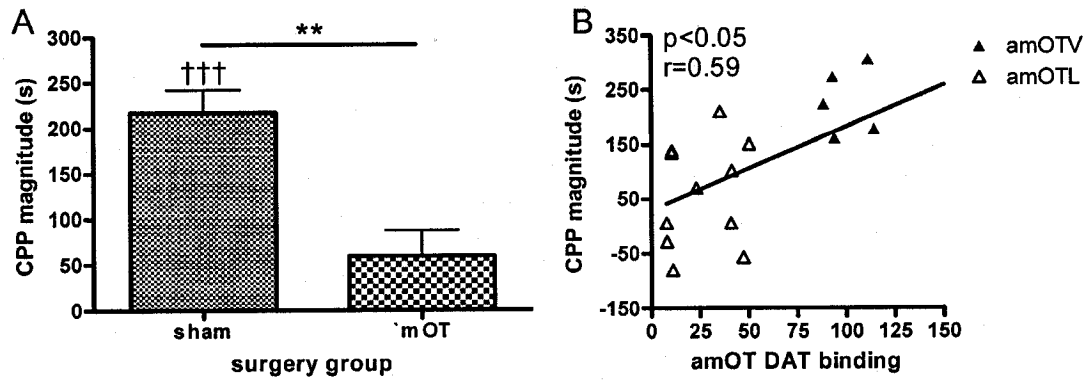

Fig. 5. Effect of 6-OHDA lesions of amOT on i.v. methylphenidate-induced CPP. Rats (n=6–11 per group) received bilateral 6-OHDA or vehicle infusions (i.e. sham lesion) into accumbens core or medial shell, and were subsequently conditioned with methylphenidate (5 mg/kg). Only sham-lesioned rats exhibited a significant CPP, which differed significantly from the CPP magnitude of anteromedial OT-lesioned rats (††† P<0.0001, one-sample t-test with Bonferroni correction; ** P<0.005, Student's t-test; panel A). CPP magnitude correlated significantly with anteromedial OT DAT binding (P<0.02; panel B). DAT binding is expressed as percent of sham-lesioned rats of the target structure being examined. Abbreviations are as follows: amOTV, amOT sham; amOTL, amOT lesion.

which a given structure (core or medial shell) was depleted in each individual animal. One drawback of this method is that it does not allow for the possibility that lesions may have caused non-uniform DAT depletions; indeed, this was the case in a subset of lesioned animals. However, since there were no consistencies as to which portion of any target structure was spared (rostrocaudally, mediolaterally or dorsoventrally), the current method of sampling appears to faithfully represent DAT depletion in ventral striatal structures. This being said, we cannot rule out the possibility that our behavioral effects resulted from damage to functionally important "hot spots" within the targeted structures.

The statistical approach adopted here is essentially correlational; however, when the present results are integrated with previous findings, causal inferences can be made with some confidence. To infer a causal link, it is important to first exclude the possibility that the lesion effects on behavior may have resulted from non-specific damage. This appears unlikely for the following reasons. First, 6-OHDA tends to destroy catecholaminergic neurons quite selectively (Jonsson, 1983) and accordingly, our lesions produced little if any change in 5-HT transporter binding levels. Second, Nissl staining revealed only slight non-specific damage in 6-OHDA lesioned animals compared with sham-operated controls; the area of non-specific damage was confined to a small region directly adjacent to the infusion site.

Methylphenidate most likely increased noradrenergic as well as DAergic transmission in our experiments (Kuczenski and Segal, 1997), and our 6-OHDA infusions almost certainly destroyed noradrenergic as well as DA terminals. We specifically avoided using desipramine pretreatment to protect noradrenergic afferents (e.g. Kelly and Iversen, 1976) since we have observed mortality rates of >25% resulting from the commonly used dose (25 mg/kg i.p.) in this strain of rat (L. H. L. Sellings, A. Constantin and P. B. S. Clarke, unpublished observations). However, loss of noradrenergic afferents is unlikely to account for our lesion effects on either behavioral measure, for the reasons given below.

In terms of psychomotor stimulant-induced locomotion, pharmacological and lesion manipulations of ventral striatal noradrenaline appear to have little or no effect in rats (Pijnenburg et al., 1975; Roberts et al., 1975). Moreover, in the present study, changes in locomotion were associated with lesions in the accumbens core, a subregion which is largely devoid of noradrenergic afferents (Berridge et al., 1997; Delfs et al., 1998).

Reward functions are more clearly associated with medial accumbens shell and amOT (see below). Although these subregions receive significant noradrenergic input (Versteeg et al., 1976; Berridge et al., 1997), several observations indicate that noradrenergic denervation probably did not significantly influence the magnitude of methylphenidate-induced CPP. First, stimulation of noradrenergic transmission does not appear to produce a CPP (Martin-Iverson et al., 1985; Subhan et al., 2000). Second, neither α nor β adrenergic receptor antagonists affect the rewarding effects of i.v. cocaine, as reflected by self-administra-

sensitization. However, all groups exhibited locomotor stimulation. Locomotor stimulation data for only the first conditioning pair (i.e. the difference between the first drug and saline exposures) were further examined. The stimulant response was smaller in the core lesioned group († P<0.05; Dunnett's test; panel B) but still significant. Locomotor response correlated positively and significantly with DAT binding in core (panel C), but not in medial shell (panel D). Sham and medial shell-lesioned groups exhibited a significant CPP, with a similar trend in the core-lesioned group ($^{@}$ P=0.06, * P<0.05, ** P<0.005, one sample t-test with Bonferroni correction, panel E). CPP magnitude did not correlate significantly with either core or medial shell DAT binding (panels F and G). DAT binding is expressed as percent of sham-lesioned rats of the target structure being examined. Values for r are obtained from single linear regression analyses; P-values, however, are from the multiple linear regression analysis. Abbreviations are as follows: CL, core lesion; CV, core vehicle (sham); SL, medial shell lesion; SV, medial shell vehicle (sham). Shell refers to medial shell. N per group: 9 (core lesion), 10 (medial shell lesion), 6 (combined sham groups).

tion behavior (Johanson and Fischman, 1989). Finally, the disruptive effects of ventral striatal 6-OHDA lesions on cocaine self-administration appear unrelated to noradrenaline depletion (Roberts et al., 1977, 1980).

