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The role of the 5-HT$_{2C}$ receptor in motivation and reward-related behaviour

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Centre for Neuroscience

Edmonton, Alberta
Spring 2009

The only proof of motivation is movement

- Walle J.H. Nauta

Abstract

The mesocorticolimbic dopamine system is important for the regulation of reward-related behaviours, and may be dysfunctional in some psychiatric disorders. Serotonin (5-hydroxytryptamine; 5-HT) also plays a role in dopamine- and reward-related behaviours. The main aim of this thesis was to explore the role of the 5-HT_{2C} receptor in reward-related behaviours, such as locomotor activity, place conditioning, and ventral tegmental area intracranial self-stimulation (ICSS); potential relationships between the 5-HT_{2C} receptor and the nicotinic acetylcholine, 5-HT_{1B}, $GABA_A$, and $GABA_B$ receptors were also investigated. Male Sprague-Dawley rats were administered drugs systemically or directly into the shell of the nucleus accumbens (NAc). The main findings were: 1) 5-HT_{2C} receptor activation, via WAY 161503, decreased basal and nicotine-induced locomotor activity but did not affect place conditioning; these effects were attenuated by the 5-HT_{2C} receptor antagonist SB 242084; 2) 5-HT_{2C} receptor activation, via TFMPP and WAY 161503, increased ICSS thresholds while intra-NAc shell WAY 161503 had no effect; the effects of TFMPP were attenuated by SB 242084; 3) 5-HT_{1B} receptor activation, via CP 94253, increased ICSS thresholds; the $5\text{-HT}_{1B/1D}$ receptor antagonist GR 127935 attenuated the effects of CP 94253 but not WAY 161503; 4) systemic administration of the $GABA_A$ receptor agonist muscimol and the antagonist picrotoxin, the $GABA_B$ receptor agonist baclofen, and WAY 161503, decreased locomotor activity; the effects of muscimol were attenuated by picrotoxin; the effects of WAY 161503 were unaltered by picrotoxin; the effects of baclofen were additive with WAY 161503; 5) intra-NAc shell muscimol increased ICSS thresholds; intra-NAc muscimol and systemic

WAY 161503 did not result in thresholds different from either treatment alone; 6) intra-NAc shell picrotoxin decreased ICSS thresholds and attenuated the effects of systemic WAY 161503; 7) systemic baclofen increased, while intra-NAc shell baclofen had no effect, on ICSS thresholds; these effects were additive when combined with WAY 161503. In summary, these data provide evidence that 5-HT_{2C} receptors may play an inhibitory role in regulating reward-related behaviours. These results may help us to better understand the reward-related circuitry which may underlie many psychiatric disorders such as depression, schizophrenia, and drug addiction, and may also assist in the development of new pharmacotherapies.

Acknowledgements

The accomplishments I have achieved, the knowledge I have gained, and the experiences I have had throughout my graduate career are due, in large part, to the support and contributions of many people.

Andy Greenshaw has been a remarkable supervisor, mentor, and friend. He provided me with the guidance needed to succeed and the space with which to do so. Because of Andy I have had many opportunities, in many different ways, to explore my interest in Neuroscience and the world at large. I am forever grateful.

My committee members, Glen Baker, Satya Kar, and Phil Tibbo, and my candidacy examiner Alan Hudson, have provided a wealth of knowledge and feedback. I am particularly indebted to Glen for his ardent editor's eye, his brilliance for all things scientific, and for his generosity and kindness.

This experience was also greatly enriched through many interactions with junior colleagues and staff members, particularly those of the Neurochemical Research Unit. In particular, I must especially thank Robert Clements, Lauren Jantzie, Erin MacKenzie, Tera Mosher, and Bernard Sowa.

Two colleagues in particular have made an enduring impact – both of which have become good friends. Adrianne Huxtable has been, and has continued to be, a colleague with a pointed mind and a tenacious work ethic; she has been a great source of inspiration, integrity, thoughtfulness, and laughs. Melissa Kelly proved to be a fantastic office mate whose positivity continually brightened the many rooms of our tenure.

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°C	degree(s) Celsius
5-HIAA	5-hydroxyindolacetic acid
5-HT	serotonin
5-HTP	5-hydroxytryptophan
ACh	acetylcholine
Amphetamine	(+) α-methylphenethylamine sulphate
AMYG	amygdala
ANOVA	analysis of variance
AP	anterior-posterior
Baclofen	(R)-4-amino-3-(4-chlorophenyl)butanoic acid
CC	corpus callosum
Cl	chlorine
cm	centimeter(s)
COMT	catechol-O-methyltrasnferase
CP 94253	5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-pyrrolo[3,2-b]pyridine hydrochloride
CPA	conditioned place aversion
CPP	conditioned place preference
CSF	cerebrospinal fluid
DA	dopamine
DAG	diacylglycerol
DC	direct current
ddH$_2$O	double-distilled water
DOPAC	3,4-dihydroxyphenylacetic acid
DRN	dorsal raphe nucleus
DV	dorsal-ventral
Fig	figure(s)
fMRI	functional magnetic resonance imaging
FR	fixed ratio
g	gram(s)
GABA	γ-aminobutyric acid
Glu	glutamate
GR 127935	N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methy 1-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide hydrochloride
h	hour(s)
HVA	homovanillic acid
Hz	hertz
IAZ	interaural zero
ICSS	intracranial self-stimulation
IP or i.p.	intraperitoneal
IV or i.v.	intravenous
kg	kilogram(s)
L	liter(s)

L-DOPA	L-3,4-dihydroxyphenylalanine
LH	lateral hypothalamus
LM	lateral-medial
M	molar
M50	rate-frequency threshold
MAO	monoamine oxidase
mCPP	1-(m-chlorophenyl)piperazine
MFB	medial forebrain bundle
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
mPFC	medial prefrontal cortex
MRN	median raphe nucleus
mRNA	messenger ribonucleic acid
Muscimol	5-aminomethyl-3-hydroxyisoxazole
n	number of subjects
Na	sodium
NAc	nucleus accumbens septi
nACh	nicotinic acetylcholine
ng	nanogram(s)
p	p-value; the probability of obtaining the observed result given that the null hypothesis is true
PBS	phosphate buffered saline
PC	place conditioning
PET	positron emission tomography
pH	negative logarithm of hydrogen ion concentration
Picrotoxin	1:1 mixture of picrotoxinin and picrotin
PIP_2	phosphatidylinositol
PO or p.o.	per os; by mouth
PR	progressive ratio
RMAX	maximum response rate
Ro 60-0175	(S)-2-(chloro-5-fluoro-indol-l-yl)-1-methylethylamine
RS 102221	8-[5-(2,4-Dimethoxy-5-(4-trifluoromethylphenylsulphonam ido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2, 4-dione hydrochloride
RU 24969	5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole
SB 242084	6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline dihydrochloride hydrate
SC or s.c.	subcutaneous
SEM	standard error of the mean
SN	substantia nigra
TFMPP	N-[3-(trifluoromethyl)phenyl] piperazine hydrochloride
TRES	total number of responses
VTA	ventral tegmental area
WAY 161503	8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-

	one hydrochloride
μA	microampere(s)
μg	microgram(s)
μl	microliter(s)
μm	micrometer(s)

Chapter 1: Introduction

Concepts related to reward

The lay concept of pleasure has always fascinated the human mind. From Plato's levels of pleasure and Epicurus' hedonism to the modern field of positive psychology, humans have always sought an enlightened understanding of the factors involved in giving us pleasure. Despite this ancient interest, neuroscience and its related disciplines have only begun to understand this concept in biological terms. In fact, virtually all of the data related to this topic have been gathered in the 136 years following the publication of Charles Darwin's *The Expression of the Emotions in Man and Animals (Darwin 1872)* – perhaps the first work to attribute a biological basis to emotion. The current focus of research on pleasure and its related topics is aimed at understanding this topic not only for its own sake, but because investigations into its neural basis have contributed greatly to our knowledge of many psychiatric disorders. A pathological loss of pleasure may be a part of many of these disorders such as depression, schizophrenia, and drug addiction (Crespo-Facorro et al. 2001; Gard et al. 2007; Janiri et al. 2005; Leventhal et al. 2008; Loas et al. 1994; Schlaepfer et al. 2008). Given the subjective nature of the lay term pleasure, most research has focused on the related concepts of motivation, reward, and reinforcement. Many of the gains in this field have come from animal research and have underscored the need to understand the neural basis of these concepts. (See Boulton A.A. et al. 1991; O'Brien and Gardner 2005; Paterson and Markou 2007 for reviews on animal models of psychiatric disorders.)

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The term motivation is used to describe the initiation, direction, persistence and vigour of goal-directed behaviour (Cofer 1972; Salamone and Correa 2002). This definition is built upon many lines of research all broadly aimed at explaining the nature and variability of goal-directed behaviour over time, within and between individuals (see Berridge, 2004 for a history of research on motivational concepts). Briefly, Clark Hull developed homeostatic drive theory (Hull 1943) by combining Walter Cannon's concept of homeostasis (Cannon 1932) with the idea that an animal's behaviour is innately directed at maintaining a stable internal state. In contrast to the notion that internal disequilibrium 'pushes' an animal to act, incentive theories suggested that animals are 'pulled' toward external stimuli or incentives (Bolles 1967; Crespi 1942; Skinner 1953). Incentive motivation theories extended these notions to include the internal state of the organism and emphasized the role of associative learning and reinforcement (Bindra 1974; 1978; Toates 1986).

Reinforcement is said to have occurred if a given behaviour is subsequently strengthened or increased following one or more stimulus presentations in the process formally known as conditioning. Ivan Pavlov noted that reinforcement may involve the strengthening of stimulus-stimulus associations (Pavlov 1927). The idea of reinforcement as the strengthening of a stimulus-response association was first noted by Herbert Spencer when he stated that "every animal habitually persists in each act which gives pleasure – and desists from each act which gives pain," (Spencer 1872); this idea was further refined by Thorndike's law of effect and B.F. Skinner's operant conditioning (Skinner 1953). As such, reinforcement involves the strengthening of stimulus-stimulus or stimulus-response associations following the presentation of a consequence (Wise

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2004). Positive reinforcement involves the presentation of a rewarding stimulus (positive reinforcer) as the consequence and negative reinforcement involves the removal of an aversive stimulus as the consequence.

Richard Solomon's opponent process theory of motivation (Solomon 1980) emphasized the role of non-associative experiences, without negating the gains made by incentive motivation theories. He suggested that following a hedonic reaction, an emotional state of opposite valance is generated in the brain. This idea extended the homeostatic drive theories by suggesting the existence of a hedonic opponent reaction which helps to maintain emotional balance. Kent Berridge and colleagues have also underscored the importance of including hedonia in modern theories of motivation and have suggested, in their theory of incentive salience, that the brain mechanisms of reward can be divided into learning, hedonic value ('liking'), and motivational incentive value ('wanting' or incentive salience) (Berridge and Robinson 1998; 2003).

In this thesis, a reward is operationally defined as a stimulus (object or event) that elicits approach responses and that an animal will work for (Wise 2004). For the present studies, the term can be used synonymously with 'positive reinforcer'; as a verb it can be used synonymously with 'to positively reinforce'. It is important to note that subjective connotations related to hedonia, positive affect, or pleasure are not invoked in the present work (Cannon and Bseikri 2004; Salamone et al. 2007). Moreover, many lines of research have identified distinct neurobiological mechanisms associated with motivation and reward; reward as a broad psychological concept can refer to brain systems regulating hedonic value, motivational incentive value and learning processes, without requiring conscious awareness (Berridge and Kringelbach 2008).

While the behavioural tests used in this thesis do not measure motivation directly, given its relationship to reward, the data gathered may be relevant to the study of motivation. As such, the term 'reward-related' will be used to connote the brain mechanisms underlying reward and motivation. The animal models commonly employed to investigate motivation and reward include drug self-administration, place conditioning and intracranial self-stimulation (ICSS). An abundance of evidence implicate the neurotransmitter dopamine (DA) in the regulation of reward-related behaviours (Schultz 2007a; Wise 2002; 2004). Recent studies have also demonstrated a role for the neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) in the regulation of DA- and reward-related behaviours (Alex and Pehek 2007; Daw et al. 2002; Rothman and Baumann 2006). Serotonin acts at many receptor subtypes (Barnes and Sharp 1999; Hannon and Hoyer 2008) and the serotonin system interacts with many other neurochemical systems (Ciranna 2006; Fink and Gothert 2007).

The overall aim of this thesis was to investigate the role of the serotonin 2C (5-HT$_{2C}$) receptor using behavioural models of motivation and reward in rats. The focus on the 5-HT$_{2C}$ receptor has been guided by its putative role in the regulation of DA in reward-related brain areas (Di Giovanni et al. 2000; Di Matteo et al. 1999) and by its potential involvement in natural reward and psychiatric disorders such as schizophrenia, depression, and drug addiction (Dremencov et al. 2005; Giorgetti and Tecott 2004; Higgins and Fletcher 2003; Hull et al. 2004; Nilsson 2006; Richtand et al. 2007; Siuciak et al. 2007). Guided by evidence from experiments and the literature, interactions among the 5-HT$_{2C}$ receptor and 5-HT$_{1B}$ receptor, nicotinic acetylcholine receptor, γ-

aminobutyric acid A (GABA$_A$) receptor and GABA$_B$ receptor were investigated to further clarify the role of the 5-HT$_{2C}$ receptor in reward-related behaviours.

Reward-related changes in behaviour were assessed following the systemic or intracranial administration of pharmacologically selective ligands (as noted in Lodish et al. 2000, a ligand is defined broadly as any molecule that binds tightly and selectively to a macromolecule to form a complex). The majority of the present studies used intracranial self-stimulation (as a measure of the rewarding and motivating effects of direct stimulation of reward-related brain circuitry), although place conditioning (as a measure of the conditioned reinforcement- and reward-related properties of drugs) and spontaneous locomotor activity (as a motivation- and motor-related behavioural model sensitive to changes in mesolimbic DA transmission) were also employed. This chapter proceeds with brief reviews on emotive brain circuitry, reward-related brain circuitry, the pharmacology of DA, 5-HT, and GABA, and the role of these neurotransmitters in motivation and reward and psychiatric disorders. The chapter ends with an overview of the general methodology used in this thesis, brief reviews on 5-HT$_{2C}$, 5-HT$_{1B}$, GABA$_A$ and GABA$_B$ receptors in behavioural models of motivation and reward, and a brief outline of thesis objectives.

Emotive brain circuitry

As reviewed in Andrew Lautin's *The Limbic Brain* (2001), an understanding of the biological basis of emotion began in 1878 when Paul Broca coined the term "le grand lobe limbique". This great limbic lobe referred to a group of brain structures – including the olfactory areas, cingulate, hippocampus, and septal nuclei, along with the tracts

connecting them – which encircled the thalami and formed the border of the cortex. Interestingly, Broca identified these areas through gross examination and posited a role in emotion with little evidence. Advances in neuroanatomy allowed James Papez to refine Broca's construct (Papez 1937). Papez' emotive circuit included all of Broca's proposed structures and added the hypothalamus, anterior nucleus of the thalamus, additional connecting tracts, and postulated the inclusion of the amygdala.

The term limbic system, as it is often referred to today, was coined by Paul MacLean in 1954. Continuing advances in the neurosciences, along with increased patient-based evidence, inspired MacLean to add the amygdala, perihippocampal areas, and frontotemporal association cortices and to suggest that the limbic system may be integral to psychiatry (Maclean 1952). While the limbic system has continued to grow, as researchers have suggested the addition of the limbic midbrain and extended amygdala (Heimer 2003; Morgane et al. 2005; Nauta 1958), some have suggested that the concept of the limbic system is too simplistic and may act as a detriment to future research (LeDoux 2000).

While our knowledge of the brain's emotion-related circuitry has grown substantially, our current understanding of reward-related brain circuitry began in 1954 with the work of Olds and Milner (Olds 1958; Olds and Milner 1954). They found that rats would self-administer electrical stimulation to many areas of the brain. This led to the notion that many of these areas may be involved in natural motivation and reward. The intracranial self-stimulation (ICSS) technique, along with pharmacological and neuroanatomical manipulations, has continued to advance our understanding of this circuitry for reviews see Ikemoto and Wise 2004; Wise 1996; 2002; 2005).

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Brain circuitry related to motivation and reward

A simplified schematic of the basic circuitry currently believed to be involved in reward-related behaviour is depicted in Fig. 1.1. The mesocorticolimbic circuit is a major component of this circuitry and consists of the ventral tegmental area (VTA), nucleus accumbens septi (NAc) and prefrontal cortex (PFC). Although not depicted in Fig. 1.1, it is important to note that the serotonergic cells of the anterior raphe nuclei (consisting of the median and dorsal raphe) project extensively throughout the limbic system (Hensler 2006; Lechin et al. 2006).

Figure 1.1 Sagittal section of the rat brain outlining the basic circuitry involved in motivation and reward. Adapted from: Ikemoto and Panksepp 1999; Kalivas and Nakamura 1999; Kalivas and Volkow 2005; Lechin et al. 2006. Note that the extensive innervation of the raphe nuclei has been omitted for simplicity.

Abbr: AMYG – amygdala; DR – dorsal raphe nucleus; MR – median raphe nucleus; NAc – nucleus accumbens; PFC – prefrontal cortex; VP – ventral pallidum; VTA – ventral tegmental area.
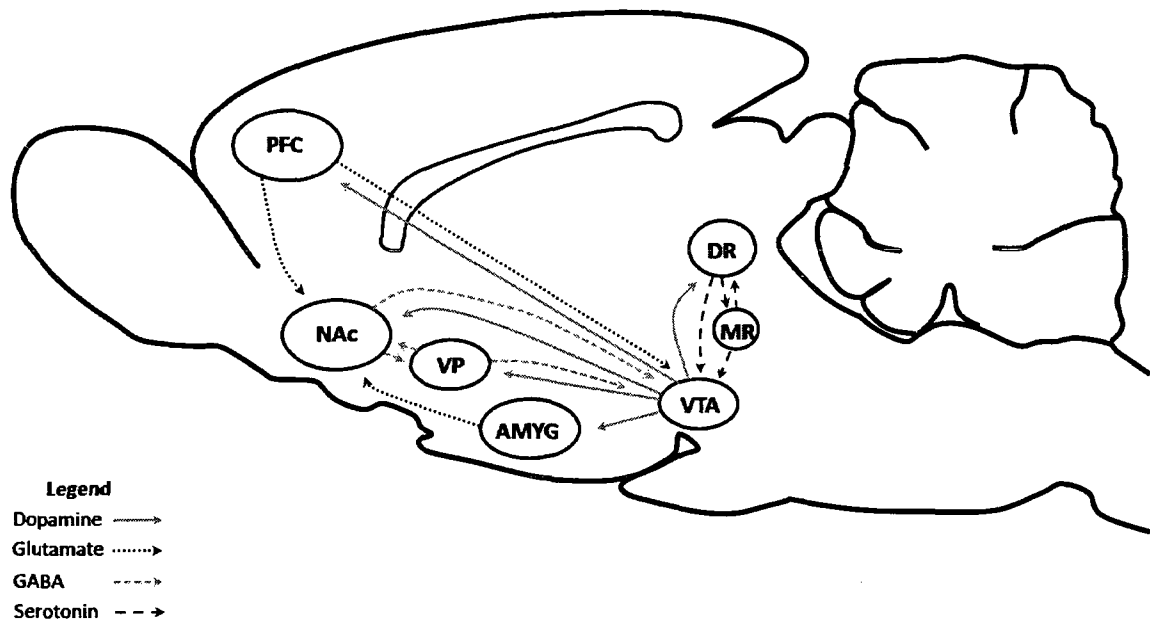
The VTA is located in the A10 region of the ventral mesencephalon (Dahlstrom and Fuxe 1964) and contains approximately 40,000 DA cells which project mainly to the nucleus accumbens septi (mesolimbic pathway) and prefrontal cortex (corticolimbic pathway) (Ikemoto and Panksepp 1999; Nair-Roberts et al. 2008). The VTA also contains a subpopulation of GABA cells which can act as interneurons to inhibit local DA cells (Bayer and Pickel 1991; Johnson and North 1992) or as afferent projections to the NAc or

PFC (Carr and Sesack 2000; Van Bockstaele and Pickel 1995). A small proportion of vesicular glutamate transporter 2-containing cells (presumably glutamatergic), which may project to the PFC and somatosensory cortex, have also been identified in the VTA although little is known about their function (Hur and Zaborszky 2005; Nair-Roberts et al. 2008). The amygdala, lateral hypothalamus, ventral pallidum and dorsal raphe also receive afferents from the VTA (Fields et al. 2007; Kalen et al. 1988). The VTA receives GABAergic inputs from the NAc (Walaas and Fonnum 1980) and ventral pallidum (Geisler and Zahm 2005), glutamatergic inputs from the PFC (Sesack and Pickel 1992), and serotonergic inputs from the anterior raphe nuclei (Del-Fava et al. 2007; Lechin et al. 2006; Phillipson 1979).

The NAc is located in the forebrain, lateral to the septum pellucidum where the head of the caudate and putamen meet, and consists mainly of GABAergic medium spiny projection neurons although a smaller number of GABAergic and cholinergic interneurons have also been identified (Chang and Kitai 1985; Meredith 1999). The work of Mogenson et al. (1980) identified the NAc as a key structure involved in integrating cortical and subcortical information, and as a proposed interface for reward-related and motor circuitry. For similar reasons, the ventral pallidum (which is reciprocally connected to the NAc) may also be important in integrating and transmitting reward- and motor-related information (Kretschmer 2000). Besides afferents from the ventral pallidum and VTA, the NAc also receives inputs from the amygdala and PFC (Groenewegen et al. 1999).

The NAc contains two main subregions consisting of the ventromedial shell and dorsolateral core which can be differentiated anatomically, pharmacologically, and

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behaviourally (Groenewegen et al. 1999; Meredith 1999; Zahm 1999; Zahm and Brog 1992). It is important to note that this area actually consists of a complex arrangement of subregions which are largely interconnected; there is evidence for rostro-caudal (Heidbreder et al. 1999; Reynolds and Berridge 2001; 2002) as well as medial-lateral and dorsal-ventral distinctions (Todtenkopf and Stellar 2000; Zahm et al. 1998). Some researchers consider the NAc shell to be a part of the interconnected extended amygdala (including the bed nucleus of the stria terminalis and central nucleus of the amygdala) which project to the VTA, lateral hypothalamus, and ventromedial ventral pallidum (Ikemoto and Panksepp 1999; Kalivas and Volkow 2005). These connections are believed to be involved in the acquisition and modulation of motivational salience as well as associative learning (Bassareo and Di Chiara 1999; Day and Carelli 2007; Sellings and Clarke 2003). The NAc core has projections similar to that of the basal ganglia in that it sends efferents to the subthalamic nucleus, substantia nigra and dorsolateral ventral pallidum (Zahm 1999). These connections are thought to play a role in responding that is elicited, or reinforced, by conditioned stimuli (Bassareo and Di Chiara 1999; Ito et al. 2004; Parkinson et al. 2000). Despite their functional differences, it is important to note that the core and shell of the NAc are anatomically connected (van Dongen et al. 2005).

Dopamine (DA)

The only compound used in this thesis believed to act as a functional DA agonist, by promoting the release of DA into the synapse, is (+)-α-methylphenethylamine ((+)-amphetamine). As such, it acted as a potent positive control in many experiments. This compound is believed to increase DA efflux primarily through its actions at the DA

transporter and vesicular monoamine transporter (Fleckenstein et al. 2007). As mentioned above, mesocorticolimbic DA is believed to play an integral role in mediating reward-related behaviours. As all of the compounds used in this thesis are believed to affect DA transmission, it is important to briefly discuss this neurotransmitter.

The synthesis and metabolism of dopamine

As reviewed in Cooper et al. (2003), DA is synthesized from the nonessential amino acid L-tyrosine which is derived mainly from conversion of the essential amino acid phenylalanine by phenylalanine hydroxylase. L-tyrosine is converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase; this is the rate limiting step in catecholamine synthesis. L-DOPA is decarboxylated by L-aromatic amino acid decarboxylase to form DA (Fig. 1.2)

Figure 1.2 The chemical structure of dopamine
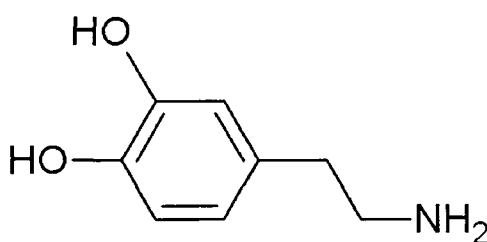
Following release, the actions of DA within the synapse are terminated by intracellular reuptake and catabolism. Reuptake is achieved via the Na^+/Cl^--dependent, 12 domain membrane-spanning, DA transporter. The catabolism of DA into its primary metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) occurs via the enzymes monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT).

Dopamine receptors

DA receptors are all putatively ligand-binding, membrane-bound, molecules. There are five known families of DA receptors which are referred to as D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) receptors (Cooper et al. 2003; Seeman and Van Tol 1993). All are believed to be metabotropic receptors coupled to guanine nucleotide-binding proteins (G-proteins) which activate (D_1-like) or inhibit (D_2-like) adenylyl cyclase. Activation of the membrane-bound enzyme adenylyl cyclase increases cyclic adenosine monophosphate (cAMP) which is involved in the regulation of the cell mainly through its actions on protein kinases (Greengard 2001; Hemmings et al. 1989). While the D_1-like receptors are mostly located postsynaptically on non-DArgic cells, D_2-like receptors are found as heteroreceptors (located presynaptically on cell types other than its own) and autoreceptors (located on its own cell type) and act to decrease neurotransmitter release (Adell and Artigas 2004; Starke et al. 1989).

Dopaminergic systems

Though the origins and terminal fields of DArgic neurons may somewhat overlap and contain many non-DArgic cells, they are often simplified into four major DA pathways (Cooper et al. 2003). The mesolimbic system generally refers to the pathway from the VTA to the NAc (and may include the olfactory tubercle which lies rostral to the NAc). The mesocortical system generally refers to the pathway from the VTA to the PFC, cingulate and entorhinal cortex. The nigrostriatal system refers to the pathway from the substantia nigra pars compacta to the dorsal striatum (Bjorklund and Dunnett 2007). The tuberoinfundibular system refers to the pathway from the arcuate nucleus of the hypothalamus to the median eminence, which regulates prolactin release from the

anterior pituitary gland – although other DArgic neurons in the hypothalamus may also be involved (Lerant et al. 2001). As discussed above, the mesocorticolimbic system is of particular interest in the context of motivation and reward.

The role of dopamine in motivation and reward

While an abundance of evidence suggests that DA plays a role in mediating motivation and reward, the exact nature of this role is unclear. The catecholamine and DA hypotheses of reward postulated an integral role for this neurotransmitter which later studies confirmed (Fibiger 1978; Wise 1989; Wise and Rompre 1989). The DA cells' exact role, however, has been difficult to understand as studies have demonstrated that they are not the 'first-stage' or principal cells activated by rewarding electrical stimulation of the brain (Wise 1996; Yeomans 1990). Alternately, studies investigating the role of noradrenalin - also involved in the catecholamine hypothesis of reward – were largely hampered by conflicting evidence and poor measures of reward; the noradrenalin hypothesis of reward, particularly in reference to ICSS, was deemed untenable (Fibiger 1978). More recent studies have, once again, implicated this neurotransmitter in reward, albeit, in a more precise capacity (Feenstra 2000; Weinshenker and Schroeder 2007).

The role of DA in motivation and reward has been recently discussed in a number of reviews; it is probable that DA does not play a singular role. DA neurons show both slow tonic activity, which maintains a low extracellular concentration of DA, and phasic burst activity, which is believed to account for fast-acting high synaptic concentrations (Arbuthnott and Wickens 2007). Phasic and tonic DArgic activity may interact to help guide goal-directed behaviours and allow for behavioural flexibility (Bratcher et al. 2005; Floresco et al. 2001; Floresco et al. 2006). Phasic burst activity follows the presentation

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of primary or conditioned rewards and may be involved in a reward-prediction error, involving the learning of anticipated rewards (Grace et al. 2007; Schultz 2007a; b).

Indeed, other researchers have underscored the potential role of DA in learning by suggesting that it is necessary to form and modulate stimulus-reward and response-reward associations (Wise 2004; 2005). Everitt and Robbins have also suggested that DA is important in learning and response maintenance, but have emphasized the role of Pavlovian conditioned stimuli in enhancing operant (or instrumental) responding (Everitt and Robbins 2005; Robbins and Everitt 1996). Dopaminergic activity may also be involved in behavioural activation, having a general energizing effect on effort and arousal (Salamone et al. 2007).

Berridge and colleagues have posited that DA is not involved directly in learning or hedonia ('liking'), but is instead involved in signaling motivational incentive salience ('wanting') (Berridge 2007). Although mesolimbic DA has been the major focus of reward-related signaling there is evidence that dopaminergic transmission in the PFC and cingulate are involved in reward-related valuation and decision making (Phillips et al. 2008; Rushworth and Behrens 2008). While early work suggested that DA might be the major neurotransmitter involved in signaling pleasure, hedonia, or 'liking' (i.e. the anhedonia hypothesis), recent work has suggested that DA may not play an important role in this regard (Pecina et al. 2006); early proponents of this idea are placing less emphasis on DA's role as a pleasure signal (Wise 2004). For additional reviews on the role of DA in motivation and reward, the reader is directed to Alcaro et al. 2007, Goto et al. 2007, Phillips et al. 2008, and Schultz 2007a, b.

Serotonin (5-HT; 5-hydroxytryptamine)

This thesis focuses on the role of the 5-HT$_{2C}$ receptor, and its potential interactions with other receptors, in reward-related behaviour. As discussed briefly below, the focus on brain 5-HT in recent years has been guided in part by its regulation of DA and its putative role in emotional regulation and psychiatric disorders. Although not discussed in this thesis, it is important to note that 5-HT is also found in the periphery, mainly in the enterochromaffin cells of the gastrointestinal tract, and is known to be involved in cardiac physiology, gastrointestinal function, gustation, platelet aggregation, and glucose and bone metabolism (Jonnakuty and Gragnoli 2008; Tecott 2007).

The synthesis and metabolism of serotonin

As reviewed in Cooper et al. (2003), brain-derived 5-HT is synthesized from the dietary essential amino acid L-tryptophan. L-tryptophan enters the brain through a large neutral amino acid transporter and is converted to 5-hydroxytrytophan (5-HTP) by tryptophan hydroxylase; this is the rate limiting step in 5-HT synthesis. 5-HTP is decarboxylated by the enzyme L-aromatic amino acid decarboxylase to form 5-HT (Fig. 1.3).

Figure 1.3 The chemical structure of serotonin
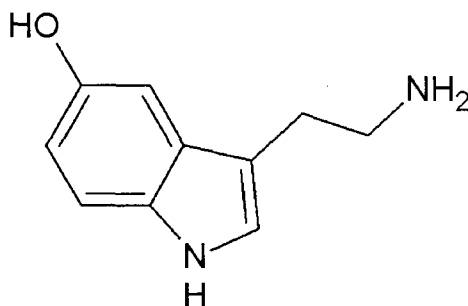
Following release, the actions of 5-HT are terminated by reuptake and catabolism. Much like DA, removal from the synapse is achieved largely via reuptake by the 5-HT transporter. In addition, the catabolism of 5-HT into its metabolites 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytrytophol is achieved by the enzymes MAO and aldehyde dehydrogenase.

Serotonin receptors (with special reference to 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors)

As reviewed recently by Hannon and Hoyer (2008), 5-HT receptors are currently divided into seven families based on structure and function. 5-HT acts on at least 13 distinct G-protein coupled receptor subtypes and one ionotropic (ligand-gated ion channel) receptor subtype. Receptors which currently lack a clearly defined physiological role are identified using lower case (i.e. 5-ht). The 5-HT$_1$ and 5-ht$_5$ receptor families are coupled to inhibitory (G$_{i/o}$) G-proteins whose activation results in the inhibition of adenylyl cyclase. In contrast, the 5-HT$_4$, 5-HT$_6$, and 5-HT$_7$ receptor families are positively coupled to adenylyl cyclase via stimulatory (G$_s$) G-proteins. The 5-HT$_2$ receptor family couples to G$_q$-proteins which stimulate the membrane-bound enzyme phospholipase C, resulting in the hydrolysis of phosphatidylinositol (PIP$_2$) to diacylglycerol (DAG) and inositol triphosphate (IP$_3$). DAG acts to activate the membrane-bound protein kinase C while cytosolic IP$_3$ acts to mobilize intracellular calcium. The ionotropic 5-HT$_3$ receptor acts as a non-selective cation channel. Table 1.1 summarizes the putative function and major localization of each 5-HT receptor subtype and provides examples of relatively selective ligands.

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Table 1.1 Summary of 5-HT receptor subtypes and related pharmacology. Adapted from Ciranna (2006) and Hannon and Hoyer (2008).

Receptor Family(subtype)	Effect	High-density localization	Agonist	Antagonist	Additional References
5-HT$_{1A}$	$G_{i/o}$ ($\downarrow$ cAMP)	C, HC, RN	8-OH-DPAT buspirone	WAY 100635 NAN 190	(Muller et al. 2007; Ogren et al. 2008)
5-HT$_{1B}$		Col, GP, HC, SN, VP	CP 94253 CP 93129	GR 127935 GR 55562	(Sari 2004; Sari et al. 1999)
5-HT$_{1D}$		Low expression in BG, DR, GP, SN, VP	PNU 109291	BRL 15572 GR 127935	(Bonaventure et al. 1998)
5-ht$_{1E}$		C, CD	-	-	-
5-ht$_{1F}$		C, CD, EC, OB, OT, RN, T	LY 334370	-	-
5-HT$_{2A}$	G_q ($\uparrow$ IP$_3$/DAG)	C, BG, EC, OB, PN	DOI DOB	MDL 100907 ketanserin	(Fletcher et al. 2007)
5-HT$_{2B}$		PN, stomach, low expression in brain	BW 723C86	SB 204741	(Bonhaus et al. 1995; Kursar et al. 1994)
5-HT$_{2C}$		AM, CP, HC, NAc, RN, SN, VTA	WAY 161503 WAY 163909 Ro 60-0175	SB 242084 RS 102221	(Berg et al. 2008; Giorgetti and Tecott 2004)
5-HT$_3$	Cation channel	AP, EC, PN	2-methyl-5-HT	MDL 72222 ondansetron granisetron	(Thompson and Lummis 2007)
5-HT$_4$	G_s ($\uparrow$ cAMP)	BG, Col, HC, PN	BIMU 8 RS 67333	GR 113808 SB 204070	(Fayyaz and Lackner 2008; King et al. 2008)
5-ht$_{5A}$	$G_{i/o}$ ($\downarrow$ cAMP)	C, CB, HC, OB	-	-	-
5-ht$_{5B}$		-	-	-	-
5-HT$_6$	G_s ($\uparrow$ cAMP)	AM, C, CD, HC, NAc, OT	-	SB 258585 Ro 63-0563	(King et al. 2008)
5-HT$_7$	G_s ($\uparrow$ cAMP)	C, HC, HT, T	8-OH-DPAT	SB 258719 SB 269970	(Cifariello et al. 2008)

Abbr: AM – amygdala; AP – area postrema; BG – basal ganglia; C – cortex; CB – cerebellum; Col –colliculi; CD – caudate putamen; CP – choroid plexus; RN – raphe nuclei; EC – entorhinal cortex; GP – globus pallidus; HC – hippocampus; HT – hypothalamus; NAc – nucleus accumbens; OB – olfactory bulb; OT – olfactory tubercle; PN – peripheral neurons; SN – substantia nigra; T – thalamus; VP – ventral pallidum; VTA – ventral tegmental area

As ligands selective for the 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors are used in this thesis, it is important to make a few additional notes regarding these receptors. The rat 5-HT$_{1B}$ receptor is homologous to the human 5-HT$_{1D\beta}$ (renamed the h5-HT$_{1B}$) receptor (Adham et al. 1992), differing only in one amino acid (Metcalf et al. 1992). 5-HT$_{1B}$ receptors are predominantly located on axon terminals (Boschert et al. 1994) and have been identified as both autoreceptors (Engel et al. 1986; Gothert et al. 1987) and heteroreceptors on cells containing acetylcholine (Cassel et al. 1995; Maura and Raiteri 1986), glutamate (Boeijinga and Boddeke 1996) and GABA (Sari et al. 1999). The highest receptor binding densities in rat brain are found in the substantia nigra, dorsal subiculum, globus pallidus and superior colliculus with lower densities reported in the caudate putamen, cortical areas, NAc and VTA (Bruinvels et al. 1993; Sari et al. 1999). 5-HT$_{1B}$ receptor knockout (KO) mice are believed to be indistinguishable from their wildtype littermates when considering their development, body weight, fertility and open field behaviour, but may be more aggressive when confronted by intruders (Ramboz et al. 1996).

5-HT$_{2C}$ receptors appear to be located postsynaptically on cholinergic (Lopez-Gimenez et al. 2001; Pasqualetti et al. 1999; Pompeiano et al. 1994), glutamatergic (Stein et al. 2000) or GABAergic cells (Bubar and Cunningham 2007; Eberle-Wang et al. 1997; Liu et al. 2007; Serrats et al. 2005) although a presynaptic localization has been hypothesized in some regions (Pasqualetti et al. 1999). In addition, a small number of DA cells in the VTA may also contain 5-HT$_{2C}$ receptors (Bubar and Cunningham 2007). 5-HT$_{2C}$ receptor mRNA appears to be confined to the central nervous system (Julius et al. 1988) and while the highest densities of this receptor are located in the choroid plexus, high levels are also noted in the amygdala, hippocampus, NAc, raphe nuclei, substantia

nigra and VTA (Barnes and Sharp 1999; Berg et al. 2008). It is also of interest that the 5-HT_{2C} receptor may function as a homodimer (Herrick-Davis et al. 2005), undergoes mRNA editing which results in putatively functional isoforms (Burns et al. 1997; Fitzgerald et al. 1999), and is known to have constitutive activity (Berg et al. 2005; Teitler et al. 2002).

Serotonergic system

Serotonergic cells are located as clusters of cells lying along the midline (or raphe) from the medulla to the midbrain (Cooper et al. 2003), as depicted in Fig. 1.4 below. Originally divided into nine cell groups (B1-B9) by Dahlstrom and Fuxe (1964), the primary ascending projections originate from the dorsal (DR; B6/7) and median (MR; B5/8) raphe nuclei and account for the majority of 5-HT innervation of the forebrain (Azmitia and Segal 1978). Innervation of the forebrain by these anterior raphe nuclei is extensive, diffuse, and overlapping, although the MR projects more heavily to the dorsal hippocampus, medial septum, NAc core, and VTA, whereas the DR projects more heavily to the amygdala, ventral hippocampus, lateral septum, NAc shell, and substantia nigra (Hensler 2006; Lechin et al. 2006). These two nuclei can be further differentiated as axons from the MR contain thick, non-varicose, fibers whereas those from the DR contain smaller fibers that branch extensively in their target areas (Hensler 2006).

Figure 1.4 Sagittal section of the rat brain outlining the projections (dotted lines) from the 5-HT raphe nuclei. The 5-HT cell groups, noted by Dahlstrom and Fuxe (1964) are labeled as B1-B9. Adapted from: Cooper et al. (2003); Murphy and Lesch 2008; Saper 2000.
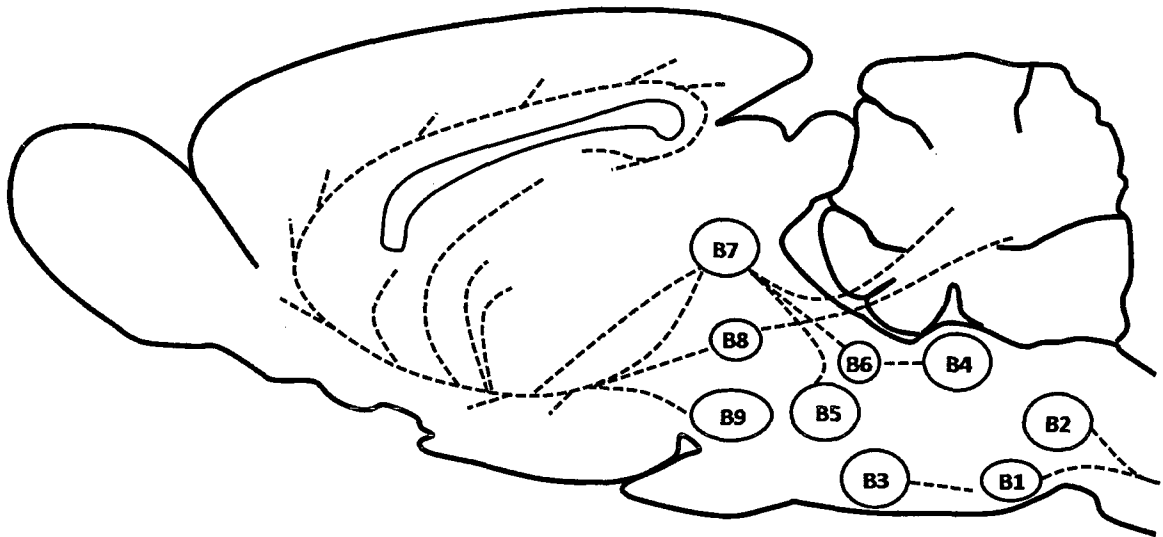
The role of serotonin in motivation and reward

The role of serotonin in motivation and reward has not been as well researched as that of DA. Increasing interest in the 5-HT system in this regard has been guided by anatomical and pharmacological evidence of its role in DA modulation (Alex and Pehek 2007; Daw et al. 2002; McBride et al. 1999; Pessia et al. 1994; Van Bockstaele et al. 1993; Van Bockstaele et al. 1994), its role in reward-related behaviours, and its putative role in psychiatric disorders (as discussed below).

Serotonin plays an integral role in reward-related behaviours as demonstrated by place conditioning, self-administration and ICSS studies. Conditioned place preferences

are seen following systemic administration of many selective serotonin reuptake inhibitors (Subhan et al. 2000), although increases in 5-HT generally correlate with decreases in self-administration behaviour (Higgins et al. 1993; Lyness 1983; Lyness et al. 1980; Yu et al. 1986). ICSS behaviour can be maintained through stimulation of the dorsal or median raphe nuclei (Broadbent and Greenshaw 1985; Van Der Kooy et al. 1978); although hampered by a limited measure of reinforcement, at least one study has demonstrated that perfusion of 5-HT close to the VTA facilitates ICSS of the medial forebrain bundle (Redgrave and Horrell 1976). Alternately, selective lesioning of serotonergic cells using the neurotoxic 5-HT analog 5,6-dihydroxytryptamine appears to facilitate ICSS (Poschel et al. 1974). These contradictory results underscore the need for studies investigating the role of individual 5-HT receptor subtypes in reward-related behaviours.

As noted above, 5-HT_{1B} receptors (Bruinvels et al. 1993; Sari et al. 1999) and 5-HT_{2C} receptors (Bubar and Cunningham 2007; Clemett et al. 2000) are found throughout the mesocorticolimbic system. While electrophysiological and microdialysis data suggest that 5-HT_{1B} receptor activation may increase DA release within this system (Boulenguez et al. 1998; Boulenguez et al. 1996; Iyer and Bradberry 1996; O'Dell and Parsons 2004; Yan and Yan 2001a; Yan et al. 2004), 5-HT_{2C} receptor activation has largely been associated with the inhibition of mesolimbic DA release (Di Giovanni et al. 2000; Di Matteo et al. 2002; Di Matteo et al. 1999; Navailles et al. 2006b; Prisco et al. 1994). For additional information on the role of 5-HT receptor subtypes in neurotransmitter regulation, see Fink and Gothert (2007). Studies on the role of 5-HT_{1B} and 5-HT_{2C} receptors in motivation and reward are reviewed below.

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γ-Aminobutyric acid (GABA)

As discussed briefly below, research on brain GABA in motivation and reward is guided by its role as a major inhibitory neurotransmitter and its putative role in emotional regulation and psychiatric disorders. In reference to this thesis, GABA transmission is particularly interesting given that many studies have identified 5-HT$_{2C}$ receptors on GABAergic cells (Bubar and Cunningham 2007; Eberle-Wang et al. 1997; Liu et al. 2007; Serrats et al. 2005) and their activation is associated with increased GABA cell activity (Bankson and Yamamoto 2004; Boothman et al. 2006; Stanford and Lacey 1996). In addition, there is evidence for GABAergic inhibition via activation of 5-HT$_{1B}$ heteroreceptors (Johnson et al. 1992; Parsons et al. 1999; Yan and Yan 2001b; Yan et al. 2004).

The synthesis and metabolism of GABA

As reviewed in Cooper et al. (2003) and Bak et al. (2006), brain-derived GABA is synthesized from the non-essential amino acid L-glutamic acid. As neurons cannot synthesize L-glutamic acid directly, they rely on the astrocytic-derived precursor glutamine which is converted into L-glutamic acid by the enzyme glutaminase. L-glutamic acid (which exists largely in its conjugate base form, glutamate, at physiological pH), is irreversibly converted to GABA (Fig. 1.5) by L-glutamic acid decarboxylase (GAD) which is found primarily in the central nervous system.

Figure 1.5 The chemical structure of γ-aminobutyric acid
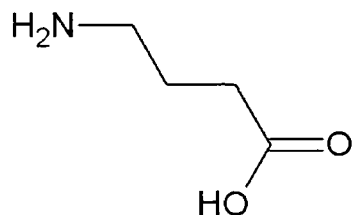
Following release, the actions of GABA are terminated by reuptake into the neuron or neighbouring astrocytes via the GABA transporter. While catecholamines are putatively taken up by a single transporter, at least four GABA transporters have been identified to date. Although reuptake by the presynaptic neuron is not understood fully, it is currently believed that the majority of GABA released is removed from the synapse by this mechanism and repackaged into vesicles for future release. GABA that is taken up by astrocytes is catabolised to the tricarboxylic acid cycle intermediate succinic semialdehyde by the enzymes GABA-transaminase and succinic semialdehyde dehydrogenase. A further intermediate of the tricarboxylic acid cycle, α-ketoglutarate, can be converted to glutamine via glutamine synthetase and released into the extracellular space where it can be taken up by neurons.

GABA receptors

There are three known GABA receptor subtypes identified as GABA$_A$, GABA$_B$, and GABA$_C$ receptors (Chebib and Johnston 2000). As reviewed recently by Schmidt (2008), activation of ionotropic GABA$_C$ receptors promotes the influx of chloride which results in inhibitory hyperpolarization of neurons. These receptors are found largely throughout the visual system (such as in the lateral geniculate nuclei and superior colliculi) and their

greatest expression is in the retina. They may also exist at low levels in the amygdala, hippocampus, cerebellum, and spinal cord, although there is some evidence that their expression may decrease in adulthood (Alakuijala et al. 2005). For these reasons, the work in this thesis focuses on the $GABA_A$ and $GABA_B$ receptors.

As reviewed by Michels and Moss (2007), Mohler (2006), and Olsen and Sieghart (2008), $GABA_A$ receptors are ionotropic receptors that, much like $GABA_C$ receptors, allow for the influx of chloride, resulting in the inhibitory hyperpolarization of neurons. Unlike $GABA_C$ receptors, however, they are located ubiquitously throughout the central nervous system. Like other ionotropic receptors, the $GABA_A$ receptor is believed to contain 5 protein subunits arranged around a central pore which acts as an ion channel. Although there are at least 16 known subunits (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ϵ, π, θ), there are less than 20 known combinations in vivo. In addition, the composition of most receptors seems to follow a $2\alpha:2\beta:1\gamma$ subunit stoichiometry and only 3 conformations ($\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$) may make up the majority of receptors in vivo (Ernst et al. 2003). Although $GABA_A$ receptors are largely located synaptically on postsynaptic cells, they may also be found presynaptically (Belenky et al. 2003) or as extrasynaptic receptors (Glykys and Mody 2007); evidence indicates that they are on cells containing acetylcholine, DA, 5-HT, and GABA (Gao et al. 1993; Ikarashi et al. 1999; Khateb et al. 1998; Laviolette and van der Kooy 2001; Tepper and Lee 2007). It is currently believed that GABA binds to the $GABA_A$ receptor at a site between the α and β subunits, and that other sites are responsible for the positive allosteric effects seen with alcohols, anesthetics, barbiturates, and benzodiazepines. For additional information on the pharmacology and related

functions of $GABA_A$ receptors, the reader is referred to Mohler et al. (2001), Rudolph and Mohler (2006), and Smith and Simpson (2003).