Ventral striatal DA and methylphenidate reward

Previous DA antagonist studies focused on the acquisition of i.p. methylphenidate CPP, and yielded only equivocal evidence for blockade (Martin-Iverson et al., 1985). In the present study, cis-flupenthixol was given either during conditioning, or on the test day, and in both cases i.v. methylphenidate CPP was blocked. At the lower effective dose (0.3 mg/kg), cis-flupenthixol would be expected to act principally on DA receptors, with only a weak antagonist effect at 5-HT2 receptors (Matsubara et al., 1993). The inhibition of methylphenidate CPP was probably not due to a disruption of memory recall, since high doses of D1 or D2 antagonists did not inhibit expression of CPP for i.p. cocaine (Cervo and Samanin, 1995).

DAergic transmission in the NAcc is considered pivotal to psychomotor stimulant reward (Koob et al., 1998; Everitt and Wolf, 2002; Di Chiara et al., 2004; Wise, 2004), and we previously reported a strong association between DA innervation of medial shell and amphetamine CPP (Sellings and Clarke, 2003). However, in the present study, methylphenidate CPP was altered by focal catecholamine depletion in neither accumbens medial shell nor core. One potential explanation of these negative findings is that our lesions were not substantial enough to produce a detectable behavioral deficit, particularly since compensatory neuroadaptations may have occurred in the 7–10 day interval between 6-OHDA infusion and behavioral testing. However, this explanation is unlikely for two reasons. First, core lesions were behaviorally significant, insofar as core-lesioned animals showed a reduced locomotor response to methylphenidate. Second, our medial shell depletions were of similar magnitude to those in a previous study where significant reductions in CPP magnitude were observed after a similar delay between lesion and CPP training (62%; Sellings and Clarke, 2003).

The main finding in the present study was the reduction in CPP magnitude observed after 6-OHDA infusions into amOT. These infusions produced a DAergic depletion of 74% in the target area, with a smaller depletion (36%) in the adjacent anterolateral OT. Importantly, DAT binding in the medial shell was virtually unchanged. This finding suggests that i.v. methylphenidate CPP depends critically on DA transmission in OT, probably in its anteromedial portion. This accords with recent evidence suggesting that the amOT plays a role in psychostimulant reward, and most likely does not represent a memory deficit, as a 6-OHDA lesion of the OT did not impair amphetamine CPP (Clarke et al., 1990). In particular, both cocaine and amphetamine are avidly self administered into anteromedial OT (Ikemoto, 2003; Ikemoto et al., 2005), and 6-OHDA lesions of the OT appear to reduce CPP for i.v. cocaine (Sellings et al., 2006). The present findings, however, do not rule out a role for other subcompartments of the OT in methylphenidate CPP.

The NAcc core and locomotor activation

There is currently no consensus on the role of core vs. shell in psychostimulant-induced locomotor activation (Boye et al., 2001; Ikemoto, 2002 and references therein). Studies employing intra-accumbens microinjection of direct or indirect DAergic agonists have implicated core, shell, or both structures. After focal administration, the relative importance of core vs. shell appears to depend on the drug in question. For example, in a recent study (Ikemoto, 2002), amphetamine acted with similar potency at either injection site, whereas cocaine stimulated locomotor activity more strongly after injection into medial shell. In contrast to published findings based on intracranial infusion, the locomotor stimulant effect of *systemically-administered* amphetamine and cocaine appears dependent on DA transmission in accumbens core rather than shell (Weiner et al., 1996; Boye et al., 2001; Sellings and Clarke, 2003; Sellings et al., 2006). The present results extend this conclusion to methylphenidate, although a contribution from ventral caudate-putamen cannot be ruled out (Campbell et al., 1997). No previous studies have, to our knowledge, examined the relative contributions of medial shell and core to methylphenidate-induced locomotion. Whether core DA transmission directly mediates the locomotor stimulant action of psychomotor stimulants drugs, or instead plays an indirect enabling role, remains a question for the future.

CONCLUSIONS

The present study suggests that the rewarding properties of i.v. methylphenidate are dependent on DA transmission, as also suggested by human imaging studies. More specifically, the anatomical site appears to be the amOT, and not the medial shell. In contrast, a role for i.v. methylphenidate-induced locomotor activity was attributable to accumbens core. These results extend our previous findings with amphetamine and cocaine, and strengthen the hypothesis that psychomotor stimulants exert their stimulant and rewarding effects via increased DA tone in functionally segregated territories within ventral striatum. The possibility that drug reward is mediated by small subregions within ventral striatum has several implications, not least for human PET studies where spatial resolution may be a limiting factor.

Acknowledgments—This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Institutes of Health Research. L.H.L.S holds an NSERC Canada Graduate Scholarship. The authors thank Robert Sorge for help with i.v. catheterization surgeries, Annie Constantin for optimizing lesion coordinates, and the National Institute on Drug Abuse for the gift of methylphenidate.

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(Accepted 20 April 2006)
(Available online 6 June 2006)