As reviewed in Bettler and Tiao (2006), Kornau (2006), and Ulrich and Bettler (2007), $GABA_B$ receptors appear to exist widely throughout the central nervous system as inhibitory metabotropic receptors which are coupled to $G_{i/o}$-proteins. The inhibition of neurotransmitter release by presynaptic $GABA_B$ auto- and heteroreceptors is primarily achieved through their indirect inhibition of Ca^{2+} channels, while postsynaptic receptors act indirectly on K^+ channels to increase conductance. There are currently 3 known receptor subunits ($GABA_{B1a}$, $GABA_{B1b}$, and $GABA_{B2}$) which appear to heterodimerize following a $GABA_{B1}$:$GABA_{B2}$ subunit stoichiometry in order to function fully in vivo (Jones et al. 1998). The $GABA_{B1a}$ and $GABA_{B1b}$ subunits show a differential distribution throughout the brain and studies in KO mice missing either subunit have suggested that they each provide a unique function in vivo. While the $GABA_{B1}$ receptor subunits may be involved in binding GABA and baclofen (the only clinically available $GABA_B$ receptor agonist), $GABA_{B2}$ receptor subunits are coupled to the G-proteins and may be targets for newer $GABA_B$ receptor modulators (Ong and Kerr 2005). $GABA_B$ receptors are located largely at extrasynaptic sites suggesting that, unlike most $GABA_A$ receptors, they may require higher synaptic concentrations of GABA to be activated; evidence indicates that they are on cells containing DA, 5-HT, GABA, noradrenaline and glutamate (Bischoff et al. 1999; Kalivas et al. 1990; Klitenick et al. 1992; Lu et al. 1999; Margeta-Mitrovic et al. 1999; Shefner and Osmanovic 1991; Tao et al. 1996; Wirtshafter and Sheppard 2001). For additional information on the pharmacology and related functions of $GABA_A$

receptors, the reader is referred to Bettler et al. (2004), Bowery (2006), and Bowery and Smart (2006).

GABAergic systems

GABAergic cells are located ubiquitously throughout the central and peripheral nervous systems (Cooper et al. 2003; Geigerseder et al. 2003; Mackie et al. 2003; Saravia-Fernandez et al. 1996; Yasumi et al. 1997). There are no primary nuclei, although some areas of the brain are more enriched than others. With particular reference to this thesis, as noted above, the NAc, ventral pallidum, and VTA contain GABAergic cells (Bayer and Pickel 1991; Carr and Sesack 2000; Geisler and Zahm 2005; Johnson and North 1992; Nair-Roberts et al. 2008; Van Bockstaele and Pickel 1995; Walaas and Fonnum 1980). In addition to the synaptic connections noted above, some GABAergic cells in the midbrain may be electrically coupled (Lassen et al. 2007).

The role of GABA in motivation and reward

Much like 5-HT, the growing interest in GABA in motivation and reward is guided by research identifying this neurotransmitter in the regulation of DA and reward-related behaviours (Bardo 1998; Ikemoto and Wise 2004; Kalivas et al. 1990; Lassen et al. 2007; McBride et al. 1999) and in its putative role in psychiatric disorders (as discussed below).

GABA plays an integral role in reward-related behaviours as demonstrated by place conditioning, self-administration, and ICSS studies. Studies have shown that systemically administered benzodiazepines and barbiturates (which can both act as positive allosteric modulators of the $GABA_A$ receptor) may produce conditioned place preferences (Bossert et al. 2003; Bossert and Franklin 2001; Gray et al. 1999; Hirai et al. 2005; Papp et al. 2002); while others have shown that some benzodiazepines produce

conditioned place aversions (Parker et al. 1998) or have no effect on place conditioning (Goeders and Goeders 2004; Le Pen et al. 2002; Matsuzawa et al. 2000; Meririnne et al. 1999; Walker and Ettenberg 2001; 2003; 2005). These compounds can also maintain self-administration behaviour (Ator and Griffiths 1987; de Wit and Griffiths 1991; Licata and Rowlett 2008; Meisch 2001) and facilitate ICSS behaviour under certain conditions (Bielajew and Harris 1991; Gomita et al. 2003; Ichimaru et al. 1983; Liebman 1985).

GABA$_A$ (Churchill et al. 1992; Okada et al. 2004; Onoe et al. 1996) and GABA$_B$ (Bischoff et al. 1999; Durkin et al. 1999; Lu et al. 1999; Margeta-Mitrovic et al. 1999; Wirtshafter and Sheppard 2001) mRNA and receptors are expressed throughout the mesolimbic system. Some studies have suggested that the release of mesolimbic DA may be under tonic inhibitory control of the GABA$_A$ receptor (Ferraro et al. 1996; Ikemoto et al. 1997a; Rahman and McBride 2002; Westerink et al. 1996; Yan 1999), while others have suggested a more complex role – due to the existence of pre- and postsynaptic receptors on various neuronal populations (Aono et al. 2008; Klitenick et al. 1992; Oakley et al. 1991; Xi and Stein 1998; Yoshida et al. 1997). GABA$_B$ receptor activation within mesolimbic areas may also inhibit DA efflux (Erhardt et al. 2002; Fadda et al. 2003; Klitenick et al. 1992; Rahman and McBride 2002; Westerink et al. 1996), although bidirectional effects on mesolimbic DA have also been noted (Cruz et al. 2004). These results underscore the need for studies investigating the role of each GABA receptor subtype in reward-related behaviours; studies to date are reviewed below.

DA, 5-HT, and GABA in psychiatric disorders

Dysfunction of DA, 5-HT, and GABA systems have been noted in psychiatric disorders such as schizophrenia, depression, and drug addiction. Aspects of motivation and reward may be impaired in individuals experiencing these disorders (Crespo-Facorro et al. 2001; Gard et al. 2007; Janiri et al. 2005; Leventhal et al. 2008; Loas et al. 1994; Murray et al. 2008a; Schlaepfer et al. 2008). A brief overview of DA, 5-HT, and GABA abnormalities in each of these disorders is provided here, with special reference to the receptors studied in this thesis.

Schizophrenia

Despite decades of research and the development of psychosocial and pharmacological therapies, the pathophysiology of schizophrenia is poorly understood. The disorder is characterized broadly by three symptom domains: positive symptoms (e.g. hallucinations, delusions, paranoia), negative symptoms (e.g. anhedonia, avolition, social withdrawal, flattened affect), and cognitive dysfunction (especially regarding attention, working memory, and decision making) (Tamminga and Holcomb 2005). Although the etiology is unknown, it is believed to be a complex disorder with many interacting environmental and genetic factors; these appear to result in changes to brain anatomy and circuitry (DeLisi 2008; Frankle 2007; Lawrie et al. 2008; van Haren et al. 2008). Monoaminergic theories of schizophrenia largely dominated early research, although current lines of evidence include the role of other neurotransmitters in this disorder.

While the role of DA-related genes is still debated (Talkowski et al. 2007), the current DA hypothesis of schizophrenia broadly states that there is evidence for increased subcortical dopaminergic function, which may be related to the positive symptoms, and

decreased cortical dopaminergic function, which may be related to the negative and cognitive symptoms (Alves et al. 2008; Kapur 2003; Laviolette 2007; Murray et al. 2008b). Support for this hypothesis comes from several lines of evidence. DA D_2 receptor antagonism by antipsychotic medications is related to their efficacy to reduce psychosis (Kapur and Mamo 2003; Seeman 1987). (+)-Amphetamine (an indirect DA agonist) potentiates striatal DA release in schizophrenic patients (Breier et al. 1997; Laruelle et al. 1996) and can induce psychotic states in healthy controls (Shoptaw et al. 2008; Yui et al. 2000) and precipitate them in patients (Lieberman et al. 1987). In addition, many changes in DA receptor expression have been noted in a number of brain regions (Frankle 2007). Nonetheless, changes in the DA system alone do not account for all aspects of schizophrenia, and other systems must be taken into consideration.

The 5-HT hypothesis of schizophrenia has evolved from its initial focus on 5-HT as a primary mediator of psychosis (due particularly to studies using lysergic acid; see Smart and Bateman 1967 for a review of early case reports) to its current role as a modulator with broad effects (Geyer and Vollenweider 2008; Iqbal and van Praag 1995). Many 5-HT receptor subtypes have been identified as playing a role in cognition and 5-HT_{2A} receptor activation is associated with the induction of positive symptoms (Aghajanian and Marek 1999; Terry et al. 2008; Vollenweider et al. 1998). Further support for the role of 5-HT in schizophrenia is that many atypical antipsychotic medications have actions at 5-HT receptors; these activities may be related to reduced negative and cognitive symptoms and reduced extrapyramidal side effects (Jones and McCreary 2008; Meltzer 1999; Meltzer et al. 2003). In particular, although the 5-HT_{2C} receptor has been identified as an important target of some atypical antipsychotics, it is

presently unclear whether its clinical effects are due to activation, blockade, or a combination of actions at multiple brain sites (Navailles et al. 2006a; Richtand et al. 2007). Studies have also suggested that 5-HT$_{2C}$ receptor gene regulation may be affected in individuals with this disorder (Castensson et al. 2005; Castensson et al. 2003; Gardiner and Du 2006; Huang et al. 2007; Reynolds et al. 2005). Selective 5-HT$_{2C}$ receptor agonists have been shown to be effective in animal models using schizophrenia endophenotypes (Dunlop et al. 2006; Wood et al. 2001).

Although most research has focused on the role of the monoamines in schizophrenia, a growing body of research is providing support for the role of GABA in this disorder. As noted above, GABAergic cells play an important role in the regulation of the DA and 5-HT systems; the direction of these effects is related to GABAergic concentration and which receptor subtypes are activated. Much of the evidence points to altered GABAergic function in the PFC and hippocampus, although other areas have also been implicated (Benes et al. 1992; Perry et al. 1979; Simpson et al. 1992). For instance, GAD and GABA transporter expression in the PFC may be reduced in schizophrenic patients (Akbarian and Huang 2006; Lewis 2000), although increased GABA$_A$ receptor and benzodiazepine binding has been noted (Benes et al. 1996b; Toru et al. 1988). Increased GABA$_A$ receptor binding has also been noted in the hippocampus (Benes et al. 1996a), while GABA$_B$ receptors may be reduced in the hippocampus of schizophrenic patients (Mizukami et al. 2000). GABA manipulations have also been effective in many animal models of schizophrenia (Wassef et al. 2003). Additional information reviewing the cellular, pharmacological, behavioural, and clinical evidence for GABA in

schizophrenia can be found in Benes (2007), Coyle (2006), Daskalakis et al. (2007), Lewis and Moghaddam (2006), Lisman et al. (2008) and Wassef et al. (2003).

Because, as noted above, there is evidence that many of the receptors noted in this thesis may directly or indirectly regulate glutamate release, it is also important to briefly mention that there is extensive literature on the role of glutamate in the pathophysiology of schizophrenia (for recent reviews, see Paz et al., 2008 and Sodhi et al., 2008).

Depression

Major depressive disorder is characterized by depressed mood and anhedonia, experienced for at least two consecutive weeks; these can be accompanied by other symptoms such as sleep disturbances, weight changes, cognitive deficits, psychomotor changes, loss of energy, and suicidal ideation (Naranjo et al. 2001; Nutt et al. 2007). Similar to research on the pathophysiology of schizophrenia, work related to major depression has focused on the monoamines; this is evidenced by the fact that most medications target these neurotransmitter systems. Despite these advances, most patients treated with first line antidepressants do not achieve remission and at least one third of patients are non-responders (Rush et al. 2006). Because of this, treatment-resistant depression is a major focus of clinical research (Shelton and Papakostas 2008; Wijeratne and Sachdev 2008). Like schizophrenia, major depression is a complex disorder which may result from many interacting environmental and genetic factors; these appear to result in changes to brain circuitry (Abosch and Cosgrove 2008; Hamet and Tremblay 2005; Sen and Sanacora 2008). It is interesting to note that some success with treatment-resistant depression has been met by surgically targeting reward-related circuitry with deep brain stimulation (Hauptman et al. 2008; Lozano et al. 2008; Schlaepfer et al. 2008).

As reviewed recently by Dunlop and Nemeroff (2007), Gershon et al. (2007), and Nutt et al. (2007), DA dysfunction is emerging as a significant factor in the pathophysiology of major depression. Animal models of depression result in decreased DA release and receptor binding which may be reversed by antidepressants or treatment with DA agonists. dopaminergic lesions may also produce depressive-like symptoms (Winter et al. 2007), For a review on animal models of depression, see McArthur and Borsini, 2006. Furthermore, some antidepressant drugs and treatments may act to increase dopaminergic tone and alter DA receptor function in human and animal studies. Although many inconsistencies remain, the data generally suggest increased DA D_2-like receptor binding and reduced DA transporter binding in depressed patients. In addition, reduced HVA (a primary metabolite of DA) levels in blood plasma and cerebrospinal fluid have also been reported. There are also numerous protein candidates, involved in the regulation of DA, which may be keys to understanding the mechanisms involved in depression (Nestler and Carlezon 2006).

Dysregulation of the 5-HT system has long been considered important in the pathophysiology of major depression (Mann 1999). Antidepressant medications, most of which act at least partly to increase 5-HT neurotransmission, have been shown to be effective in many animal studies of depression (McArthur and Borsini 2006). Many studies on the role of 5-HT in mood regulation have been conducted in humans; 5-HT is reduced in people experiencing mania, aggression, and depression resulting in suicide (Cools et al. 2008). Some studies have shown reduced levels of the 5-HT metabolite 5-HIAA in the brains and cerebrospinal fluid of depressed patients, particularly those who were unmedicated at the time of death (Ferrier et al. 1986; Mann 1999). Studies which

deplete 5-HT stores through acute tryptophan depletion or the use of the 5-HT releaser para-chlorophenylalanine have shown that these treatments do not, or very mildly, affect the mood of healthy controls although they do depress mood in healthy controls with a family history of major depression, in drug-free patients in remission for major depression, and in medicated patients in remission (Bell et al. 2005; Cools et al. 2008; Ruhe et al. 2007). Another approach has suggested that reduced 5-HT may be related to changes in the metabolism of its precursor tryptophan caused by stress-related mechanisms (Miura et al. 2008). In addition, known genetic polymorphisms in 5-HT-related genes (such as the 5-HT transporter) have led to recent approaches focused on identifying patients who will respond best to antidepressants (Lotrich and Pollock 2005; Rasmussen-Torvik and McAlpine 2007).

Some 5-HT receptors may also be affected in patients with major depression (Stockmeier 2003). Chronic treatment with selective serotonin reuptake inhibitors (SSRIs) may down regulate or desensitize $5-HT_{1B}$ autoreceptors and related mRNA (Anthony et al. 2000; Blier et al. 1988; Neumaier et al. 1996; O'Connor and Kruk 1994). Studies using animal models have shown that blockade of $5-HT_{1B}$ receptors may potentiate the antidepressant effects of SSRIs while $5-HT_{2C}$ receptor activation or blockade may result in antidepressant effects (Cryan and Lucki 2000; Cryan et al. 2005; Dekeyne et al. 2008; Dremencov et al. 2005). The latter is supported by data indicating that some antidepressants may act at, or alter the expression of, $5-HT_{2C}$ receptors (Jenck et al. 1994; Jenck et al. 1993; Ni and Miledi 1997; Yamada and Sugimoto 2001). Clinically, agomelatine (a melatonin receptor agonist and $5-HT_{2C}$ receptor antagonist) may be an effective antidepressant with few unwanted side effects (Eser et al. 2007;

Zupancic and Guilleminault 2006). (It is important to note that melatonin is a hormone whose central actions are important in the regulation of circadian rhythms; for more information on melatonin see Pandi-Perumal et al. 2008.) It is also interesting to note that altered $5\text{-}HT_{2C}$ receptor mRNA editing has been noted in the brains of depressed patients and in some animal models of depression (Gurevich et al. 2002; Iwamoto and Kato 2003; Iwamoto et al. 2005; Tohda et al. 2006).

As reviewed in Kalueff and Nutt (2007) and Sanacora and Saricicek (2007), increasing evidence supports a role for GABA in depression. Animal models of depression have suggested that GABAergic transmission is reduced following depressive-like behaviours and that both $GABA_A$ and $GABA_B$ receptors may be involved. Animal models have generally pointed to $GABA_A$ receptor agonists and $GABA_B$ receptor antagonists as having antidepressant-like properties (Cryan and Kaupmann 2005). Interestingly, interpretation of the $GABA_B$ receptor data may be complicated by the fact that $GABA_B$ receptor agonists have differential effects on tests of anxiety. Human studies have revealed decreased GABAergic cells in the frontal cortex and decreased GABA concentrations in the plasma, cerebrospinal fluid, and occipital and frontal cortices of depressed patients. Preliminary evidence suggests that polymorphisms in the enzyme GAD or $GABA_A$ receptor subunits may also be involved, although these data are at present inconclusive. Many antidepressants have been shown to increase GABA levels in the brains of humans and animals and some GABA-mimetic compounds have demonstrated antidepressant properties or usefulness in augmentation therapies with other antidepressants. In addition, stress is considered a major trigger for the onset of a depressive episode and changes in the GABAergic system, such as decreased GABA

synthesis and GABA$_A$ receptor binding and expression, have been noted following acute and chronic stressors.

Drug addiction

Clinically, although the forthcoming edition may alter this fact, the term addiction is not used in the current Diagnostic and Statistical Manual of Mental Disorders (O'Brien et al. 2006). Instead, clinicians are asked to consider drug use on a continuum: social use, abuse, and dependence. Briefly, drug abuse is characterized by one or more symptoms regarding recurrent drug use despite threatened social and/or physical wellbeing. Drug dependence is characterized by the presence of three or more symptoms, including: tolerance, withdrawal, drug use that is more than intended, high motivation to obtain drug, activities are replaced by drug use, and drug use that continues despite negative consequences.

Although drugs of abuse act through a wide array of mechanisms, they all appear to increase mesocorticolimbic DA neurotransmission (Wise 1996). In general, research to date suggests that the activity of the mesolimbic pathway is involved in the acute reinforcing effects of drugs of abuse as well as conditioning to drug-related stimuli, whereas the activity of the corticolimbic system is related to the executive control over drug seeking behaviour (Kalivas and Volkow 2005; Volkow et al. 2004). A great deal of addiction-related information has been gathered in the past few decades and there are currently a number of biopsychological theories of addiction. The neurotransmitter systems most relevant to this thesis are all believed to be involved in mediating the effects of drugs of abuse, as discussed briefly below. Recent reviews by Goodman et al.

(2008), Feltenstein et al. (2008), Moal and Koob (2007), and Olmstead (2006) provide excellent overviews on addiction theories and the neurobiology of addiction.

Drugs of abuse, such as (+)-amphetamine, cocaine, ethanol, nicotine, and opiates, increase DA release in the NAc (Di Chiara and Imperato 1988) and may support reward-related behaviours following injection into the VTA, NAc, and PFC (McBride et al. 1999). dopaminergic drugs injected into the NAc shell, compared to the core, are more effective in supporting reward-related behaviours (Carlezon and Wise 1996; Ikemoto and Wise 2004; Sellings and Clarke 2003; Sellings et al. 2006). While much of the data implicating altered DA transmission in the pathophysiology of addiction has come from animal models, studies in humans generally agree with these data (Franken et al. 2005). Nonetheless, treatments which selectively target the DA system have not been successful in combating addiction (Amato et al. 2007; Nutt and Lingford-Hughes 2008), underscoring the need for further investigation regarding other neurotransmitter systems.

The 5-HT system is not currently considered to be primarily involved in the pathophysiology of addiction. However, manipulations of this system can alter the reinforcing effects of drugs of abuse; it is believed that these effects are due to the 5-HT system's modulatory role in DA transmission, as discussed above. For instance, self-administration of many drugs of abuse in animals is altered following 5-HT_{1A} receptor activation (Roberts et al. 1998; Wilson et al. 1998), 5-HT_{1B} receptor activation (Fletcher et al. 2002a; Parsons et al. 1998), 5-HT_{2C} receptor activation or blockade (Fletcher et al. 2002b; Grottick et al. 2001; Grottick et al. 2000; Tomkins et al. 2002), and 5-HT_6 receptor blockade (Frantz et al. 2002). In particular, 5-HT_{2C} receptor agonists have been proposed as potential treatments for drug addiction (Giorgetti and Tecott 2004; Higgins

and Fletcher 2003). The roles of the 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors in self-administration are reviewed further below. Although there are many incongruities within the literature, some human studies indicate a role for SSRIs in assisting the cessation of alcohol and (+)-amphetamine dependence (Janiri et al. 1996; Pettinati et al. 2000; Srisurapanont et al. 2001). Bupropion – an atypical antidepressant which acts as a DA and norepinephrine reuptake inhibitor and nicotinic acetylcholine receptor antagonist that indirectly increases 5-HT release – is used effectively in nicotine cessation (Hurt et al. 1997; Jorenby et al. 1999). Overall, however, treatments selectively targeting the 5-HT system have not been successful in treating drug addiction (Ciraulo et al. 2005; Hughes et al. 2007).

It is interesting to note, as discussed above, that the effects of 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors on the DA system may be indirectly mediated by the GABAergic system. The role of the GABAergic system, and in particular the GABA$_A$ receptor, in ethanol addiction has been well studied in animals and humans; ethanol dependence is thought to be related, in part, to its actions as a positive allosteric modulator at this receptor (Enoch 2008). GABA$_A$ receptor antagonists may be effective in reducing the reinforcing effects of ethanol (Hyytia and Koob 1995; Nowak et al. 1998). It has been postulated that increased activity in the DA system may lead to a compensatory suppression of GABAergic activity – which may be important in the development of dependence for some drugs of abuse (Ivanov et al. 2006). In support of this hypothesis, the GABA$_B$ receptor agonist baclofen may be effective in decreasing the reinforcing effects of (+)-amphetamine, cocaine, and nicotine in animals (Brebner et al. 2005; Di Ciano and Everitt 2003; Paterson et al. 2004; Slattery et al. 2005) and humans (Cousins et al. 2001; Haney et al. 2006; Heinzerling et al. 2006; Shoptaw et al. 2003).

General methodology

Before reviewing the literature on the roles of $5\text{-}HT_{2C}$, $5\text{-}HT_{1B}$, $GABA_A$ and $GABA_B$ receptors in locomotor activity and models of motivation and reward, an outline of the general methodology used in this thesis is provided. The drugs used, as well as the methods of locomotor activity, place conditioning, nicotine-induced locomotor activity and place conditioning, intracranial self-stimulation, and food intake will be discussed. Although differences in parameters may exist, the methodology used in this thesis is fundamentally similar to studies throughout the literature providing the reader with a general overview of these methods.

Drugs

All drugs used in this thesis were administered systemically (subcutaneously, s.c.; intraperitoneally, i.p.) or centrally into the NAc shell via microinjection. Parameters pertaining to each study are included in their respective *Materials & methods* sections within each subsequent chapter; drug preparation is described in further detail in Appendix A; artificial cerebrospinal fluid preparation for microinjections is described in Appendix B. The $5\text{-}HT_{2C}$ receptor agonist, WAY 161503 • HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-one hydrochloride], the $5\text{-}HT_{1A/1B/2A/2B/2C}$ receptor agonist TFMPP • HCl [N-[3-(trifluoromethyl)phenyl] piperazine hydrochloride], the $5\text{-}HT_{1B}$ receptor agonist CP 94253 • HCl [5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-pyrrolo[3,2-b]pyridine hydrochloride], the $5\text{-}HT_{1B/1D}$ receptor antagonist GR 127935 • HCl [N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'- (5-methyl-1,2,4-oxadiazol-3-yl) -1,1'-biphenyl-4-carboxamide hydrochloride], the $GABA_A$ receptor agonist muscimol [5-aminomethyl-3-hydroxyisoxazole] and the

antagonist picrotoxin [1:1 mixture of picrotoxinin and picrotin], and the GABA$_B$ receptor agonist (R)-baclofen [(R)-4-Amino-3-(4-chlorophenyl)butanoic acid] (baclofen) were purchased from Tocris Cookson Inc. (Ellisville, MO, USA). (+) α-Methylphenethylamine ((+)-amphetamine) sulphate, which was purchased from Health and Welfare Canada, and (–)-nicotine hydrogen tartrate salt (nicotine) and the 5-HT$_{2C}$ receptor antagonist SB 242084 · 2HCl [6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline dihydrochloride hydrate] were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). The chemical structures of these compounds are shown in Fig. 1.6.

Figure 1.6 The chemical structures of WAY 161503, TFMPP, CP 94253, GR 127935, muscimol, picrotoxin, baclofen, (+)-amphetamine, and SB 242084.
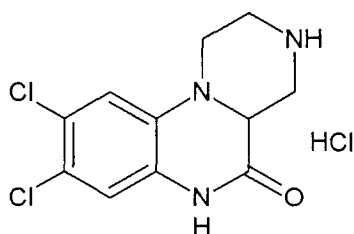
8,9-Dichloro-2,3,4,4a-tetrahydro-1H-pyrazino[1,2-a]quin oxalin-5(6H)-one
hydrochloride
(WAY 161503)
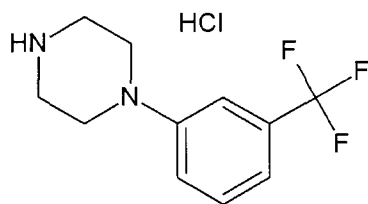
N-[3-(trifluoromethyl)phenyl] piperazine hydrochloride

(TFMPP)
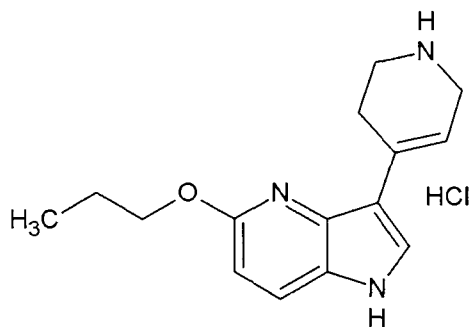
5-Propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-pyrrolo [3,2-b]pyridine

hydrochloride

(CP 94253)
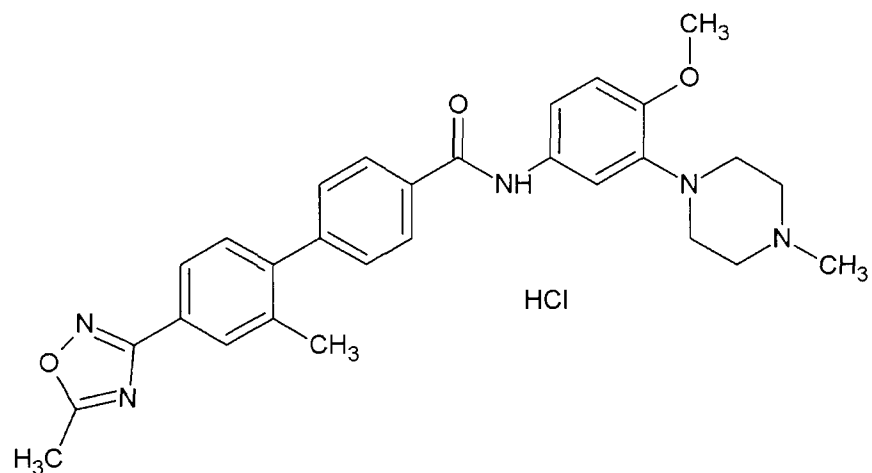
N-[4-Methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methy 1-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide hydrochloride

(GR 127935)
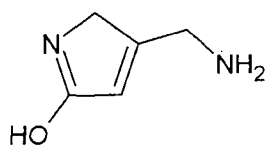
5-Aminomethyl-3-hydroxyisoxazole

(Muscimol)
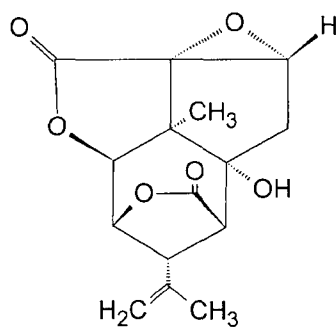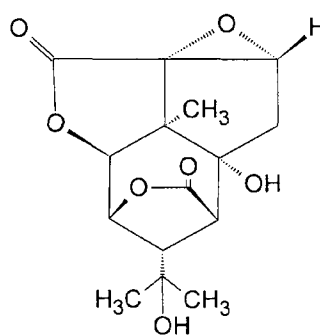
Picrotoxinin   Picrotin

1:1 mixture

(Picrotoxin)

41
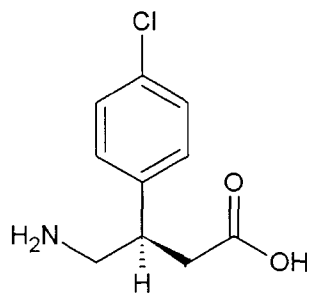
(R)-4-Amino-3-(4-chlorophenyl) butanoic acid

(Baclofen)
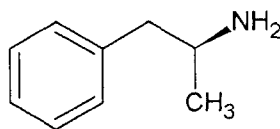
(+) α-Methylphenethylamine sulphate

((+)-amphetamine)

$2C_4H_6O_6$
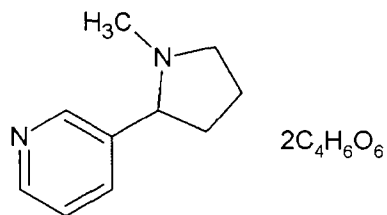
(−)-Nicotine hydrogen tartrate

(Nicotine)
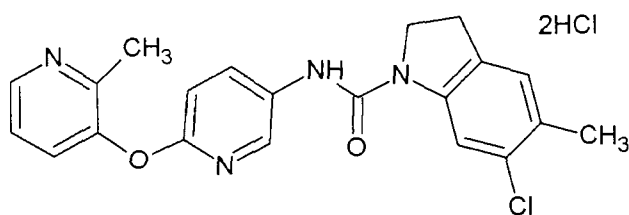
6-Chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline
dihydrochloride
(SB 242084)

Locomotor activity

Locomotor activity is used widely as a general measure of the stimulant or depressant properties of drugs. Exploratory behaviour is a complex behaviour affected by the organisms' external and internal environment, sensory processes, motor processes, and motivational processes (Kelley et al. 1989). The mesocorticolimbic DA system is also believed to play a pivotal role in the mediation of locomotor activity (Beninger 1983; Steketee and Kalivas 1992). Throughout this thesis, locomotor activity was used to compare the potential motor effects of drugs and to provide an initial dose-response curve.

Throughout the experiments in this thesis, spontaneous locomotor activity was measured using computer–monitored photobeam boxes (I. Halvorsen System Design, Phoenix, AZ, USA) consisting of a clear Plexiglas test cage (43 cm L x 43 cm W x 30 cm H) with a 12 x 12 photobeam grid located 2.5 cm above the floor. These beams measured horizontal activity (measured by the number of infrared beams broken) as well as consecutive beam breaks (repeat activity; repetitive breaking of one photobeam). Vertical activity (or rearing activity, measured by infrared beams broken following rears on the

hind legs) was measured using 12 additional photobeams located 12 cm above the floor. Upon arrival at the colony, all animals were allowed to acclimatize for at least two days. This was followed by two days of handling (5 min/animal/day). All animals were habituated to the locomotor activity boxes for two consecutive days (60 min/day). They subsequently received randomized and counterbalanced injections with three drug-free days between injections. All locomotor activity was monitored over a 30 or 60 min time course, with counts recorded every 5 min for the duration of testing.

It is important to briefly note that there is extensive descending 5-HT innervation of the mammalian spinal cord which is integral to locomotor function (for a review of the role of 5-HT in locomotor circuits in the spinal cord see Schmidt and Jordan, 2000). 5-HT cells in the parapyramidal region appear to be especially involved in activating locomotor central pattern generator activity; furthermore, this activity may be mediated by 5-HT_{2A} and 5-HT_7 receptors (Jordan et al. 2008). While the animals in the present thesis were not directly observed (their locomotor activity was monitored by a computer, as outlined above), there is no indication that any of the compounds used in this thesis produced any of the adverse symptoms associated with excessive 5-HT function characteristic of serotonin syndrome (Kalueff et al. 2008).

Place conditioning

Place conditioning is a widely used behavioural test to assess the conditioned reinforcing effects of a stimulus. Briefly, the primary motivational properties of a stimulus (i.e. unconditioned stimulus) are paired repeatedly with neutral stimuli within the environment until, following conditioning, they may develop secondary motivational properties and serve as conditioned stimuli. Standard place conditioning apparatus contain at least two

distinct environments which are differentially paired with the unconditioned stimulus under investigation and a control stimulus, respectively. When the unconditioned stimulus is positively reinforcing, a conditioned place preference may develop whereby the animal spends more time in the environment previously paired with that stimulus. When the unconditioned stimulus is aversive, a conditioned place aversion may develop whereby the animal spends less time in the environment previously paired with that stimulus. For more information, the reader is referred to the recently published comprehensive review on place conditioning studies by Thomas Tzschentke (2007).

Throughout the experiments in this thesis, place conditioning was assessed using an apparatus (I. Halvorsen System Design, Phoenix, AZ, USA) consisting of a rectangular Plexiglas box divided into two compartments (30 cm L x 30 cm W x 25 cm H). The compartments differed only in floor texture: 14 horizontal bars positioned 1.25 cm apart compared with 1-cm square grate wire flooring. The compartments were separated by a white plastic divider, which contained a tunnel (7.5 cm long) allowing access to both compartments that could be obstructed with removable doors during conditioning.

Upon arrival at the colony, all animals were allowed to acclimatize for at least two days. This was followed by two days of handling (5 min/animal/day). The procedure consisted of three phases. *Phase 1* (Pre-Conditioning): Animals were habituated to the place conditioning apparatus for three consecutive days, during which animals had free access to both compartments for 15 min. On the third day of pre-conditioning, the amount of time spent in each compartment was recorded. Animals were assigned to drug groups such that the average time initially spent in each compartment (bar vs. grate) was equal

between and within each group (unbiased design) or such that each animal was conditioned to the compartment in which it spent the least time, as determined on pre-conditioning day three (biased design). *Phase 2* (Conditioning): On alternate days, animals received drug and vehicle treatments and were confined to the drug-paired or vehicle-paired compartment for 30 min. Animals were conditioned for eight consecutive days during which they received four drug treatments. *Phase 3* (Post-Conditioning): During retention testing, animals were placed in the apparatus in a drug-free state and allowed free access to both compartments for 15 min. The amount of time spent in each compartment was recorded. A simplified schematic of this procedure is provided in Fig. 1.7.

Figure 1.7 Simplified schematic of the place conditioning procedure. Adapted from Clements (2005).
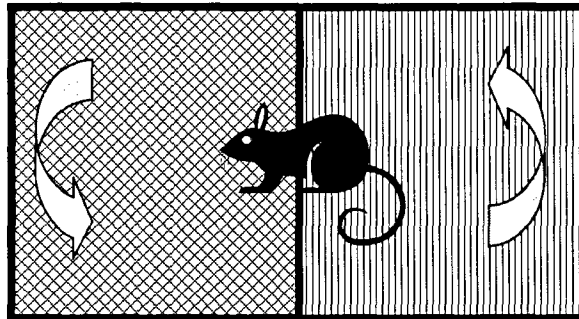
Phase 1: Pre-conditioning

Free access to both compartments for 3 days (15 min/day)
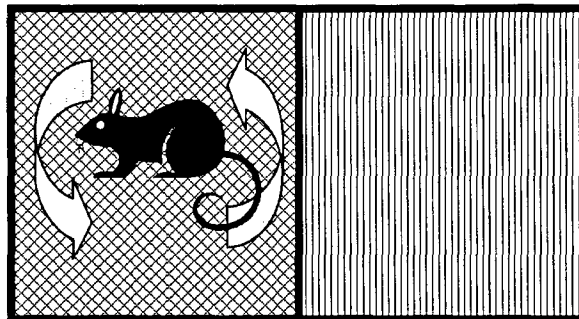
Phase 2: Conditioning

On alternate days animals are confined to single compartment following injection with drug of interest or vehicle (30 min/day for 8 days)
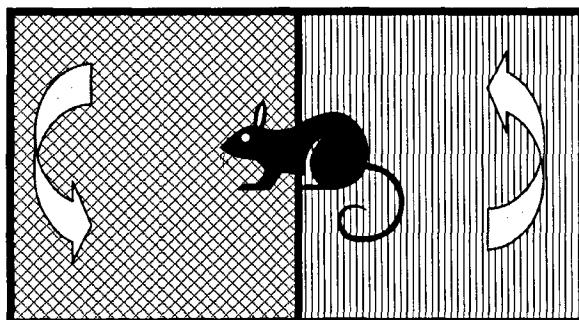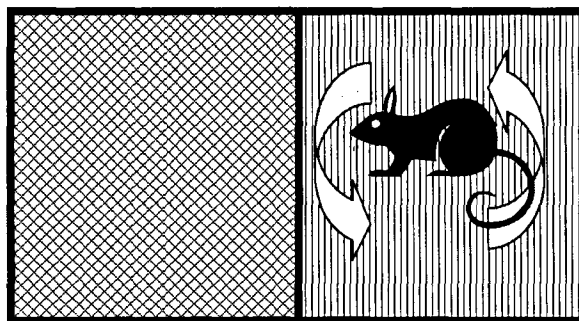
Phase 3: Post-conditioning

Free access to both compartments (15 min)

Nicotine-induced increases in locomotor activity

Nicotine, a potent nicotinic acetylcholine receptor agonist, can act as an effective reinforcer of drug-seeking and drug-taking behaviour in animal and human studies (Le Foll and Goldberg 2006). Systemic, intra-NAc, and intra-VTA administration of nicotine increases DA release in the NAc; nicotinic acetylcholine receptors in the VTA appear to be especially important in this regard (Nisell et al. 1994a; b; Pontieri et al. 1996). Ferrari et al. (2002) found that systemic or intra-VTA, but not intra-NAc, administration of nicotine caused an increase in locomotion. Many studies have demonstrated the locomotor stimulant effects of repeated exposure to nicotine (Arnold et al., 1995; Imperato et al., 1986; Clarke et al., 1988) and it is thought that these effects may be related to increased DA release in the NAc (Imperato et al., 1986; Clarke et al., 1988). In agreement with these ideas, nicotine-induced increases in locomotion have been blocked by systemic administration of the nicotinic receptor antagonist mecamylamine and by the DA D_2-like receptor antagonist haloperidol (Clarke and Kumar 1983b; Arnold et al. 1995). For more information on nicotine's mechanism of action and use in behavioural studies, see Di Chiara (2000), Janhunen and Ahtee (2007), and Wonnacott et al. (2005).

In the experiments in this thesis, nicotine-sensitized locomotor activity was used as a DA-related behavioural model to assess the potential role of the $5\text{-}HT_{2C}$ receptor, as $5\text{-}HT_{2C}$ receptor stimulation is known to decrease mesolimbic DA release (Di Giovanni et al. 2000; Di Matteo et al. 1999) and locomotor activity (Mosher et al. 2005). Two sets of experiments were carried out using this method. For the initial set, animals were habituated to the locomotor activity boxes for 14 days, during which they were injected daily with nicotine (0.6 mg/kg; to establish behavioural sensitization) or vehicle.

Following the 14 day sensitization period, the animals received randomized counterbalanced injections with the compound of interest. Three days were allowed between each treatment; during these days, animals continued to receive respective nicotine or vehicle injections and locomotor activity was measured. Locomotor activity was measured over a 60 min time course. In the second set, animals from nicotine-induced place conditioning experiments (experiencing 4 injections of 0.6 mg/kg nicotine) were assessed in a single locomotor activity session following a combination of vehicle, nicotine, and/or 5-HT$_{2C}$ receptor agonist drug injections.

Nicotine-induced place conditioning

Nicotine has also been shown to positively affect reward-related behaviour as demonstrated in intracranial self-stimulation (Clarke and Kumar 1984; Druhan et al. 1989; Huston-Lyons and Kornetsky 1992; Ivanova and Greenshaw 1997; Pradhan and Bowling 1971) and self-administration behaviour (Corrigall and Coen 1989; Corrigall et al. 1994; Lang et al. 1977; Smith and Roberts 1995; Tessari et al. 1995). Despite early controversies using systemically administered nicotine in the place conditioning method – as some groups noted place preferences, place aversions, or the absence of place conditioning – it is now accepted that systemic nicotine-induced place preference is reliable over a range of doses, under well-defined parameters, and with the use of a biased place conditioning design (for review see Le Foll & Goldberg, 2005).

In this thesis, nicotine-induced conditioned place preference was used as a DA- and reward-related behavioural model to assess the potential role of the 5-HT$_{2C}$ receptor, as 5-HT$_{2C}$ receptor stimulation is known to decrease mesolimbic DA release (Di Giovanni et al. 2000; Di Matteo et al. 1999) and nicotine-induced increases in DA release

(Di Matteo et al. 2004) without inducing place conditioning on its own (Mosher et al. 2005) – although 5-HT$_{2C}$ receptor activation may induce a state-dependent conditioned place aversion (Mosher et al. 2006). In this context, it is important to note that at least one group has suggested that the reinforcing effects of nicotine may be largely DA-independent (Laviolette and van der Kooy 2003). The parameters used to obtain nicotine-induced conditioned place preference are identical to those described above in the *place conditioning* section; some factors involved in this model are discussed in further detail in chapter 2.

Intracranial self-stimulation (ICSS)

Intracranial self-stimulation (ICSS) – sometimes referred to as brain stimulation reward – is an operant paradigm whereby animals learn to self-administer electrical stimulation to specific brain areas through surgically implanted electrodes (Olds and Milner 1954). ICSS has been used to map areas of the brain associated with aversive and reward-related behaviour, and can also be used to assess the reward-related effects of drugs (Kenny 2007; Wise 2002; 2005). There are a number of different ICSS methodologies, each with their own parameters (Yeomans 1990). The experiments within this thesis use electrical stimulation of the VTA to drive self-stimulation behaviour in rats, as the VTA is the origin of mesocorticolimbic DA projections and stimulation of this area results in DA release in the NAc (Blaha and Phillips 1990; Fiorino et al. 1993) – making VTA ICSS a sensitive and directed model of reward-related behaviour.

The ICSS method is used as a robust model of reward-related behaviour because it is resistant to satiation effects seen in other models of motivation and reward (e.g. food- or drug-maintained operant behaviours; access to sexual partners); it acts via primary

stimulation of reward-related circuitry as opposed to indirect stimulation via Pavlovian conditioning (e.g. place conditioning), and the animal's performance can be extremely stable over a time period of months (Carlezon and Chartoff 2007). Given the number of parameters involved, however, it is important to note that studies using the ICSS method must always be interpreted with caution. Potential nonspecific effects of drugs on general arousal, sensory or motor systems, attention, or memory may confound ICSS results under some conditions – particularly as DA systems are believed to play a role in regulating these phenomena (Coull 1998; Laviolette 2007; Wise 2004; 2005).

The ICSS method employed in the present thesis is termed 'curve-shift rate-frequency (Hz) threshold analysis' but may be referred to as simply the 'rate-frequency' or 'curve-shift' method throughout the literature, although the latter should not be confused with 'rate-current (µA) curve-shift threshold analysis' (Kornetsky et al. 1979). The rate-frequency threshold method measures operant responses to stepwise changes in frequency while the rate-current threshold method uses stepwise changes in current; either threshold measure is currently considered superior to other ICSS methods (Konkle et al. 2001). Changes in frequency (i.e. rate-frequency threshold analysis) are used within the present thesis, compared to changes in current, as changes in current are positively correlated to the number of stimulated cells while changes in frequency have been shown to alter the firing rates of neurons without largely affecting the population of cells activated (Ranck 1975; Yeomans 1990). With this procedure, M50 is the threshold frequency at which half-maximal response rates occur; RMAX is the maximal rate of responding in a session; TRES is the total responses in a given session. While M50 is a measure of reward sensitivity (which is dissociable from non-specific changes in

behaviour), RMAX is a measure of response performance (see Gallistel and Karras, 1984; Greenshaw and Wishart, 1987). Additional factors which are important to consider include pulse length and type, train length, electrode tip size, and training parameters.

While there are various potential approaches and parameters for electrical stimulation of the brain, we used monopolar electrodes to deliver cathodal stimulation to the VTA. We chose monopolar electrodes (compared to bipolar electrodes) because they are smaller in diameter and allow for increased precision with minimal tissue damage. We chose cathodal stimulation (compared to anodal stimulation) because it allows for the use of lower stimulation parameters (e.g. lower current levels are required compared to anodal stimulation) and produces less tissue damage due to the emission of metal ions from the electrode tip. To minimize the probability of tissue damage due to high current density at the electrode tip, 0.5 mm of the insulating resin was shaved in order to increase the surface area of the exposed electrode tip. The VTA was chosen in order to increase the probability of orthodromically stimulating the mesocorticolimbic DA cells, although it is important to note that cells containing other neurotransmitters, as well as nearby axons, may also be stimulated. For extensive reviews regarding the parameters of, and other issues surrounding, electrical stimulation of neural tissue, see Carlezon and Chartoff, 2007; Ranck, 1975; Roth, 1994; Tehovnik, 1996; Yeomans, 1990.

All measures (i.e. M50, RMAX, and TRES) are reported as a percentage of baseline responding, calculated by dividing the respective value collected on the treatment day divided by the mean of the 3 preceding drug-free days and converting to percent. M50 values for individual animals were calculated daily using linear regression. While these measurements are communicated in histograms throughout this thesis, group-

averaged rate-frequency curves were also created for illustrative purposes. These were derived by converting response rate per frequency to a percentage of maximum response rate for each animal and then averaging these values for each frequency by treatment group. It is important to note that while rate-frequency curves are usually concordant with data obtained following linear regression, discrepancies may exist due to the loss of data integrity (or 'smoothing out' of the data) following non-linear regression. Fig. 1.8 provides an example of a rate-frequency curve.

Figure 1.8 Example of a rate-frequency curve demonstrating the rate-frequency threshold (M50) and the maximal response rate (RMAX). Note that a curve shift to the right (an increase in M50 threshold), following a drug treatment, suggests a decrease in reward.
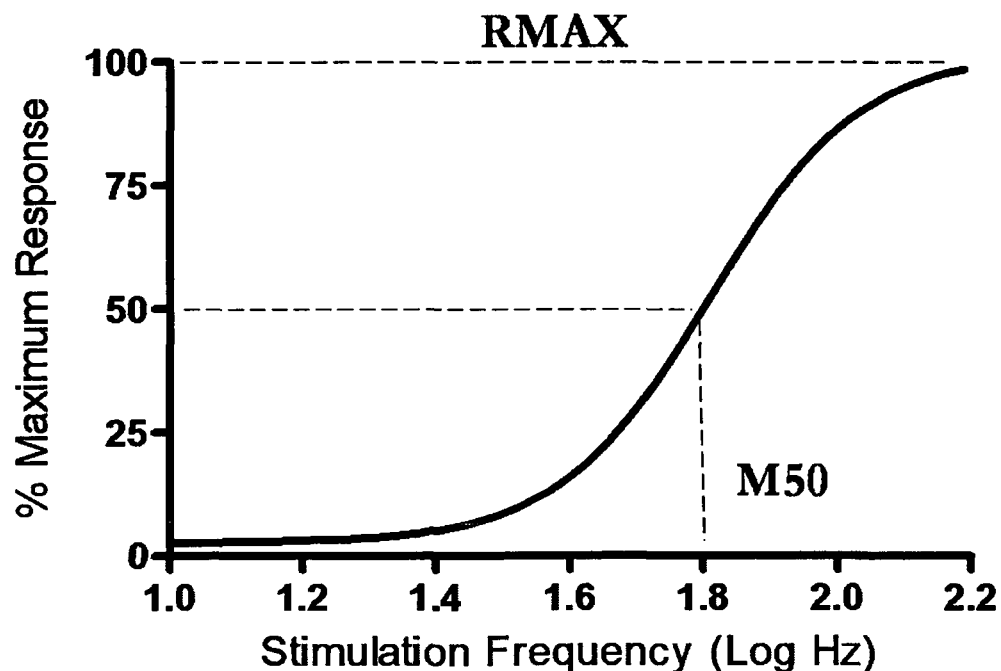
Upon arrival at the colony, all animals were allowed to acclimatize for at least two days before surgery. Unlike the locomotor and place conditioning experiments, the animals were not specifically handled over the course of 2 days, as the ICSS procedure requires daily handling for at least 3 weeks before drug testing begins. A standard operating procedure for central implantation of electrodes and cannulae is provided in Appendix C. Briefly, using a previously described procedure (Greenshaw 1993), each animal was anesthetized using halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Halocarbon Laboratories, River Edge, NJ, USA) or isoflurane [2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane; Halocarbon Laboratories, River Edge, NJ, USA] and placed in a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA). Animals were implanted with a stainless steel, monopolar, stimulating electrode (E363/2; tip diameter 200 μm; Plastics One Ltd., Roanoke, VA) directed to the VTA. A large silver indifferent electrode in the skull served as the relative ground. Animals receiving subsequent microinjections were also implanted with bilateral cannulae (22 gauge) directed to the rostral shell of the NAc. Stereotaxic coordinates were [mm]: VTA – AP +2.6, L +0.5, V +1.8; NAc shell – AP +11.0, L +0.4, V +2.8, from inter-aural zero, with the incisor bar set at 2.4 mm below the inter-aural line (Paxinos and Watson 1998). These coordinates were interpolated from the target site for an angle of 20° lateral and anterior for the VTA and 16° lateral for the NAc shell (Greenshaw 1997). The guide cannulae were placed 1 mm above the actual injection sites. Electrode and cannulae placements were verified at the end of the experiment by microscopic inspection of flash-frozen coronal brain sections (40 μm) stained with cresyl violet; flash-freezing was achieved

using isopentane cooled on dry ice. Only animals with VTA and NAc placements were included in the analysis. The histology protocol is described in Appendix D.

The apparatus consisted of a ventilated operant chamber (24 cm L x 30 cm W x 29 cm H) within a sound-attenuating chamber (Coulbourn Instruments Ltd., Lehigh Valley, PA, USA). Monopolar stimulation of the VTA was provided from constant current DC stimulators (cathodal monophasic pulse width of 200 μs; initial training frequency of 100 Hz; train length of 1 s) connected to each animal via a gold-track slip ring. Between pulses the active electrode and indifferent electrode were connected through a 10 kΩ resistor to cancel any effects of electrode polarisation (Greenshaw 1986).

Following at least one week of recovery, each animal was initially trained to lever-press for electrical stimulation of the VTA using a constant frequency of 100 Hz and the minimum current (80-400 μA; although the majority of currents used were between 80-160 μA) required to maintain responding. Once established, animals underwent daily sessions on continuous reinforcement schedules designed to determine the current that produced half-maximal responding, with frequency held constant at 100 Hz. This continued until the half-maximal current varied less than 15% over three consecutive days. Animals then underwent daily sessions designed to determine the stimulation frequency (0-160 Hz; 1.0-2.2 log Hz) that produced an M50 threshold that varied less than 15% over three consecutive days. Animals then began a randomized counterbalanced sequence of drug treatments with 3 days of baseline frequency testing between each treatment. One rate-frequency function was generated per day, using stepwise changes in frequency with all other parameters remaining constant. Responding

was initiated at 160 Hz and decreased stepwise each minute by 0.1 log Hz until 0 Hz (or until responding ceased for the duration of an entire available frequency), upon which, the frequency values increased stepwise. Each current or frequency trial began with three priming pulses which served as a discriminative stimulus, indicating the availability of stimulation.

Food intake

Adapted from previously described procedures (Reynolds and Berridge 2001; Stratford and Kelley 1997), animals were placed in standard Plexiglas laboratory cages (free from wood shavings) immediately following the VTA ICSS session. A pre-weighed amount of food, placed in a container identical to that found in their home cage, was made available along with water 25 min after the initiation of each VTA ICSS session (each session is a maximum of 25 min). At the end of a 30 min session (55 min post injection), food intake was calculated by subtracting the initial weight of the food and container from the final weight. Animals were habituated for three days prior to the beginning of microinjection treatments. This procedure was subsequently performed on each microinjection treatment day to determine total food intake (measured in grams) in a 30 min session following intra-NAc $GABA_A$ receptor (muscimol) or $GABA_B$ receptor (baclofen) agonists. This procedure was used only following intra-NAc microinjections of these compounds, in order to provide an additional positive control. The literature on food reward is extensive and beyond the scope of this thesis; as such, the interested reader is referred to recent reviews by Figlewicz and Benoit (2008), Olszewski et al. (2008), and Yamamoto (2008).

General review of 5-HT$_{2C}$, 5-HT$_{1B}$, GABA$_A$, and GABA$_B$ receptors

in behavioural models of motivation and reward

The aim of this thesis was to investigate the role of the 5-HT$_{2C}$ receptor in reward-related behaviour. To this end, potential interactions between the 5-HT$_{1B}$, GABA$_A$, and GABA$_B$ receptors were also investigated. To provide context to this endeavour, a brief review of studies examining the effects of ligands for these receptors in behavioural models of motivation and reward (e.g. place conditioning, ICSS, and self-administration) is presented here. While there are many studies which have used 'mixed' compounds (displaying pharmacological activity at a number of receptor subtypes) the present review has attempted, for clarity, to include mainly studies that used ligands relatively selective for one receptor subtype or those that used mixed drugs whose behavioural effects are strongly associated with activity at one receptor subtype. In addition, studies using techniques that may result in broader, non-selective, effects on neurotransmitter systems (e.g. lesion studies or studies using neurotransmitter releasers or reuptake inhibitors) are not included here.

5-HT$_{2C}$ receptors in motivation and reward

Place conditioning

Few studies have investigated the reinforcing effects of 5-HT$_{2C}$ receptor ligands in place conditioning. The mixed 5-HT$_{1B/2C}$ receptor agonist mCPP did not induce place conditioning on its own, but was able to block the conditioned place aversion induced by mianserin (a mixed 5-HT$_2$ antagonist) and eltoprazine (a mixed 5-HT$_{1B}$ receptor agonist & 5-HT$_{2C}$ receptor antagonist) (Rocha et al. 1993). The notion that 5-HT$_{2C}$ receptor

compounds do not appear to induce place conditioning in rats was further supported using the mixed 5-HT$_{2C/1A/1B}$ receptor agonist TFMPP and the selective 5-HT$_{2C}$ receptor agonist WAY 161503 (Mosher et al. 2005). However, WAY 161503 may induce a state-dependent place aversion (Mosher et al. 2006).

In addition, one report has shown that administration of the selective 5-HT$_{2C}$ receptor agonist Ro 60-0175 attenuates Δ 9-tetrahydrocannibanol-induced place conditioning and also suggested that 5-HT$_{2C}$ receptor activity may inhibit nicotine-induced place preference – although this hypothesis was not directly tested (Ji et al. 2006). In general, these studies have suggested that 5-HT$_{2C}$ receptor stimulation alone may not induce place conditioning, except under state-dependent conditions, although it may attenuate the reinforcing and aversive properties of other compounds under some conditions.

Intracranial self-stimulation

Repeated administration of the 5-HT$_{2C}$ receptor agonists Ro 60-0175 and Ro 60-0332, in the chronic stress-induced model of anhedonia (i.e. animals are exposed daily to mild stressors such as food or water deprivation, resulting in anhedonia-like behaviours; for more information regarding this model see Willner et al. (1992)), prevented decreases in VTA ICSS reward typically seen with this model; in contrast, Ro 60-0175 was ineffective in non-stressed animals (Moreau et al. 1996). Interestingly, repeated administration of the 5-HT$_{2A/C}$ receptor antagonist mianserin also prevented decreases in VTA ICSS reward measures in this model; this treatment produced decreases in reward in non-stressed animals with stable VTA ICSS responses prior to the onset of injections (Moreau et al. 1994). In addition, systemic administration of mCPP (a mixed 5-HT$_{1B/2C}$ receptor

agonist) dose-dependently increased current-thresholds for medial forebrain bundle ICSS (Borisenko et al. 1996). These somewhat variable results underscore the need for further examination of selective 5-HT$_{2C}$ receptor ligands using frequency-threshold measures of ICSS.

Self-administration

Although the self-administration method is not employed in this thesis, it is a valuable method for investigating the effects of drugs or other reinforcers. Briefly, a variety of schedules of reinforcement can be used (as is also true, but less common, in ICSS behaviour) and may provide convergent verification of drug effects, although they may also reveal important differences in self-administration behaviour. Briefly, the fixed-ratio (FR) schedule involves a fixed number of responses required for drug administration (e.g. FR 5 means that the animal must respond 5 times before receiving reinforcement), while the fixed-interval (FI) schedule requires a given amount of time to pass before access to the reinforcer is granted. It is important to note that self-administration on these schedules usually results in an inverted U-shaped dose-response function, as the animal responds to maintain ideal blood concentration levels of the compound. Second-order schedules can also be employed, whereby a conditioned reinforcer (e.g. a light or tone cue) is used to signal or replace a primary reinforcer. The progressive ratio (PR) schedule requires incremental increases in responding to achieve drug administration. It is suggested that this schedule is best at determining the rank-order effectiveness for reinforcers, as determined mainly by the breaking point (the point at which the animal will no longer respond). Further discussion of these schedules and other issues involved in the self-

administration model is covered elsewhere (Panlilio and Goldberg 2007; Shippenberg and Koob 2002).

Studies investigating the role of 5-HT$_{2C}$ receptors in self-administration have been largely limited by the number of pharmacologically specific compounds available. Mice lacking the 5-HT$_{2C}$ receptor gene (i.e. 'knock-out mice') were shown to press an active lever approximately twice as often as their wildtype counterparts in order to self-administer cocaine on a PR schedule of reinforcement (Rocha et al. 2002). Cocaine responding and reinstatement was reduced by the 5-HT$_{2C}$ receptor agonist Ro 60-0175 in rats trained on FR 5 (with a one minute time-out period between injections) and PR schedules of reinforcement; nicotine self-administration was also reduced on the same FR 5 schedule (Grottick et al. 2001; Grottick et al. 2000). Fletcher et al. (2002b) showed that rats trained on a PR schedule of reinforcement for cocaine showed a dose-dependent increase in breaking points when pretreated with the selective 5-HT$_{2C}$ receptor antagonist SB 242084. In addition, SB 242084 was shown to enhance cocaine reinstatement following a two-week extinction period. In a separate study, Fletcher et al. (2004) showed that intra-VTA administration of Ro 60-0175 decreased responding for cocaine on an FR 5 and PR schedule of reinforcement and these effects were attenuated by SB 242084. Alternately, Filip (2005) showed that the 5-HT$_{2C}$ receptor antagonist SDZ SER-082 had no effect on the maintenance of cocaine self-administration, priming, or cue-induced reinstatement in rats trained on a FR 5 schedule of reinforcement.

Regarding ethanol self-administration, the mixed 5-HT$_{2C/1A/1B}$ receptor agonist TFMPP decreased responding in rats trained on a continuous and second-order schedule of reinforcement (Wilson et al. 2000; Wilson et al. 1998). In another study,

administration of the 5-HT$_{2C}$ receptor antagonist SB 242084 dose-dependently increased responding for low ethanol drinking rats (rats that did not acquire pharmacologically relevant levels of ethanol; <0.3 g/kg) trained to self-administer 12% ethanol on a FR 4 schedule of reinforcement (Tomkins et al. 2002). Though a similar enhancement was observed, these effects were not significant in rats selected for high levels of ethanol drinking. Overall, these studies suggest that the 5-HT$_{2C}$ receptor may act to tonically and phasically inhibit reward-related behaviour induced by drugs of abuse.

5-HT$_{1B}$ receptors in motivation and reward

Place conditioning

The selective 5-HT$_{1B}$ receptor agonist CP 94253 has been shown to induce conditioned place aversion (CPA) on its own and conditioned place preference (CPP) when co-administered with a subthreshold dose of cocaine (Cervo et al. 2002; Mosher 2006). These effects were attenuated using the 5-HT$_{1B/1D}$ receptor antagonist GR 127935, although this compound had no effect on place conditioning when administered alone. Consistent with these effects, viral-mediated gene transfer – resulting in the overexpression of 5-HT$_{1B}$ receptors in NAc projections to the VTA – produced conditioned place preference in rats given a subthreshold dose of cocaine and enhanced the aversive effects of high doses of cocaine resulting in a CPA (Barot et al. 2007; Neumaier et al. 2002). The authors believe that the effects seen are due to 5-HT$_{1B}$ heteroreceptors located on the terminals of GABA cells within the VTA. They believe that increased 5-HT$_{1B}$ receptors on GABA cells may contribute to the hyperpolarization of these cells, resulting in the disinhibition of DA cells projecting from the VTA.

Intracranial self-stimulation

Only one study to date has suggested a role for the $5-HT_{1B}$ receptor in ICSS, demonstrating decreased reward (measured by increases in lateral hypothalamic current thresholds) following systemic administration of the $5-HT_{1A/1B/1D/2C}$ mixed agonist RU 24969 (whose behavioural effects are often suggested to be $5-HT_{1B}$ receptor-mediated) and subsequent attenuation by the $5-HT_{1B/1D}$ receptor antagonist GR 127935. This same study also found that RU 24969 attenuated the threshold-reducing (reward-enhancing) effects of cocaine (Harrison et al. 1999). GR 127935 did not show any effects when administered alone under these conditions.

Self-administration

In general, selective $5-HT_{1B}$ receptor agonists seem to enhance the effects of cocaine, while tonic activity of the $5-HT_{1B}$ receptor may not be required for these effects as antagonists appear to have no effect on their own. Systemic administration of the selective $5-HT_{1B}$ receptor agonist CP 94253, in rats trained to self-administer cocaine on FR 5 and PR schedules of reinforcement, dose-dependently enhanced the reinforcing effects of cocaine; GR 127935 attenuated these effects. Intracerebroventricular injections of another $5-HT_{1B}$ receptor agonist, CP 93129, showed similar results in this study (Parsons et al. 1998). It is interesting to note that this same study demonstrated that systemic CP 94253, but not RU 24969, could sustain self-administration behaviour on the FR 5 schedule of reinforcement, although this effect was seen only at a single mid-range dose and self-administration was not maintained longer than 90 minutes. In agreement, Przegalinski et al. (2007) also found that systemic CP 94253 enhanced the reinforcing

effects of cocaine in rats trained on a FR 5 schedule of reinforcement and that these effects were blocked by the $5-HT_{1B}$ receptor antagonist SB 216641 which showed no intrinsic activity. Interestingly, one study has demonstrated that systemic injections of the DA D_1 receptor antagonist SCH 23390 or $5-HT_{1B/1D}$ receptor antagonist GR 127935 were able to attenuate the reinforcing effects of cocaine in mice trained to self-administer cocaine into the VTA by discriminating between two arms of a Y-maze (David et al. 2004). In addition, mice lacking the $5-HT_{1B}$ receptor, and their wildtype counterparts, may self-administer cocaine in a similar fashion under a FR 2 schedule of reinforcement (Rocha et al. 1997). However, under a progressive ratio schedule $5-HT_{1B}$ receptor knock-out mice showed significantly higher breakpoints then their wildtype counterparts, an effect not altered by pretreatment with GR 127935 in either group (Castanon et al. 2000; Rocha et al. 1998). The authors suggested that this discrepancy may be due to compensatory mechanisms which may have arisen during the development of the $5-HT_{1B}$ receptor knock-out mouse.

Systemic administration of the $5-HT_{1A/1B/1D/2C}$ mixed agonist RU 24969 non-specifically reduced fluid intake in a two-bottle, free choice, ethanol self-administration test in rats (Silvestre et al. 1998). Pretreatment with the selective $5-HT_{1B}$ receptor agonist CP 94253 reduced orally administered 10% ethanol-reinforced responding on a FR 1 schedule of reinforcement, although this effect was seen only at the highest dose tested. The authors indicated that this may suggest a nonselective disruption of operant behaviour (Maurel et al. 1999). Tomkins and O'Neill (2000) challenged this position by showing that RU 24969 decreased ethanol self-administration for animals trained on a FR 4 schedule of reinforcement and that this effect was attenuated by GR 127935 (although

not at the highest dose of RU 24969 tested), but not by 5-HT$_{1A}$ receptor antagonists. In addition, GR 127935 appeared to have no effect when administered alone. In contrast, viral-mediated over-expression of NAc shell 5-HT$_{1B}$ receptors in rats led to increases in ethanol consumption over control subjects (Hoplight et al. 2006). In addition, two studies investigating the effects of ethanol self-administration in 5-HT$_{1B}$ receptor knock-out mice have shown that these mice may (Crabbe et al. 1996) or may not (Risinger et al. 1999) consume more ethanol than their wildtype littermates.

Fletcher and Korth (1999) showed that (+)-amphetamine self-administration and (+)-amphetamine-potentiated responding for a conditioned reinforcer (light/tone stimulus previously paired with water availability) are reduced following administration of RU 24969. These effects were reversed by GR 127935 but not by the 5-HT$_{1A}$ receptor antagonist WAY 100635. In a separate study, intra-NAc injections of 5-HT and the 5-HT$_{1B}$ receptor agonist CP 93129 (but not the non-selective 5-HT$_{1A/7}$ receptor agonist 8-OHDPAT or the 5-HT$_2$ receptor agonist DOI) reduced responding on a PR schedule of reinforcement for (+)-amphetamine. These effects were attenuated by systemically administered GR 127935, although it had no effect when administered alone. The authors suggest that these data point to a role for 5-HT$_{1B}$ receptors within the nucleus accumbens in inhibiting the reinforcing properties of (+)-amphetamine (Fletcher et al. 2002a).

These data appear to indicate a differential role for 5-HT$_{1B}$ receptors in the reinforcing effects of various self-administered compounds. In addition, there are incongruities within each area which should be addressed by future studies. Overall, stimulation of the 5-HT$_{1B}$ receptor may lead to a decrease in reward, as seen with place conditioning, self-stimulation, and ethanol and (+)-amphetamine self-administration. 5-

HT$_{1B}$ receptor stimulation may also play a role in the enhancement of cocaine's reinforcing effects. In addition, there is little evidence for tonic activity of the 5-HT$_{1B}$ receptor across studies.

GABA$_A$ receptors in motivation and reward

Place conditioning

Intra-VTA injections of the GABA$_A$ receptor agonist muscimol, or antagonist bicuculline, produced a robust CPP. The effects of muscimol were blocked with the DA D$_{1/2}$ receptor antagonist α-flupenthixol, although it did not affect bicuculline-induced CPP (Laviolette and van der Kooy 2001). Intra-VTA injections of muscimol and bicuculline showed biphasic effects on morphine-induced CPP. A low dose of muscimol attenuated, while a higher dose potentiated, morphine-induced CPP; the opposite was true for bicuculline (Sahraei et al. 2005). Injections of muscimol into the rostral portion of the NAc shell induced CPP, whereas injections into the medial or caudal shell produced CPA (Reynolds and Berridge 2002). Muscimol administration into the median raphe, but not the dorsal raphe nucleus, induced a CPP; these effects were blocked by the GABA$_A$ receptor antagonist picrotoxin and by pretreatment with the DA D$_1$-like receptor antagonist SCH 23390 (Liu and Ikemoto 2007). Intra-basolateral amygdala injections of muscimol or bicuculline did not produce place conditioning, although muscimol significantly decreased the acquisition (but not the expression) of morphine-induced CPP; intra-basolateral amygdala bicuculline produced CPP in combination with a subthreshold dose of morphine (Macedo et al. 2006; Zarrindast et al. 2004).

Studies have shown that systemically administered benzodiazepines and barbiturates (which can both act as positive allosteric modulators of the $GABA_A$ receptor) may also produce effects in place conditioning. Systemic diazepam, pentobarbital, triazolam, and systemic and intracerebroventricular sodium barbital all induced a CPP (Bossert et al. 2003; Bossert and Franklin 2001; Gray et al. 1999; Hirai et al. 2005; Papp et al. 2002). Interestingly, the effects of pentobarbital were blocked by bicuculline but not picrotoxin (Bossert and Franklin 2001) and the effects of diazepam were blocked with glutamate receptor antagonists (Gray et al. 1999; Papp et al. 2002). Systemic chlordiazepoxide produced a CPA (Parker et al. 1998), while alprazolam, diazepam, and oxazepam may have no effect in place conditioning (Goeders and Goeders 2004; Le Pen et al. 2002; Matsuzawa et al. 2000; Meririnne et al. 1999; Walker and Ettenberg 2001; 2003; 2005). Alprazolam was able to enhance systemic and intra-VTA heroin-induced CPP (Walker and Ettenberg 2001; 2003; 2005), while diazepam attenuated (+)-amphetamine- and cocaine-induced CPP (Leri and Franklin 2000; Meririnne et al. 1999), and oxazepam attenuated meth(+)-amphetamine-induced CPP (Goeders and Goeders 2004). The differential effects of these compounds may be related to their affinity for $GABA_A$/benzodiazepine receptors containing different subunit combinations (see *GABA receptors* section for discussion and review references).

Intracranial self-stimulation

Electrophysiologically characterized VTA GABAergic cells have been implicated in the mediation of ICSS in the rat (Steffensen et al. 2001; Steffensen et al. 1998); electrically coupled GABA cells may be involved (Lassen et al. 2007). In addition, Cheer et al.

(2005) found that both contingent and non-contingent stimulation of the medial forebrain bundle produced NAc core DA release as well as time-locked, $GABA_A$ receptor-mediated, inhibitions in a subset of NAc cells, supporting a role for mesolimbic GABA cells in ICSS.

The systemically administered $GABA_A$ receptor agonist muscimol decreased, while the antagonist picrotoxin increased, current thresholds in rats trained to self-stimulate the medial forebrain bundle (Zarevics and Setler 1981). In agreement, Porrino and Coons (1980) showed that systemic administration of picrotoxin produced increases in lateral hypothalamic frequency thresholds. The systemically administered barbiturate pentobarbital decreased rate-frequency thresholds in medial forebrain bundle ICSS, comparable to the effects of (+)-amphetamine (Bossert and Franklin 2003). Systemically administered benzodiazepines, such as bromazepam, chlordiazepoxide, diazepam, triazolam, and the anxiolytic meprobamate, all facilitated responding for lateral hypothalamic stimulation on a number of schedules of reinforcement (Gomita et al. 2003; Greenshaw et al. 1983; Ichimaru et al. 1983; Liebman 1985).

Intra-VTA injections of muscimol may have no effect on rate-current thresholds or performance effects in lateral hypothalamic-medial forebrain bundle ICSS behaviour (Willick and Kokkinidis 1995), although Panagis and Kastellakis (2002) showed that intra-VTA muscimol produced an increase in rate-frequency thresholds for ventral pallidal ICSS. Singh et al. (1997) investigated the effects of ipsilateral intra-VTA-substantia nigra and intra-lateral hypothalamic-medial forebrain bundle injections of $GABA_A$ receptor antagonists on VTA-substantia nigra and lateral hypothalamic-medial forebrain bundle ICSS (where current and frequency were held constant at a

predetermined M50). Intra-VTA bicuculline had no effect on either VTA or lateral hypothalamic ICSS, but did produce robust decreases in response rates for both sites when injected into the lateral hypothalamus. Intra-VTA picrotoxin produced decreases in response rates for VTA and lateral hypothalamic ICSS, and also produced decreases in response rates for lateral hypothalamic ICSS when injected into the lateral hypothalamus.

Waraczynski (2008) showed that injecting muscimol into the sublenticular central extended amygdala produced increases in frequency-thresholds and decreases in motor performance for medial forebrain bundle ICSS, while bicuculline showed no effect. In a separate study, Waraczynski (2007) showed that muscimol injected into the lateral and medial septum and the vertical limb of the diagonal band of Broca had no effect on medial forebrain bundle ICSS, while injections into the horizontal limb of the diagonal band of Broca or the magnocellular preoptic area resulted in slight increases in frequency-thresholds and large decreases in motor performance. In addition, intra-basolateral amygdala administration of muscimol produced increases in frequency-thresholds in conjunction with decreases in motor performance for VTA ICSS (Simmons et al. 2007). It is important to reiterate that incongruent results across these studies may involve many methodological issues including the use of non-specific measures of reward, and the absence of motor performance measures, as discussed above.

Self-administration

Muscimol is readily self-administered into the posterior, but not anterior, VTA in rats trained on FR1 and FR3 schedules of reinforcement; these effects are blocked by co-administration of picrotoxin (Ikemoto et al. 1998). Alternately, the $GABA_A$ receptor

antagonists picrotoxin and bicuculline are self-administered into the anterior VTA, but not into the posterior VTA, substantia nigra or lateral hypothalamus; these effects are attenuated by muscimol (Ikemoto et al. 1997b). Rats will also self-administer muscimol into the median or dorsal raphe nucleus, although the reinforcing effects of muscimol appeared to be greater when administered into the median raphe nucleus; these effects were attenuated by coadministration of picrotoxin and by pretreatment with the DA D_1 receptor antagonist SCH 23390 (Liu and Ikemoto 2007). Muscimol, but not bicuculline, is also self-administered by mice into the medial septal division (including the medial septal nucleus and the diagonal band of Broca); these effects were blocked by systemic or intra-VTA administration of SCH 23390 or systemic administration of the DA $D_{2/3}$ receptor antagonist sulpiride. Interestingly, muscimol was not self-administered into the NAc shell in this study, and in fact, mice developed a preference for the previously neutral arm of a Y-maze which avoided muscimol administration (Gavello-Baudy et al. 2008).

Systemic administration of muscimol, the benzodiazepine midazolam, and the barbiturate pentobarbital, all decrease cocaine self-administration for rats trained on a FR 5 (with 20 s time-outs) schedule of reinforcement (Barrett et al. 2005). Anterior, but not posterior, intra-VTA injection of muscimol reduced cocaine responding on FR 1, and increased breakpoints on PR, schedules of reinforcement – suggesting an enhancement of cocaine reward; picrotoxin had no effect (Lee et al. 2007). In contrast, intra-VTA injection of muscimol had no effect on cocaine self-administration in rats trained on a FR 5 schedule of reinforcement in another study, although picrotoxin attenuated cocaine responding (Backes and Hemby 2008). Intra-VTA muscimol also greatly reduced

nicotine, without significant effects on cocaine, self-administration for animals trained on a FR 5 schedule of reinforcement (Corrigall et al. 2000). It is possible that the discrepancy between some of these studies lies in the rostral-caudal differences in the VTA. In addition, intra-pedunculopontine tegmental nucleus injections of muscimol attenuates responding for nicotine, but not cocaine, self-administration in rats trained on a FR 5, but not a PR, schedule of reinforcement (Corrigall et al. 2001).

Similar to the place conditioning and ICSS data, numerous self-administration studies indicate that benzodiazepines and barbiturates are generally reinforcing. Systemically administered self-administration can be maintained with alprazolam, amobarbital, diazepam, lorazepam, methohexital, midazolam, pentobarbital, phenobarbital, secobarbital, triazolam under various FI and PR schedules of reinforcement (see Ator 2005, Licata and Rowlett 2008, and Meisch 2001 for reviews). In addition, ethanol also positively modulates the $GABA_A$ receptor and is involved in motivation and reward-related behaviour. As there are a large number of studies in this regard, and those related specifically to the $5\text{-}HT_{2C}$ and $5\text{-}HT_{1B}$ receptors are noted above, the reader is referred to reviews covering this broad literature (Chester and Cunningham 2002; Crabbe et al. 2006; Enoch 2008; Koob 2004).

Overall, these studies suggest that the GABA system is involved in reward-related behaviour and that limbic structures such as the VTA, NAc, and lateral hypothalamus may be particularly important in this regard. Although studies employing the systemic injection of GABAergic compounds can be useful, they should be interpreted with caution. It is also important to remember that changes in reward-related behaviours,

following administration of GABA$_A$ receptor ligands, is probably due to a functional balance between activation at pre-, post-, and extra-synaptic receptors.

GABA$_B$ receptors in motivation and reward

Place conditioning

Systemic administration of the selective GABA$_B$ receptor agonist baclofen did not produce place conditioning alone but blocked fentanyl- and morphine-induced CPP (Kaplan et al. 2003; Suzuki et al. 2005), as well as the acquisition and expression of meth(+)-amphetamine-induced CPP (Li et al. 2001) and nicotine-induced CPP (Le Foll et al. 2008), but did not affect ethanol-induced CPP (Chester and Cunningham 1999). Systemic administration of the GABA$_B$ receptor positive allosteric modulator GS 39783 also blocked the acquisition, but not expression, of nicotine-induced CPP (Mombereau et al. 2007).

Intra-VTA baclofen did not produce place conditioning when administered alone, although it did attenuate the CPP induced following intra-VTA injections of the GABA$_A$ receptor agonist muscimol (Laviolette and van der Kooy 2001). Intra-VTA baclofen also showed biphasic effects for, although the GABA$_B$ receptor antagonist CGP 35348 blocked, morphine-induced CPP (Sahraei et al. 2005). In a follow-up study, Sahraei et al. (2008) showed that intra-VTA baclofen attenuated the expression of morphine-induced CPP at low and high doses, whereas a midrange dose enhanced CPP; all doses attenuated the acquisition of CPP. In addition, CGP 35348 reduced the expression and acquisition of morphine-induced CPP. Intra-VTA baclofen also effectively blocked ethanol-induced CPP (Bechtholt and Cunningham 2005). Intra-dorsal hippocampal baclofen reduced the

acquisition and expression of morphine-induced CPP, while the $GABA_B$ receptor antagonist phaclofen enhanced only the acquisition (Zarrindast et al. 2006).

Intracranial self-stimulation

Early studies showed that systemically administered baclofen reduced responding rates for low-current lateral hypothalamic ICSS (Fenton and Liebman 1982; Liebeman and Prowse 1980). Systemic administration of the selective $GABA_B$ receptor agonist CGP 44532 did not affect measures of performance or rate-frequency thresholds in animals implanted with lateral hypothalamic electrodes, although it was effective in attenuating the reward-enhancing effects of cocaine (Dobrovitsky et al. 2002). In contrast, Macey et al. (2001) found that systemic administration of CGP 44532 (using a higher dose than used in the study by Dobrovitsky et al. 2000) and the $GABA_B$ receptor antagonists CGP 56433A and CGP 51176 produced increases in rate-current thresholds. Co-administration of either antagonist with the agonist produced additive increases in thresholds. The authors suggest that these effects may be due to complex interactions of pre- vs. post-synaptic $GABA_B$ receptors, although interactions with extrasynaptic receptors cannot be ruled out. Slattery et al. (2005) showed that neither systemic administration of the positive allosteric $GABA_B$ receptor modulator GS 39783 nor baclofen had any effects alone on lateral hypothalamic ICSS current thresholds, although both compounds attenuated the current threshold lowering (reward-enhancing) effects of cocaine in a dose-dependent manner. The highest dose of baclofen used in this study did raise ICSS current thresholds, although this effect may be related to an inhibition of motor performance.

Willick and Kokkinidis (1995) found that intra-VTA baclofen produced an increase in current thresholds, without affecting maximal response rates, in lateral hypothalamic-medial forebrain bundle ICSS. Intra-VTA injections of baclofen also increased frequency thresholds for ventral pallidal-stimulated ICSS without affecting measures of performance (Panagis and Kastellakis 2002). Waraczynski (2008) showed that injecting baclofen into the sublenticular central extended amygdala produced increases in frequency thresholds, although this was accompanied by decreases in motor performance, for medial forebrain bundle ICSS; the $GABA_B$ receptor antagonist phaclofen showed no effect.

Self-administration

Many studies have investigated the effects of systemically administered $GABA_B$ receptor ligands on drug-maintained self-administration behaviour, as baclofen itself is not readily self-administered (Griffiths et al. 1991). Baclofen decreases breakpoints for cocaine responding on a PR schedule of reinforcement, but may only decrease responding in animals trained on a FR 1 schedule of reinforcement at low to moderate doses of cocaine (Brebner et al. 2002; Brebner et al. 2000a; Roberts et al. 1996). Brebner et al. (2002) also showed that the $GABA_B$ receptor antagonist CGP 56433A had no effect on cocaine or heroin self-administration, although the highest dose of CGP 56433A used did attenuate the cocaine-reduced responding by baclofen on the FR 1 and PR schedules. Baclofen also decreased responding in animals trained to self-administer cocaine on a FR 5 schedule of reinforcement that is limited to 10 min discrete trials separated by 20 min time-out periods (Roberts and Andrews 1997), or those separated by 6 min time-out periods

(Shoaib et al. 1998), and for animals trained on FR 1 and FR 5 (with 20 s time-outs) schedules of reinforcement (Barrett et al. 2005; Campbell et al. 1999). In addition, repeated administration of baclofen, over 3 and 5 days, produced gradual decreases in cocaine-maintained responding (Shoaib et al. 1998). The $GABA_B$ receptor agonists, baclofen and SKF 97541, and the $GABA_B$ receptor positive allosteric modulator, CGP 7930, attenuated cocaine self-administration in rats trained on a FR 5 schedule of reinforcement; the $GABA_B$ receptor antagonist SCH50911 did not alter responding (Filip et al. 2007). Smith et al. (2004) showed that the positive allosteric modulators CGP 7930 and GS 39783 decreased breaking points for rats trained on a PR schedule of reinforcement, and along with baclofen, decreased cocaine-maintained responding when administered on a discrete trial schedule (three 10 min trials per hour over 24 hours) at the beginning of the dark phase of the animals' light cycle. Systemic administration of baclofen and CGP 44532 also reduced cocaine responding in baboons trained on a FR 10 schedule of reinforcement (Weerts et al. 2005). Under a second-order schedule of reinforcement, systemically administered baclofen decreased responding for cocaine-associated responding under a FR 1 schedule of reinforcement (Di Ciano and Everitt 2003).

In a separate study, Brebner et al. (2000b) showed that baclofen injected into the VTA, NAc, and dorsal striatum significantly reduced breakpoints for cocaine administration in rats trained on a PR schedule of reinforcement. Self-administration on a FR 5 (with 20 s time-outs) schedule of reinforcement was also attenuated by intra-NAc or intra-VTA administration of baclofen, while intra-dorsal striatum baclofen had no effect (Shoaib et al. 1998). Another study showed that intra-VTA administration of baclofen

also reduced responding for cocaine in rats trained on a FR 5 schedule of reinforcement (Backes and Hemby 2008).

Regarding heroin self-administration, systemically administered baclofen attenuated the reinforcing effects of heroin for rats trained on FR 1 and PR schedules of reinforcement, although the $GABA_B$ receptor antagonist was unable to block these effects (Brebner et al. 2002). Another group found that while two relatively low doses of baclofen had no effect on heroin self-administration on a FR 1 schedule, it did dose-dependently block the reinstatement of heroin self-administration following a priming dose (Spano et al. 2007). Under a second-order schedule of reinforcement, systemically administered baclofen decreased responding for heroin-associated stimuli under FR 1 and PR schedules of reinforcement (Di Ciano and Everitt 2003). Intra-VTA, but not intra-NAc, injections of baclofen decreased heroin self-administration for rats trained on a FR 1 schedule of reinforcement (Xi and Stein 1999).

Systemically administered baclofen attenuated the self-administration of ethanol in ethanol-nondependent and -dependent rats trained on FR 1 and PR schedules of reinforcement (Walker and Koob 2007). Baclofen also reduced ethanol intake in selectively bred alcohol-preferring rats (Maccioni et al. 2005). A separate study showed that the $GABA_B$ positive allosteric modulators, CGP 7930 and GS 39783, also reduced ethanol intake in alcohol-preferring rats (Orru et al. 2005). Systemic administration of both baclofen and CGP 7930 dose-dependently reduced ethanol responding in alcohol-preferring rats trained on a FR 3 schedule of reinforcement; in addition, a combination of two subthreshold doses of these drugs was also effective in reducing operant responding for alcohol (Liang et al. 2006).

Systemically administered GABA$_B$ receptor agonists, such as baclofen and CGP 44532, decreased nicotine self-administration for rats trained on FR 5 and PR schedules of reinforcement (Paterson et al. 2004). In a separate study, repeated administration of CGP 44532 decreased nicotine self-administration on a FR 5 schedule of reinforcement (Paterson et al. 2005). Baclofen also decreased nicotine self-administration in mice trained on a FR 1 schedule of reinforcement (Fattore et al. 2002). Intra-pedunculopontine tegmental nucleus injections of baclofen attenuates responding for nicotine, but not cocaine, self-administration in rats trained on a FR 5, but not a PR, schedule of reinforcement (Corrigall et al. 2001). Intra-VTA baclofen also greatly reduced nicotine, without significant effects on cocaine, self-administration for animals trained on a FR 5 schedule of reinforcement (Corrigall et al. 2000). Differential results between this study and that of Backes and Hemby (2008), noted above, may be due to the use of lower concentrations of cocaine per injection and a higher dose of baclofen in that study.

Lastly, systemic baclofen reduced responding for meth(+)-amphetamine in rats trained on a PR schedule of reinforcement (Ranaldi and Poeggel 2002) and also decreases (+)-amphetamine self-administration in rats trained on FR 1 and PR schedules of reinforcement (Brebner et al. 2005).

Much like the GABA$_A$ receptor data discussed above, studies investigating the role of the GABA$_B$ receptor in motivation and reward-related behaviours suggest that this receptor may play an inhibitory role and that limbic structures such as the VTA and NAc may be particularly important in this regard. Unlike the GABA$_A$ receptor data, studies focused on the GABA$_B$ receptor suggest that it may not be as important in mediating the effects of natural reinforcers, but instead, may be especially involved in modulating the

activity of drugs of abuse. Once again, although studies employing the systemic injection of GABAergic compounds can be useful, they should be interpreted with caution. It is also important to remember that changes in reward-related behaviours, following administration of $GABA_B$ receptor ligands, is probably due to a functional balance between activation at pre-, post-, and extrasynaptic receptors (with a growing emphasis on extrasynaptic receptors).

Thesis objectives

As outlined in this chapter, the $5-HT_{2C}$ receptor has been implicated in the pathophysiology of many psychiatric disorders, such as schizophrenia, depression, and drug addiction. These disorders all have in common a dysregulation of DA- and reward-related circuitry and behaviour. The $5-HT_{2C}$ receptor may be involved in the regulation of DA- and reward-related circuitry and behaviour, as activation of this receptor may inhibit, while antagonism may increase, mesolimbic DA release and modulate reward-related behaviours. While the $5-HT_{2C}$ receptor's role in this regard is still unclear, there is evidence that its regulation of DA- and reward-related behaviours may involve interactions with other neurotransmitters and receptors.

As discussed above, there is evidence for interconnections between the DA, 5-HT, and GABA systems. While the interaction between these systems is of great complexity, there are some clear points of interaction, such as the fact that $5-HT_{2C}$, $5-HT_{1B}$, $GABA_A$, $GABA_B$, and nicotinic acetylcholine receptors have all been identified on GABAergic cells and all affect DA regulation in some way. The main aim of this thesis was to explore the role of the $5-HT_{2C}$ receptor in reward-related behaviours. The potential

relationships between the 5-HT$_{2C}$ receptor and other receptors investigated in this thesis will be discussed in further detail throughout the discussion sections of each respective chapter as well as in the final general discussion.

Briefly, the following chapters will explore hypotheses related to the 5-HT$_{2C}$ receptor's role in reward-related behaviours by investigating:

- The role of 5-HT$_{2C}$ receptor stimulation in nicotine-induced locomotor activity and place conditioning (as nicotine-related reward is believed to be mediated, in part, by changes in DA which occur following the targeted activation of the nicotinic acetylcholine receptor), as previous data have demonstrated that 5-HT$_{2C}$ receptor activation alone decreases locomotor activity but does not affect place conditioning – except under state-dependent conditions (chapter 2).

- The role of systemically administered, and intra-NAc shell, 5-HT$_{2C}$ receptor compounds on ventral tegmental area ICSS, as electrical stimulation of the VTA results in NAc DA release and the NAc has been identified as a potential site for the inhibition of 5-HT$_{2C}$ receptor-related DA efflux (chapter 3).

- The potential interaction between the 5-HT$_{2C}$ and 5-HT$_{1B}$ receptors on VTA ICSS, as both receptors may be localized on GABA cells within reward-related circuitry and may be involved in mediating ICSS behaviour (chapter 4).

- The role of systemically administered $GABA_A$ receptor compounds on locomotor activity and VTA ICSS, intra-NAc shell administration of $GABA_A$ receptor compounds on VTA ICSS, and the potential interaction between the $GABA_A$ and $5\text{-}HT_{2C}$ receptors on VTA ICSS, as $5\text{-}HT_{2C}$ receptors may be located on GABA cells (chapter 5).

- The role of a systemically administered $GABA_B$ receptor agonist on locomotor activity and VTA ICSS, intra-NAc shell administration of a $GABA_B$ receptor agonist on VTA ICSS, and the potential interaction between the $GABA_B$ and $5\text{-}HT_{2C}$ receptors on VTA ICSS, as $5\text{-}HT_{2C}$ receptors may be located on GABA cells (chapter 6).

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Chapter 2: Differential effects of 5-HT$_{2C}$ receptor activation by WAY 161503 on nicotine-induced place conditioning and locomotor activity in rats

(A version of this chapter has been published. Hayes DJ, Mosher TM, Greenshaw AJ. 2008. Differential effects of 5-HT(2C) receptor activation by WAY 161503 on nicotine-induced place conditioning and locomotor activity in rats. Behavioural Brain Research. Sept 2, Epub ahead of print, DOI: 10.1016/j.bbr.2008.08.034. The initial experiments on locomotor activity with WAY 161503 and nicotine were conducted by T. Mosher and were reported in thesis form (Mosher, 2006). The representation and discussion of the Mosher data – Fig. 2.1 and 2.2 – have been prepared by the present author with full acknowledgement of the source of those data.)

Introduction

The mesocorticolimbic dopamine (DA) system plays an important role in mediating motivated and reward-related behaviours (Ikemoto and Wise 2004; Tzschentke 1998) and the reinforcing properties of many drugs of abuse (Self 2004; Volkow et al. 2004; Wise 1996). Although the precise role of dopaminergic cells has not been established, they may carry reward-related signals involved in reward valuation, prediction, incentive salience and conditioning (Berridge and Robinson 1998; Pessiglione et al. 2006; Schultz 1998; 2006; Stefani and Moghaddam 2006; Tobler et al. 2005). The midbrain raphe serotonergic system shows extensive connectivity with DA-containing areas suggesting a role of serotonin (5-HT) in the control of these cells (McBride et al. 1999; Van Bockstaele et al. 1993; Van Bockstaele et al. 1994). There is evidence for many distinct structural and pharmacological subtypes of 5-HT receptors with subtype-dependent

excitatory or inhibitory effects (Barnes and Sharp 1999). Serotonin 2C (5-HT$_{2C}$) receptor activation may inhibit the release of mesolimbic DA (Di Giovanni et al. 2000; Di Matteo et al. 1999).

Nicotine, a potent nicotinic acetylcholine receptor agonist, may facilitate DA-related behaviours including drug self-administration, place conditioning, intracranial self-stimulation and locomotion (Di Chiara 2000; Ivanova and Greenshaw 1997; Wonnacott et al. 2005). Many studies have demonstrated nicotinic-serotonergic interactions, generally finding increased 5-HT release following nicotinic receptor stimulation (Seth et al. 2002). In particular, 5-HT$_{2C}$ receptor activation may attenuate nicotine-induced mesolimbic DA release (Di Matteo et al. 2004; Pierucci et al. 2004). Given the evidence for functional relationships between 5-HT$_{2C}$ receptors and the cholinergic and dopaminergic systems in the context of reward-related behaviours, it is possible that the 5-HT$_{2C}$ receptor may play a role in nicotine-mediated behaviours – an idea that has been previously suggested from studies involving locomotion and drug self-administration (Batman et al. 2005; Fletcher et al. 2006; Grottick et al. 2001).

Few studies have investigated the reinforcing effects of 5-HT$_{2C}$ receptor ligands in place conditioning. The mixed 5-HT$_{1B/2C}$ receptor agonist mCPP did not induce place conditioning on its own, but was able to block the conditioned place aversion induced by mianserin (a mixed 5-HT$_2$ antagonist) and eltoprazine (a mixed 5-HT$_{1B}$ receptor agonist & 5-HT$_{2C}$ receptor antagonist) (Rocha et al. 1993). Recently, Mosher et al. (2005) showed that systemic administration of the mixed 5-HT$_{1A/1B/2C}$ receptor agonist TFMPP and the selective 5-HT$_{2C}$ receptor agonist WAY 161503 did not induce place conditioning. Nevertheless, in a separate study, WAY 161503 did produce a state-

dependent place aversion (Mosher et al. 2006). Under these conditions, both compounds reduced spontaneous locomotor activity (Mosher et al. 2005); these findings are in agreement with previous locomotion studies demonstrating an inhibitory role for the 5-HT_{2C} receptor (Gleason et al. 2001; Higgins et al. 2001; Kennett et al. 2000; Kennett et al. 1997; Lucki et al. 1989; Martin et al. 2002).

A recent paper has suggested that nicotine-induced place conditioning could be inhibited through the indirect activation of the 5-HT_{2C} receptor (Ji et al. 2006). Though there is still some debate (Laviolette and van der Kooy 2003), there is evidence that nicotine's rewarding effects may be related to direct activation of DA cells in the ventral tegmental area (VTA) as well as its desensitization effects on GABA cells (Ferrari et al. 2002; Mansvelder et al. 2002; Nisell et al. 1994; Pontieri et al. 1996; Sziraki et al. 2002). Dopaminergic cell lesions within the mesolimbic system and DA antagonists both attenuate nicotine-induced locomotor activity and self-administration (Clarke et al. 1988; Corrigall and Coen 1994; Corrigall et al. 1992; Singer et al. 1982). Though many authors have reported conflicting results regarding the peripheral administration of nicotine, a recent review of the literature and subsequent systematic study has clearly demonstrated that nicotine may induce robust place preference conditioning, over a range of doses, under well-defined parameters (Le Foll and Goldberg 2005).

The present study investigated the role of the 5-HT_{2C} receptor in nicotine-induced place conditioning and locomotor activity. Nicotine-induced (0.6 mg/kg) place conditioning was compared to the well-established effects of (+)-amphetamine (McBride et al. 1999; Spyraki et al. 1982). In addition to place conditioning, nicotine was also used as a locomotor stimulant, as many studies have demonstrated that repeated exposure to

nicotine produces locomotor sensitization (Arnold et al. 1995; Clarke et al. 1988; Imperato et al. 1986) and at least one study has found that the 5-HT$_{2C}$ receptor may be involved in attenuating nicotine-induced locomotion in rats (Grottick et al. 2001). Given that 5-HT$_{2C}$ receptor activation has been shown to attenuate nicotine-induced mesolimbic DA release, and both spontaneous locomotor activity and place conditioning are affected by mesolimbic DA transmission, the authors hypothesized that 5-HT$_{2C}$ receptor activation by WAY 161503 would attenuate the behavioural effects of nicotine.

Materials & methods

Subjects

One hundred and thirty four male Sprague-Dawley rats (Health Sciences Laboratory Animal Services, University of Alberta) weighing 200-250 g were housed individually in standard Plexiglas laboratory cages at 20°C and 50 % humidity, with a 12-hr light/dark cycle (lights from 0700 h -1900 h) with food and water freely available. All testing took place under red light during the light phase of the light/dark cycle. All apparatus were cleaned between animals with diluted (1:6) ammonia-based window cleaner (No Name® Glass Cleaner with ammonia). The care and use of animals were in accordance with guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

Drugs

The 5-HT$_{2C}$ receptor agonist WAY 161503 · HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-one hydrochloride] was purchased from Tocris Cookson Inc. (Ellisville, MO, USA). (+) α-Methylphenethylamine ((+)-amphetamine)

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sulphate was purchased from Health and Welfare Canada. (–)-Nicotine hydrogen tartrate (nicotine) and the 5-HT$_{2C}$ receptor antagonist SB 242084 · HCl [6-chloro-5-methyl-1-[[2-(2methylpyrid-3-yloxy)pyrid-5-yl] carbamoyl] indoline dihydrochloride] were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). Nicotine was dissolved in saline; all other compounds were dissolved in double-distilled water (ddH$_2$O). SB 242084 was injected intraperitoneally (i.p.) and all other drugs were injected subcutaneously (s.c.) in a volume of 1.0 ml/kg. Nicotine was given immediately before testing; (+)-amphetamine and WAY 161503 were both given 10 min before testing; SB 242084 was given 30 min before testing. All drug doses are expressed as free-base.

Place Conditioning

The place conditioning apparatus (I. Halvorsen System Design, Phoenix, AZ, USA) consisted of a rectangular Plexiglas box divided into two compartments (30 cm L x 30 cm W x 25 cm H). The compartments differed only in floor texture: 14 horizontal bars positioned 1.25 cm apart compared with 1-cm square grate wire flooring. The compartments were separated by a white plastic divider, which contained a tunnel (7.5 cm long) allowing access to both compartments that could be obstructed with removable doors during conditioning.

The procedure consisted of three phases. *Phase 1* (Pre-Conditioning): Animals were habituated to the place conditioning apparatus for three consecutive days, during which animals had free access to both compartments for 15 min. On the third day of pre-conditioning, the amount of time spent in each compartment was recorded. Animals were assigned to drug groups such that each animal was conditioned to the compartment in which it spent the least time, as determined on pre-conditioning day three (biased design).

Phase 2 (Conditioning): On alternate days, animals received drug and vehicle treatments and were confined to the drug-paired or vehicle-paired compartment for 30 min. Animals were conditioned for eight consecutive days during which they received four drug treatments. *Phase 3* (Post-Conditioning): During retention testing, animals were placed in the apparatus in a drug-free state and allowed free access to both compartments for 15 min. The amount of time spent in each compartment was recorded. Each dose of WAY 161503 (1.0, 3.0 mg/kg) was tested in a separate experiment to allow for a replication of the nicotine-induced place preference.

Spontaneous Locomotor Activity

Apparatus: Spontaneous locomotor activity was measured using computer–monitored photobeam boxes (I. Halvorsen System Design, Phoenix, AZ, USA). The locomotor apparatus consisted of a clear Plexiglas test cage (43 cm L x 43 cm W x 30 cm H) with a 12 x 12 photobeam grid located 2.5 cm above the floor. These beams measured horizontal activity as well as consecutive beam breaks. Vertical activity was measured using 12 additional photobeams located 12 cm above the floor.

Procedure: For the initial locomotor experiments, animals were habituated to the locomotor activity boxes for 14 days, during which, they were injected daily with nicotine (0.6 mg/kg; to establish behavioural sensitization) or saline vehicle. Following the 14 day sensitization period, the animals received randomized counterbalanced injections with the compound of interest. Three days were allowed between each treatment; during these days, animals continued to receive respective nicotine or vehicle injections and locomotor activity was measured. Locomotor activity was measured over a 60 min time course.

To ensure that the differential effects seen with WAY 161503 on nicotine-related locomotor activity and place conditioning behaviour were not due to subject or design variability, animals from the completed place conditioning experiments described above were randomly assigned to one of four treatment groups: vehicle + vehicle; vehicle + nicotine; WAY 161503 + vehicle; WAY 161503 + nicotine. The dose of the nicotine challenge was 0.6 mg/kg, while the WAY 161503 dose was 1.0 mg/kg. Only animals with prior nicotine exposure (i.e. sensitized to nicotine) in place conditioning were assigned to the locomotor groups containing nicotine. Locomotor activity was monitored over 30 min in order to explore the time course of drug effects.

Statistical Analysis

Paired samples t-tests were used to analyze place conditioning effects, comparing time spent in the drug-paired side on pre- versus post-conditioning days; to compare initial preferences for the bar vs. grate compartments for all animals ($p \leq 0.05$). Experimental effects on the initial spontaneous locomotor activity experiments were determined using three-way (WAY 161503 x time x group) or four-way (agonist x antagonist x time x group) repeated measures analysis of variance (ANOVA) with drug treatment group – defined as animals who have received nicotine injections vs. those who have not – as a between subjects factor. For the single-day locomotor activity experiment, following place conditioning, a three-way (nicotine x WAY 161503 x time) ANOVA with repeated measures on one factor (time) was conducted. Where appropriate, analysis of time course data was performed using one-way ANOVA across treatments for each 5 min time interval. A significant F ratio ($p \leq 0.05$) on a 5 min interval was followed by Newman-Keuls post hoc tests ($\alpha = 0.05$). As the results of the analyses of consecutive and vertical

activity paralleled those for horizontal locomotor activity counts, only the latter results are reported. For experiments involving repeated measures, Greenhouse-Geisser corrected degrees of freedom were used as a conservative estimate of the F-ratio. All statistical analyses were completed using SPSS statistical software (SPSS 14.0, SPSS Inc., Chicago, U.S.A.).

Results

Effects of systemic WAY 161503 on basal and nicotine-induced locomotor activity

WAY 161503 (1.0 mg/kg) significantly decreased spontaneous locomotor activity as well as nicotine-induced hyperactivity [Fig. 2.1A, $F_{(1.53, 21.35)} = 39.73$, $p<0.05$]. There were significant effects of time [$F_{(5.51, 77.08)} = 50.01$, $p<0.05$] and group [$F_{(1, 14)} = 50.59$, $p<0.05$]; interactions of WAY 161503 x group [$F_{(1.53, 21.35)} = 25.23$, $p<0.05$], time x group [$F_{(5.51, 77.08)} = 15.57$, $p<0.05$] and WAY 161503 x time [$F_{(5.54, 77.58)} = 4.97$, $p<0.05$]. Local time course analysis revealed that WAY 161503 (1.0 mg/kg) reduced locomotor activity in vehicle-treated animals during the first 10 min of testing (Fig. 2.1B) and during the first 30 min, and again at 40 and 45 min, of testing for the nicotine-sensitized animals (Fig. 2.1C).

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Figure 2.1A-C Locomotor effects of WAY 161503 (WAY; 0-1.0 mg/kg) in vehicle- and nicotine-treated (0.6 mg/kg) rats **(A)** (n = 8/group). Time course activity over 60 min in vehicle-treated **(B)** and nicotine-treated **(C)** rats. The term 'baseline' refers to recorded activity measured in vehicle-treated animals in the control group and nicotine-treated animals in the nicotine group. Data shown are means ± SEM. * Significant at p<0.05 following Newman-Keuls post hoc tests.
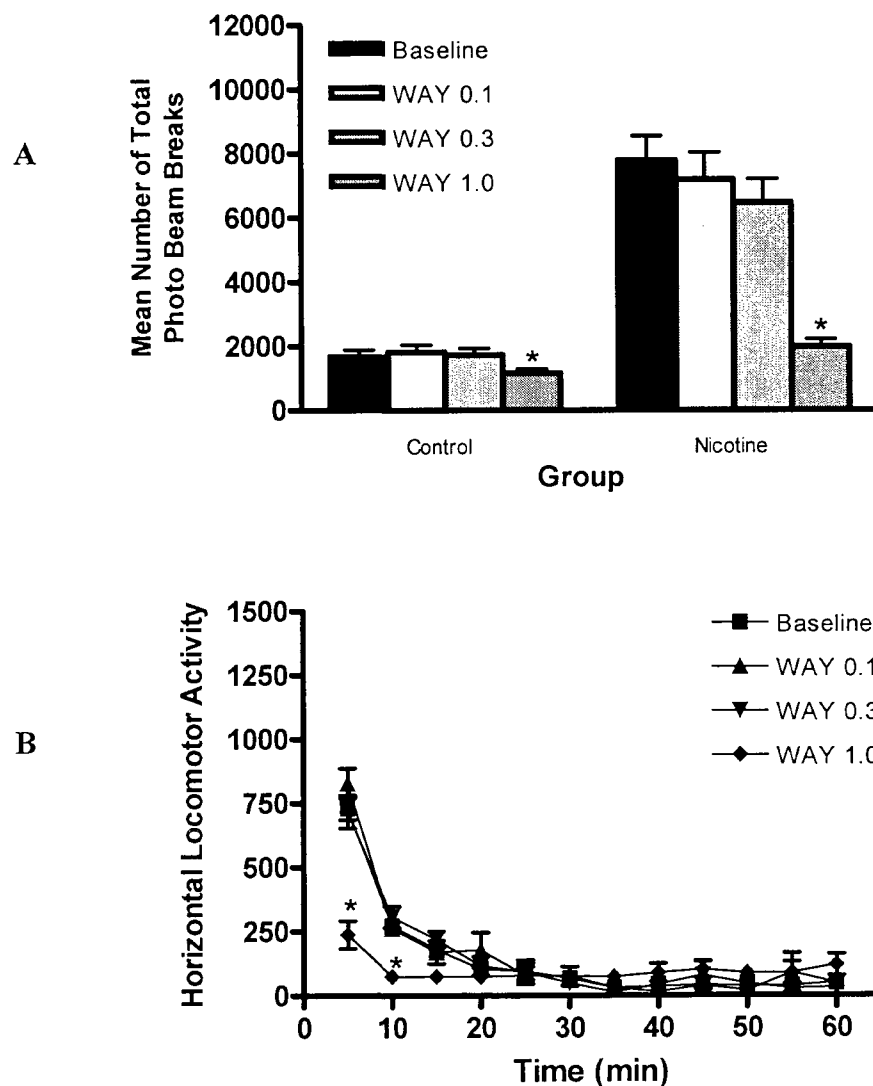
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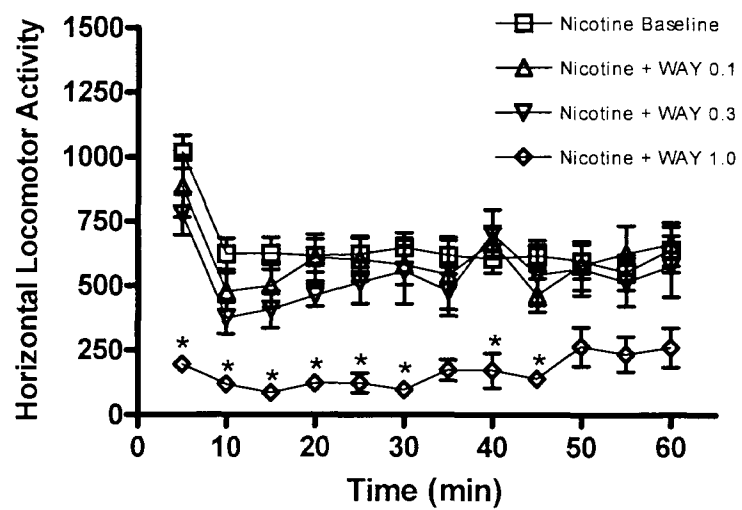
WAY 161503 and SB 242084 on basal and nicotine-induced locomotor activity

WAY 161503 (1.0 mg/kg) significantly decreased locomotor activity in vehicle- and nicotine-treated animals [Fig. 2.2A, $F_{(1, 14)} = 39.93$, $p < 0.05$]. There was an effect of SB 242084 (1.0 mg/kg) [$F_{(1, 14)} = 93.31$, $p < 0.05$], time [$F_{(4.05, 56.68)} = 35.43$, $p < 0.05$] and group [$F_{(1, 14)} = 80.98$, $p < 0.05$]; interactions of WAY 161503 x SB 242084 [$F_{(1, 14)} = 19.13$, $p < 0.05$], WAY 161503 x group [$F_{(1, 14)} = 36.23$, $p < 0.05$], SB 242084 x time [$F_{(1, 14)} = 33.31$, $p < 0.05$], time x group [$F_{(4.05, 56.68)} = 7.17$, $p < 0.05$], WAY 161503 x time [$F_{(4.77, 66.75)} = 8.51$, $p < 0.05$], SB 242084 x time [$F_{(5.56, 77.77)} = 2.16$, $p < 0.05$] and WAY 161503 x SB 242084 x time [$F_{(4.45, 62.28)} = 2.57$, $p < 0.05$] (Fig. 2.2A). Local time course analysis revealed that WAY 161503 decreased locomotor activity in vehicle-treated animals during the first 15 min of testing (Fig. 2.2B) and during the first 35 min, and again at 45 and 55 min, of testing for nicotine-treated animals (Fig. 2.2C). SB 242084 (1.0 mg/kg) attenuated the reduction in locomotor activity seen with vehicle-treated animals (Fig. 2.2B; with the exception of the first 5 min) and nicotine-treated animals (Fig. 2.2C; with the exception of the 15, 20 and 30 min test points). SB 242084 did not significantly affect locomotor activity when administered alone.

Figure 2.2A-C Locomotor effects of WAY 161503 (WAY; 1.0 mg/kg) and SB 242084 (1.0 mg/kg) alone and in combination in vehicle- and nicotine-treated (0.6 mg/kg) rats (A) (n = 8/group). Time course activity over 60 min in vehicle-treated (B) and nicotine-treated (C) rats. The term 'baseline' refers to recorded activity measured in vehicle-treated animals in the control group and nicotine-treated animals in the nicotine group. Data shown are means ± SEM. * Significant at $p<0.05$ following Newman-Keuls post hoc tests.
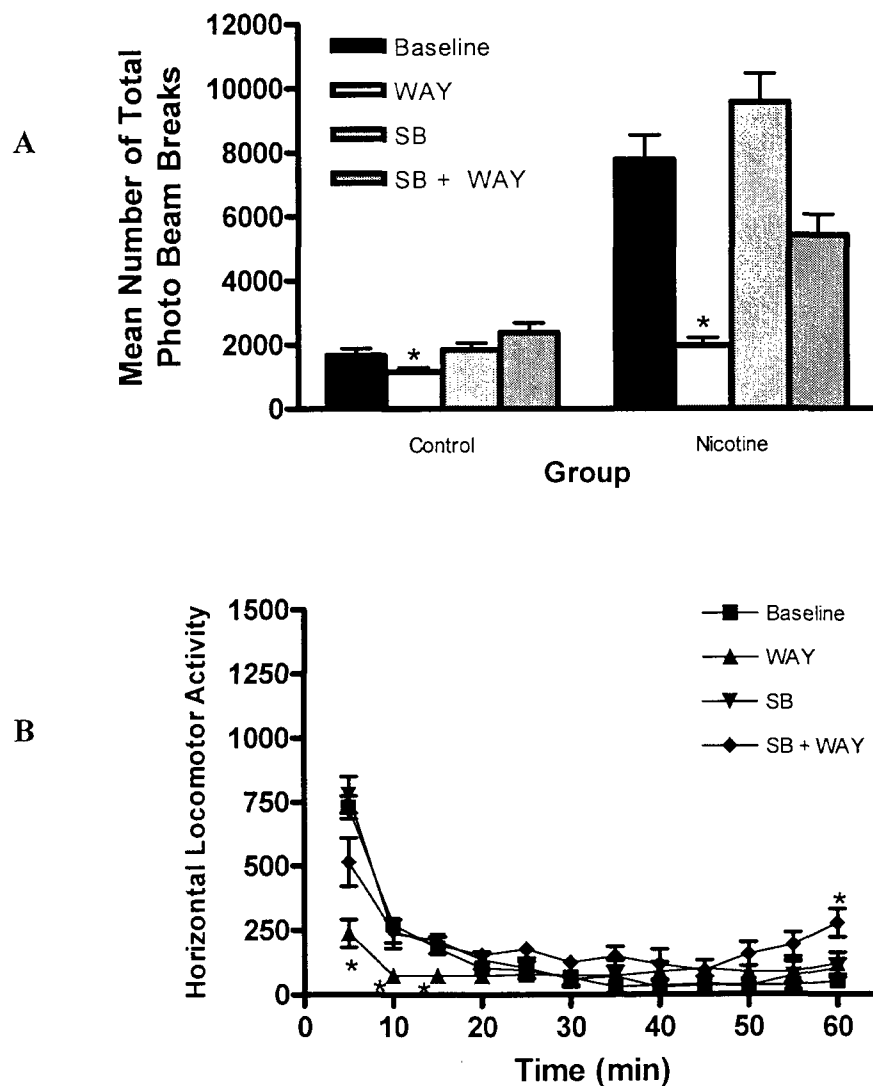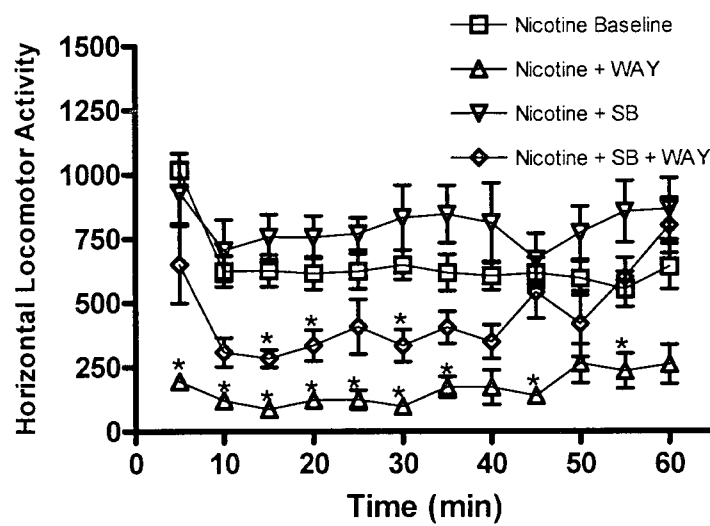
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Verification of nicotine-induced place conditioning

Using a biased place conditioning design, both 0.6 mg/kg nicotine [t (7) = 4.47, p<0.05] and 1.0 mg/kg (+)-amphetamine [t (7) = 4.54, p<0.05] produced conditioned place preferences, indicating a significant difference in time spent in the conditioned compartment on post- over pre-conditioning days (Fig. 2.3). Though each animal was conditioned to the compartment in which it spent the least time on pre-conditioning day 3 (i.e. biased design), as a group, animals showed no initial preference for either compartment (which differed only in bar vs. grate flooring) [395 ± 14 sec; 391 ± 13 sec; t (89) = 0.15, p>0.05].

Figure 2.3 Verification of the biased place conditioning design using (+) (+)-amphetamine (1.0 mg/kg) as a positive control compared to nicotine (0.6 mg/kg) (n = 16). Data shown are means ± SEM. * Significant at $p<0.05$ following paired samples t-test.
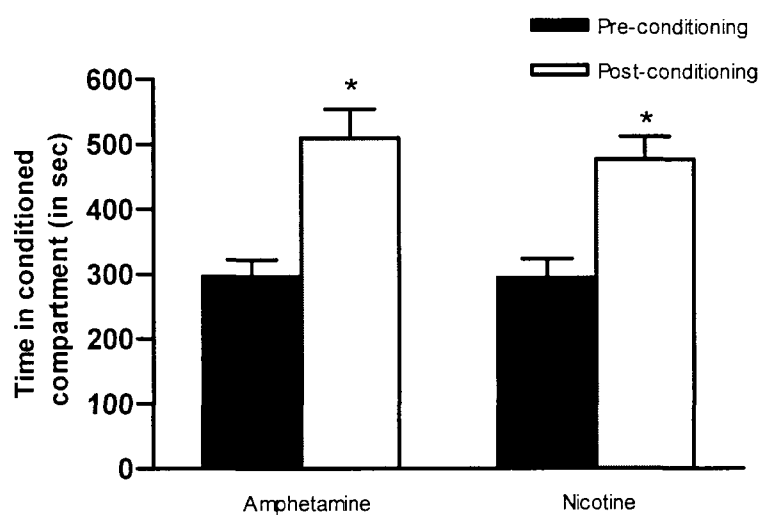
WAY 161503 and nicotine in place conditioning

Nicotine (0.6 mg/kg) induced a place preference [Fig. 2.4A, t (11) = 2.36, p<0.05; Fig. 2.4B, t (9) = 3.36, p<0.05]; WAY 161503 (1.0, 3.0 mg/kg) did not induce place conditioning [Fig. 2.4A, t (11) = 1.77, p>0.05; Fig. 2.4B, t (9) = 2.13, p>0.05] and did not influence nicotine-induced place conditioning [Fig. 2.4A, t (11) = 4.73, p<0.05; Fig. 2.4B, t (9) = 4.59, p<0.05].

Figure 2.4 Place conditioning effects of **(A)** nicotine (0.6 mg/kg) and WAY 161503 (WAY; 1.0 mg/kg) (n = 24); **(B)** nicotine (0.6 mg/kg) and WAY 161503 (WAY; 3.0 mg/kg) (n = 30). Data shown are means ± SEM. * Significant at $p < 0.05$ following paired samples t-test.
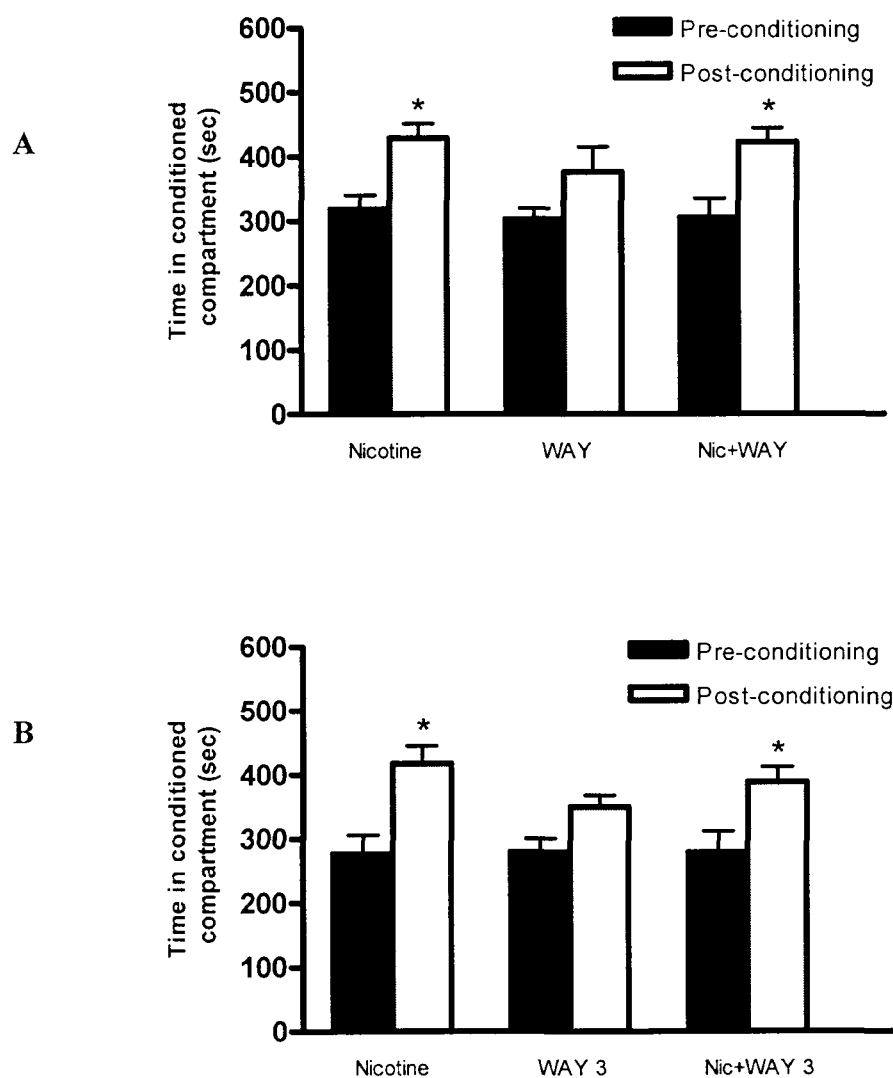
WAY 161503 and nicotine in locomotor activity following place conditioning

Following the nicotine-induced place preference experiments above, the effects of WAY 161503 on spontaneous and nicotine-induced locomotor activity of those animals was examined. Testing took place over 30 min as the initial locomotor experiments indicated that the effects of WAY 161503 on basal locomotor activity occur within this time period. Three-way ANOVA revealed a significant interaction for nicotine (0.6 mg/kg) x WAY 161503 (1.0 mg/kg) x time [Fig. 2.5A, $F_{(21.12)} = 6.43$, $p<0.05$]. To investigate this interaction, one-way ANOVA was performed for each 5 min interval; following significant one-way ANOVA, Newman-Keuls post hoc tests ($\alpha = 0.05$) showed that 1.0 mg/kg WAY 161503 reduced locomotor activity during the first 20 min of testing, as did the nicotine and WAY 161503 combination. Nicotine significantly increased locomotor activity, over saline-treated animals, from 15 to 30 min of testing (Fig. 2.5B). Three-way ANOVA revealed an interaction for nicotine (0.6 mg/kg) x WAY 161503 (3.0 mg/kg) x time [Fig. 2.5C, $F_{(9.53)} = 17.01$, $p<0.05$]. Locomotor activity was reduced for the group receiving 3.0 mg/kg WAY 161503 during the first 10 to 25 min of testing and for the group receiving WAY 161503 and nicotine for the first 20 min. The group that received nicotine showed increased activity during the first 10 min of testing (Fig. 2.5D).

Figure 2.5A-D Spontaneous locomotor activity, following place conditioning experiments, for **(A)** nicotine (Nic; 0.6 mg/kg) and WAY 161503 (WAY; 1.0 mg/kg) (n = 10/group); **(C)** nicotine (Nic; 0.6 mg/kg) and WAY 161503 (WAY 3; 3.0 mg/kg) (n = 9/group). Time course activity over 30 min for rats receiving **(B)** nicotine and WAY 161503 (1.0 mg/kg) **(D)** and nicotine and WAY 161503 (3.0 mg/kg). Data shown are means ± SEM. * Significant at $p < 0.05$ following Newman-Keuls post hoc tests.
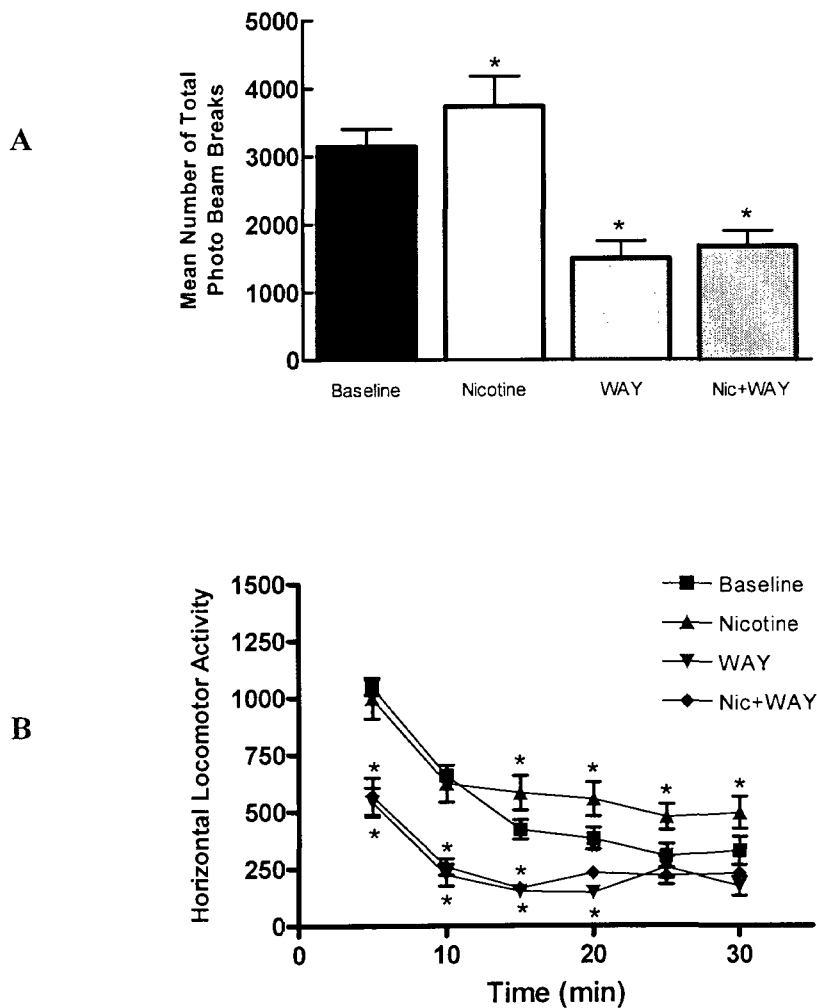
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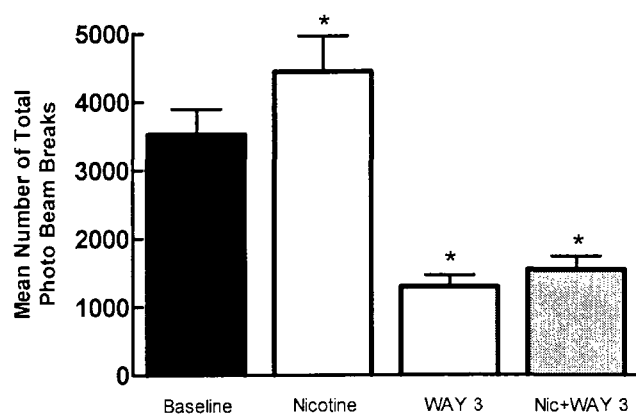
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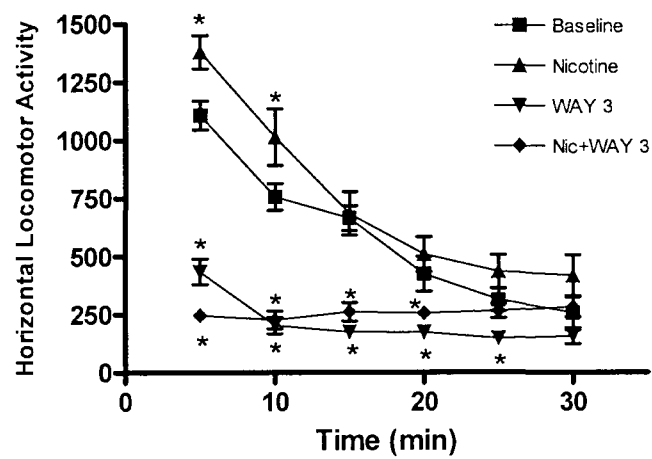
Discussion

This study investigated the effects of 5-HT$_{2C}$ receptor activation on spontaneous locomotor activity and nicotine-induced place conditioning. Consistent with prior data (Grottick et al. 2001), 5-HT$_{2C}$ receptor activation decreased locomotion when administered alone and in combination with nicotine (0.6 mg/kg; Fig. 2.1A-C) and these effects were attenuated by the selective 5-HT$_{2C}$ receptor antagonist SB 242084 (1.0 mg/kg; Fig. 2.2A-C). These data further support an inhibitory role for the 5-HT$_{2C}$ receptor in basal and nicotine-induced locomotor activity. A floor effect may be responsible for the fact that the selective 5-HT$_{2C}$ receptor agonist WAY 161503 (1.0 mg/kg) only decreased basal locomotor activity in the first 10-15 min of testing (Fig. 2.1B & 2.2B), given that this same dose is effective in attenuating nicotine-induced activity over at least a 45 min time period (Fig. 2.1C & 2.2C). As all relevant drug effects are noted within the first 30 min, subsequent locomotor testing focused on this time period.

Nicotine (0.6 mg/kg) induced a conditioned place preference comparable to the well-established effects of (+)-amphetamine (Fig. 2.3A, 2.4A & 2.4B) (McBride et al. 1999; Spyraki et al. 1982). These data are consistent with a number of studies indicating that systemic nicotine-induced place preference is reliable over a range of doses, under well-defined parameters, and with the use of a biased place conditioning design (for review see Le Foll & Goldberg, 2005). Some researchers have suggested that factors such as age, strain, timing and route of administration may contribute to the varying results seen across studies (Horan et al. 1997; Le Foll and Goldberg 2005; Philibin et al. 2005; Wilkinson and Bevins 2008), as some groups have reported a place preference (Ashby et

al. 2002; Dewey et al. 1999; Forget et al. 2006; Horan et al. 1997), place aversion (Fudala and Iwamoto 1987; Jorenby et al. 1990) or absence of place conditioning (Acquas et al. 1989; Carboni et al. 1989; Shram et al. 2006) following systemic nicotine administration using an unbiased design. Nonetheless, the vast majority of studies demonstrating a nicotine-induced place preference used a biased design (Le Foll and Goldberg 2005).

It is a possibility that the place preference induced by nicotine in the biased design is not a reward-related effect, but rather the result of a reduction of an aversive state related to the initially non-preferred compartment – suggesting a potentially anxiolytic effect of nicotine. This is a rather unlikely hypothesis as nicotine's effects on anxiety vary largely based on route and timing of administration, the behavioural model used, and subject variability (Picciotto et al. 2002) and a number of behavioural studies have demonstrated the affects of 5-HT$_{2C}$ receptor compounds on anxiety, yet none of these compounds have been shown to induce place conditioning (Kennett et al. 1989; Kennett et al. 1997; Martin et al. 2002). Another possible concern involves the definition of a biased design (Cunningham et al. 2003; Tzschentke 2007). The term 'biased design' often incorrectly elicits the notion of a biased apparatus (e.g. rats naturally prefer darker compartments), though an apparatus which produces a general bias for one compartment over others may be a detriment to the investigation of reward-related effects (Cunningham et al. 2003; Le Foll and Goldberg 2005; Roma and Riley 2005; Tzschentke 1998). The biased design (or 'biased compartment assignment') involves the assignment of individual animals to the compartment in which they initially spent the least, or most, amount of time. As a group there is no compartment preference; as such, the biased design may allow for greater sensitivity in detecting the reinforcing effects of drugs

(Cunningham et al. 2003; Tzschentke 2007). The apparatus used in the present study differed only in floor texture (bar vs. grate) and as a group, animals showed no preference for either side.

We are aware of one report that 5-HT$_{2C}$ receptor activity may inhibit nicotine-induced place preference (Ji et al. 2006). These authors proposed that the tumor suppressor molecule, PTEN, complexes with the 5-HT$_{2C}$ receptor and tonically inhibits its activity in vivo. The putative PTEN:5-HT$_{2C}$ receptor complex was disrupted using a Tat-conjugated interfering peptide and it was suggested that this may mimic the effects of 5-HT$_{2C}$ receptor activation. It is difficult to assess the effects of 5-HT$_{2C}$ receptor activity on nicotine-induced place preference from this study because it is not clear to what extent the PTEN molecule affects 5-HT$_{2C}$ receptor activity. Though the authors used a relatively selective 5-HT$_{2C}$ receptor agonist (RO 600175) to attenuate tetrahydrocannibanol-induced place conditioning, they did not use the same compound to directly assess nicotine-induced place conditioning.

The present study is, to the author's knowledge, the first to investigate the effects of a specific 5-HT$_{2C}$ receptor agonist (WAY 161503) on nicotine-induced place conditioning (Fig. 2.4A & 2.4B). No place conditioning was seen under the present conditions using systemic doses of WAY 161503 (1.0, 3.0 mg/kg) that are behaviourally active in locomotor activity (Mosher et al. 2005); this observation is consistent with previous results indicating that compounds with 5-HT$_{2C}$ receptor activity do not induce place conditioning when testing occurs in a drug-free state (Mosher et al. 2005; Rocha et al. 1993). Contrary to the initial hypothesis, WAY 161503 did not attenuate nicotine's ability to induce a place preference. This was unexpected because 5-HT$_{2C}$ receptor

activation attenuates nicotine-induced mesolimbic DA release (Di Matteo et al. 2004; Pierucci et al. 2004), locomotor activity and self-administration (Grottick et al. 2001). While the current place conditioning results do not support the notion that 5-HT$_{2C}$ receptor activation attenuates all nicotine-induced behaviours, it must be noted that testing in the current place conditioning study took place in a drug-free state, while the attenuation of nicotine's effects by 5-HT$_{2C}$ receptor stimulation in locomotion and self-administration were drug-dependent. It is also important to note that the present study investigated the effects of a single dose of nicotine (0.6 mg/kg) that was above the previously reported threshold dose of 0.1 mg/kg for producing a CPP (Le Foll and Goldberg 2005). While it is possible that 5-HT$_{2C}$ receptor stimulation may have attenuated the effects of a lower dose of nicotine, the fact that other nicotine-induced activities (using comparable or higher doses of nicotine) are attenuated by 5-HT$_{2C}$ receptor stimulation remains (Grottick et al. 2001).

Previous studies have demonstrated 5-HT$_{2C}$ receptor-related decreases in both basal (Higgins et al. 2001; Lucki et al. 1989; Martin et al. 2002; Mosher et al. 2005) and nicotine-induced (Batman et al. 2005; Fletcher et al. 2006; Grottick et al. 2001) locomotor activity. Because of the differential effects observed between the place conditioning and locomotor activity experiments, the subjects used in the current place conditioning experiments were subsequently tested in the locomotor apparatus over thirty min (Fig. 2.5A-D). The results of these locomotor experiments were consistent with all previous demonstrations showing that nicotine-induced increases in locomotor activity are attenuated through 5-HT$_{2C}$ receptor activation. Based on the current results, it is possible that this 5-HT$_{2C}$ receptor agonist-induced effect may not be influenced by the

duration of nicotine exposure (14 treatments in the initial locomotor studies versus 4 treatments during the place conditioning studies; Fig. 2.1A-C and Fig. 2.5A-D, respectively). In addition, future studies should address the possibility that the effects of WAY 161503 on nicotine-induced locomotor activity may be non-specific, as subthreshold doses of WAY 161503 failed to attenuate nicotine-induced locomotion and 5-HT$_{2C}$ receptor activity has been shown to affect both basal locomotion and drug-induced increases in locomotor activity (Filip and Cunningham 2003; Fletcher et al. 2006; Higgins et al. 2001; Kennett et al. 2000; McMahon et al. 2001).

While 5-HT$_{2C}$ receptor activation may inhibit other DA-related behaviours such as cocaine and ethanol self-administration (Fletcher et al. 2004; Rocha et al. 2002; Tomkins et al. 2002), nicotine-induced locomotion and self-administration (Grottick et al. 2001), we are not aware of any other studies regarding 5-HT$_{2C}$ receptor activation on nicotine-induced place conditioning. It is of interest that these results are consistent with reports suggesting DA-independence of the place preference-inducing effects of intra-ventral tegmental nicotine (Laviolette and van der Kooy 2003). Indeed, nicotinic receptor-stimulated increases in nucleus accumbens DA levels and locomotor activity do not always correspond with the establishment of place preference conditioning (Janhunen and Ahtee 2004; Janhunen et al. 2005) and some direct manipulations of DA signalling may differentially affect nicotine-induced place preference and locomotor activity (Le Foll et al. 2005). 5-HT$_{2C}$ receptor stimulation may differentially affect nicotine-induced increases in DA release from the nigrostriatal versus mesolimbic systems (Di Matteo et al. 2004; Pierucci et al. 2004). Also, other studies investigating the effects of dopaminergic or serotonergic manipulations on the effects of drugs of abuse show that

the dissociation between place conditioning and locomotor effects are not unique to 5-HT_{2C} receptor activation and nicotine (Baker et al. 1998; Baker et al. 1996; Ferguson et al. 2008; Khroyan et al. 1998; Le et al. 1997; Suzuki et al. 1992a; Suzuki et al. 1992b). The current results suggest that the 5-HT_{2C} receptor may play an inhibitory role in nicotine-induced locomotor activity without having effects on nicotine-induced place conditioning under the present experimental conditions. The complex role of the 5-HT_{2C} receptor in nicotine-mediated and DA-related reward may be currently underappreciated and future studies will be needed to determine the precise roles of DA, acetylcholine and 5-HT in this regard.

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Chapter 3: Effects of systemic and intra-nucleus accumbens 5-HT$_{2C}$ receptor compounds on ventral tegmental area self-stimulation thresholds in rats

(A version of this chapter has been published. Hayes DJ, Clements R, Greenshaw AJ. 2009. Effects of systemic and intra-nucleus accumbens 5-HT$_{2C}$ receptor compounds on ventral tegmental area self-stimulation thresholds in rats. Psychopharmacology 203: 579-88. The previously unpublished data involving TFMPP – Fig. 3.2 – were collected by R. Clements. The analysis, representation, and discussion of the Clements data have been prepared by the present author with full acknowledgement of the source of those data.)

Introduction

Activity of the mesocorticolimbic dopamine (DA) system is important for the regulation of motivation and reward-related behaviours (Wise 2004). Anatomical, pharmacological and behavioural data have all demonstrated the ventral tegmental area (VTA) and nucleus accumbens (NAc) as key brain areas involved in mediating natural and drug-induced reward (Ikemoto and Wise 2004; Kalivas and Volkow 2005), and this circuitry may be involved in depression, schizophrenia and drug abuse (Kalivas and Volkow 2005; Laviolette 2007; Nestler and Carlezon 2006). Though the exact role for dopaminergic cells is still under investigation, evidence indicates DA release in mesolimbic areas may be associated with behavioural activation, reward valuation, prediction, incentive salience and conditioning (Nicola et al. 2005; Robbins 1997; Salamone et al. 2007; Tobler et al. 2005; Wyvell and Berridge 2000). Many of these findings are consistent with neuroimaging studies in humans (Cooper and Knutson 2008; Murray et al. 2008; O'Doherty 2004). For more information on the putative roles of mesolimbic DA, readers

are referred to the recent special edition of Psychopharmacology (DA – revised, 2007, 191(3)). Place conditioning, self-administration and intracranial self-stimulation (ICSS) are often used as animal models of reward-related behaviour (McBride et al. 1999; Tzschentke 2007; Wise 2002). Electrical stimulation of the VTA drives self-stimulation behaviour in rats and results in DA release in the NAc (Fiorino et al. 1993) – making VTA ICSS a sensitive and directed model of reward-related behaviour.

Serotonin (5-HT) is thought to play a role in the regulation of DA and reward-related behaviours (Benloucif et al. 1993; Hetey and Drescher 1986; Muramatsu et al. 1988). Previous studies have suggested both inhibitory and excitatory roles for 5-HT in ICSS behaviour (Broadbent and Greenshaw 1985; Poschel et al. 1974; Redgrave and Horrell 1976; Van Der Kooy et al. 1978) and these differential effects are likely related to the existence of many 5-HT receptor subtypes (Alex and Pehek 2007; Barnes and Sharp 1999). The 5-HT_{2C} receptor is expressed throughout the mesocorticolimbic system (Bubar and Cunningham 2007; Clemett et al. 2000) and is of interest as a potential target for antidepressants (Chanrion et al. 2007) and atypical antipsychotics (Reynolds et al. 2005). Studies have shown that 5-HT_{2C} receptor activation may inhibit the release of mesolimbic DA (Di Giovanni et al. 2000; Di Matteo et al. 1999). Behavioural studies generally agree with an inhibitory role for the 5-HT_{2C} receptor in reward-related behaviours. Activation of 5-HT_{2C} receptors attenuates nicotine-induced locomotion, food intake and self-administration (Grottick et al. 2001). Functional antagonism of the 5-HT_{2C} receptor also increases cocaine and ethanol responding in rodents (Fletcher et al. 2002; Rocha et al. 2002; Tomkins et al. 2002). While 5-HT_{2C} receptor compounds do not affect

the expression of place conditioning on their own (Mosher et al. 2005), they may produce state-dependent place aversion conditioning (Mosher et al. 2006).

Due to the relatively recent availability of pharmacologically selective 5-HT$_{2C}$ receptor compounds, few studies have investigated the role of the 5-HT$_{2C}$ receptor in ICSS; compounds with 5-HT$_2$ receptor (5-HT$_{2A/B/C}$) activity have been used in some ICSS studies. Sinden and Atrens (1978) suggested a reward-enhancing effect for lateral hypothalamic ICSS following administration of the mixed agonist 5-methoxy-*NN*-dimethyltryptamine, while Stark et al. (1964) demonstrated that another mixed agonist, bromlysergic acid diethylamide, showed biphasic effects in dogs responding for hypothalamic ICSS. Studies using VTA ICSS have suggested a possible inhibitory, and antidepressant, role for 5-HT$_2$ receptors (Grottick et al. 1997; Moreau et al. 1996) . All of these ICSS studies have been limited by the use of non-selective compounds as well as restricted measurements of reward – underscoring the need for more studies in this context.

This study tested the hypothesis that 5-HT$_{2C}$ receptor activation would result in increases in VTA ICSS thresholds using systemic administration of the mixed 5-HT$_{1A/1B/2C}$ receptor agonist TFMPP (0.3 mg/kg), the selective 5-HT$_{2C}$ receptor agonist WAY 161503 (0.3, 0.6, 1.0 mg/kg) and the selective 5-HT$_{2C}$ receptor antagonist SB 242084 (1.0 mg/kg). WAY 161503 and SB 242084 were chosen for their high selectivity at the 5-HT$_{2C}$ receptor (Kennett et al. 1997; Rosenzweig-Lipson et al. 2006; Schlag et al. 2004). TFMPP was chosen as many studies have suggested its behavioural effects to be 5-HT$_{2C}$ receptor-mediated (Kennett and Curzon 1988b; Lucki et al. 1989; Mora et al. 1997). As the NAc shell has been identified as a potential site for the inhibition of 5-HT$_{2C}$

receptor-related DA efflux (Navailles et al. 2006b), the hypothesis that 5-HT$_{2C}$ receptors in the NAc shell are involved in regulating VTA ICSS behaviour was tested using bilateral microinjections of WAY 161503 (0-1.5 µg/side).

Materials & methods

Subjects

Twenty-nine male Sprague-Dawley rats (Health Sciences Laboratory Animal Services, University of Alberta) weighing 200-300g were housed individually in standard Plexiglas laboratory cages at 20°C and 50% humidity, with a 12-hr light/dark cycle with food and water freely available. All apparatus were cleaned between experiments with individual animals with diluted (1:6) ammonia-based window cleaner (No Name® Glass Cleaner with ammonia). The care and use of animals were in accordance with guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

Drugs

The 5-HT$_{2C}$ receptor agonist WAY 161503 · HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-one hydrochloride] and the 5-HT$_{1A/1B/2A/2B/2C}$ receptor agonist TFMPP · HCl [N-[3-(trifluoromethyl)phenyl] piperazine hydrochloride] were purchased from Tocris Cookson Inc. (Ellisville, MO, USA). The 5-HT$_{2C}$ receptor antagonist SB 242084 · 2HCl [6-chloro-5-methyl-1-[[2-(2-methylpyrid-3-yloxy)pyrid-5-yl]carbamoyl]indoline dihydrochloride hydrate] was purchased from Sigma Chemical Company (St. Louis, MO, USA). (+)-α-Methylphenethylamine ((+)-amphetamine) sulphate was purchased from Health and Welfare Canada. TFMPP was dissolved in 0.9%

saline; all other compounds were dissolved in double-distilled water. All compounds were administered in a volume of 1.0 ml/kg. WAY 161503 (0-1 mg/kg) and TFMPP (0.3 mg/kg) were administered subcutaneously, 10 minutes prior to testing; SB 242084 (1.0 mg/kg) was administered intraperitoneally 30 minutes prior to testing. Artificial cerebrospinal fluid was freshly prepared (Elliott and Lewis 1950) and drug solutions made daily (pH 6.0–7.0). All drug doses are expressed as free-base.

Surgery & histology

Using a previously described procedure (Greenshaw 1993), each animal (n = 9 for the WAY 161503 dose response experiment; n = 11 for the TFMPP and SB 242084 experiment; n = 9 for the intra-NAc WAY 161503 experiment) was implanted with a stainless steel, monopolar, stimulating electrode (E363/2; tip diameter 200 μm; Plastics One Ltd., Roanoke, VA) directed to the VTA. A large silver indifferent electrode in the skull served as the relative ground. Animals used for microinjection were also implanted with bilateral cannulae (22 gauge) directed to the shell of the NAc. Stereotaxic coordinates were [mm]: VTA – AP +2.6, L +0.4-0.5, V +1.8-2.2; NAc shell – AP +11.0, L +0.4, V +2.8; from inter-aural zero, with the incisor bar set at 2.4 mm below the inter-aural line (Paxinos and Watson 1998). These coordinates were interpolated from the target site for an angle of 20°, 20° lateral and anterior for the VTA and 16° lateral for the NAc shell (Greenshaw 1997). The guide cannulae were placed 1 mm above the actual injection sites. Electrode and cannulae placements were verified at the end of the experiment by microscopic inspection of flash-frozen coronal brain sections (40 μm); flash-freezing was achieved using isopentane cooled on dry ice. Only animals with VTA and NAc placements were included in the analysis.

Intracranial self-stimulation (ICSS)

Monopolar stimulation of the VTA was provided from constant current DC stimulators (cathodal monophasic pulse width of 200 µs; initial training frequency of 100 Hz; train length of 1 s) connected to each animal via a gold-track slip ring. Between pulses the active electrode and indifferent electrode were connected through a resistor to cancel any effects of electrode polarisation (Greenshaw 1986). The apparatus and rate-frequency analysis were as described by Ivanová et al (1997). With this procedure, M50 is the threshold frequency at which half-maximal response rates occur; RMAX is the maximal rate of responding in a session. While M50 is a measure of reward sensitivity (which is dissociable from non-specific changes in behaviour), RMAX is a measure of response performance (see Gallistel and Karras, 1984; Greenshaw and Wishart, 1987). Group-averaged rate-frequency regression curves are used to illustrate the shifts in M50 thresholds.

Microinjection of drugs

Rats with bilateral cannulae in the NAc shell received a counterbalanced sequence of five treatments: artificial cerebrospinal fluid (CSF), WAY 161503 (0-1.5 µg/side) and (+)-amphetamine (1.0 µg/side), with at least three days between each microinjection. Each intra-NAc injection was administered in a total volume of 0.5µL at a pump-controlled rate of 0.2µL per minute (Beehive controller, Bioanalytical Systems, Inc.) and the injection cannulae remained in place for a further minute to allow for drug absorption. Immediately following each set of microinjections, each animal was tested for VTA self-stimulation. The highest dose of WAY 161503 chosen was based on its maximal solubility in water.

Statistical analysis

All effects of treatments were assessed using repeated measures analysis of variance (ANOVA) followed by Newman-Keuls post hoc tests ($\alpha = 0.05$) where appropriate. All data are presented as a percentage of average baseline performance of each animal. Statistical analyses were completed using statistical software (SPSS Inc., Chicago, IL, USA).

Results

Effects of systemic 5-HT$_{2C}$ receptor agonist WAY 161503

Systemic administration of the selective 5-HT$_{2C}$ receptor agonist WAY 161503 (0.3, 0.6, 1.0 mg/kg) resulted in a main effect for M50 thresholds [Fig. 3.1A, $F (2, 13) = 7.72$, $p<0.05$], RMAX values [Fig. 3.1B, $F (2, 15) = 4.76$, $p<0.05$], and TRES values [Fig. 3.1C, $F (2, 15) = 12.50$, $p<0.05$]. Further analysis with Newman-Keuls revealed that the highest dose of WAY 161503 (1.0 mg/kg) produced a significant increase in M50 (Fig. 3.1A) and TRES (Fig. 3.1C) values; no dose affected RMAX values (Fig. 3.1B). Group-averaged rate-frequency regression curves are included to illustrate the dose-dependent rightward shift in M50 seen with WAY 161503 (indicating a decrease in reward) (Fig. 3.1D).

Figure 3.1A-D The effects of WAY 161503 (W; 0-1.0 mg/kg) on **(A)** rate-frequency thresholds (M50 values), **(B)** maximal response rates (RMAX values), **(C)** and total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the shifts in M50. Data shown are means ± SEM expressed as a percentage of baseline performance. *Denotes significance from control at $p<0.05$ following Newman-Keuls post hoc tests.

C

D

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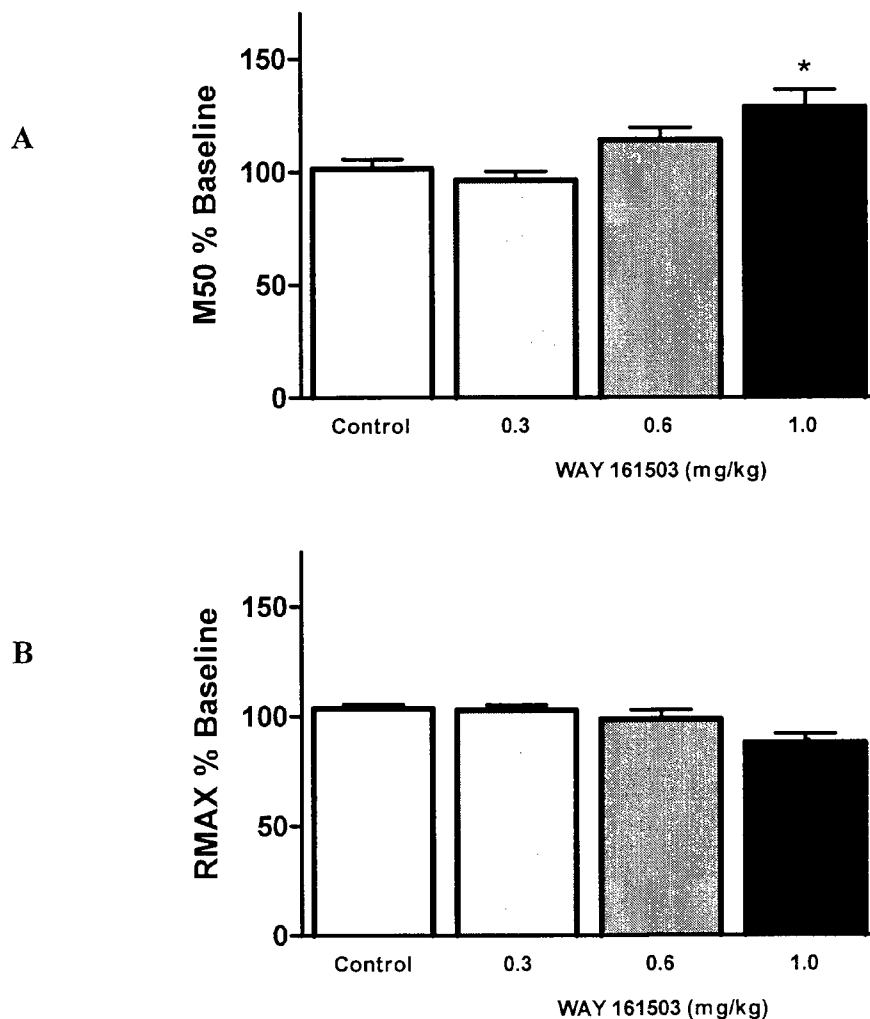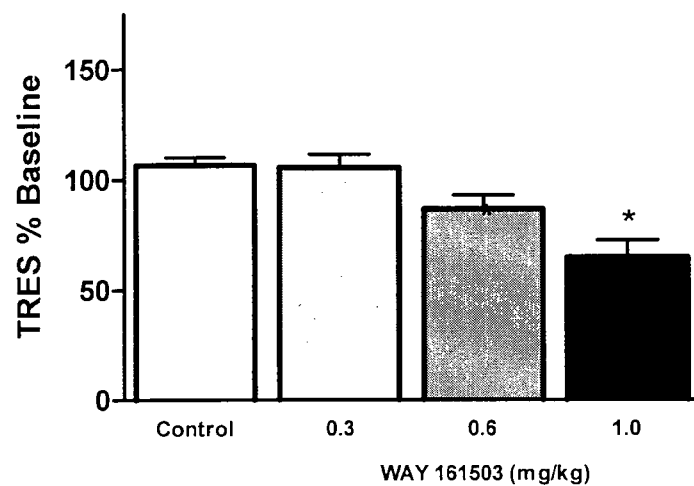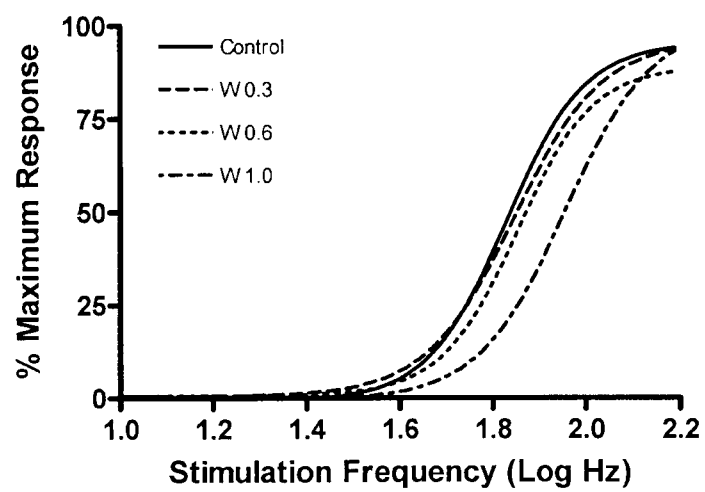
Effects of systemic TFMPP attenuated by SB 242084

Based on pilot data in our lab indicating significant increases in M50 values without effects on RMAX values, the dose of 0.3 mg/kg of TFMPP was chosen for this study. Analysis of M50 values revealed a main effect of TFMPP [F (1, 10) = 16.33, p<0.05] and SB 242084 [F (1, 10) = 11.17, p<0.05] and an interaction between TFMPP and SB 242084 [Fig. 3.2A; F (1, 10) = 5.29, p<0.05]. Newman-Keuls post hoc tests revealed that TFMPP produced a significant increase in M50 thresholds while SB 242084 blocked this effect, without having any effects on its own. None of the treatments showed significant changes in RMAX values (Fig. 3.2B). Analysis of TRES values revealed a main effect of TFMPP [F (1, 10) = 18.52, p<0.05] and SB 242084 [F (1, 10) = 5.90, p<0.05] but no interaction between TFMPP and SB 242084 (Fig. 3.2C). Group-averaged rate-frequency regression curves are included to illustrate the rightward shift in M50 seen with TFMPP (indicating a decrease in reward) and attenuation of TFMPP by SB 242084 (Fig. 3.2D).

Figure 3.2A-D The effects of TFMPP (0.3 mg/kg) and SB 242084 (SB; 1.0 mg/kg) on **(A)** rate-frequency thresholds (M50 values), **(B)** maximal response rates (RMAX values), **(C)** and total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the shifts in M50. Data shown are means ± SEM expressed as a percentage of baseline performance. *Denotes significance from control at $p < 0.05$ following Newman-Keuls post hoc tests.
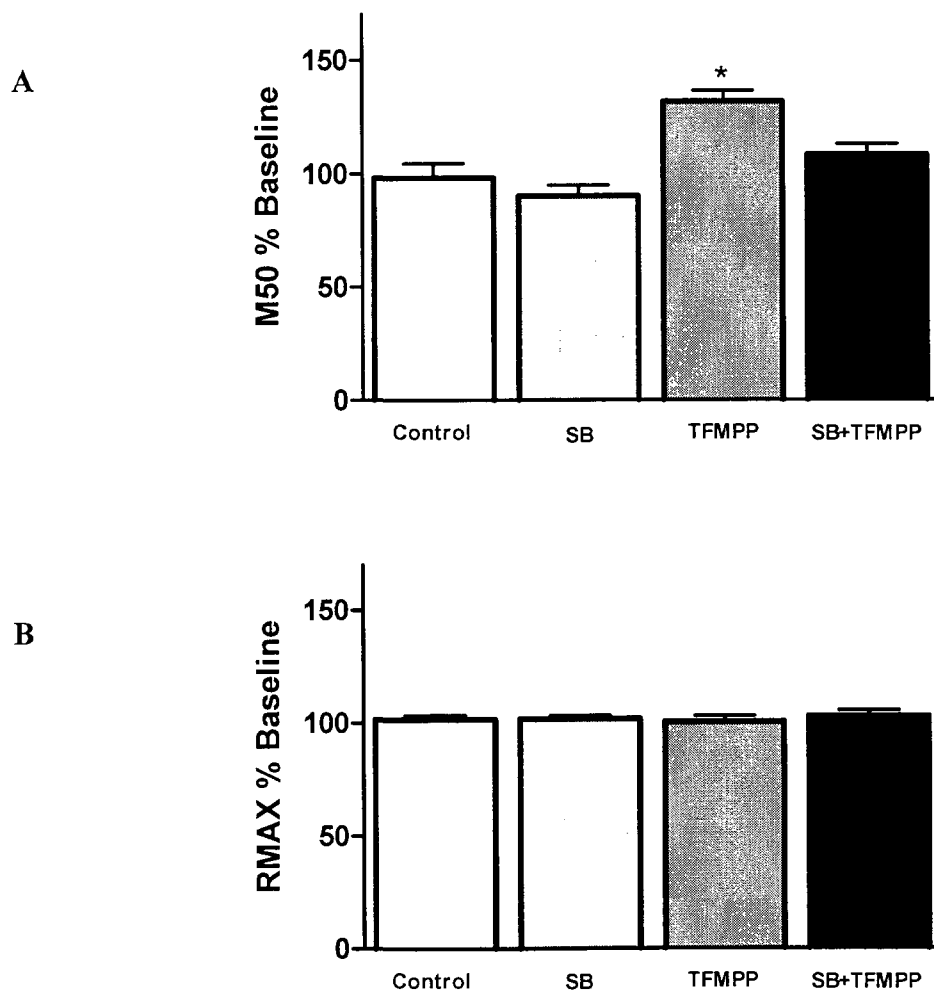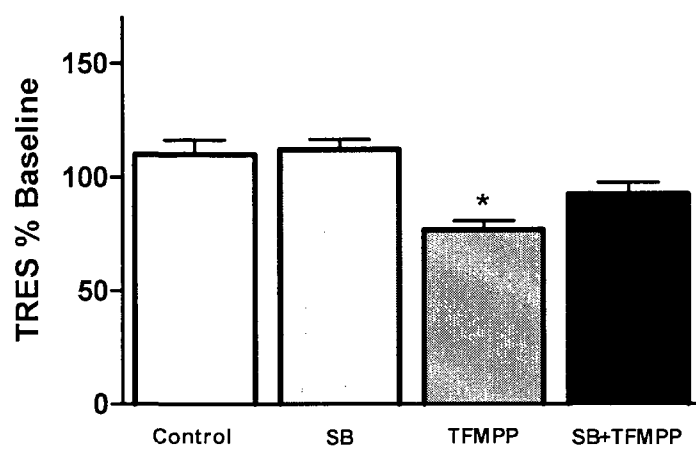
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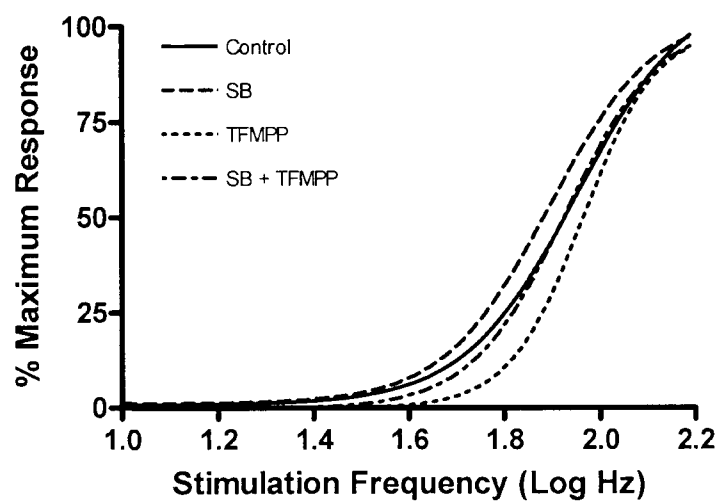
Effects of intra-NAc shell WAY 161503

Intra-NAc shell microinjections of WAY 161503 (0.15, 0.5, 1.5 µg/side) showed no change in M50 (Fig. 3.3A), RMAX (Fig. 3.3B), or TRES values (Fig. 3.3C). The positive control (+)-amphetamine (1.0 µg/side) significantly decreased M50 values [Fig. 3.3A; $F_{(1, 8)} = 26.19$, $p < 0.05$], and increased TRES values [Fig. 3.3C; $F_{(1, 8)} = 30.07$, $p < 0.05$], without affecting RMAX values (Fig. 3.3B). Group-averaged rate-frequency regression curves are included to compare the leftward shift in M50 seen with (+)-amphetamine (indicating an increase in reward) to the absence of effects by WAY 161503 (Fig. 3.3D).

Only rats with electrode placements in the VTA and bilateral cannulae in the NAc shell were included in the analysis. Representative photomicrographs of VTA stimulation sites and NAc shell microinjection sites are seen in Fig. 3.4A & C, respectively. Histological locations of electrode terminal sites and NAc shell microinjection sites are represented in Fig. 3.4B and D, respectively.

Figure 3.3A-D The effects of intra-NAc shell microinjections of WAY 161503 (WAY; 0-1.5 µg/side) and (+)-amphetamine (AMPH; 1.0 µg/side) compared to injections of artificial cerebrospinal fluid (Control) on **(A)** rate-frequency thresholds (M50 values), **(B)** maximal response rates (RMAX values), **(C)** and total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the shifts in M50. Data shown are means ± SEM expressed as a percentage of baseline performance. *Denotes significance from control at p<0.05 following Newman-Keuls post hoc tests.
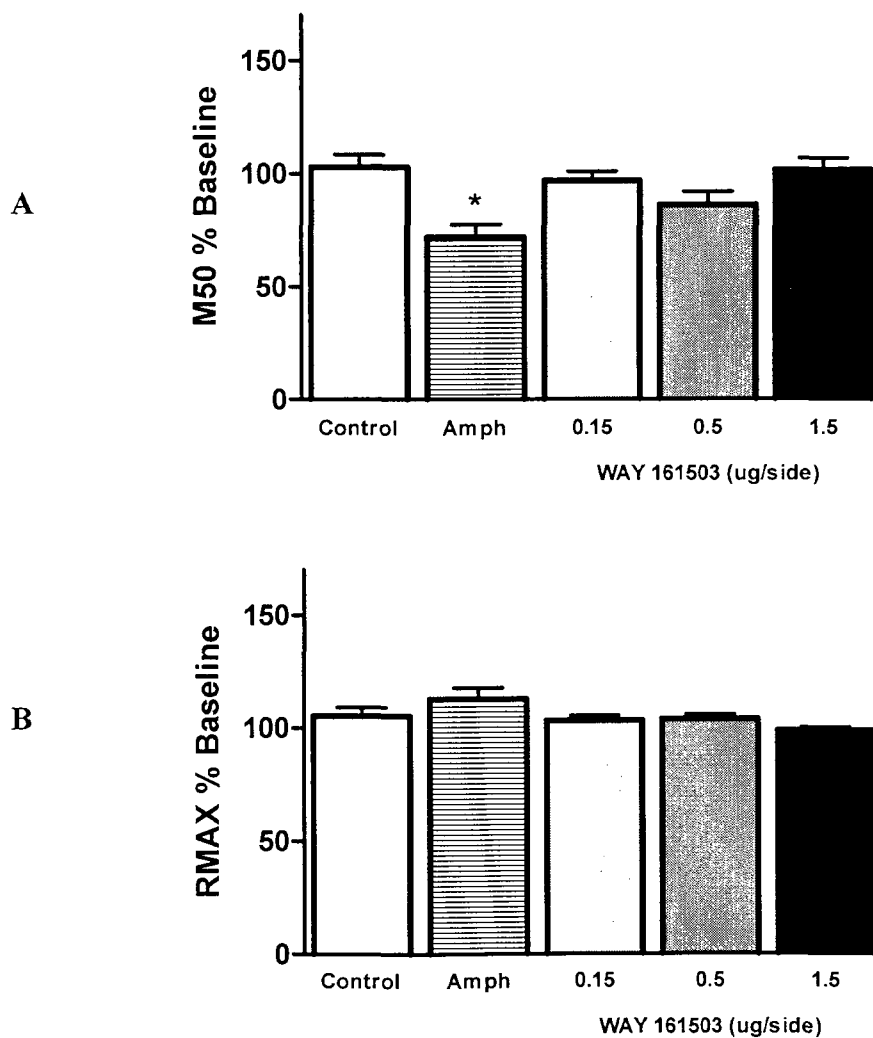
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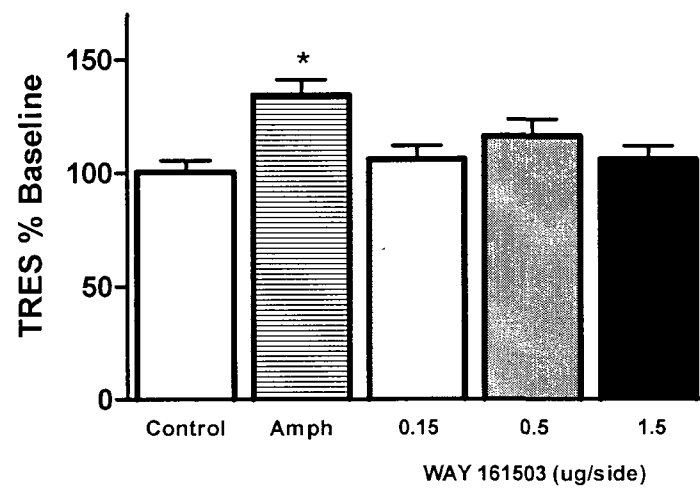
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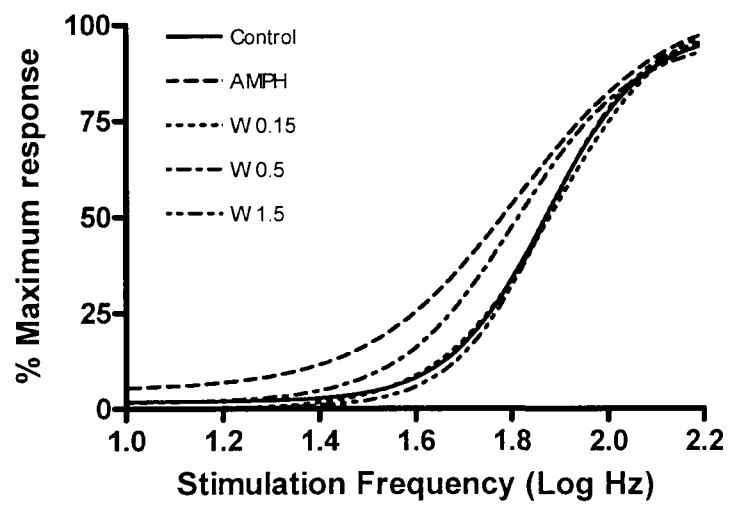
Figure 3.4A-D Histological verification of VTA and NAc shell sites. **(A)** Representative photomicrograph (circle identifies VTA electrode terminal) and **(B)** histological locations of VTA stimulation sites. **(C)** Representative photomicrograph and **(D)** histological locations of NAc shell microinjection sites. Brain diagrams from Paxinos & Watson (1998).

A

B

Interaural 4.20

Interaural 3.80

Interaural 3.70

Interaural 3.40

nteraural 3.20

nteraural 2.96

Interaural 2.97

C

D

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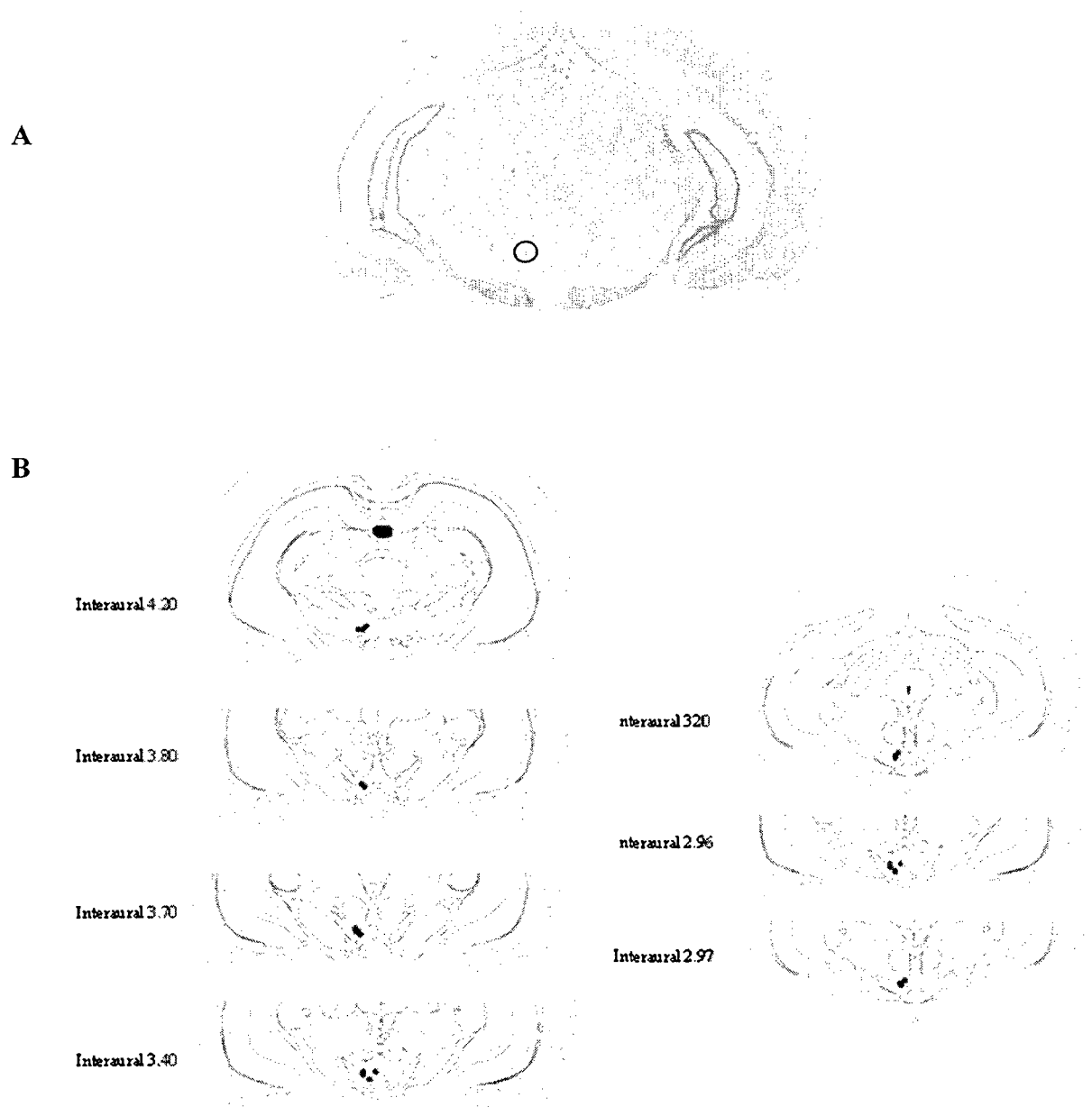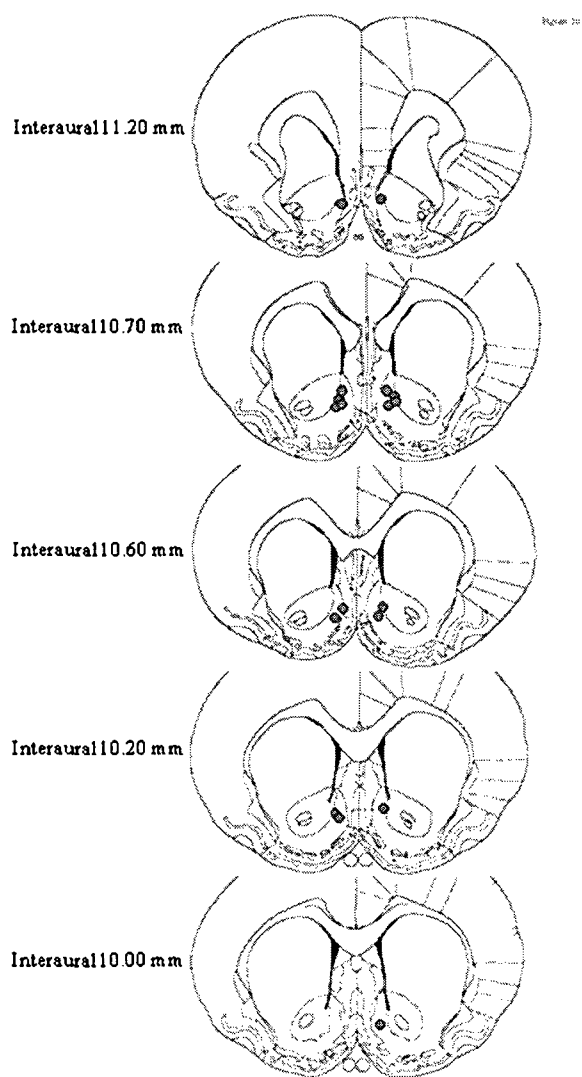
Discussion

The increase in rate-frequency thresholds (i.e. M50 values) following systemically administered WAY 161503 and TFMPP (Fig. 3.1A and 3.2A, respectively), without significant effects on maximal response rates (RMAX; indicating no overall motor effects) (Fig. 3.1B & 3.2B, respectively), supports the hypothesis that $5-HT_{2C}$ receptor activation plays an inhibitory role in VTA ICSS behaviour. The attenuation of TFMPP's effects by the highly selective $5-HT_{2C}$ receptor antagonist, SB 242084, supports the notion that these effects are $5-HT_{2C}$ receptor-mediated (Fig. 3.2A).

Systemic TFMPP has been shown to stimulate 5-HT release in the NAc (Baumann et al. 2005), while intra-VTA mCPP (a mixed $5-HT_{1B/2C}$ receptor agonist) reduced the firing rate of DA cells in this region (Prisco et al. 1994). Though TFMPP and mCPP are mixed 5-HT receptor agonists with only modest $5-HT_{2C}$ receptor binding (Barnes and Sharp 1999; Berg et al. 1998) a number of studies have suggested that their behavioural effects are $5-HT_{2C}$ receptor-mediated (Dalton et al. 2006; Hayashi et al. 2005; Kennett and Curzon 1988b; Lucki et al. 1989; Mora et al. 1997). Alternately, some studies have identified behavioural effects for TFMPP that appear to be $5-HT_{1B}$ receptor-mediated (Kennett and Curzon 1988a; Rodriguez-Manzo et al. 2002; Schechter 1988). As activation of the $5-HT_{1B}$ receptor has also been shown to increase current thresholds in lateral hypothalamic and VTA ICSS experiments (Harrison et al. 1999; Hayes et al. 2006), the present results demonstrating an attenuation of TFMPP's effects by SB 242084 provide evidence that TFMPP's effects on VTA ICSS are largely $5-HT_{2C}$ receptor-mediated. Nonetheless, it is important to note that there is some evidence for $5-HT_{1B}$ and $5-HT_{2C}$ receptor interactions (Clifton et al. 2003; Dalton et al. 2006; Nonogaki

et al. 2007; Wang et al. 2008). SB 242084 had no effect on VTA ICSS (Fig. 3.2A) which is interesting as previous studies have shown that SB 242084 can increase the firing rates of VTA DA cells and increase DA release in the NAc (Di Giovanni et al. 2000; Di Matteo et al. 1999). In addition, SB 242084 has been shown to potentiate cocaine- and ethanol-induced self-administration in rats (Fletcher et al. 2002; Tomkins et al. 2002).

Increased M50 values in ICSS following the 1.0 mg/kg dose of WAY 161503 (Fig. 3.1A) are in agreement with other studies investigating 5-HT$_{2C}$ receptor stimulation using behavioural models of motivation and reward as demonstrated in state-dependent place conditioning (Mosher et al. 2006) and cocaine-, nicotine-, ethanol- and food-maintained operant responding (Fletcher et al. 2004; Grottick et al. 2001; Grottick et al. 2000; Tomkins et al. 2002). Doses of WAY 161503 and TFMPP used in the present experiments (and in previous unpublished replications) do not affect RMAX values, while higher doses of WAY 161503 (Hayes and Greenshaw 2005) and TFMPP (results not shown) may eliminate all responding. These data are consistent with in vivo studies demonstrating that 5-HT$_{2C}$ receptor agonists preferentially affect DA efflux in mesolimbic over nigrostriatal regions (Di Giovanni et al. 2000; Di Matteo et al. 1999), as these two regions are broadly associated with regulating motivated and sensorimotor aspects of behaviour, respectively (O'Doherty 2004; Salamone 1996; White 1986; Wise 2002).

WAY 161503 had no effect on VTA ICSS when microinjected into the NAc shell (Fig. 3.3A-C), in contrast to the well established reward-enhancing effects of (+)-amphetamine (Colle and Wise 1988; Schaefer and Michael 1988). This suggests that NAc shell 5-HT$_{2C}$ receptor activation may not play a primary role in regulating VTA ICSS

behaviour. This was unexpected as VTA ICSS results in an increase of DA release in the NAc shell (Fiorino et al. 1993) and systemically administered 5-HT$_{2C}$ receptor agonists decrease DA efflux in the NAc which is attenuated with systemically administered, intra-NAc, and intra-VTA, 5-HT$_{2C}$ receptor antagonists (Di Giovanni et al. 2000; Di Matteo et al. 1999; Navailles et al. 2006b). In addition, 5-HT$_{2C}$ receptor activation in the VTA inhibits cocaine-induced DA release while intra-NAc 5-HT$_{2C}$ receptor activation produces biphasic effects on cocaine-induced DA release (Navailles et al. 2008). In a behavioural study, Filip & Cunningham (2002) showed that 5-HT$_{2C}$ receptor activation in the NAc shell potentiated cocaine-induced increases in locomotion and increased the discriminability of subthreshold doses of cocaine.

Though intra-NAc shell administration of 5-HT$_{2C}$ receptor agonists appears to potentiate cocaine-induced behaviour, studies to date have reported no effects on basal DA efflux or behaviour following intra-NAc 5-HT$_{2C}$ receptor stimulation (Filip and Cunningham 2002; Navailles et al. 2008). Because of the relatively recent development of WAY 161503 as a selective ligand for the 5-HT$_{2C}$ receptor (Rosenzweig-Lipson et al. 2006; Schlag et al. 2004) there are no other studies reporting the effects of intra-cranial administration and most studies have relied on other agonists to stimulate this receptor. As such, future replications of the present study may benefit from the use of other selective 5-HT$_{2C}$ receptor agonists. Nonetheless it remains that no 5-HT$_{2C}$ receptor agonist to date, including WAY 161503 – a compound that acts as a full 5-HT$_{2C}$ receptor agonist in stimulating 5-HT$_{2C}$ receptor-coupled inositol phosphate formation and calcium mobilization (Rosenzweig-Lipson et al. 2006) and whose activity in behavioural studies is currently believed to be 5-HT$_{2C}$ receptor mediated (Cryan and Lucki 2000; Egashira et

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al. 2007; Hayes et al. 2008; Mosher et al. 2005) – demonstrates effects on its own following intra-NAc shell administration.

As the systemically administered 5-HT$_{2C}$ receptor agonists produced increases in M50 thresholds and 5-HT$_{2C}$ receptors appear to be located exclusively in the central nervous system (Barnes and Sharp 1999), reward-related circuitry may be involved in the mediation of these effects. The VTA, dorsal raphe and prefrontal cortex are good candidates in this regard – though others such as the amygdala cannot be ruled out (Simmons et al. 2007) – as these areas support ICSS and contain 5-HT$_{2C}$ receptors that alter DA efflux in the NAc (Broadbent and Greenshaw 1985; Bubar and Cunningham 2007; De Deurwaerdere and Spampinato 1999; Fiorino et al. 1993; Liu et al. 2007; Phillips and Fibiger 1978). Neurotransmitter interactions should also be taken into consideration as recent studies have identified 5-HT$_{2C}$ receptors on GABA interneurons in the dorsal raphe (Serrats et al., 2005), and on GABA-containing cells within the VTA (Bubar and Cunningham 2007; Di Giovanni et al. 2001); it has been suggested that 5-HT$_{2C}$ receptor activation decreases DA efflux indirectly through an excitation of GABAergic cells (Boothman et al. 2006; Di Giovanni et al. 2001; Serrats et al. 2005).

5-HT$_{2C}$ receptors are proposed to play a role in many psychiatric disorders that demonstrate altered mesocorticolimbic function and/or structure. In this context it is notable that some antipsychotic and antidepressant drugs may act upon 5-HT$_{2C}$ receptors (Chanrion et al. 2007; Navailles et al. 2006a; Rauser et al. 2001); these findings, together with other reports of 5-HT$_{2C}$ receptor involvement in appetitive behaviour and drug abuse suggest that the 5-HT$_{2C}$ receptor may be a useful target for the treatment of schizophrenia, depression and possibly drug abuse (Dremencov et al. 2005; Hill and

Reynolds 2007; Nilsson 2006; Siuciak et al. 2007). In addition, given that mesolimbic DA may be involved in a diverse array of behavioural functions such as action selection, decision making, and Pavlovian conditioning (Bassareo et al. 2007; Nicola 2007; Pattij et al. 2007; Phillips et al. 2007), the 5-HT$_{2C}$ receptor may also be of interest in these contexts. Indeed, at least one recent study has proposed a role for the 5-HT$_{2C}$ receptor in decision making and impulsive behaviour (Fletcher et al. 2007). The present results provide support for the hypothesis that 5-HT$_{2C}$ receptor activation may regulate VTA ICSS behaviour. While the precise circuitry has not been mapped, it appears that 5-HT$_{2C}$ receptor activation in the NAc shell does not play a primary role in the mediation of VTA ICSS under the present experimental conditions.

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Chapter 4: Effects of systemic 5-HT$_{1B}$ and 5-HT$_{2C}$ receptor compounds on ventral tegmental area intracranial self-stimulation thresholds in rats

Introduction

Interactions between serotonin (5-HT; 5-hydroxytryptamine) and dopamine (DA) have received increased attention in the context of regulating cognitive, motor and emotional functions (Alex and Pehek 2007; Daw et al. 2002). The midbrain raphe serotonergic system shows extensive connectivity with DA-containing areas of the brain, suggesting an important role for serotonin in the regulation of these cells (McBride et al. 1999; Van Bockstaele et al. 1993; Van Bockstaele et al. 1994). DA has long been associated with reward functions in the brain, though its exact role is still debated (Nicola et al. 2005; Robbins 1997; Schultz 1998; Tobler et al. 2005; Wyvell and Berridge 2000). The importance of research in this area is underscored by evidence indicating that DA functioning and reward-associated behaviour are altered in schizophrenia, depression and drug abuse (Juckel et al. 2006; Kalivas and Volkow 2005; Laviolette 2007; Nestler and Carlezon 2006; Wise 2002).

Serotonin plays an integral role in reward behaviours as demonstrated by place conditioning, self-administration and intracranial self-stimulation (ICSS) studies. Conditioned place preferences are seen following systemic administration of many

selective serotonin reuptake inhibitors (Subhan et al. 2000), though increased 5-HT transmission generally correlates with decreases in self-administration behaviour (Higgins et al. 1993; Lyness 1983; Lyness et al. 1980; Yu et al. 1986). ICSS behaviour can be maintained through stimulation of the major serotonergic nuclei innervating the brain (i.e. dorsal and median raphe nuclei) (Broadbent and Greenshaw 1985; Van Der Kooy et al. 1978). Although hampered by a limited measure of reinforcement, at least one study has demonstrated that perfusion of 5-HT close to the ventral tegmental area (VTA) facilitates ICSS of the medial forebrain bundle (Redgrave and Horrell 1976). Alternately, serotonergic cell lesioning may facilitate ICSS, indicating an inhibitory role for 5-HT (Poschel et al. 1974).

Conflicting results from these studies can be explained by the fact that 5-HT acts differentially through a number of receptor subtypes (Barnes and Sharp 1999); some of these have been shown to play a role in DA regulation (Alex and Pehek 2007). Serotonin 1B (5-HT$_{1B}$) receptors are found in a number of reward-related brain areas (Bruinvels et al. 1993; Sari et al. 1999) and their activation may increase DA release in the mesocorticolimbic system (Boulenguez et al. 1998; Boulenguez et al. 1996; Iyer and Bradberry 1996; O'Dell and Parsons 2004; Yan and Yan 2001a; Yan et al. 2004). 5-HT$_{1B}$ receptors are linked to inhibitory G-proteins (Hamblin and Metcalf 1991) and may exist as terminal autoreceptors (Engel et al. 1986; Gothert et al. 1987) or as heteroreceptors on cells containing acetylcholine (Cassel et al. 1995), glutamate (Boeijinga and Boddeke 1996) or γ-aminobutyric acid (GABA) (Sari et al. 1999). Some researchers have provided evidence for GABAergic inhibition via 5-HT$_{1B}$ heteroreceptors (Hurley et al. 2008; Johnson et al. 1992; Parsons et al. 1999; Yan and Yan 2001b; Yan et al. 2004).

The 5-HT$_{2C}$ receptor is also expressed throughout the mesocorticolimbic system (Bubar and Cunningham 2007; Clemett et al. 2000), but unlike the 5-HT$_{1B}$ receptor, studies have shown that 5-HT$_{2C}$ receptor activation may inhibit the release of mesolimbic DA (Di Giovanni et al. 2000; Di Matteo et al. 1999). The 5-HT$_{2C}$ receptor is coupled to excitatory G-proteins (Lucaites et al. 1996) and studies suggest that it may be located postsynaptically on cholinergic (Lopez-Gimenez et al. 2001; Pasqualetti et al. 1999; Pompeiano et al. 1994) or GABAergic cells (Bubar and Cunningham 2007; Liu et al. 2007; Serrats et al. 2005). In support of the latter, electrophysiological studies have demonstrated that 5-HT$_{2C}$ receptor activation results in increased GABA cell activity (Bankson and Yamamoto 2004; Boothman et al. 2006; Stanford and Lacey 1996).

At the behavioural level, activation of the 5-HT$_{1B}$ receptor induces a conditioned place aversion but enhances the reinforcing effects of cocaine (Barot et al. 2007; Cervo et al. 2002; Neumaier et al. 2002). While 5-HT$_{1B}$ receptor activation may also increase cocaine self-administration (Parsons et al. 1998; Przegalinski et al. 2007), it has been shown to decrease responding for ethanol (Maurel et al. 1999; Tomkins and O'Neill 2000) and (+)-amphetamine (Fletcher et al. 2002a; Fletcher and Korth 1999). At least one study has suggested an inhibitory role for the 5-HT$_{1B}$ receptor in ICSS, demonstrating decreased reward (measured by increases in lateral hypothalamic current thresholds) following systemic administration of the 5-HT$_{1A/1B/1D/2C}$ agonist RU 24969 and subsequent attenuation by the 5-HT$_{1B/1D}$ receptor antagonist GR 127935. This same study also found that RU 24969 attenuated the threshold-reducing (reward-enhancing) effects of cocaine (Harrison et al. 1999).

Activation of the 5-HT_{2C} receptor may also increase ICSS rate-frequency thresholds (Hayes and Greenshaw 2005) and inhibit drug-induced reward (Fletcher et al. 2002b; Grottick et al. 2001; Rocha et al. 2002; Tomkins et al. 2002), although some exceptions may exist (Filip and Cunningham 2002; Hayes et al. 2008). 5-HT_{2C} receptor compounds do not affect the expression of place conditioning on their own (Mosher et al. 2005; Rocha et al. 1993) but may produce a state-dependent conditioned place aversion (Mosher et al. 2006). The $5\text{-HT}_{1B/2C}$ receptor agonist mCPP also attenuated the conditioned place aversions induced by mianserin (5-HT_2 receptor and α_1- and α_2-adrenoceptor antagonist) and eltoprazine ($5\text{-HT}_{1A/1B}$ receptor agonist and 5-HT_{2C} receptor antagonist) (Rocha et al. 1993).

The present study tested the hypothesis that 5-HT_{1B} receptors play an excitatory role in VTA ICSS behaviour by investigating the effects of the 5-HT_{1B} receptor agonist CP 94253 (0-5.0 mg/kg) and antagonist GR 127935 (10.0 mg/kg), alone and in combination. As some behavioural studies have provided evidence for 5-HT_{1B} and 5-HT_{2C} receptor interactions (Clifton et al. 2003; Dalton et al. 2006; Nonogaki et al. 2007; Wang et al. 2008), and given their proposed localization on GABA cells within reward-related circuitry, the effects of GR 127935 (10.0 mg/kg) and the 5-HT_{2C} receptor agonist WAY 161503 (0.3, 1.0 mg/kg) were also investigated in VTA ICSS. It was hypothesized that 5-HT_{2C} receptor stimulation and 5-HT_{1B} receptor antagonism, together, would potentiate increases in VTA ICSS thresholds.

Materials & methods

Subjects

Twenty male Sprague-Dawley rats (Health Sciences Laboratory Animal Services, University of Alberta) weighing 200-300g were housed individually in standard Plexiglas laboratory cages at 20°C and 50% humidity, with a 12-hr light/dark cycle with food and water freely available. All apparatus were cleaned between animals with diluted (1:6) ammonia-based window cleaner (No Name® Glass Cleaner with ammonia). The care and use of animals were in accordance with guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

Drugs

The 5-HT$_{2C}$ receptor agonist WAY 161503 · HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1H-pyrazino[1,2-a]quinoxalin-5(6H)-one hydrochloride], the 5-HT$_{1B}$ receptor agonist CP 94253 · HCl [5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-pyrrolo[3,2-b]pyridine hydrochloride] and the 5-HT$_{1B/1D}$ receptor antagonist GR 127935 · HCl [N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide hydrochloride] were purchased from Tocris Cookson Inc. (Ellisville, MO, USA). All compounds were dissolved in double-distilled water (ddH$_2$O) and were administered subcutaneously in a volume of 1.0 ml/kg. WAY 161503 (0.3, 1.0 mg/kg) was administered 10 min prior to testing; CP 94253 (0-5.0 mg/kg) was administered 20 min prior to testing; GR 127935 (10.0 mg/kg) was administered 40 min prior to testing. All drug doses are expressed as free-base.

Intracranial self-stimulation (ICSS)

Surgery & histology: Using a previously described procedure (Greenshaw 1993), each animal was implanted with a stainless steel, monopolar, stimulating electrode (E363/2; tip diameter 200 µm; Plastics One Ltd., Roanoke, VA) directed to the VTA. A large silver electrode in the skull served as the relative ground. Stereotaxic coordinates were [mm]: VTA – AP +2.6, L +0.5, V +1.8 from inter-aural zero, with the incisor bar set at 2.4 mm below the inter-aural line (Paxinos and Watson 1998). These coordinates were interpolated from the target site for angles of 20° lateral and 20° anterior (Greenshaw 1997). Electrode placements were verified at the end of the experiment by microscopic inspection of flash-frozen, cresyl violet stained, coronal brain sections (40 µm). Flash-freezing was achieved using isopentane cooled on dry ice. Only animals with VTA placements were included in the analysis.

Apparatus & Procedure: Animals in the CP 94253 dose-response experiment (n = 11), CP 942543 + GR 127935 (n = 8) experiment, and WAY 161503 + GR 127935 (n = 9) were trained in ICSS using monopolar stimulation of the VTA provided from constant current DC stimulators (cathodal monophasic pulse width of 200 µs; initial training frequency of 100 Hz; train length of 1 s) connected to each animal via a gold-track slip ring. Between pulses the active electrode and indifferent electrode were connected through a resistor to cancel any effects of electrode polarisation (Greenshaw 1986). The apparatus and rate-frequency analysis were as described by Ivanová and Greenshaw (1997). With this procedure, M50 is the threshold frequency at which half-maximal response rates occur (i.e. rate-frequency threshold); RMAX is the maximal rate of responding in a session. While M50 is a measure of reward sensitivity (which is

dissociable from non-specific changes in behaviour), RMAX is a measure of response performance (see Gallistel and Karras, 1984; Greenshaw and Wishart, 1987). Animals received a randomized counterbalanced sequence of treatments with 3 days of baseline frequency testing between each treatment. The doses chosen for GR 1279835 (10.0 mg/kg) and WAY 161503 (0.3, 1.0 mg/kg) were based on prior behavioural studies (Cervo et al. 2002; Hayes and Greenshaw 2005; Maurel et al. 1998; Mosher et al. 2005). To minimize the use of animals, animals with stable implants and ICSS behaviour at the end of each experiment were used in additional experiments.

Statistical Analysis

Experimental effects in the ICSS experiments were determined using repeated measures ANOVA followed by Newman-Keuls post hoc tests ($\alpha = 0.05$) where appropriate. All ICSS data are presented as a percentage of average baseline performance of each animal. Greenhouse-Geisser corrected degrees of freedom are used as a conservative estimate of the F-ratio. Statistical analyses for all experiments were completed using statistical software (SPSS Inc., Chicago, IL, USA).

Results

Effects of CP 94253 on ICSS

Analysis following systemic administration of CP 94253 (0-5.0 mg/kg) revealed significant main effects for M50 thresholds [Fig. 4.1A; $F_{(2.64, 26.44)} = 11.22$, $p < 0.05$], RMAX values [Fig. 4.1B; $F_{(1.56, 15.57)} = 4.14$, $p < 0.05$] and TRES values [Fig. 4.1C; $F_{(2.08, 20.83)} = 13.54$, $p < 0.05$]. Further analysis using Newman-Keuls post hoc tests ($\alpha = 0.05$) revealed that only the highest dose (5.0 mg/kg) produced a significant increase in

M50 thresholds (Fig. 4.1A) and decrease in TRES values (Fig. 4.1C) compared to control and that none of the doses resulted in significant changes in RMAX values (Fig. 4.1B). Given the appearance of motor effects at the highest dose of CP 94253 the non-transformed (raw) RMAX data were also analysed; no RMAX effects were noted (results not shown). Group-averaged rate-frequency regression curves are included to illustrate the dose-dependent rightward shift in M50 seen with CP 94253 (indicating a decrease in reward) (Fig. 4.1D).

Figure 4.1A-D. Effects of CP 94253 (0-5.0 mg/kg) on rate-frequency thresholds (M50 values), maximal response rates (RMAX values) and total responses (TRES values) for VTA ICSS. **(A)** The highest dose of CP 94253 tested (5.0 mg/kg) produced a significant increase in M50 values; **(B)** without affecting RMAX values. **(C)** CP 94253 (5.0 mg/kg) decreased TRES values. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the dose-dependent rightward shift in M50 seen with CP 94253 (indicating a decrease in reward). Data shown are means ± SEM. *Significant at $p < 0.05$ following Newman-Keuls post hoc tests.
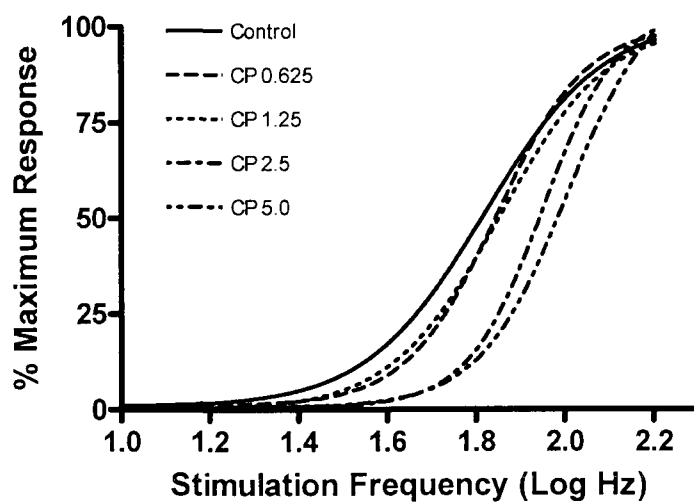
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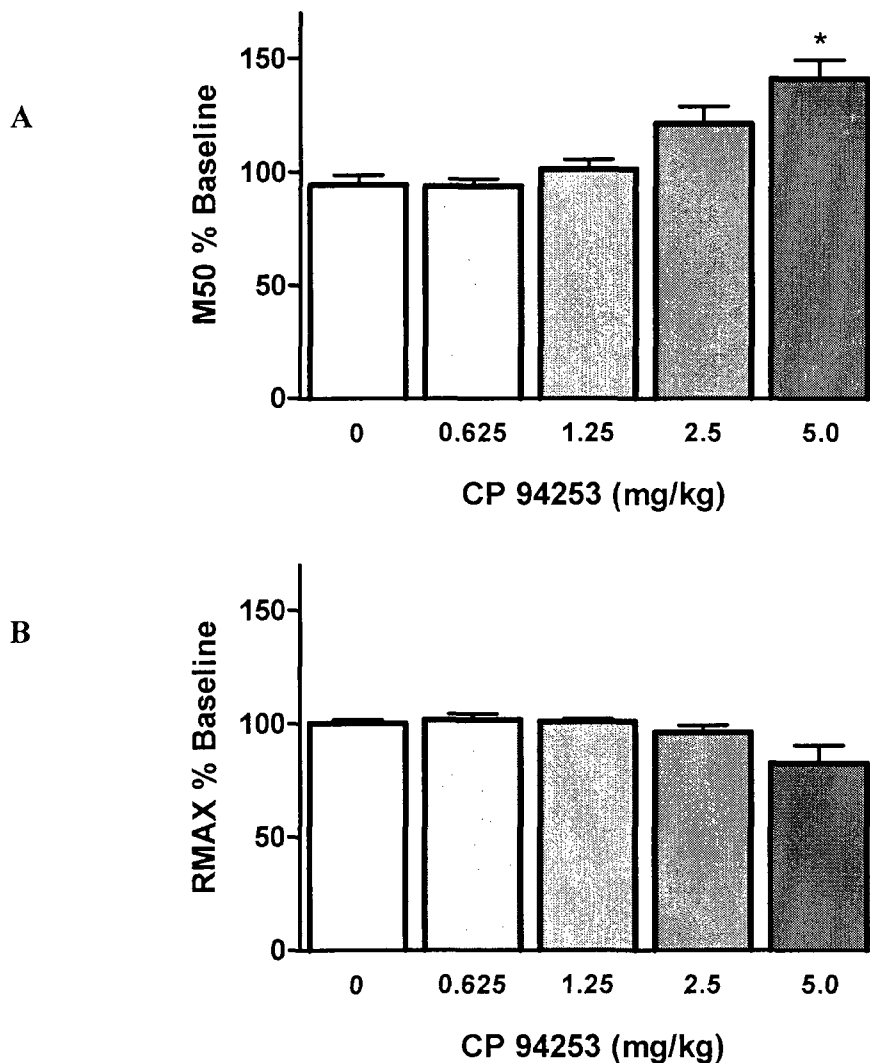
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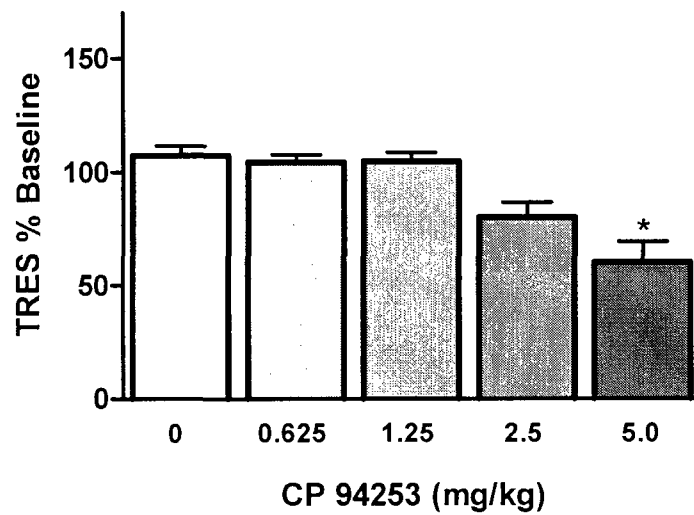
Effects of CP 94253 and GR 127935 on ICSS

To further characterize the 5-HT$_{1B}$ receptor-related behavioural component of CP 94253, the effects of the 5-HT$_{1B/1D}$ receptor antagonist GR 127935 (10.0 mg/kg), alone and in combination with CP 94253 (5.0 mg/kg), were investigated (Fig. 4.2A & 4.2B). A main effect of CP 94253 was seen with M50 thresholds [Fig. 4.2A; $F(1, 7) = 44.11$, $p < 0.05$] and TRES values [Fig. 4.2C; $F(1, 7) = 28.82$, $p < 0.05$] though not with RMAX values. A main effect of GR 127935 was seen with M50 and TRES values [$F(1, 7) = 17.08$, $p < 0.05$; $F(1, 7) = 11.67$, $p < 0.05$] though not with RMAX values. An interaction was seen for M50 and TRES values [$F(1, 7) = 6.24$, $p < 0.05$; $F(1, 7) = 7.18$, $p < 0.05$] though not for RMAX. Newman-Keuls post hoc tests revealed that the 5.0 mg/kg dose of CP 94253 produced a significant increase in M50 and TRES values compared to all other treatments; the combination of CP 94253 and GR 127935 was not different from control. Group-averaged rate-frequency regression curves are included to further contrast the rightward shift in M50 seen with CP 94253 (indicating a decrease in reward) to the other treatments, which are similar to control (Fig. 4.2D).

Figure 4.2A-D. Effects of CP 94253 (C; 5.0 mg/kg) and GR 127935 (G; 10.0 mg/kg) on rate-frequency thresholds (M50 values), maximal response rates (RMAX values) and total responses (TRES values) for VTA ICSS. **(A)** The highest dose of CP 94253 tested (5.0 mg/kg) produced a significant increase in M50 values, while GR 127935 had no effect on M50 values. The effects of CP 94253 on frequency thresholds were attenuated by GR 127935. **(B)** None of the treatments affected RMAX values. **(C)** CP 94253 (5.0 mg/kg) produced a decrease in TRES values, while GR 127935 attenuated these effects without having effects on its own. **(D)** Group-averaged rate-frequency regression curves are included to further contrast the rightward shift in M50 seen with CP 94253 (indicating a decrease in reward) to all other treatments. Data shown are means ± SEM. *Significant at p < 0.05 following Newman-Keuls post hoc tests.

A
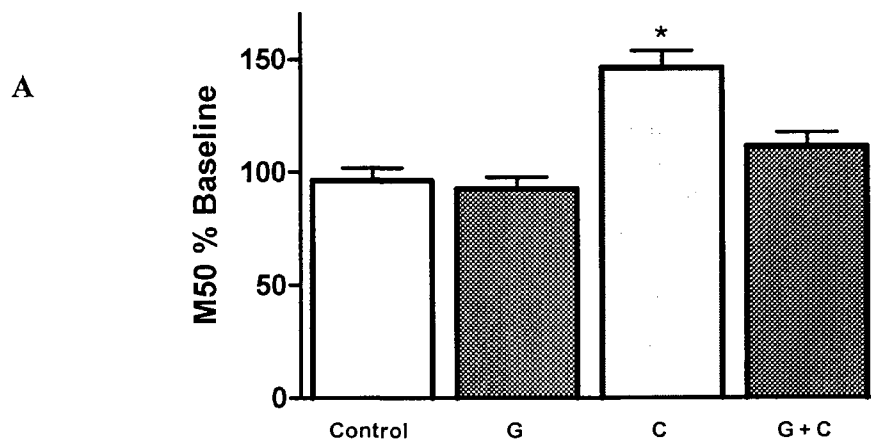
B
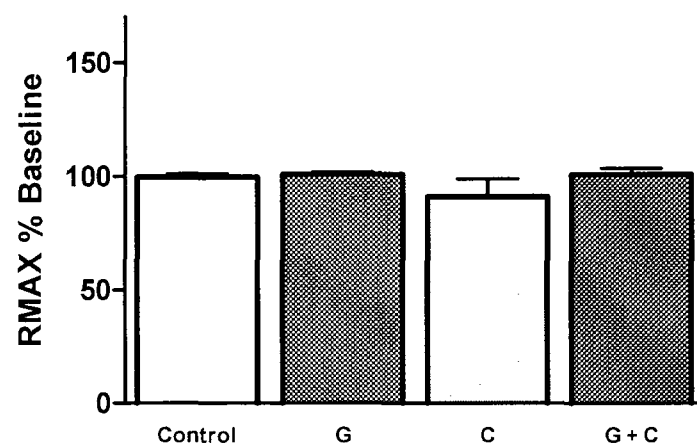
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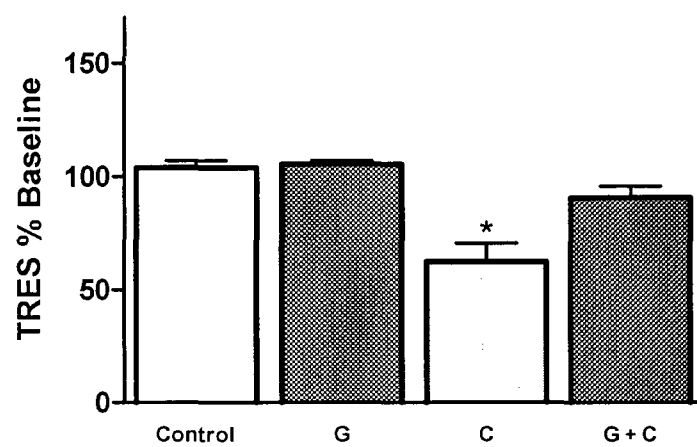
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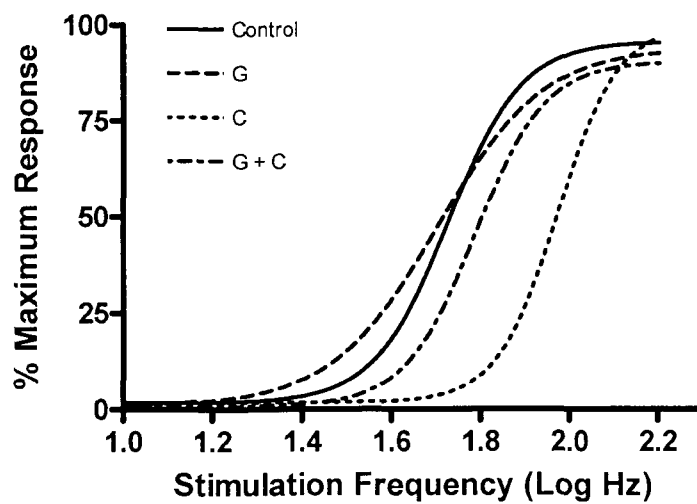
Effects of WAY 161503 and GR 127935 on ICSS

Analysis following systemic administration of WAY 161503 (0.3, 1.0 mg/kg) and GR 127935 (10.0 mg/kg) revealed significant main effects of WAY 161503 for M50 thresholds and TRES values [Fig. 4.3A, $F_{(1.83, 12.78)} = 18.22$, $p < 0.05$; Fig. 4.3C $F_{(1.64, 13.08)} = 14.69$, $p < 0.05$], but not for RMAX values. There were no main effects for GR 127935 across M50, RMAX or TRES values, respectively (Fig. 4.3A-C) nor were any interactions noted between WAY 161503 and GR 127935. Further analysis of WAY 161503, following the collapse of data across GR 127395, revealed that the 1.0 mg/kg dose was significant from control for M50 (Fig. 4.3D) and TRES (Fig. 4.3E). Group-averaged rate-frequency regression curves are included to illustrate the rightward shift in M50 seen with WAY 161503 (indicating a decrease in reward) (Fig. 4.3F).

Only rats with electrode placements in the VTA were included in the analysis. A representative photomicrograph of a VTA stimulation site is seen in Fig. 4.4A. Histological locations of VTA stimulation sites are represented in Fig. 4.4B.

Figure 4.3A-F The effects of GR 127935 (G; 10.0 mg/kg) and WAY 161503 (W; 0.3, 1.0 mg/kg) on rate-frequency thresholds (M50), maximal response rates (RMAX) and total responses (TRES) for VTA ICSS. **(A, C)** WAY 161503 showed a main effects for M50 and TRES values. **(B)** None of the treatments affected RMAX values. **(D)** WAY 161503 (1.0 mg/kg) increased M50 thresholds **(E)** and decreased TRES values, as noted following the collapse of data across GR 127935. **(F)** Group-averaged rate-frequency regression curves are included to illustrate the dose-dependent rightward shift (indicating a decrease in reward) in M50 thresholds seen with treatments containing WAY 161503. Data shown are means ± SEM expressed as a percentage of baseline performance. *Significant from control at $p < 0.05$ following Newman-Keuls post hoc tests.

A
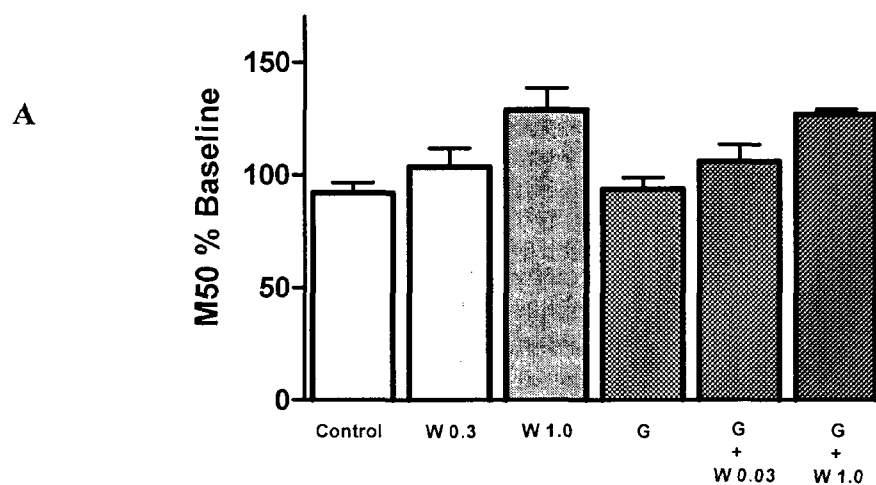
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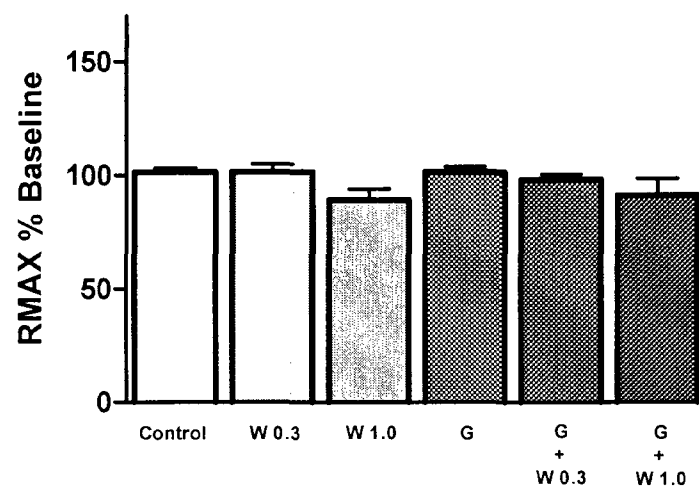
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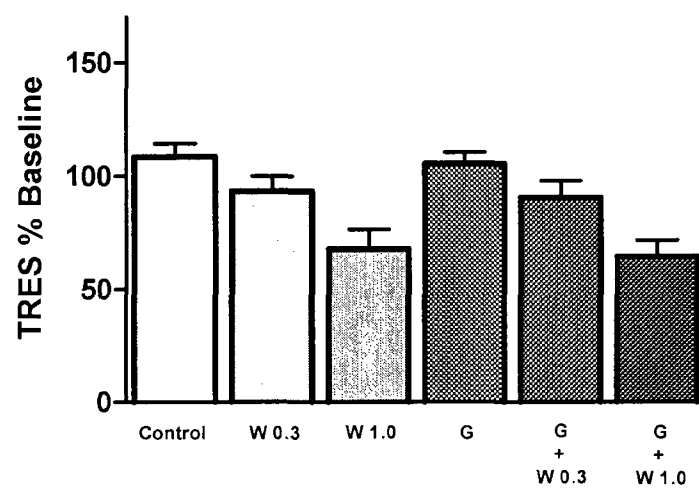
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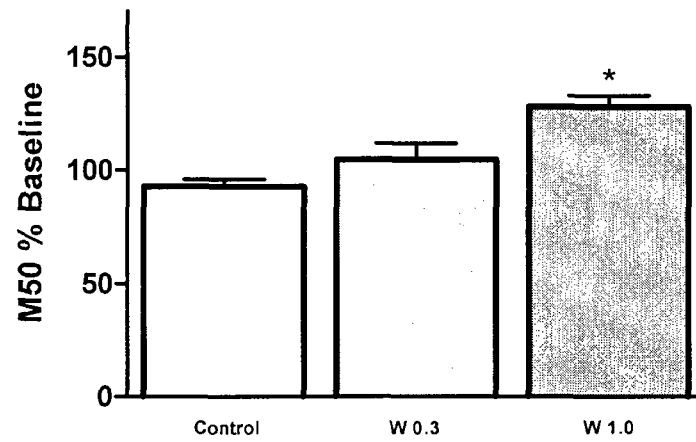
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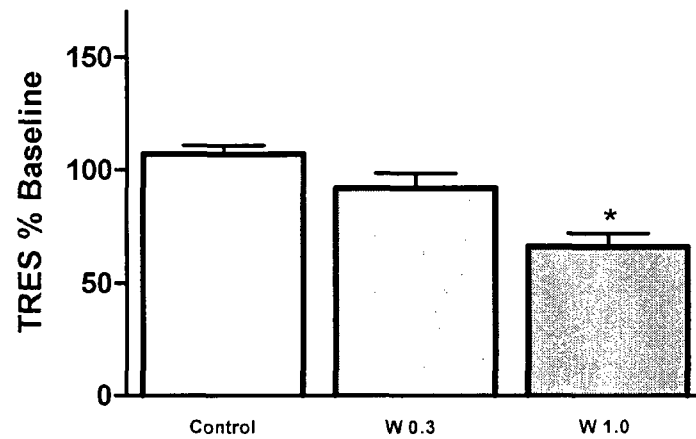
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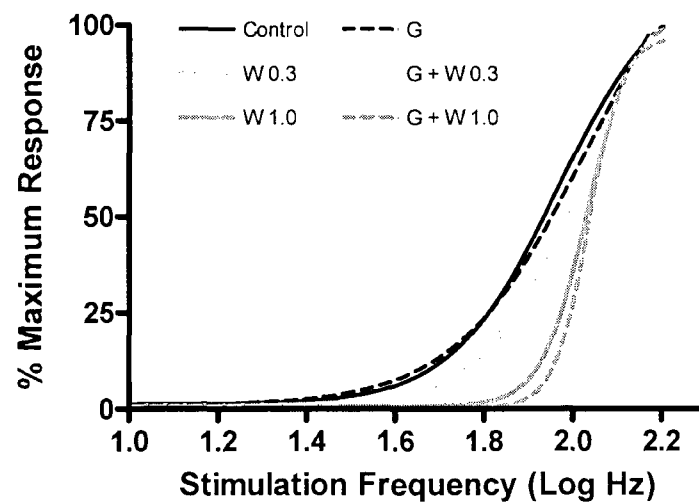
Figure 4.4A, B Histological verification of VTA sites. (a) Representative photomicrograph of VTA stimulation site (circle identifies VTA electrode terminal) and (b) Histological locations of VTA stimulating electrode sites. Brain diagrams from Paxinos & Watson (1998).

A

B
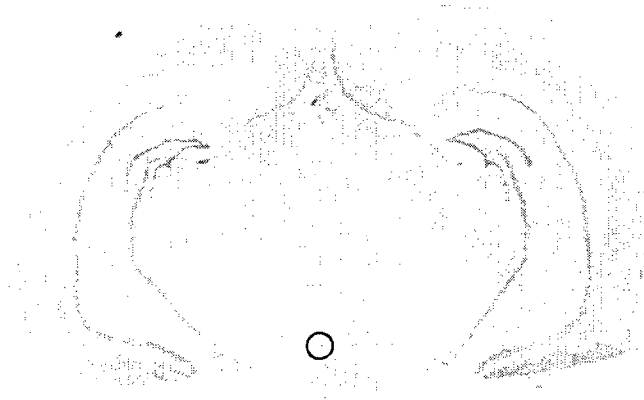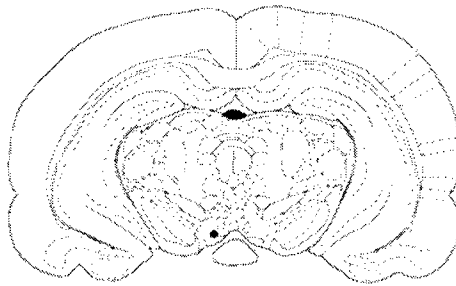
Interaural 3.70
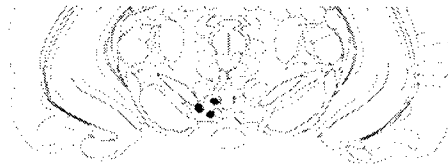
Interaural 3.40
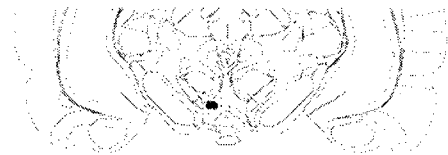
Interaural 3.20

Interaural 2.96

Interaural 2.70

Interaural 2.28

Discussion

Contrary to the initial hypothesis, the selective 5-HT$_{1B}$ receptor agonist CP 94253 (5.0 mg/kg) resulted in an increase in rate-frequency thresholds (M50 values; Fig. 4.1A & D) without effects on RMAX values (Fig. 4.1B), suggesting an inhibitory effect on reward. These results are congruent with previous behavioural studies demonstrating CP 94253-induced conditioned place aversion (Cervo et al. 2002) and RU 24969-induced increases in lateral hypothalamic ICSS rate-current thresholds (Harrison et al. 1999). However, these data do not support the hypothesis that 5-HT$_{1B}$ receptors alter VTA ICSS behaviour through their activity as GABAergic heteroceptors (Hurley et al. 2008; Johnson et al. 1992; Parsons et al. 1999; Yan and Yan 2001b; Yan et al. 2004) whose activation leads to an increase in DA efflux (Boulenguez et al. 1998; Boulenguez et al. 1996; Iyer and Bradberry 1996; O'Dell and Parsons 2004; Yan and Yan 2001a; Yan et al. 2004).

The attenuation of CP 94253-induced increases in M50 values by the 5-HT$_{1B/D}$ receptor antagonist GR 127935 (10 mg/kg; Fig. 4.2A & D) without effects on RMAX values (Fig. 4.2B) further supports the hypothesis that the reward-decreasing effects of CP 94253 are 5-HT$_{1B}$ receptor-mediated. These results are consistent with the ability of GR 127935 to attenuate the behavioural effects of 5-HT$_{1B}$ receptor activation (Cervo et al. 2002; Harrison et al. 1999; Maurel et al. 1998; Parsons et al. 1998; Tomkins and O'Neill 2000). The fact that GR 127935 has no effect on ICSS alone is consistent both with Harrison et al.'s (1999) work and the notion that reward-related behaviours do not appear to be under tonic control of the 5-HT$_{1B}$ receptor (Cervo et al. 2002; Fletcher et al. 2002a; Fletcher and Korth 1999; Parsons et al. 1998; Tomkins and O'Neill 2000).

5-HT$_{1B}$ receptors are found mainly as presynaptic auto- and heteroreceptors throughout the brain (Sari 2004; Sari et al. 1999). It seems unlikely that stimulation of 5-HT$_{1B}$ autoreceptors is involved in the reward-decreasing effects of CP 94253 as stimulation of presynaptic 5-HT$_{1A}$ autoreceptors, via systemic or intra-raphe injection of 8-OH-DPAT, induces a robust conditioned place preference (Fletcher et al. 1993; Papp and Willner 1991; Shippenberg 1991) and decreases M50 values in VTA ICSS following intra-raphe injections of 8-OH-DPAT (Ahn et al. 2005). However, the involvement of a decrease in serotonergic tone in areas other than those involved in 8-OH-DPAT's rewarding effects cannot be excluded. One current line of evidence suggests that 5-HT$_{1B}$ heteroreceptors on GABA-containing cells may be involved in regulating DA- and reward-related behaviours (Johnson et al. 1992; Parsons et al. 1999; Stanford and Lacey 1996). Many non-dopaminergic cells within the VTA contain GABA and these cells can act as interneurons or project to the ventral striatum and other cortical regions (Steffensen et al. 1998). Major targets of these GABA cells within the rat VTA appear to be DA cells (Bayer and Pickel 1991). 5-HT$_{1B}$ receptor stimulation can reduce VTA GABA release (Yan and Yan 2001b) and increase nucleus accumbens and VTA DA release (Yan et al. 2004).

The present results support an inhibitory role for the 5-HT$_{2C}$ receptor in VTA ICSS, as WAY 161503 (1.0 mg/kg) increased M50 thresholds (Fig. 4.3A, D & F) without affecting RMAX values (Fig. 4.3B), consistent with previous results (Hayes and Greenshaw 2005). 5-HT$_{2C}$ receptors may act as excitatory postsynaptic receptors on GABAergic cells (Bubar and Cunningham 2007; Liu et al. 2007; Serrats et al. 2005) whose activation results in an increase of GABA release (Bankson and Yamamoto 2004;

Boothman et al. 2006; Stanford and Lacey 1996) and ultimately leads to reduced DA transmission (Di Giovanni et al. 2000; Di Matteo et al. 1999). Given the inverse actions of 5-HT_{1B} and 5-HT_{2C} receptor activation on DA functioning, and the relationship between these receptors noted in other behavioural studies (Clifton et al. 2003; Dalton et al. 2006; Nonogaki et al. 2007; Wang et al. 2008), the present study hypothesized that 5-HT_{2C} receptor activation with WAY 161503 and 5-HT_{1B} receptor antagonism with GR 127935 would result in the potentiation of M50 thresholds.

That the 5-$HT_{1B/1D}$ receptor antagonist had no affect on WAY 161503-induced increases in M50 thresholds (Fig. 4.3A & D) further supports the notion that WAY 161503 may act at postsynaptic 5-HT_{2C} receptors to increase GABAergic activity while 5-HT_{1B} receptor activation may inhibit VTA ICSS through some other mechanism. As 5-HT_{1B} heteroreceptors are found on a number of cell types, including those containing GABA (Johnson et al. 1992; Parsons et al. 1999; Yan and Yan 2001b; Yan et al. 2004), glutamate (Boeijinga and Boddeke 1996) and acetylcholine (Cassel et al. 1995), it will be difficult to map the precise circuitry involved in reward-related behaviour. A full understanding must include an accurate interpretation of data regarding the differential effects seen following 5-HT_{1B} receptor stimulation on the activity of drugs of abuse as well as the current data suggesting that 5-HT_{1B} receptors are involved in modulating, but not mediating, the effects of these drugs (Barot et al. 2007; Cervo et al. 1996; Fletcher et al. 2002a; Fletcher and Korth 1999; Hoplight et al. 2006; Maurel et al. 1999; Parsons et al. 1998; Przegalinski et al. 2007; Tomkins and O'Neill 2000). In addition, given the relationship between DA, reward-related behaviour, and the 5-HT_{1B} receptor, creating a

precise map is also constrained by our limited understanding of the exact role of DA in reward (Salamone et al. 2005; Schultz 2007a; b).

Though 5-HT$_{1B}$ receptor activation increases DA release throughout the mesocorticolimbic system (Boulenguez et al. 1998; Boulenguez et al. 1996; Iyer and Bradberry 1996; O'Dell and Parsons 2004; Yan and Yan 2001a; Yan et al. 2004), this increase in extracellular DA does not consistently lead to increases in reward-related behaviour. If changes in DA are related to the inhibition of reward-related behaviours seen in the present study, one possible explanation relies on data demonstrating DA release during exposure to aversive stimuli (Guarraci and Kapp 1999; Salamone 1994). Though the activity of DA cells is quite different in response to aversive- versus reward-related stimuli (Schultz 2007a), increases in the release of DA would still be noted in both conditions. Another possibility is that the aversive effects of 5-HT$_{1B}$ receptor stimulation are not reward-related but instead may be related to its anxiogenic effects, as noted in behavioural studies of anxiety (Benjamin et al. 1990; Lin and Parsons 2002). This idea is supported by studies which have demonstrated conditioned place aversions or increases in ICSS M50 values following administration of known anxiogenic compounds such as yohimbine or picrotoxin (Acquas et al. 1990; File 1986; Hayes et al. 2006); however it is important to note that some anxiogenic compounds may not produce aversive behaviours in all behavioural tests (Alves et al. 2004; Kennett et al. 1989; Mosher et al. 2005). Also, 5-HT$_{1B}$ receptor stimulation does not always result in anxiety-related behaviours and may in fact be anxiolytic under some conditions (Bell et al. 1995; Chojnacka-Wojcik et al. 2005).

The present results support previous studies indicating that 5-HT_{1B} and 5-HT_{2C} receptors are involved in the inhibition of reward-related behaviour. While the existence of 5-HT_{2C} receptors as GABAergic postsynaptic receptors is supported by the present findings, the activity of CP 94253 at presynaptic 5-HT_{1B} receptors on GABA cells is questioned. Nonetheless, while it seems unlikely that these two receptors are affecting VTA ICSS through similar neuronal populations, they may affect reward behaviour through activity at different GABA cells. 5-HT_{1B} receptors have been proposed to play a role in aggressive behaviour and psychiatric disorders that demonstrate altered mesocorticolimbic function and/or structure such as drug addiction, depression and anxiety (Alex and Pehek 2007; Olivier and van Oorschot 2005; Sari 2004). 5-HT_{2C} receptors may also be useful targets for the treatment of schizophrenia, depression and possibly drug abuse (Dremencov et al. 2005; Hill and Reynolds 2007; Nilsson 2006; Siuciak et al. 2007). Together, these findings suggest that research on 5-HT_{1B} and 5-HT_{2C} receptors may be important for understanding DA-related behaviours and some psychiatric disorders.

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Chapter 5: Effects of GABA$_A$ and 5-HT$_{2C}$ receptor compounds on ventral tegmental area intracranial self-stimulation thresholds and locomotor activity in rats

(A version of this chapter has been submitted to Neuropsychopharmacology. Hayes DJ, Hoang J, Greenshaw AJ. Effects of GABA$_A$ and 5-HT$_{2C}$ receptor compounds on ventral tegmental area intracranial self-stimulation thresholds and locomotor activity in rats.)

Introduction

Activity of the mesocorticolimbic dopamine (DA) system is important for the regulation of motivation and reward-related behaviours; place conditioning, self-administration and intracranial self-stimulation (ICSS) are commonly used animal models (McBride et al. 1999; Tzschentke 2007; Wise 2002; 2004). Electrical stimulation of the ventral tegmental area (VTA) drives ICSS behaviour in rats and results in DA release in the nucleus accumbens (NAc) (Fiorino et al. 1993). Locomotor activity is also sensitive to changes in DA and can be used to compare the potential motor effects of drugs (Di Chiara and Imperato 1988; Mogenson et al. 1980; Pijnenburg et al. 1975). Serotonin (5-HT) and γ-aminobutyric acid (GABA) have emerged as important neurotransmitters in the regulation of DA- and reward-related behaviours, yet the mechanisms involved are still unclear (Bardo 1998; Kalivas et al. 1990; McBride et al. 1999; Van Bockstaele et al. 1993; Van Bockstaele et al. 1994; Van Bockstaele and Pickel 1995). 5-HT and GABA act at numerous receptor subtypes and understanding how these neurotransmitter systems interact may help to elucidate the mechanisms of natural reward, drug addiction, and many psychiatric disorders (Feltenstein and See 2008; Goodman 2008; Ikemoto and Wise 2004; Kalueff and Nutt 2007; Laviolette 2007; Meltzer 1999; Shirayama and Chaki 2006;

Wrase et al. 2006). Recent studies have suggested that 5-HT_{2C} and GABA_A receptors within reward-related circuitry may be of particular interest (Boothman et al. 2006; Di Giovanni et al. 2001; Huidobro-Toro et al. 1996; Serrats et al. 2005; Stanford and Lacey 1996).

The 5-HT_{2C} receptor is expressed throughout the mesolimbic system (Bubar and Cunningham 2007; Clemett et al. 2000) and activation of this receptor may inhibit the release of mesolimbic DA (Di Giovanni et al. 2000; Di Matteo et al. 1999). Behavioural studies generally agree with an inhibitory role for the 5-HT_{2C} receptor in locomotion and reward-related behaviours. Activation of 5-HT_{2C} receptors attenuates nicotine-induced locomotion, food intake and self-administration (Grottick et al. 2001). Functional antagonism of the 5-HT_{2C} receptor also increases responding for cocaine and ethanol in rodents (Fletcher et al. 2002; Rocha et al. 2002; Tomkins et al. 2002). While selective 5-HT_{2C} receptor compounds do not affect the expression of place conditioning on their own (Mosher et al. 2005), they may produce a state-dependent conditioned place aversion (Mosher et al. 2006). Selective activation of the 5-HT_{2C} receptor using the agonist WAY 161503 increases ICSS rate-frequency thresholds (indicating a decrease in reward) without affecting measures of motor performance (Hayes et al. 2008a). 5-HT_{2C} receptor activation has been shown to decrease basal locomotor activity (Gleason et al. 2001; Higgins et al. 2001; Kennett et al. 2000; Kennett et al. 1997; Lucki et al. 1989; Martin et al. 2002; Mosher et al. 2005).

The GABA_A receptor is also expressed throughout the mesolimbic system (Churchill et al. 1992; Kalivas 1993; Onoe et al. 1996), although its precise role in DA- and reward-related behaviours is less clear than that of the 5-HT_{2C} receptor. Some studies

have suggested that the release of mesolimbic DA may be under tonic inhibitory control of the $GABA_A$ receptor (Ferraro et al. 1996; Ikemoto et al. 1997; Rahman and McBride 2002; Westerink et al. 1996; Yan 1999), while others have suggested a more complex role (Aono et al. 2008; Klitenick et al. 1992; Oakley et al. 1991; Xi and Stein 1998; Yoshida et al. 1997). Reports using ICSS rate-frequency thresholds have indicated a role for the $GABA_A$ receptor in ICSS behaviour (Cheer et al. 2005; Panagis and Kastellakis 2002), although the precise role may depend on the location of $GABA_A$ receptors in the brain (Simmons et al. 2007; Waraczynski 2007; 2008). $GABA_A$ receptor agonists and antagonists decreased locomotion when administered systemically (Mukhopadhyay and Poddar 1995; Sienkiewicz-Jarosz et al. 2003), increased locomotion following intra-VTA administration (Kalivas et al. 1990; Oakley et al. 1991; Schwienbacher et al. 2002) and decreased and increased locomotor activity, respectively, following intra-NAc injections (Austin and Kalivas 1989; Morgenstern et al. 1984; Plaznik et al. 1990; Pycock and Horton 1979).

Reciprocal connections exist between GABAergic and serotonergic cells (Bagdy et al. 2000; Wang et al. 1992). $5-HT_{2C}$ receptors have been identified on GABAergic cells in the VTA, prefrontal cortex and raphe nuclei (Bubar and Cunningham 2007; Liu et al. 2007; Serrats et al. 2005) and their activation has been associated with increasing GABA cell activity (Bankson and Yamamoto 2004; Boothman et al. 2006; Stanford and Lacey 1996). Activation of anterior raphe nuclei $GABA_A$ receptors decreased, while intra-dorsal raphe administration of $GABA_A$ receptor antagonists increased, 5-HT release in the raphe and NAc (Tao and Auerbach 2000; Tao et al. 1996). Activation of $GABA_A$ or $5-HT_{2C}$ receptors can inhibit 5-HT cell firing; the $5-HT_{2C}$ receptor-related inhibition was

attenuated by the $GABA_A$ receptor antagonist picrotoxin (Boothman et al. 2006; Judge et al. 2006). At least one study has shown evidence of direct modulation of $GABA_A$ receptors by $5-HT_{2C}$ receptors (Huidobro-Toro et al. 1996). Behaviourally, intra-raphe $GABA_A$ receptor agonists sustain self-administration, induce a conditioned place preference (Liu and Ikemoto 2007) and increase feeding (Bendotti et al. 1986; Klitenick and Wirtshafter 1989).

The present study tested the hypotheses that the $GABA_A$ and $5-HT_{2C}$ receptors play inhibitory roles in locomotor activity and reward-related behaviours. These hypotheses were examined by investigating the effects of the selective $5-HT_{2C}$ receptor agonist WAY 161503 (0-3.0 mg/kg) and the $GABA_A$ receptor agonist muscimol (0-4.0 mg/kg) and antagonist picrotoxin (0-1.0 mg/kg), alone and in combination, on locomotor activity and ICSS behaviour. The NAc shell has been identified as a potential site for the behavioural effects of $GABA_A$ receptor activation (Lopes et al. 2007; Reynolds and Berridge 2001; 2002; Stratford and Kelley 1997). Given evidence that $GABA_A$ and $5-HT_{2C}$ receptors may work through common pathways, the hypotheses that NAc shell $GABA_A$ receptor activation tonically inhibits VTA ICSS, and that $5-HT_{2C}$ receptor-related changes in VTA ICSS are mediated by a $GABA_A$ receptor-related mechanism, were tested using bilateral microinjections of muscimol (0-225 ng/side) and picrotoxin (125 ng/side) alone and in combination with systemically administered WAY 161503 (1.0 mg/kg). Food intake was also measured and used as a positive control as previous studies have demonstrated increased feeding following intra-NAc muscimol (Lopes et al. 2007; Stratford and Kelley 1997; Znamensky et al. 2001).

Materials & methods

Subjects

Fifty-six male Sprague-Dawley rats (Health Sciences Laboratory Animal Services, University of Alberta) weighing 200-250 g were housed individually in standard Plexiglas laboratory cages at 20°C and 50 % humidity, with a 12-hr light/dark cycle (lights from 0700 h -1900 h) with food and water freely available. All testing took place in the dark or under red light during the light phase of the light/dark cycle. All apparatus were cleaned between animals with diluted (1:6) ammonia-based window cleaner (No Name® Glass Cleaner with ammonia). The care and use of animals were in accordance with guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

Drugs

The $5\text{-}HT_{2C}$ receptor agonist WAY 161503 · HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1H-pyrazino[1,2-a]quinoxalin-5(6H)-one hydrochloride], the $GABA_A$ receptor agonist muscimol [5-aminomethyl-3-hydroxyisoxazole] and the antagonist picrotoxin [1:1 mixture of picrotoxinin and picrotin] were purchased from Tocris Cookson Inc. (Ellisville, MO, USA). (+)-α-Methylphenethylamine ((+)-amphetamine) sulphate was purchased from Health and Welfare Canada. All systemically administered compounds were dissolved in double-distilled water (ddH$_2$O) and injected subcutaneously, 10 min prior to testing, in a volume of 1.0 ml/kg. Artificial cerebrospinal fluid was freshly prepared (Elliott and Lewis 1950) and drug solutions made daily (pH 6.0–7.0). All drug doses are expressed as free-base.

Spontaneous Locomotor Activity

Apparatus: Spontaneous locomotor activity was measured using computer–monitored photobeam boxes (I. Halvorsen System Design, Phoenix, AZ, USA). The locomotor apparatus consisted of a clear Plexiglas test cage (43 cm L x 43 cm W x 30 cm H) with a 12 x 12 photobeam grid located 2.5 cm above the floor. These beams measured horizontal activity as well as consecutive beam breaks. Vertical activity was measured using 12 additional photobeams located 12 cm above the floor.

Procedure: Animals (n = 8/experiment) were habituated to the locomotor activity boxes for two consecutive days (60 min/day). They subsequently received randomized and counterbalanced injections with three drug-free days between injections. All locomotor activity was monitored over 30 min.

Intracranial self-stimulation (ICSS)

Surgery & histology: Using a previously described procedure (Greenshaw 1993), each animal (10 for each dose response experiment; 8 for each intracranial microinjection experiment) was implanted with a stainless steel, monopolar, stimulating electrode (E363/2; tip diameter 200 μm; Plastics One Ltd., Roanoke, VA) directed to the VTA. A large silver indifferent electrode in the skull served as the relative ground. Animals used for microinjection were also implanted with bilateral cannulae (22 gauge) directed to the rostral shell of the NAc. Stereotaxic coordinates were [mm]: VTA − AP +2.6, L +0.5, V +1.8; NAc shell − AP +11.0, L +0.4, V +2.8, from inter-aural zero, with the incisor bar set at 2.4 mm below the inter-aural line (Paxinos and Watson 1998). These coordinates were interpolated from the target site for an angle of 20° lateral and anterior for the VTA and 16° lateral for the NAc shell (Greenshaw 1997). The guide cannulae were placed 1

mm above the actual injection sites. Electrode and cannulae placements were verified at the end of the experiment by microscopic inspection of flash-frozen coronal brain sections (40 μm); flash-freezing was achieved using isopentane cooled on dry ice. Only animals with VTA and NAc placements were included in the analysis.

Apparatus & Procedure: Monopolar stimulation of the VTA was provided from constant current DC stimulators (cathodal monophasic pulse width of 200 μs; initial training frequency of 100 Hz; train length of 1 s) connected to each animal via a gold-track slip ring. Between pulses, the active electrode and indifferent electrode were connected through a resistor to cancel any effects of electrode polarisation (Greenshaw 1986). The apparatus and rate-frequency analysis were as described by Ivanová et al (1997). With this procedure, M50 is the threshold frequency at which half-maximal response rates occur; RMAX is the maximal rate of responding in a session. While M50 is a measure of reward sensitivity (which is dissociable from non-specific changes in behaviour), RMAX is a measure of response performance (see Gallistel and Karras, 1984; Greenshaw and Wishart, 1987).

Microinjection of drugs: Rats with bilateral cannulae in the NAc shell received randomly assigned, counterbalanced treatments separated by at least three days between each microinjection. Depending on the experiment, treatments included intra-NAc shell microinjections of artificial cerebrospinal fluid (CSF), muscimol (0-225 ng/side), picrotoxin (125 ng/side) and (+)-amphetamine (1.0 μg/side) administered in a total volume of 0.5μL at a pump-controlled rate of 0.2μL per minute (Beehive controller, Bioanalytical Systems, Inc.); the injection cannulae remained in place for a further

minute to allow for drug absorption. Immediately following each set of microinjections, each animal was tested for VTA ICSS.

Food intake

Adapted from previously described procedures (Reynolds and Berridge 2001; Stratford and Kelley 1997), animals were placed in standard Plexiglas laboratory cages (free from wood shavings) immediately following the VTA ICSS session. A pre-weighed amount of food, placed in a container identical to that found in their home cage, was made available along with water 25 min after the initiation of each VTA ICSS session (each session is a maximum of 25 min). At the end of a 30 min session (55 min post injection), food intake (corrected for spillage) was calculated by subtracting the initial weight of the food and container from the final weight. Animals were habituated for three days prior to the beginning of microinjection treatments. This procedure was subsequently performed on each microinjection treatment day to determine total food intake (measured in grams) in a 30 min session following intra-NAc muscimol (0-225 ng/side).

Statistical Analysis

Experimental effects in the ICSS and spontaneous locomotor activity were determined using repeated measures analysis of variance (ANOVA). A significant F ratio ($p \leq 0.05$) was followed by Newman-Keuls post hoc tests ($\alpha = 0.05$) where appropriate. As the results of the analyses of consecutive and vertical activity paralleled those for horizontal locomotor activity, only the latter results are reported. All ICSS data are presented as a percentage of average baseline performance of each animal. Greenhouse-Geisser corrected degrees of freedom are used as a conservative estimate of the F-ratio. Statistical

analyses for all experiments were completed using statistical software (SPSS Inc., Chicago, IL, USA).

Results

Effects of systemic GABA$_A$ receptor ligands on locomotor activity

Muscimol (0-0.75 mg/kg) significantly decreased locomotor activity at all doses tested, as revealed by Newman-Keuls post hoc tests (α = 0.05) following repeated measured ANOVA [Fig. 5.1A, $F(2.96, 20.75)$ = 5.39, p < 0.05]. No effect of drug over time was noted. Two-way repeated measures ANOVA (drug dose x time) also revealed a significant main effect of picrotoxin [Fig. 5.1B, $F(2.09, 14.64)$ = 11.82, p < 0.05] without an interaction with time. Post hoc tests revealed that picrotoxin decreased locomotor activity at the two highest doses tested (0.5, 1.0 mg/kg). There was a significant interaction between muscimol (0.10 mg/kg) and picrotoxin (0.25, 0.50 mg/kg) [Fig. 5.1C, $F(1.79, 12.54)$ = 4.87, p < 0.05] without an interaction with time. Post hoc tests revealed that muscimol (0.10 mg/kg) and picrotoxin (0.50 mg/kg) significantly reduced locomotor activity while combinations of muscimol and picrotoxin were not significant from baseline.

Figure 5.1A-C. Effects of **(A)** muscimol (Musc; 0-0.75 mg/kg), **(B)** picrotoxin (P; 0-1.0 mg/kg), and **(C)** their combination on spontaneous locomotor activity measured over a 30 min time course. Data shown are means ± SEM. *Significant from control at $p < 0.05$ following Newman-Keuls post hoc tests.

C

Mean Number of Total Photo Beam Breaks

Legend:
- Baseline
- Musc (0.10 mg/kg)
- P (0.25 mg/kg)
- P (0.50 mg/kg)
- Musc + P (0.25 mg/kg)
- Musc + P (0.50 mg/kg)
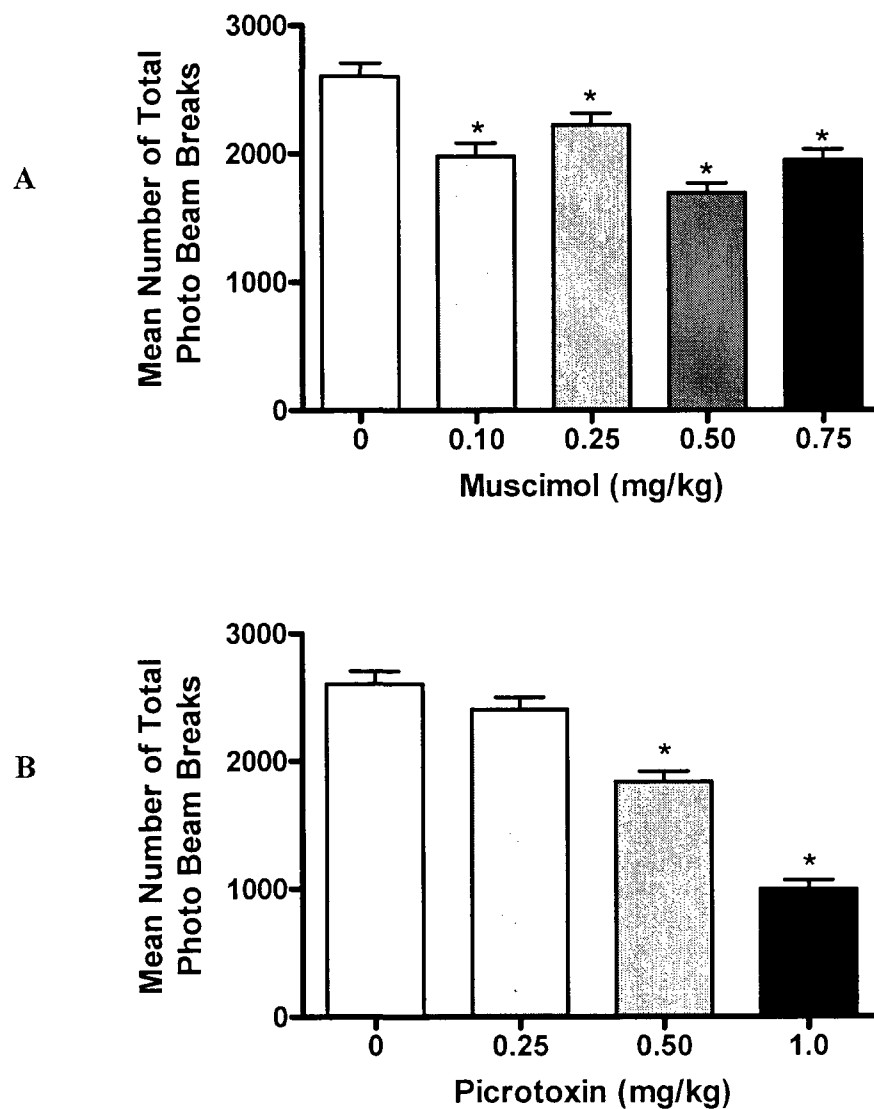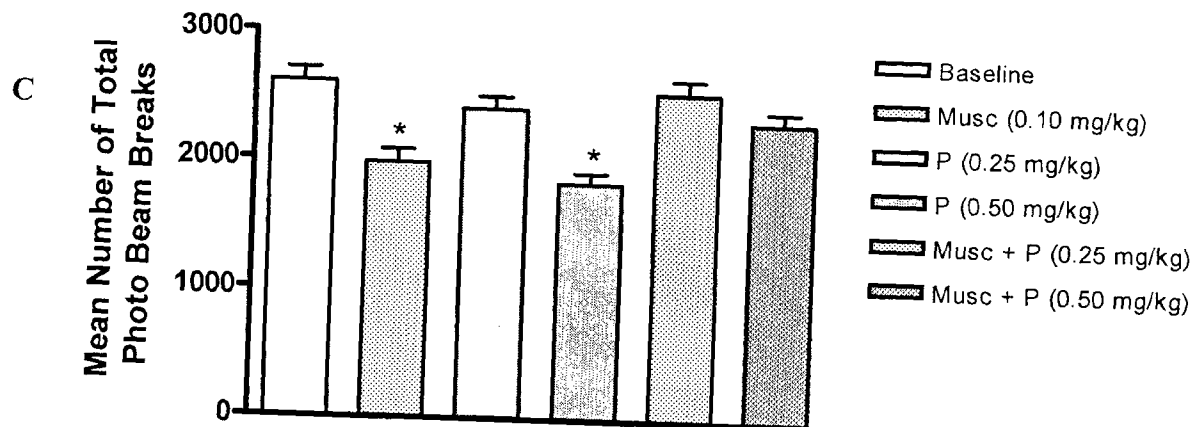
Effects of WAY 161503 and picrotoxin on locomotor activity

The 5-HT$_{2C}$ receptor agonist WAY 161503 significantly decreased locomotor activity at all doses tested, as revealed by Newman-Keuls post hoc tests following repeated measures ANOVA [Fig. 5.2, $F(2.01, 14.07) = 26.12$, $p < 0.05$]. This effect was also significant over time [$F(4.46, 31.21) = 24.19$, $p < 0.05$]. There was no main effect of picrotoxin or picrotoxin x time and no interaction with (WAY x picrotoxin x time) or without (WAY x picrotoxin) time.

Figure 5.2 Effects of WAY 161503 (W; 0-3.0 mg/kg) and picrotoxin (P; 0.25 mg/kg) on spontaneous locomotor activity measured over a 30 min time course. Data shown are means ± SEM. There was no interaction between WAY 161503 and picrotoxin. *Significant from control at $p < 0.05$ following simple effects of WAY 161503 and Newman-Keuls post hoc tests.
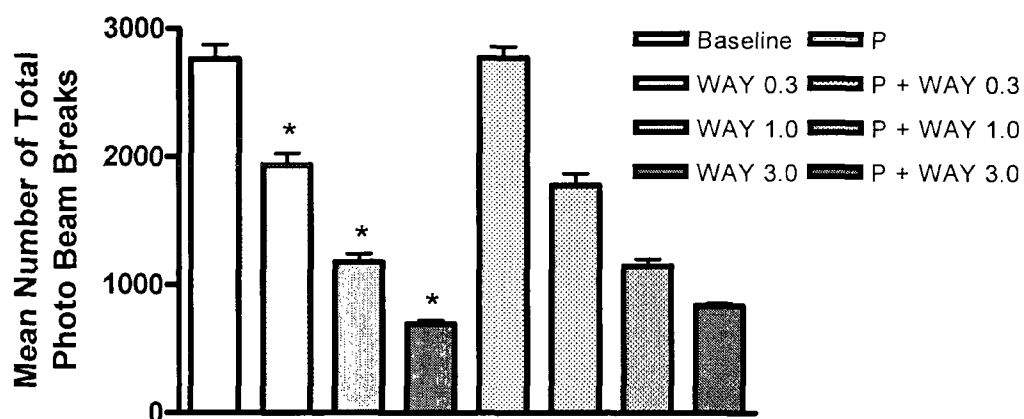
Effects of systemic GABA$_A$ receptor ligands on ICSS

Analysis following systemic administration of muscimol (0-4.0 mg/kg) revealed significant main effects for M50 thresholds [Fig. 5.3A, F (3.24, 29.18) = 7.13, p < 0.05], RMAX values [Fig. 5.3B, F (2.77, 24.95) = 53.39, p < 0.05], and TRES values [Fig. 5.3C, F (3.18, 28.65) = 24.16, p < 0.05]. Further analysis using Newman-Keuls post hoc tests revealed that only the highest dose (4.0 mg/kg) produced a significant increase in M50 (indicating a decrease in reward), RMAX (indicating a decrease in motor performance) compared to control, and TRES values. A main effect of picrotoxin (0-1.0 mg/kg) was also seen with M50 thresholds [Fig. 5.4A, F(2.04, 18.36) = 5.75, p < 0.05], RMAX values [Fig. 5.4B, F(1.54, 13.88) = 6.47, p < 0.05], and TRES values [Fig. 5.4C, F(2.10, 18.88) = 10.59, p < 0.05], although post hoc tests revealed that the highest dose tested produced a significant increase in only M50 thresholds and decrease in TRES. Following administration of the 4.0 mg/kg dose of muscimol in combination with the subthreshold doses of picrotoxin, no interaction was noted for any measure (Fig. 5.5A-C), although there was a main effect of muscimol for M50 [F(1, 7) = 8.54, p < 0.05] and main effects for both muscimol and picrotoxin for RMAX [F(1, 7) = 84.30, p < 0.05; F(1.99, 13.90) = 5.47, p < 0.05] and TRES values [F(1, 7) = 143.08, p < 0.05; F(1.66, 11.65) = 7.45, p < 0.05]. Group-averaged rate-frequency regression curves are included to illustrate the rightward shift in M50 seen with muscimol and picrotoxin (indicating a decrease in reward) (Fig. 5.3D, 5.4D, 5.5D).

Figure 5.3A-D. The effects of muscimol (0-4.0 mg/kg) on **(A)** rate-frequency thresholds (M50), **(B)** maximal response rates (RMAX), and **(C)** total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the rightward shift (indicating a decrease in reward) in M50 seen with muscimol. Data shown are means ± SEM expressed as a percentage of baseline performance. *Significant from control at $p < 0.05$ following Newman-Keuls post hoc tests.

A

B
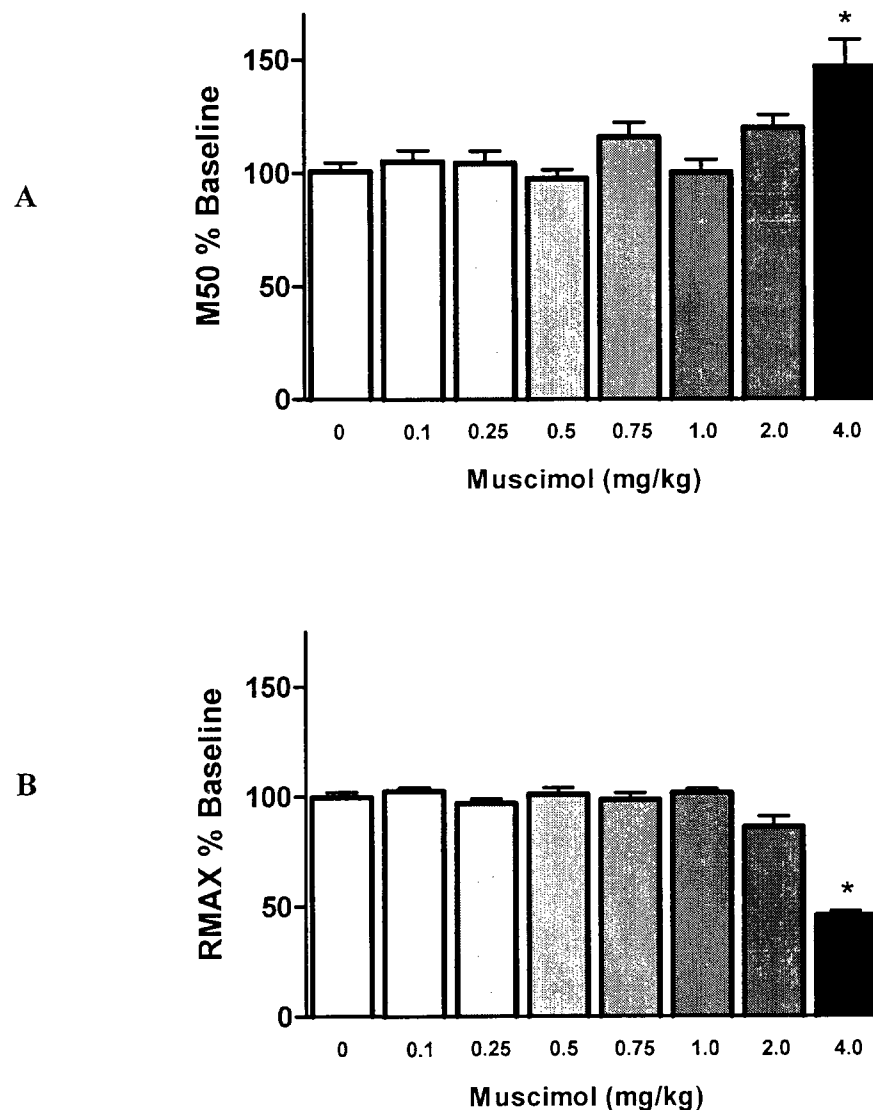
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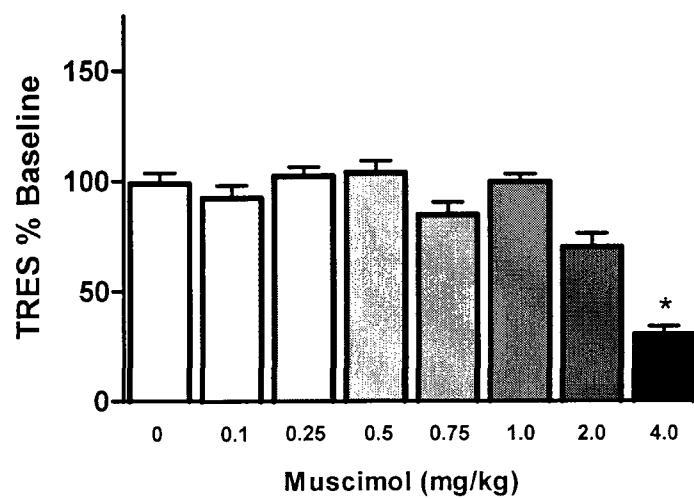
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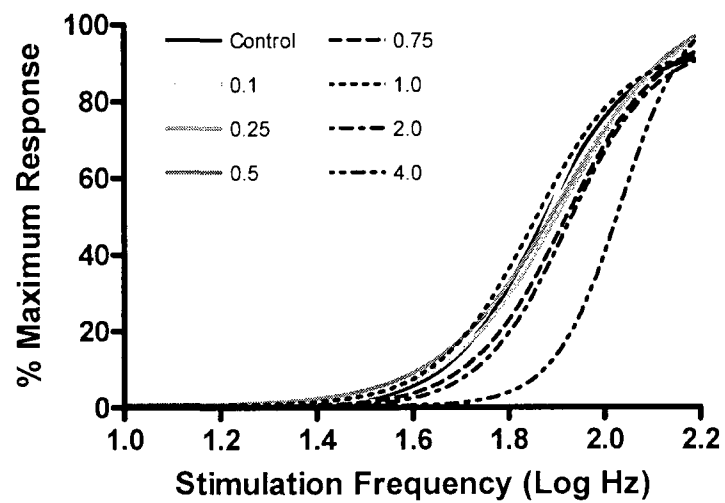
Figure 5.4A-D. The effects of picrotoxin (0-1.0 mg/kg) on **(A)** rate-frequency thresholds (M50), **(B)** maximal response rates (RMAX), and **(C)** total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the rightward shift (indicating a decrease in reward) in M50 seen with muscimol. Data shown are means ± SEM expressed as a percentage of baseline performance. *Significant from control at p < 0.05 following Newman-Keuls post hoc tests.

A

B

C

D

Figure 5.5A-D. The effects of muscimol (M; 4.0 mg/kg) and picrotoxin (P; 0.25, 0.5 mg/kg) on **(A)** rate-frequency thresholds (M50), **(B)** maximal response rates (RMAX), and **(C)** total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the rightward shift (indicating a decrease in reward) in M50 seen with muscimol. Data shown are means ± SEM expressed as a percentage of baseline performance.

A

B

C

D
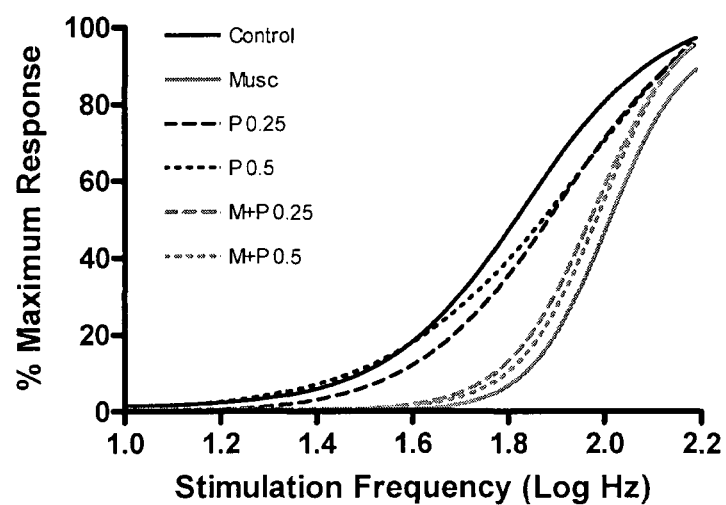
Effects of intra-NAc shell muscimol on ICSS

Intra-NAc shell muscimol (25, 75, 225 ng/side) produced a significant increase in M50 thresholds [Fig. 5.6A, $F(1.89, 13.21) = 8.63$, $p < 0.05$], and decrease in TRES values [Fig. 5.6C, $F(2.28, 15.95) = 5.27$, $p < 0.05$], without effects on RMAX values (Fig. 5.6B). Newman-Keuls post hoc tests revealed that the highest dose of muscimol (225 ng/side) produced an increase in M50 thresholds (Fig. 5.6A). The positive control (+)-amphetamine (1.0 μg/side) significantly decreased M50 values [Fig. 5.6A, $F(1, 7) = 9.43$, $p<0.05$], and increased TRES values [Fig. 5.6C, $F(1, 7) = 11.34$, $p<0.05$], without affecting RMAX values (Fig. 5.6B). Group-averaged rate-frequency regression curves are included to compare the leftward shift in M50 seen with (+)-amphetamine (indicating an increase in reward) to the rightward shift seen with muscimol (Fig. 5.6D).

Figure 5.6A-D. Effects of intra-NAc shell (+)-amphetamine (Amph; 1.0 µg/side) and muscimol (0-225 ng/side) on **(A)** rate-frequency thresholds (M50), **(B)** maximal response rates (RMAX), and **(C)** total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the leftward shift (indicating an increase in reward) in M50 seen with (+)-amphetamine compared to the rightward shift (indicating a decrease in reward) in M50 seen with muscimol. Data shown are means ± SEM expressed as a percentage of baseline performance. *Significant from control at p < 0.05 following Newman-Keuls post hoc tests.
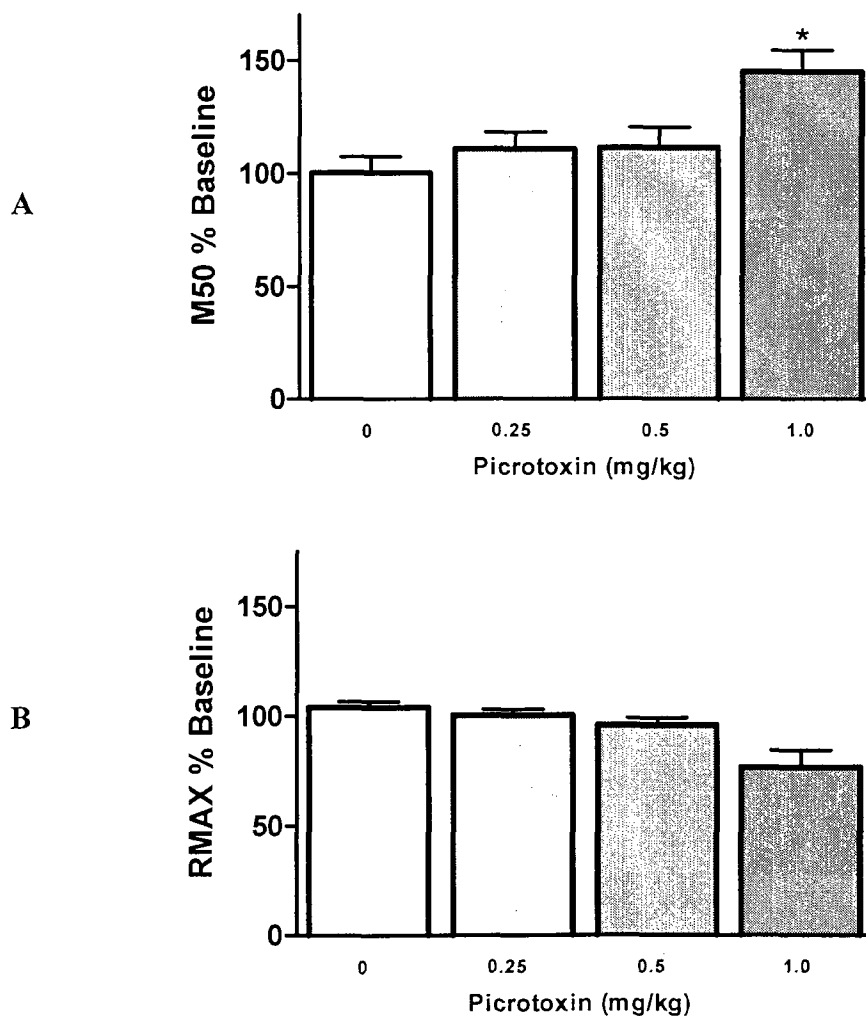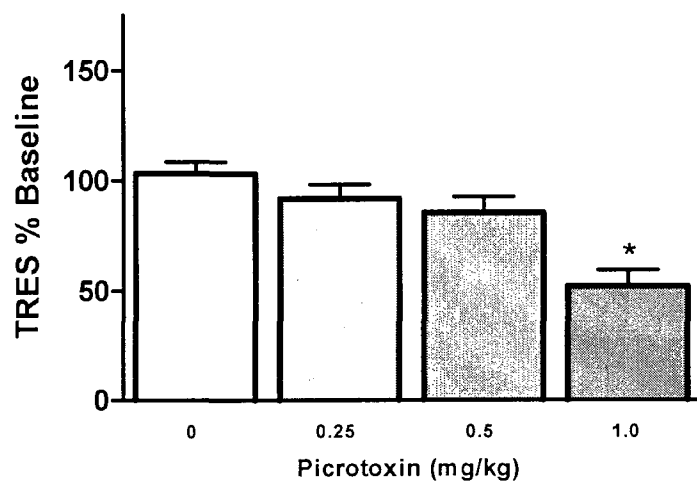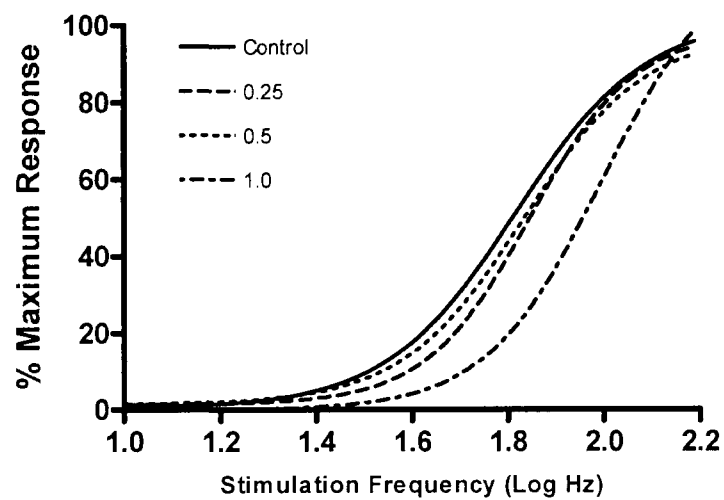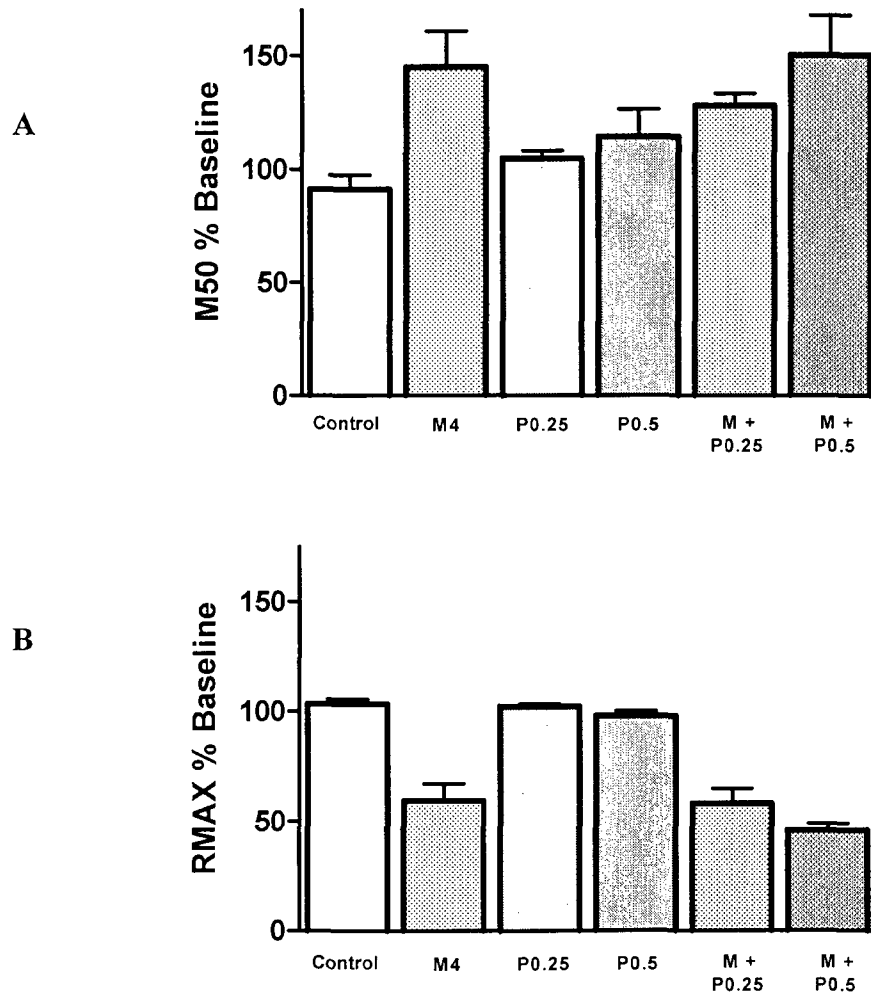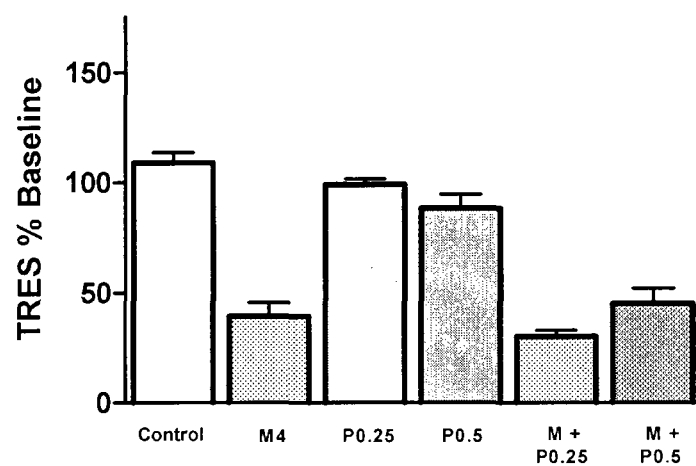
C

D
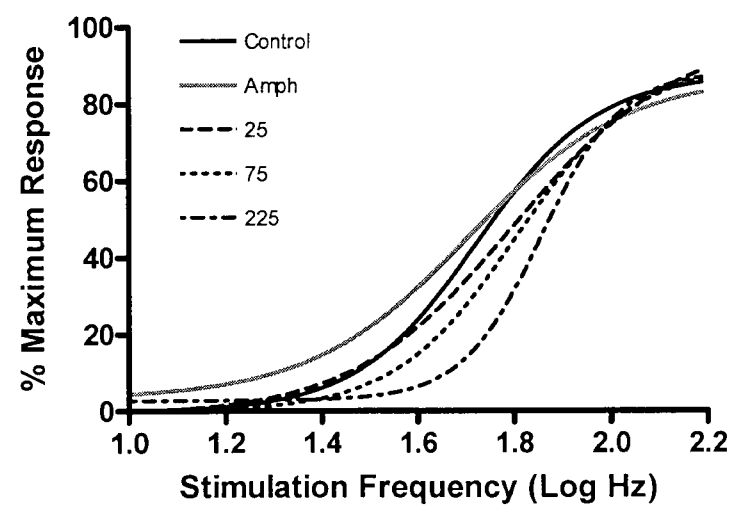
Effects of intra-NAc shell muscimol and systemic WAY 161503 on ICSS

Intra-NAc shell muscimol (225 ng/side), once again, produced a significant increase in M50 thresholds [Fig. 5.7A, $F(1, 7) = 5.92$, $p < 0.05$], and a decrease in TRES values [Fig. 5.7C, $F(1, 7) = 6.68$, $p < 0.05$], without having an effect on RMAX values (Fig. 5.7B); WAY 161503 (1.0 mg/kg) also increased M50 thresholds [Fig. 5.7A, $F(1, 7) = 6.84$, $p < 0.05$] and had no effect on RMAX or TRES values (Fig. 5.7B & C). The combination of WAY 161503 and muscimol did not result in a significant interaction for any measure. Group-averaged rate-frequency regression curves are included to compare the rightward shift in M50 seen with muscimol and WAY 161503 (Fig. 5.7D).

Figure 5.7A-D. Effects of intra-NAc shell muscimol (225 ng/side) and systemic WAY 161503 (1.0 mg/kg) on **(A)** rate-frequency thresholds (M50 values), **(B)** maximal response rates (RMAX values), and **(C)** total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the rightward shifts (indicating a decrease in reward) in M50. Data shown are means ± SEM expressed as a percentage of baseline performance. *Main effect at $p < 0.05$.
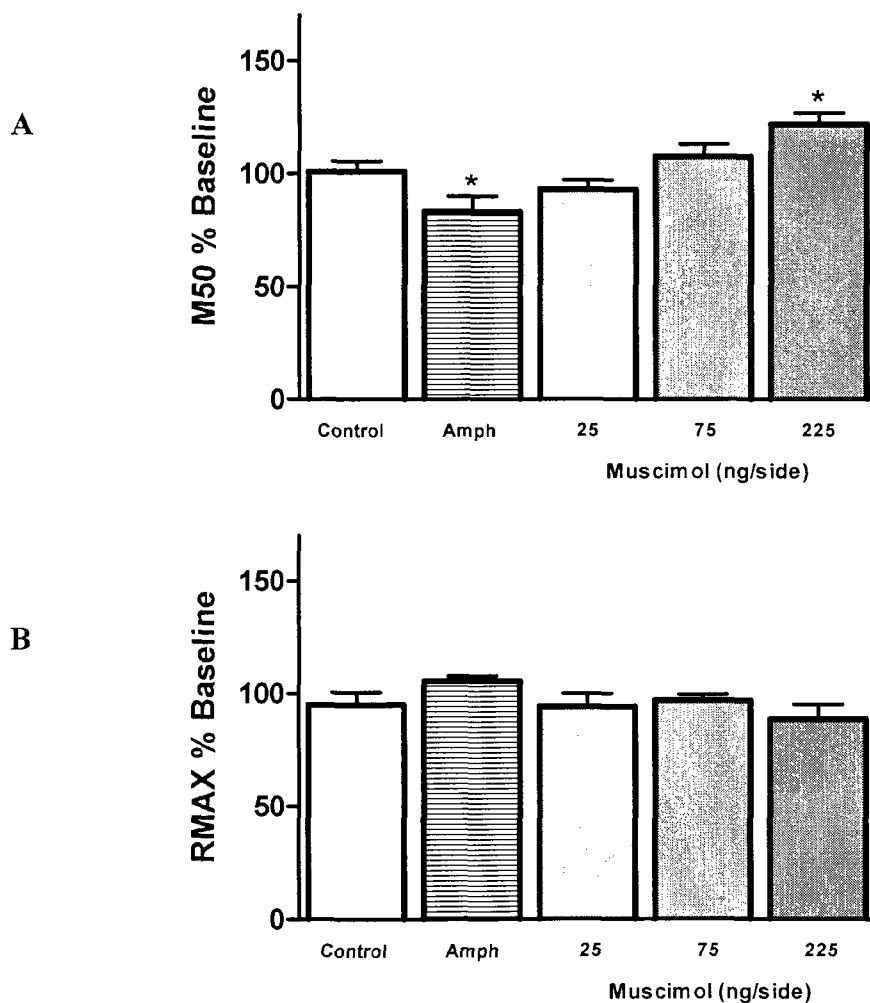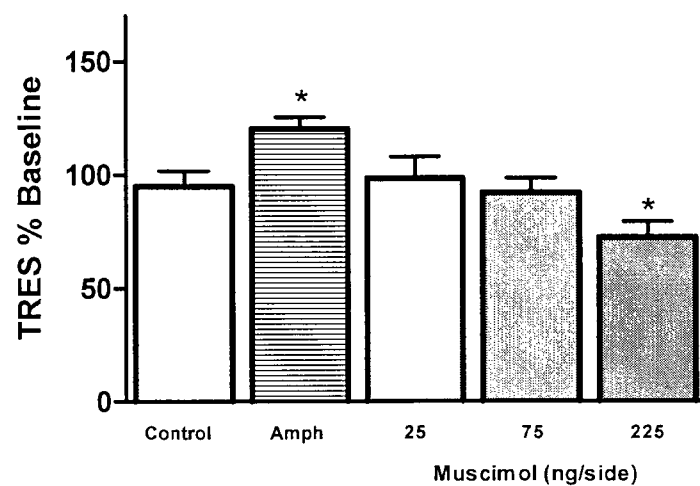
C

D

243
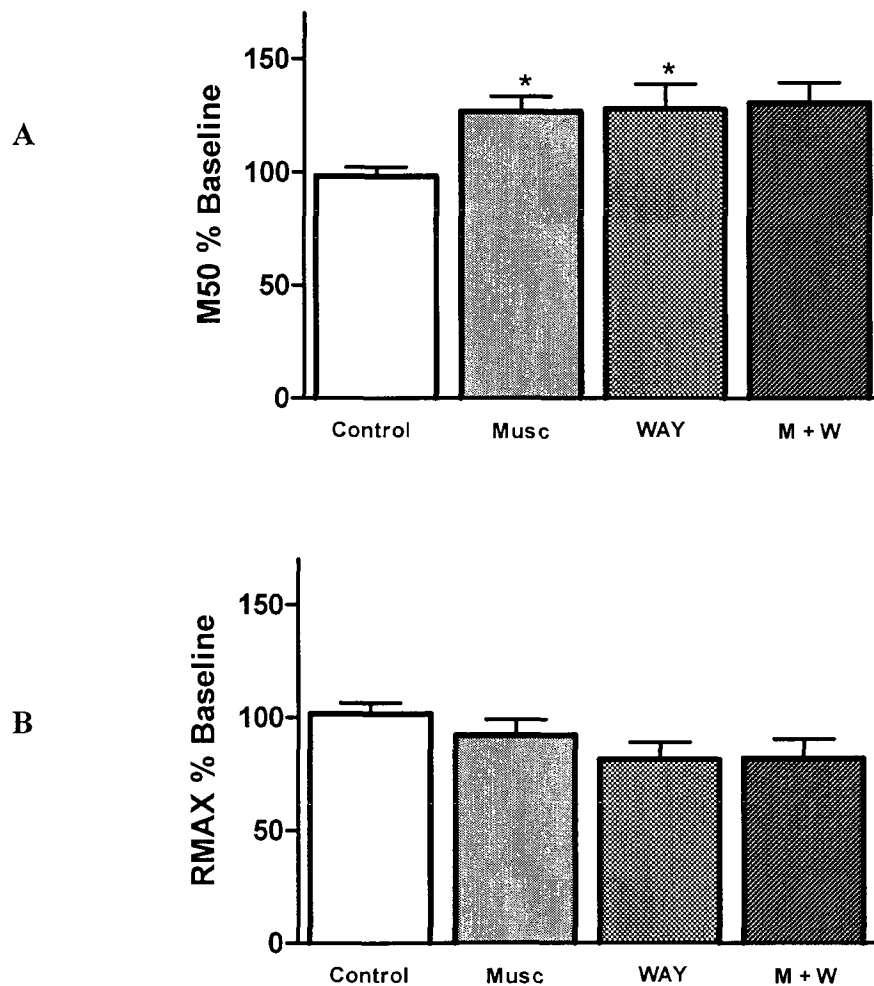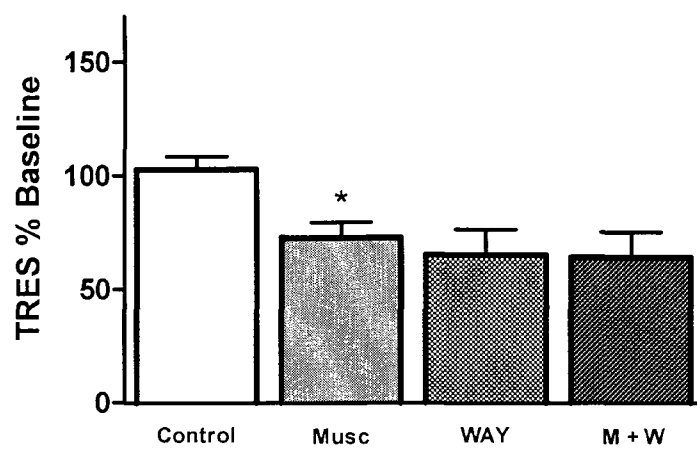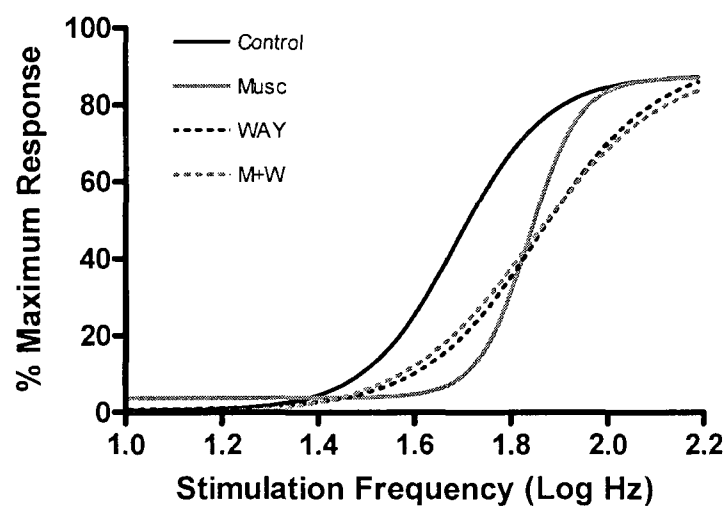
Effects of intra-NAc shell picrotoxin and systemic WAY 161503 on ICSS

Intra-NAc shell picrotoxin (125 ng/side) produced a significant decrease in M50 thresholds [Fig. 5.8A, $F(1, 7) = 13.58$, $p < 0.05$] without having an effect on RMAX or TRES values (Fig. 5.8B & C); WAY 161503 (1.0 mg/kg) significantly increased M50 thresholds [Fig. 5.8A, $F(1, 7) = 24.05$, $p < 0.05$] and decreased RMAX and TRES values [Fig. 5.8B, $F(1,7) = 11.47$, $p < 0.05$; Fig. 5.8C, $F(1,7) = 19.28$, $p < 0.05$]. The combination of WAY 161503 and picrotoxin did not result in a significant interaction for any measure. Group-averaged rate-frequency regression curves are included to compare the leftward shift in M50 seen with picrotoxin to the rightward shift seen with WAY 161503 (Fig. 5.8D).

Figure 5.8A-C. Effects of intra-NAc shell picrotoxin (125 ng/side) and systemic WAY 161503 (1.0 mg/kg) on **(A)** rate-frequency thresholds (M50), **(B)** maximal response rates (RMAX), and **(C)** total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the leftward shift (indicating an increase in reward) in M50 seen with picrotoxin, the rightward shift (indicating a decrease in reward) in M50 seen with WAY 161503, and the return to control values following the combination. Data shown are means ± SEM expressed as a percentage of baseline performance. *Main effect at $p < 0.05$.

245

C

D
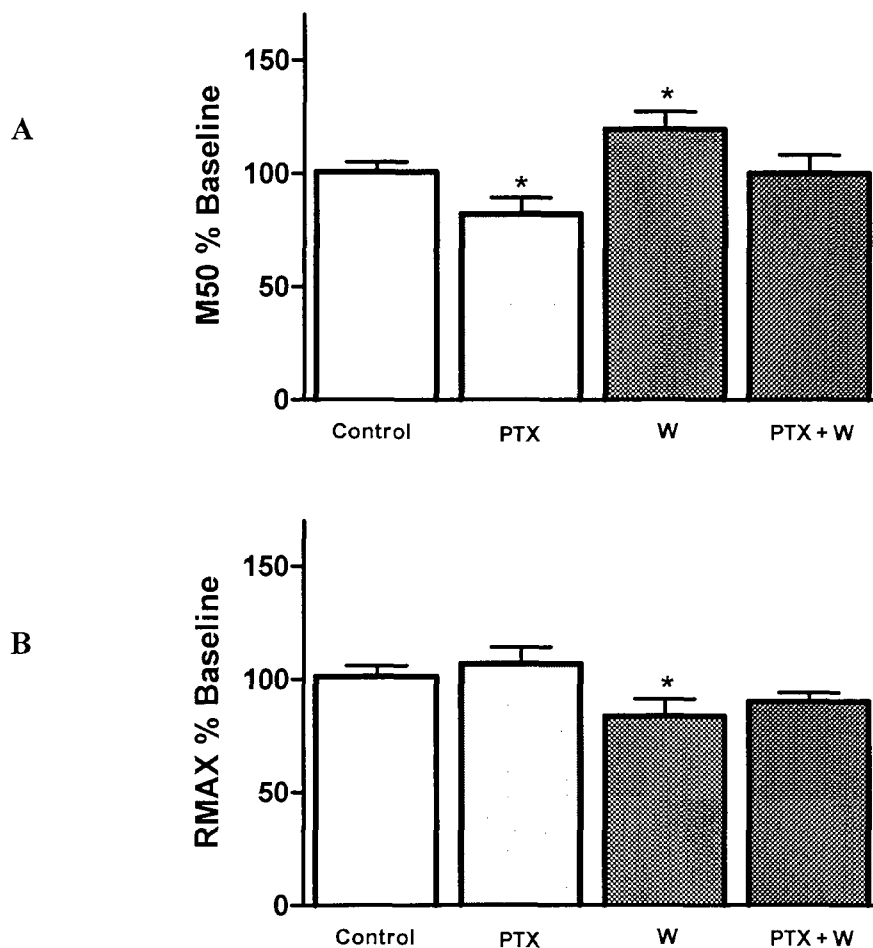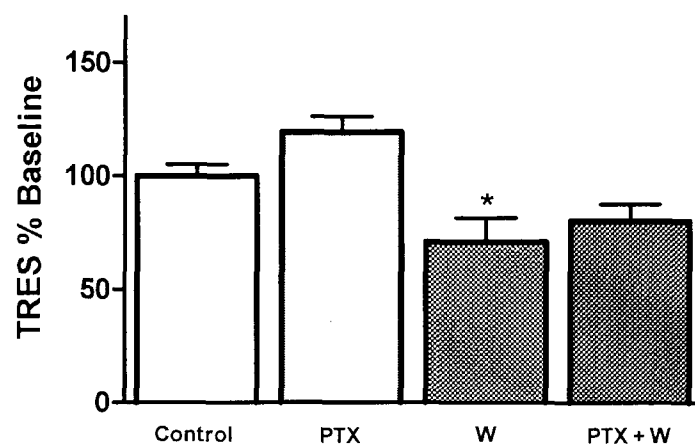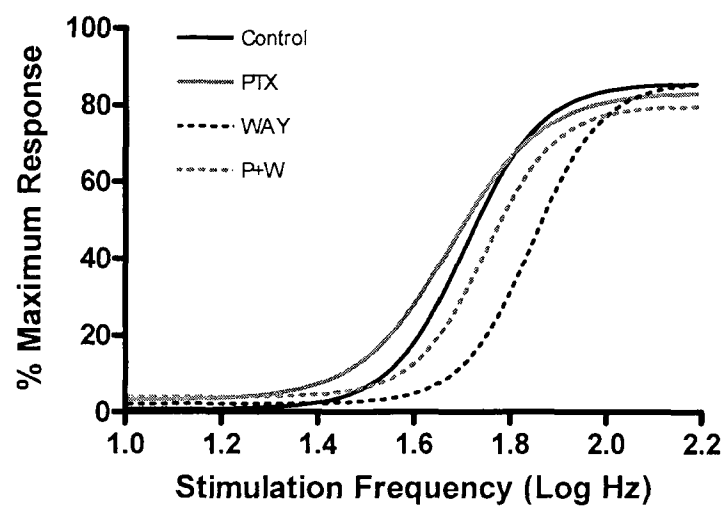
Effects of intra-NAc shell muscimol on food intake

Intra-NAc shell muscimol (25, 75, 225 ng/side) did not significantly affect feeding [Fig. 5.9., $F(1.47, 10.31) = 1.95$, $p > 0.05$).

Representative photomicrographs of VTA stimulation sites and NAc shell microinjection sites are seen in Fig. 5.10A & C, respectively. Histological locations of electrode terminal sites and NAc shell microinjection sites are represented in Fig. 5.10B & D, respectively.

Figure 5.9 Effects of intra-NAc shell muscimol (0-225 ng/side) on food intake (measured in grams) over 30 min. Data shown are means ± SEM.
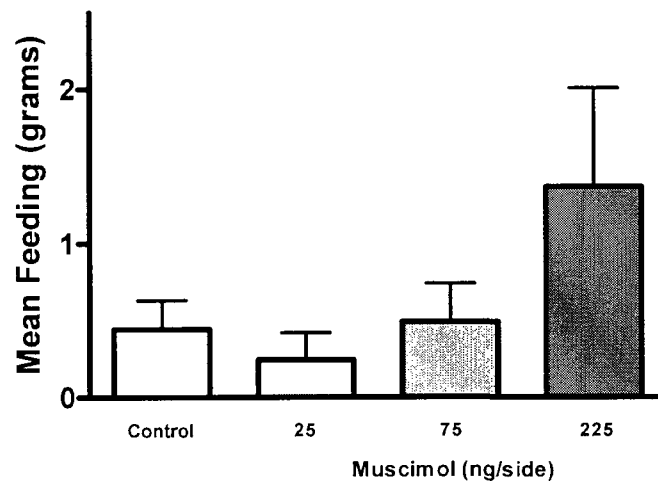
Figure 5.10A-D Histological verification of VTA and NAc shell sites. **(A)** Representative photomicrograph (circle identifies VTA electrode terminal) and **(B)** histological locations of VTA stimulation sites. **(C)** Representative photomicrograph and **(D)** histological locations of NAc shell microinjection sites. Brain diagrams from Paxinos & Watson (1998).

A

B

Interaural 3.70

Interaural 3.40

Interaural 3.20

Interaural 2.96

Interaural 2.70

C

D
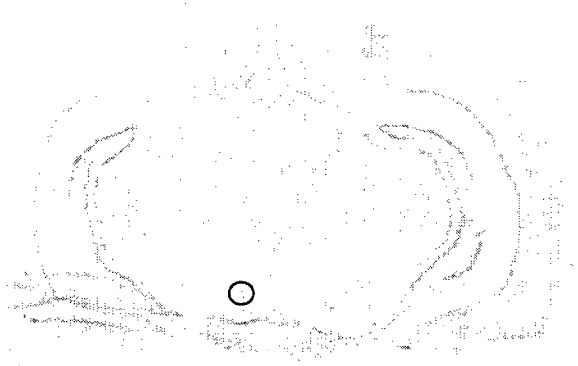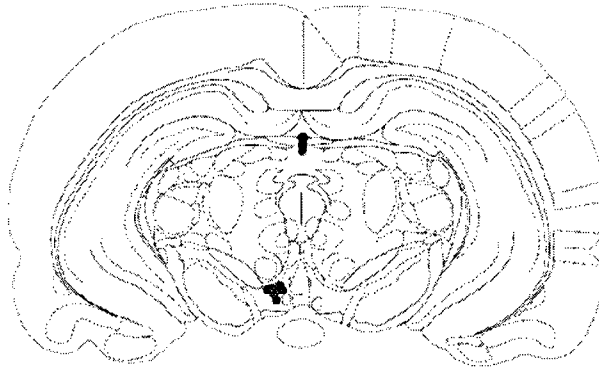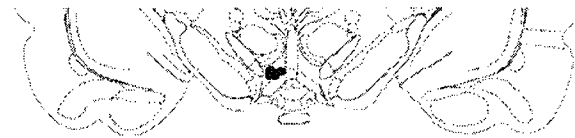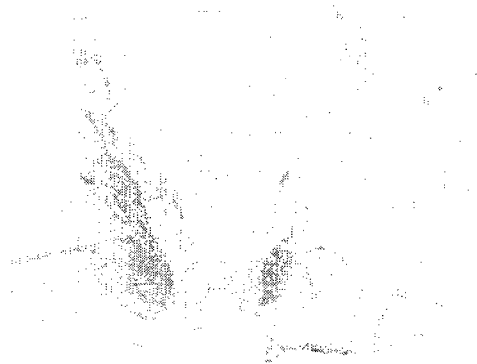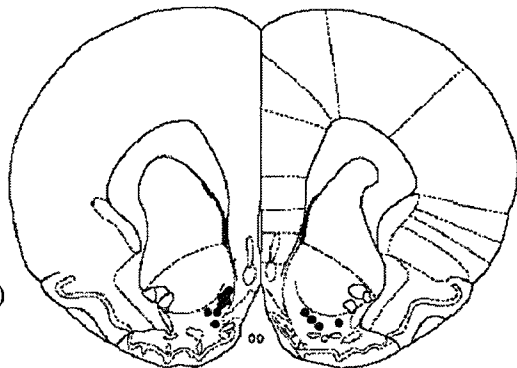
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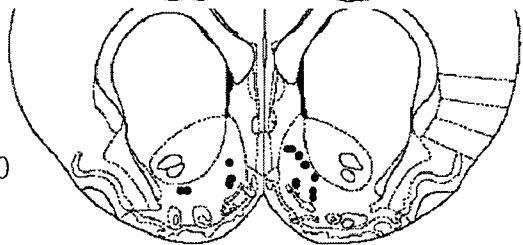
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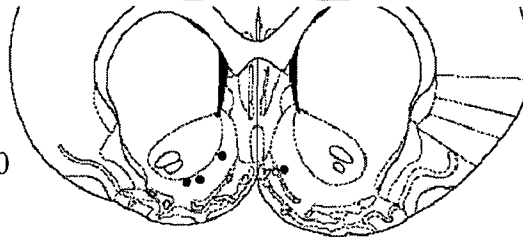
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Interaural 10.20
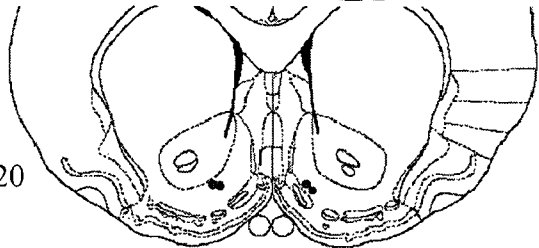
Discussion

This study investigated the role of the $GABA_A$ and $5\text{-}HT_{2C}$ receptors in spontaneous locomotor activity and ICSS. Consistent with prior data (Mukhopadhyay and Poddar 1995; Sienkiewicz-Jarosz et al. 2003), systemically administered $GABA_A$ receptor ligands produced a decrease in locomotor activity (Fig. 5.1A & B). These effects appear to be $GABA_A$ receptor-specific as the locomotor decreasing effects of the $GABA_A$ receptor agonist muscimol (0.10 mg/kg) were blocked by sub- (0.25 mg/kg) and supra- (0.50 mg/kg) threshold doses of the $GABA_A$ receptor antagonist picrotoxin (Fig. 5.1C). The similar effects of muscimol and picrotoxin may be related to the balance of activated $GABA_A$ receptors, as varying subunit compositions are found throughout the brain (Mohler 2007; Pirker et al. 2000) and are known to have differential effects on locomotion (Austin and Kalivas 1989; Hauser et al. 2005; Morgenstern et al. 1984; Oakley et al. 1991; Plaznik et al. 1990; Reynolds et al. 2003; Schwienbacher et al. 2002; Yee et al. 2005). It is also possible that the decrease in locomotor activity at higher doses of picrotoxin may be related to its anxiogenic effects following systemic administration (Epstein et al. 2003; Sienkiewicz-Jarosz et al. 2003).

The effects of the selective $5\text{-}HT_{2C}$ receptor agonist WAY 161503 (0-3.0 mg/kg) and $GABA_A$ receptor antagonist picrotoxin (0.25 mg/kg) were investigated as some studies have proposed a relationship between $GABA_A$ and $5\text{-}HT_{2C}$ receptors. $5\text{-}HT_{2C}$ receptor activation produced an inhibition of $GABA_A$ receptors in Xenopus oocytes (Huidobro-Toro et al. 1996) and picrotoxin was found to attenuate the reduction of 5-HT cell firing by WAY 161503 (Boothman et al. 2006). WAY 161503 (Fig. 5.2) decreased locomotor activity, consistent with reports using this compound (Hayes et al. 2008b;

Mosher et al. 2005) and other 5-HT$_{2C}$ receptor ligands (Higgins et al. 2001; Kennett et al. 1997; Lucki et al. 1989; Martin et al. 2002). A dose of picrotoxin (0.25 mg/kg) that blocked the locomotor effects of muscimol did not alter the effects of WAY 161503 (Fig. 5.2). Though these results suggest that GABA$_A$ and 5-HT$_{2C}$ receptors do not inhibit locomotor activity through a similar mechanism it is important to note that the 0.25 mg/kg dose of picrotoxin used in the present study was not employed in the study by Boothman et al. (2006). They found that 0.5 and 1.0 mg/kg of picrotoxin attenuated the inhibition of 5-HT cell firing induced by WAY 161503 (0.125-1.0 mg/kg). Nonetheless, it remains that a dose of picrotoxin that blocked the behavioural effects of muscimol did not affect the 5-HT$_{2C}$ receptor-related decrease in locomotor activity under the present experimental conditions.

This study investigated the effects of systemically administered muscimol and picrotoxin using ICSS with reward-sensitive, rate-frequency threshold, measures. Although a number of studies have demonstrated the effects of GABA$_A$ receptor ligands on ICSS, they have been hampered by reward insensitive measures and have often lacked a measurement of motor performance (Kent and Fedinets 1976; Nazzaro and Gardner 1980; Porrino and Coons 1980; Singh et al. 1997; Willick and Kokkinidis 1995; Zarevics and Setler 1981). The present study demonstrated increases in rate-frequency thresholds (M50 values) following systemically administered muscimol (Fig. 5.3A & 5.3D; 0-4.0 mg/kg) and picrotoxin (Fig. 5.4A and 5.4D; 0-1.0 mg/kg) which may be related to factors other than reward. The dose of muscimol (4.0 mg/kg) that increased M50 thresholds also produced a substantial decrease in RMAX values (Fig. 5.3B; indicating impaired motor performance). These effects are consistent with the sedative and myorelaxant properties

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of GABA$_A$ receptor activation (Crestani et al. 2001; Mohler et al. 2001); however, as its effects were not attenuated by picrotoxin (Fig. 5.5A-D), it is possible that they are not GABA$_A$ receptor-specific. The dose of picrotoxin (1.0 mg/kg) that increased M50 thresholds did not result in a significant decrease in RMAX values (Fig. 5.4B). However, this dose may produce increased anxiety and freezing behaviour (Dalvi and Rodgers 1996; Sienkiewicz-Jarosz et al. 2003) which is not observed at lower doses (Dalvi and Rodgers 2001; Dombrowski et al. 2006) – making any determination of effects on reward difficult. These effects are not unique to picrotoxin, as other anxiogenic compounds have demonstrated conditioned place aversions or increases in ICSS M50 values (Acquas et al. 1990; File 1986; Gallistel and Freyd 1987).

While there are difficulties in interpreting ICSS results following systemic administration of GABAergic compounds, GABA$_A$ receptor compounds have been shown to effectively alter reward-related measures. Intra-VTA muscimol produced an increase in ventral pallidal-stimulated M50 thresholds without effects on RMAX (Panagis and Kastellakis 2002). Decreased NAc cell firing, following medial forebrain bundle ICSS, is inhibited by the GABA$_A$ receptor antagonist bicuculline (Cheer et al. 2005). Effects of GABA$_A$ receptors on ICSS may be site specific as GABA$_A$ receptor activation in the horizontal limb of the diagonal band of Broca, magnocellular preoptic area and basolateral amygdala do not result in reward-specific changes in behaviour (Simmons et al. 2007; Waraczynski 2007; 2008). Beyond ICSS, rats will self-administer GABA$_A$ receptor agonists into the anterior raphe nuclei, supramammillary nucleus and rostral VTA (Ikemoto 2005; Liu and Ikemoto 2007), although intra-rostral VTA administration attenuates cocaine self-administration (Lee et al. 2007). Rostral-caudal differences have

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also been noted in the NAc shell, as GABA$_A$ receptor agonists injected into the rostral portion increased feeding behaviour, induced conditioned place preference and increased a hedonic response to sucrose (Lopes et al. 2007; Reynolds and Berridge 2001; 2002; Stratford and Kelley 1997). These studies underscore the need to investigate the GABAergic system using a central approach and to interpret systemic data with caution.

Intra-NAc muscimol (225 ng/side) increased M50 thresholds (Fig. 5.6A & 5.6D), in contrast to the well established reward-enhancing effects of (+)-amphetamine (1.0 µg/side) (Colle and Wise 1988; Schaefer and Michael 1988). These effects likely represent a specific decrease in reward as RMAX values were not affected (Fig. 5.6B) and intra-NAc muscimol does not affect muscle tone (Stefanski et al. 1990). Although intra-NAc shell muscimol did not affect feeding behaviour (Fig. 5.9), this may be the result of procedural differences between the present study and others (Reynolds and Berridge 2001; Stratford and Kelley 1997) – as this procedure did not allow the feeding test to begin until nearly 30 min post-injection. Nonetheless, animals may show a tendency to increased feeding at the highest dose of muscimol; this dose was effective in altering ICSS behaviour. In addition, although intra-NAc muscimol may be anxiolytic (Lopes et al. 2007), anxiolytic drugs are more likely to positively affect reward-related behaviour (Gomita et al. 2003; Koob 2008; Lupica et al. 2004). These data suggest that GABA$_A$ receptors in the rostral NAc shell may play an inhibitory role in VTA ICSS behaviour. This is in contrast to their proposed role in feeding, place conditioning and the hedonic response to sucrose, mentioned above, but is not inconsistent with a differential role for receptors across reward-related behaviours (Backes and Hemby 2008; Hayes et al. 2008b; Martin-Fardon et al. 2007).

Interestingly, microdialysis studies investigating the effects of intra-NAc muscimol have shown either an increase or no change in basal DA release (Aono et al. 2008; Yan 1999; Yoshida et al. 1997). However, a strict interpretation of these data and comparison to the ICSS data are difficult given that these studies did not differentiate between rostral-caudal or shell-core portions of the NAc. It is unlikely that the present results are related to misdirected cannulae as the verified rostral NAc shell cannulae placements (Fig. 5.10C & D) are similar to those in other studies (Lopes et al. 2007; Reynolds and Berridge 2001; 2002; Stratford and Kelley 1997). In addition, the rapid onset of behavioural effects, slow injection rates and small injection volumes helped to minimize the spread of drug and suggest that the current results are due to activity at the site of injection.

The hypothesis that $5\text{-}HT_{2C}$ and $GABA_A$ receptors may act through a similar mechanism to regulate VTA ICSS behaviour was supported in the present study. The increase in M50 thresholds seen with the selective $5\text{-}HT_{2C}$ receptor agonist WAY 161503 (1.0 mg/kg), as demonstrated previously (Hayes et al. 2008a), is comparable to that seen with intra-NAc muscimol (225 ng/side) (Fig. 5.7A & D), without significant effects on RMAX values (Fig. 5.7B) – further supporting the notion that these are reward-specific changes. Together, intra-NAc muscimol and systemic WAY 161503 produced an increase in M50 thresholds indistinguishable from either treatment alone. In addition, the effects of WAY 161503 were attenuated by $GABA_A$ receptor antagonism via intra-NAc picrotoxin (125 ng/side) (Fig. 5.8A & D), results consistent with in vivo data by Boothman et al. (2006). Picrotoxin decreased M50 thresholds when administered alone, and these effects are likely due to a specific increase in reward as picrotoxin did not

affect RMAX values (Fig. 5.8B) and the dose used was subconvulsive and does not affect locomotor activity (Bast et al. 2001; Plaznik et al. 1990; Swerdlow et al. 1990).

It is possible that the similar effects of WAY 161503, muscimol, and their combination, on M50 thresholds are due to a ceiling effect. This is unlikely as these treatments produced an approximate 30% shift in M50 values; larger shifts are possible and have been noted by others (Hayes et al. 2006; Morissette and Boye 2008; Sonnenschein and Franklin 2008; Vlachou et al. 2005). Instead, a similar shift in M50 values by each treatment and their combination could be explained if both treatments worked through a similar mechanism. In addition, the attenuation of WAY 161503's effects by picrotoxin further supports this notion. Although the current study aimed to use as few microinjections as possible (in order to maintain the integrity of the sites), future studies should include subthreshold doses of the $GABA_A$ and $5\text{-}HT_{2C}$ receptor compounds to provide a clearer interpretation of the relationship between these receptors. It should also be noted that the authors believe that the slight decrease in RMAX by WAY 161503 (Fig. 5.8B; the experiment combining picrotoxin and WAY 161503) is an artifact given that numerous replications have shown this same dose of WAY 161503 to be ineffective (Hayes et al. 2008a).

The present data are consistent with the hypothesis that $5\text{-}HT_{2C}$ and $GABA_A$ receptors act through a similar mechanism to regulate VTA ICSS behaviour. That intra-NAc muscimol increases, and picrotoxin decreases, M50 values is consistent with the notion that GABAergic transmission in the NAc may inhibit the reward signal (Rahman and McBride 2002; Yan 1999). This is also consistent with data investigating the effects of intra-NAc GABA ligands on locomotor activity (Austin and Kalivas 1989;

Morgenstern et al. 1984; Plaznik et al. 1990; Pycock and Horton 1979). It is not clear why NAc GABA$_A$ receptor activation results in an increase in NAc DA release in some studies (Aono et al. 2008; Yoshida et al. 1997), although this may be explained by a differential activation of pre- vs. post-synaptic (Belenky et al. 2003) or synaptic vs. extrasynaptic (Glykys and Mody 2007) GABA$_A$ receptors.

5-HT$_{2C}$ receptors and their mRNA largely appear to be on non-dopaminergic cells (Clemett et al. 2000; Eberle-Wang et al. 1997; Lopez-Gimenez et al. 2001; Pasqualetti et al. 1999). Studies have identified 5-HT$_{2C}$ receptors on GABAergic cells of the dorsal raphe (Serrats et al., 2005), prefrontal cortex (Liu et al. 2007) and VTA (Bubar and Cunningham 2007; Di Giovanni et al. 2001), and each of these areas has projections to the NAc (Kalivas and Volkow 2005; Lechin et al. 2006). It has been suggested that 5-HT$_{2C}$ receptor activation decreases DA efflux indirectly by increasing GABAergic activity (Boothman et al. 2006; Di Giovanni et al. 2001; Serrats et al. 2005; Stanford and Lacey 1996) and electrophysiological studies have supported this notion by demonstrating that 5-HT$_{2C}$ receptor activation excites GABAergic cells in the VTA, substantia nigra and raphe nuclei (Di Giovanni et al. 2001; Invernizzi et al. 2007; Liu et al. 2000). As WAY 161503 was systemically administered, the present study cannot comment directly on the location of 5-HT$_{2C}$ receptors which affect VTA ICSS. However, these results, in combination with those in the literature, support the notion that 5-HT$_{2C}$ receptor activation may inhibit VTA ICSS behaviour through a GABAergic mechanism and that GABA$_A$ receptors in the NAc shell may be of particular importance. Furthermore, these results are in line with evidence underscoring the GABAergic system

as integral in regulating ICSS behaviour (Cheer et al. 2005; Ishida et al. 2001; Lassen et al. 2007; Steffensen et al. 2001).

In summary, this study provides support for the hypothesis that 5-HT$_{2C}$ and GABA$_A$ receptor activation regulate locomotion and VTA ICSS behaviour. Under the present conditions, this study has provided evidence that these receptors may regulate VTA ICSS through a similar mechanism. Activation of GABA$_A$ receptors in the rostral NAc shell may inhibit VTA ICSS and 5-HT$_{2C}$ receptors may inhibit VTA ICSS by increasing GABA release in the NAc shell, although the precise circuitry remains unknown. Further research is needed to elucidate the precise mechanisms involved as each of these receptors, and their interaction, may be involved in regulating natural reward, drug addiction and many psychiatric disorders (Dremencov et al. 2005; Goodman 2008; Hill and Reynolds 2007; Kalueff and Nutt 2007; Nilsson 2006; Siuciak et al. 2007; Stratford and Kelley 1997).

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Chapter 6: Effects of GABA$_B$ and 5-HT$_{2C}$ receptor compounds on ventral tegmental area intracranial self-stimulation thresholds and locomotor activity in rats

(A version of this chapter has been submitted to European Journal of Neuroscience. Hayes DJ, Greenshaw AJ. Effects of GABA$_B$ and 5-HT$_{2C}$ receptor compounds on ventral tegmental area intracranial self-stimulation thresholds and locomotor activity in rats.)

Introduction

Activity of the mesocorticolimbic dopamine (DA) system is important for the regulation of motivation and reward-related behaviours (McBride et al. 1999; Tzschentke 2007; Wise 2002; 2004). Serotonin (5-HT) and γ-aminobutyric acid (GABA) have also emerged as important neurotransmitters in the regulation of dopamine- and reward-related behaviours, yet the mechanisms involved are still unclear (Bardo 1998; Kalivas et al. 1990; McBride et al. 1999; Van Bockstaele et al. 1993; Van Bockstaele et al. 1994; Van Bockstaele and Pickel 1995). Reciprocal connections exist between GABAergic and serotonergic cells (Bagdy et al. 2000; Wang et al. 1992) and the actions of their neurotransmitters are through numerous receptor subtypes (Barnes and Sharp 1999; Mohler 2006; Ulrich and Bettler 2007). Understanding how these neurotransmitter systems interact may help elucidate the mechanisms of natural reward, drug addiction and many psychiatric disorders (Feltenstein and See 2008; Goodman 2008; Kalueff and Nutt 2007; Laviolette 2007; Meltzer 1999; Shirayama and Chaki 2006; Wrase et al. 2006).

The 5-HT$_{2C}$ receptor is expressed throughout the mesolimbic system (Bubar and Cunningham 2007; Clemett et al. 2000) and activation of this receptor may inhibit the release of mesolimbic dopamine (Di Giovanni et al. 2000; Di Matteo et al. 1999).

Selective activation of the 5-HT$_{2C}$ receptor using the agonist WAY 161503 increases ventral tegmental area (VTA) intracranial self-stimulation (ICSS) rate-frequency thresholds (indicating a decrease in reward) without affecting measures of motor performance (Hayes et al. 2008a). While selective 5-HT$_{2C}$ receptor compounds do not affect the expression of place conditioning on their own (Mosher et al. 2005), they may produce a state-dependent conditioned place aversion (Mosher et al. 2006). The 5-HT$_{2C}$ receptor may play an inhibitory role in mediating the rewarding properties of drugs of abuse (Fletcher et al. 2004; Fletcher et al. 2002; Grottick et al. 2001; Rocha et al. 2002; Tomkins et al. 2002) although it may not affect nicotine-induced place conditioning (Hayes et al. 2008b). 5-HT$_{2C}$ receptor activation has been shown to decrease basal locomotor activity (Gleason et al. 2001; Higgins et al. 2001; Kennett et al. 2000; Kennett et al. 1997; Lucki et al. 1989; Martin et al. 2002; Mosher et al. 2005). Though some studies have demonstrated place conditioning as an exception, studies generally agree with an inhibitory role for the 5-HT$_{2C}$ receptor in dopamine regulation, locomotion and reward-related behaviours.

The GABA$_B$ receptor is located extensively throughout the brain (Margeta-Mitrovic et al. 1999) and activation of these receptors within mesolimbic areas may inhibit dopamine efflux (Erhardt et al. 2002; Fadda et al. 2003; Klitenick et al. 1992; Rahman and McBride 2002; Westerink et al. 1996). Behaviourally, the picture is more complex. Systemically administered GABA$_B$ receptor agonists and antagonists may both increase (Macey et al. 2001; Slattery et al. 2005), or have no effect (Dobrovitsky et al. 2002), on ICSS thresholds. GABA$_B$ receptor ligands do not appear to induce place conditioning or support self-administration on their own, however GABA$_B$ receptor

activation may attenuate drug-induced conditioned place preferences (Bechtholt and Cunningham 2005; Le Foll et al. 2008; Li et al. 2001; Sahraei et al. 2008; Suzuki et al. 2005; Zarrindast et al. 2006) and decrease responding for drugs of abuse (Brebner et al. 2005; Brebner et al. 2000a; b; Colombo et al. 2002; Di Ciano and Everitt 2003; Markou et al. 2004; Paterson et al. 2004). The $GABA_B$ receptor agonist baclofen has also been shown to decrease basal locomotor activity when administered systemically (Agmo and Giordano 1985; Cryan et al. 2004; Gianutsos and Moore 1978; McManus and Greenshaw 1991; Nissbrandt and Engberg 1996), intra-ventricularly (Agmo and Giordano 1985) and intra-nucleus accumbens (NAc) (Plaznik et al. 1990; Wachtel and Anden 1978; Wong et al. 1991), although some studies have reported biphasic (Hotsenpiller and Wolf 2003; Le Foll et al. 2008) or no (Chester and Cunningham 1999; Phillis et al. 2001) effects on locomotion following systemic baclofen.

Reciprocal connections exist between GABAergic and serotonergic cells (Bagdy et al. 2000; Wang et al. 1992) and there is evidence that $5-HT_{2C}$ and $GABA_B$ receptors are involved in the communication between these two neurotransmitter systems. $5-HT_{2C}$ receptors have been identified on some GABAergic cell populations (Bubar and Cunningham 2007; Liu et al. 2007; Serrats et al. 2005) and their activation has been associated with increasing GABA cell activity (Bankson and Yamamoto 2004; Boothman et al. 2006; Stanford and Lacey 1996). Activation of $GABA_B$ receptors on 5-HT cells in the anterior raphe nuclei results in a biphasic effect on 5-HT release (Abellan et al. 2000a; Abellan et al. 2000b; Serrats et al. 2003; Tao et al. 1996). Injections of the $GABA_B$ receptor agonist baclofen into the median raphe results in increased locomotor activity and food and water intake (Wirtshafter et al. 1993). As there is some evidence

that the inhibitory actions of the 5-HT$_{2C}$ receptor are mediated through a GABAergic mechanism, and that these receptors may interact in some contexts (Masse et al. 2007; Reilly et al. 2008; Stanford and Lacey 1996), it is possible that 5-HT$_{2C}$ and GABA$_B$ receptors work through a similar mechanism to regulate locomotion and reward-related behaviours.

The present study tested the hypothesis that GABA$_B$ and 5-HT$_{2C}$ receptors play inhibitory roles in locomotor activity and ICSS behaviour by investigating the effects of the selective 5-HT$_{2C}$ receptor agonist WAY 161503 (0-1.0 mg/kg) and the GABA$_B$ receptor agonist baclofen (0-2.5 mg/kg), alone and in combination. As the NAc shell has been identified as a potential site for some of the behavioural effects of GABA$_B$ receptor activation (Lopes et al. 2007; Plaznik et al. 1990; Stratford and Kelley 1997), the hypothesis that NAc shell GABA$_B$ receptor activation would inhibit VTA ICSS behaviour was tested using bilateral microinjections of baclofen (0-225 ng/side). Food intake was also measured and used as a positive control as previous studies have demonstrated increased feeding following intra-NAc baclofen (Lopes et al. 2007; Stratford and Kelley 1997; Ward et al. 2000; Znamensky et al. 2001).

Materials & methods

Subjects

Twenty-six male Sprague-Dawley rats (Health Sciences Laboratory Animal Services, University of Alberta) weighing 200-250 g were housed individually in standard Plexiglas laboratory cages at 20°C and 50 % humidity, with a 12-hr light/dark cycle (lights from 0700 h -1900 h) with food and water freely available. All testing took place

in the dark or under red light during the light phase of the light/dark cycle. All apparatus were cleaned between animals with diluted (1:6) ammonia-based window cleaner (No Name® Glass Cleaner with ammonia). The care and use of animals were in accordance with guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

Drugs

The 5-HT$_{2C}$ receptor agonist WAY 161503 · HCl [8,9-dichloro-2,3,4,4a-tetrahydro-1H-pyrazino[1,2-a]quinoxalin-5(6H)-one hydrochloride]and the GABA$_B$ receptor agonist (R)-baclofen [(R)-4-amino-3-(4-chlorophenyl)butanoic acid] were purchased from Tocris Cookson Inc. (Ellisville, MO, USA). (+) α-Methylphenethylamine ((+)-amphetamine) sulphate was purchased from Health and Welfare Canada. All systemically administered compounds were dissolved in double-distilled water (ddH$_2$O) in a volume of 1.0 ml/kg. WAY 161503 and (+)-amphetamine were injected subcutaneously, 10 min prior to testing, while baclofen was injected intraperitoneally, 20 min prior to testing. Artificial cerebrospinal fluid was freshly prepared (Elliott and Lewis 1950) and drug solutions made daily (pH 6.0–7.0). All drug doses are expressed as free-base.

Spontaneous Locomotor Activity

Apparatus: Spontaneous locomotor activity was measured using computer–monitored photobeam boxes (I. Halvorsen System Design, Phoenix, AZ, USA). The locomotor apparatus consisted of a clear Plexiglas test cage (43 cm L x 43 cm W x 30 cm H) with a 12 x 12 photobeam grid located 2.5 cm above the floor. These beams measured horizontal activity (measured by the number of infrared beams broken) as well as consecutive beam breaks (repeat activity). Vertical activity (or rearing activity, measured

by infrared beams broken following rears on the hind legs) was measured using 12 additional photobeams located 12 cm above the floor.

Procedure: Animals (n = 8) were habituated to the locomotor activity boxes for two consecutive days (60 min/day). They subsequently received randomized and counterbalanced injections with three drug-free days between injections. All locomotor activity was monitored over a 30 min time course.

Intracranial self-stimulation (ICSS)

Surgery & histology: Using a previously described procedure (Greenshaw 1993), each animal (10 for the systemic dose-response, 8 for the baclofen x WAY 161503 experiment; 8 for the intracranial microinjection experiment) was implanted with a stainless steel, monopolar, stimulating electrode (E363/2; tip diameter 200 µm; Plastics One Ltd., Roanoke, VA) directed to the VTA. A large silver indifferent electrode in the skull served as the relative ground. Animals used for microinjection were also implanted with bilateral cannulae (22 gauge) directed to the rostral shell of the NAc. Stereotaxic coordinates were [mm]: VTA – AP +2.6, L +0.5, V +1.8; NAc shell – AP +11.0, L +0.4, V +2.8, from inter-aural zero, with the incisor bar set at 2.4 mm below the inter-aural line (Paxinos and Watson 1998). These coordinates were interpolated from the target site for an angle of 20° lateral and anterior for the VTA and 16° lateral for the NAc shell (Greenshaw 1997). The guide cannulae were placed 1 mm above the actual injection sites. Electrode and cannulae placements were verified at the end of the experiment by microscopic inspection of flash-frozen coronal brain sections (40 µm); flash-freezing was achieved using isopentane cooled on dry ice. Only animals with VTA and NAc placements were included in the analysis.

Apparatus & Procedure: Monopolar stimulation of the VTA was provided from constant current DC stimulators (cathodal monophasic pulse width of 200 μs; initial training frequency of 100 Hz; train length of 1 s) connected to each animal via a gold-track slip ring. Between pulses the active electrode and indifferent electrode were connected through a resistor to cancel any effects of electrode polarisation (Greenshaw 1986). The apparatus and rate-frequency analysis were as described by Ivanová et al (1997). With this procedure, M50 is the threshold frequency at which half-maximal response rates occur; RMAX is the maximal rate of responding in a session. While M50 is a measure of reward sensitivity (which is dissociable from non-specific changes in behaviour), RMAX is a measure of response performance (see Gallistel and Karras, 1984; Greenshaw and Wishart, 1987). Animals received a randomized counterbalanced sequence of treatments with 3 days of baseline frequency testing between each treatment. To minimize the use of animals, eight animals from the baclofen dose response experiment were subsequently used in the baclofen x WAY 161503 experiment.

Microinjection of drugs: Rats with bilateral cannulae in the NAc shell received randomly assigned, counterbalanced, treatments [artificial cerebrospinal fluid, baclofen (0-225 ng/side), and (+)-amphetamine (1.0 μg/side)] with at least three days between each microinjection. Each treatment was administered in a total volume of 0.5μL at a pump-controlled rate of 0.2μL per minute (Beehive controller, Bioanalytical Systems, Inc.); the injection cannulae remained in place for a further minute to allow for drug absorption. Immediately following each set of microinjections, each animal was tested for VTA ICSS.

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Food intake

Adapted from previously described procedures (Reynolds and Berridge 2001; Stratford and Kelley 1997), animals were placed in standard Plexiglas laboratory cages (free from wood shavings) immediately following the VTA ICSS session. A pre-weighed amount of food, placed in a container identical to that found in their home cage, was made available along with water 25 min after the initiation of each VTA ICSS session (each session is a maximum of 25 min). At the end of a 30 min session (55 min post injection), food intake (corrected for spillage) was calculated by subtracting the initial weight of the food and container from the final weight. Animals were habituated for three days prior to the beginning of microinjection treatments. This procedure was subsequently performed on each microinjection treatment day to determine total food intake (measured in grams) in a 30 min session following intra-NAc baclofen (0-225 ng/side).

Statistical Analysis

Experimental effects for all behavioural measures were determined using repeated measures analysis of variance (ANOVA). A significant F ratio ($p \leq 0.05$) was followed by Newman-Keuls post hoc tests ($\alpha = 0.05$) where appropriate. As the results of the analyses for repeat (consecutive) activity paralleled those for horizontal locomotor activity, only the latter results are discussed. All ICSS data are presented as a percentage of average baseline performance of each animal. Greenhouse-Geisser corrected degrees of freedom are used as a conservative estimate of the F-ratio. Statistical analyses for all experiments were completed using statistical software (SPSS Inc., Chicago, IL, USA).

Results

Effects of WAY 161503 and baclofen on locomotor activity

Horizontal activity: Three-way repeated measures ANOVA revealed that WAY 161503 (0-1.0 mg/kg) decreased horizontal locomotor activity [Fig. 6.1A, $F_{(2.04, 14.25)}$ = 20.20, $p < 0.05$], an effect significant across time [$F_{(4.48, 31.36)}$ = 6.70, $p < 0.05$]. A main effect of baclofen (1.25 mg/kg) was also found [$F_{(1, 7)}$ = 61.01, $p < 0.05$] and was significant over time [$F_{(2.80, 19.61)}$ = 17.91, $p < 0.05$]. There was no interaction between WAY 161503 and baclofen. Further analysis of WAY 161503, following the collapse of data across baclofen and time, revealed that the dose of 1.0 mg/kg was significant from control (Fig. 6.1B).

Rearing activity: Three-way repeated measures ANOVA revealed that WAY 161503 (0-1.0 mg/kg) decreased locomotor activity [Fig. 6.1C, $F_{(2.18, 15.26)}$ = 18.79, $p < 0.05$], an effect not significant across time. Baclofen (1.25 mg/kg) significantly decreased rearing activity across time [$F_{(2.65, 18.57)}$ = 4.89, $p < 0.05$]. There was no interaction between WAY 161503 and baclofen. Further analysis of WAY 161503, following the collapse of data across baclofen and time, revealed that the 0.3 and 1.0 mg/kg doses were significant from control (Fig. 6.1D).

Repeat activity: Three-way repeated measures ANOVA revealed that WAY 161503 (0-1.0 mg/kg) decreased locomotor activity [Fig. 6.1E, $F_{(2.11, 14.74)}$ = 5.63, $p < 0.05$], an effect not significant across time. Baclofen (1.25 mg/kg) significantly decreased repeat activity [$F_{(1, 7)}$ = 44.25, $p < 0.05$], an effect not significant across time. There was no interaction between WAY 161503 and baclofen. Further analysis of WAY 161503,

following the collapse of data across baclofen and time, revealed that the 1.0 mg/kg dose

was significant from control (Fig. 6.1F).

Figure 6.1A-F Effects of systemic WAY 161503 (W; 0-1.0 mg/kg) and baclofen (B; 1.25 mg/kg) on spontaneous locomotor activity measured over 30 min. **(A)** Horizontal locomotor activity. **(B)** Horizontal locomotor activity, as seen with data collapsed across baclofen and time. **(C)** Rearing (vertical) locomotor activity. **(D)** Rearing locomotor activity, as seen with data collapsed across baclofen and time. **(E)** Repeat (consecutive) locomotor activity. **(F)** Repeat locomotor activity, as seen with data collapsed across baclofen and time. Data shown are means ± SEM. *Significant from control at $p < 0.05$ following Newman-Keuls post hoc tests.

C

D

E

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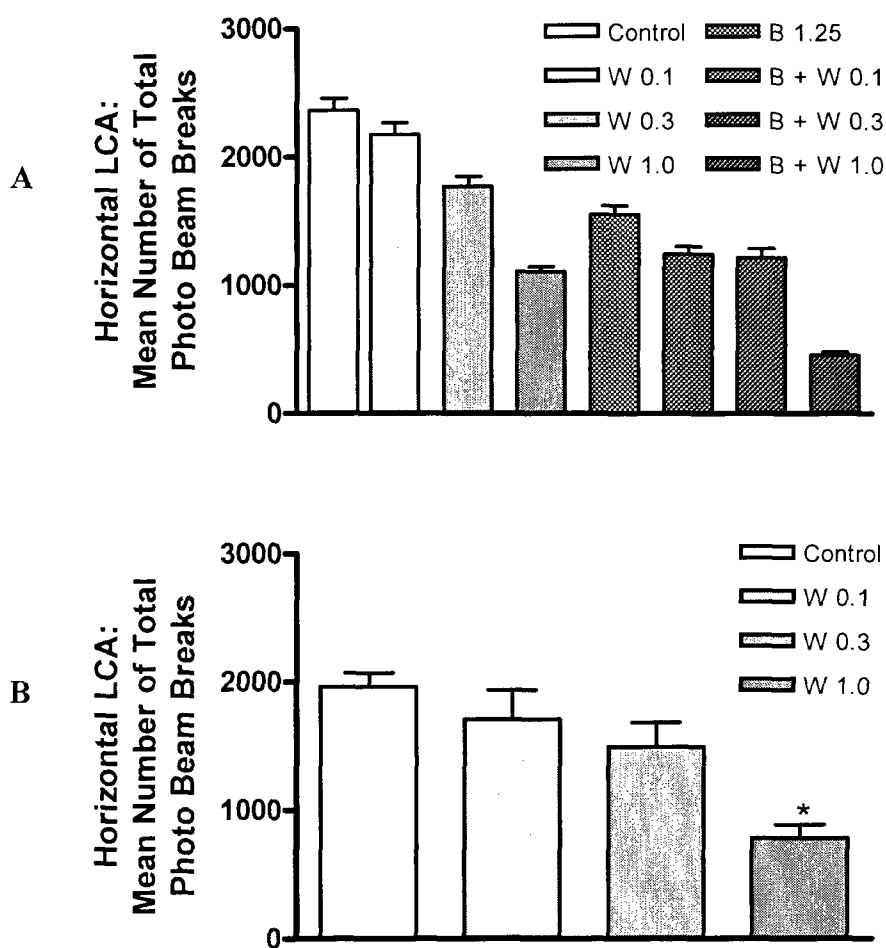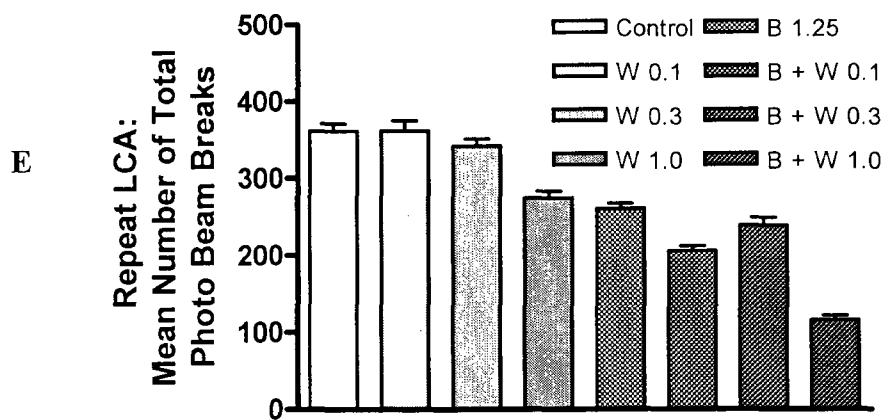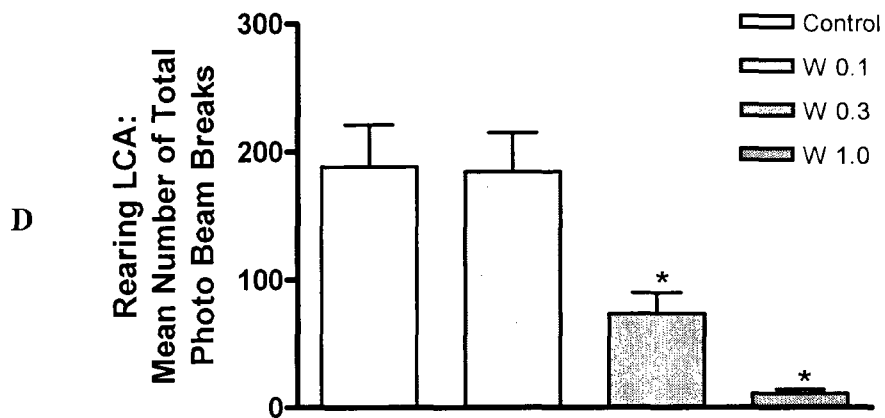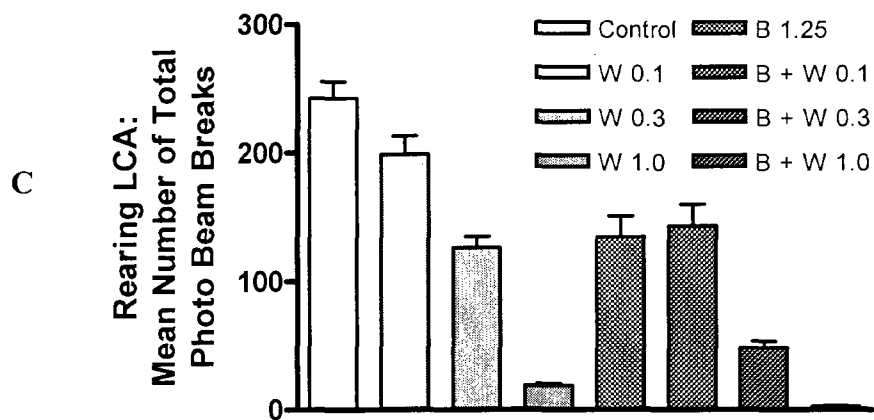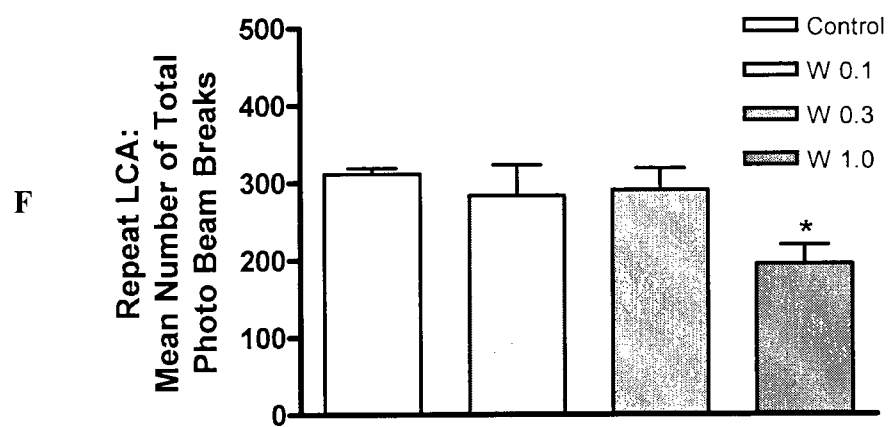
Effects of systemic baclofen on ICSS

Analysis following systemic administration of baclofen (0-2.5 mg/kg) revealed significant main effects for M50 thresholds [Fig. 6.2A, $F_{(2.69, 24.21)} = 16.04$, $p < 0.05$], RMAX values [Fig. 6.2B, $F_{(1.32, 11.86)} = 12.31$, $p < 0.05$] and TRES values [Fig. 6.2C, $F_{(2.08, 18.60)} = 28.68$, $p < 0.05$]. Further analysis using Newman-Keuls post hoc tests ($\alpha = 0.05$) revealed that the 1.25 and 2.5 mg/kg doses produced a significant increase in M50 thresholds (Fig. 6.2A), and decrease in TRES values (Fig. 6.2C), compared to control. The highest dose of baclofen (2.5 mg/kg) decreased RMAX values (Fig. 6.2B). Group-averaged rate-frequency regression curves are included to illustrate the dose-dependent rightward shift in M50 seen with baclofen (indicating a decrease in reward) (Fig. 6.2D).

Figure 6.2A-D The effects of baclofen (0-2.5 mg/kg) on **(A)** rate-frequency thresholds (M50), **(B)** maximal response rates (RMAX), and **(C)** total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the rightward shift in M50 seen with baclofen (indicating a decrease in reward). Data shown are means ± SEM expressed as a percentage of baseline performance. *Denotes significance from control at $p<0.05$ following Newman-Keuls post hoc tests.
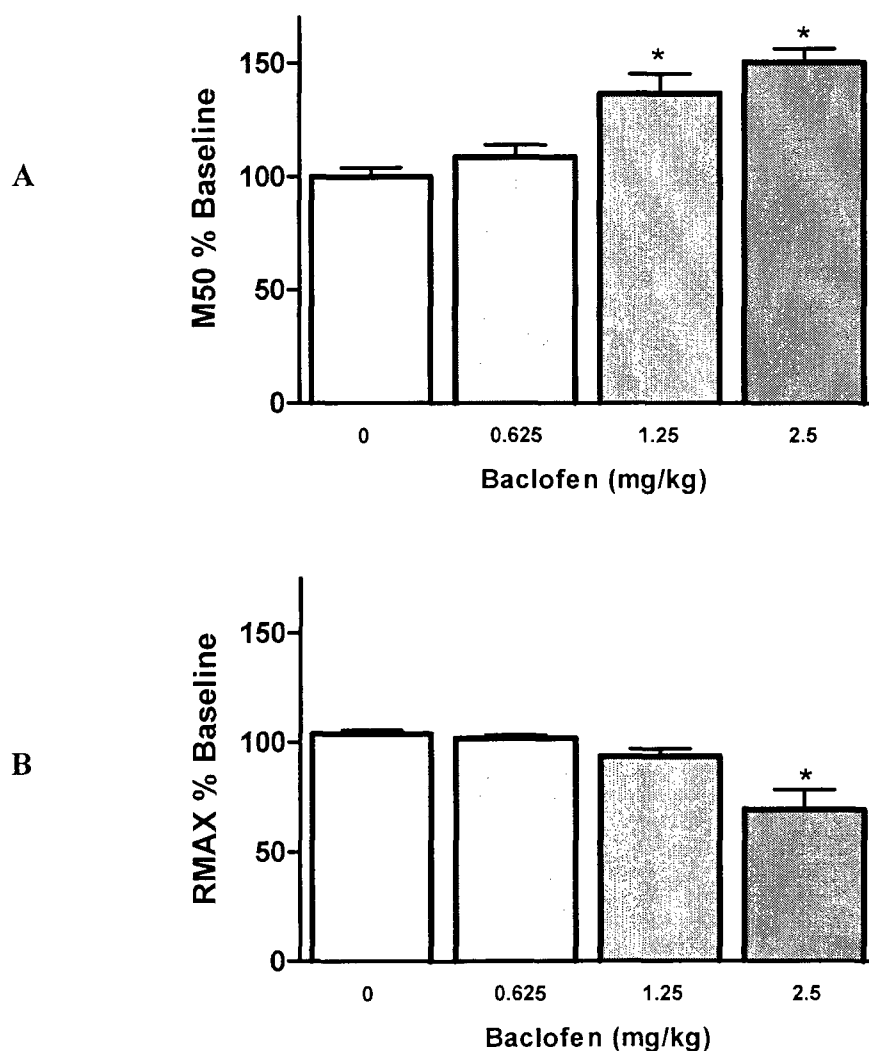
C
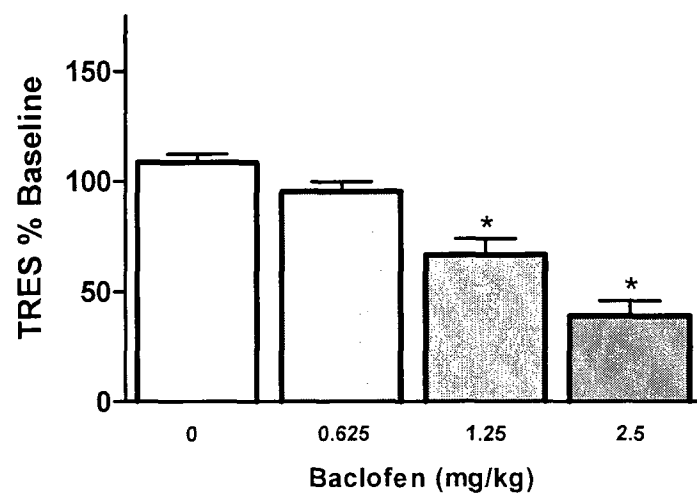
D
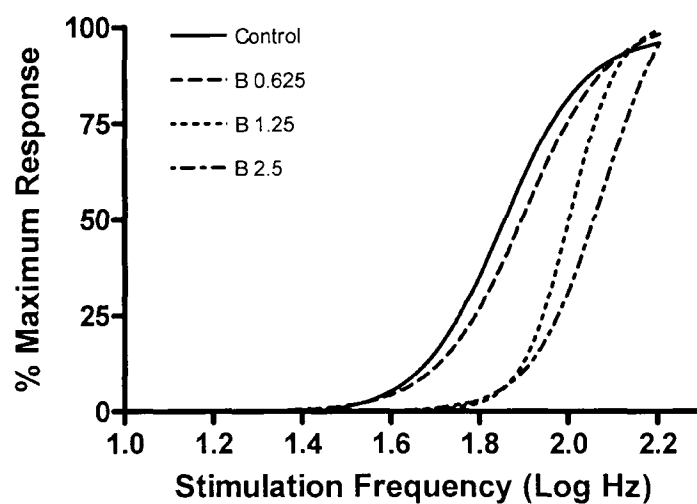
Effects of systemic WAY 161503 and baclofen on ICSS

Analysis following systemic administration of WAY 161503 (0.3, 1.0 mg/kg) and baclofen (0.625, 1.25 mg/kg) revealed significant main effects of WAY 161503 for M50 thresholds [Fig. 6.3A, $F_{(1.83, 12.78)} = 18.22$, $p < 0.05$], RMAX values [Fig. 6.3B, $F_{(1.26, 8.82)} = 10.19$, $p < 0.05$] and TRES values [Fig. 6.3C, $F_{(1.41, 9.86)} = 34.06$, $p < 0.05$]. For baclofen, significant main effects were seen for M50 thresholds [Fig. 6.3A, $F_{(1.60, 11.20)} = 25.14$, $p < 0.05$] and TRES values [Fig. 6.3C, $F_{(1.14, 7.97)} = 29.99$, $p < 0.05$] but not for RMAX values. WAY 161503 and baclofen did not interact on any measure. Further analysis of WAY 161503, following the collapse of data across baclofen, revealed that the 1.0 mg/kg dose was significant from control for M50 (Fig. 6.3D) and TRES (Fig. 6.3F), while no differences were noted for RMAX values (Fig. 6.3E). Group-averaged rate-frequency regression curves are included to illustrate the rightward shifts in M50 seen with WAY 161503 and baclofen (indicating a decrease in reward) (Fig. 6.3G).

Figure 6.3A-G The effects of baclofen (B; 0.625, 1.25 mg/kg) and WAY 161503 (W; 0.3, 1.0 mg/kg) on **(A)** rate-frequency thresholds (M50), **(B)** maximal response rates (RMAX), and **(C)** total responses (TRES) for VTA ICSS. **(D-F)** WAY 161503 (1.0 mg/kg) increased M50 and decreased TRES without affects on RMAX values, following the collapse of data across baclofen. **(G)** Group-averaged rate-frequency regression curves are included to illustrate the rightward shift (indicating a decrease in reward), and additive effects, in M50 seen with baclofen and WAY 161503. Data shown are means ± SEM expressed as a percentage of baseline performance. *Significant from control at $p <$ 0.05 following Newman-Keuls post hoc tests.

B

C

D

WAY 161503 (mg/kg)

E

F

G

291
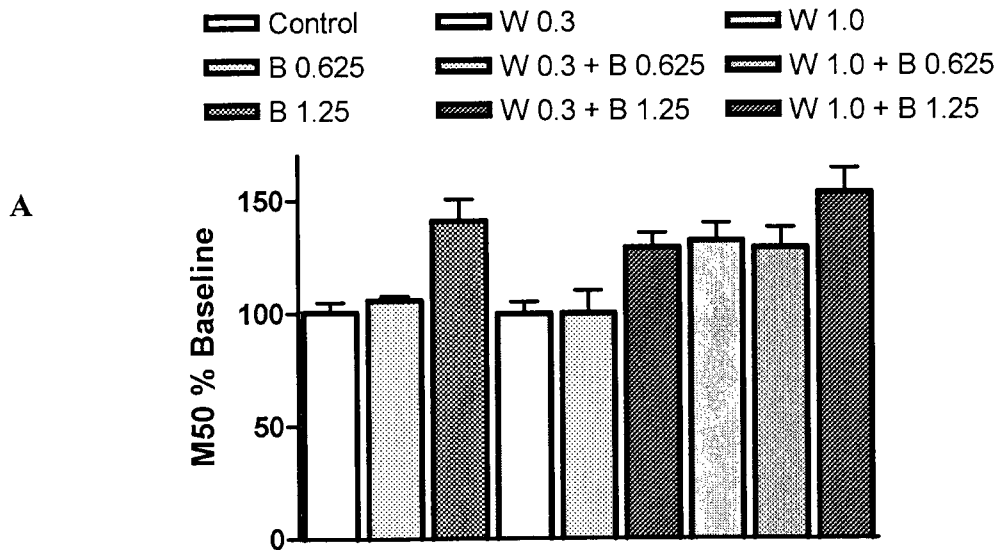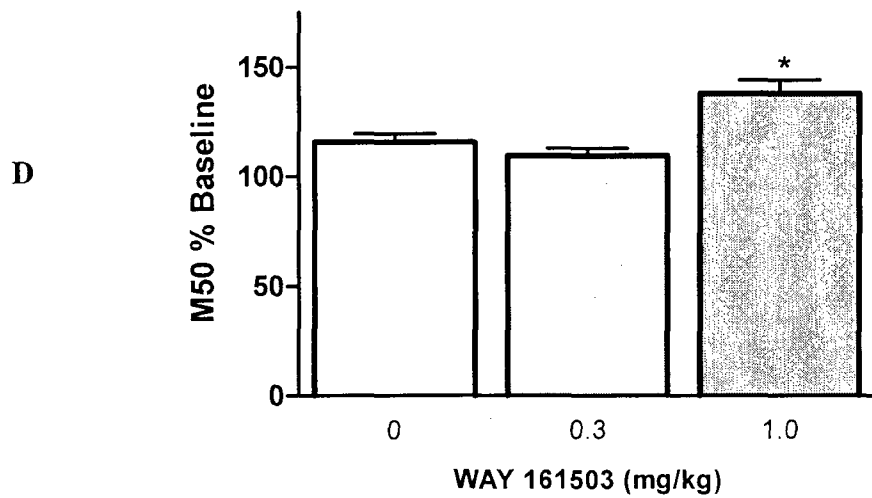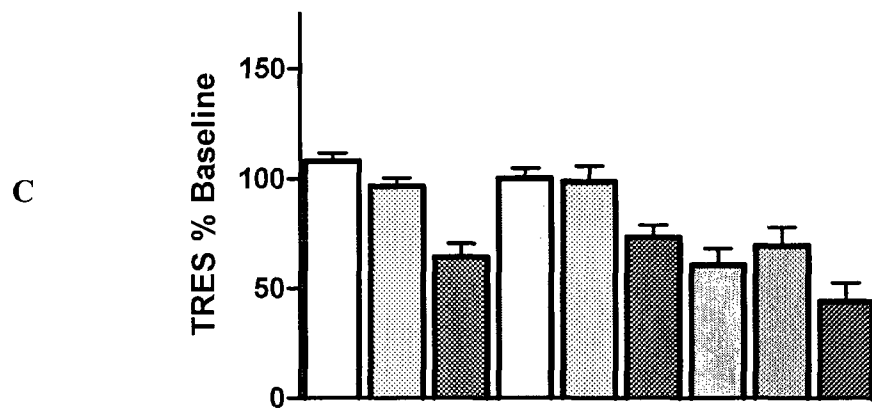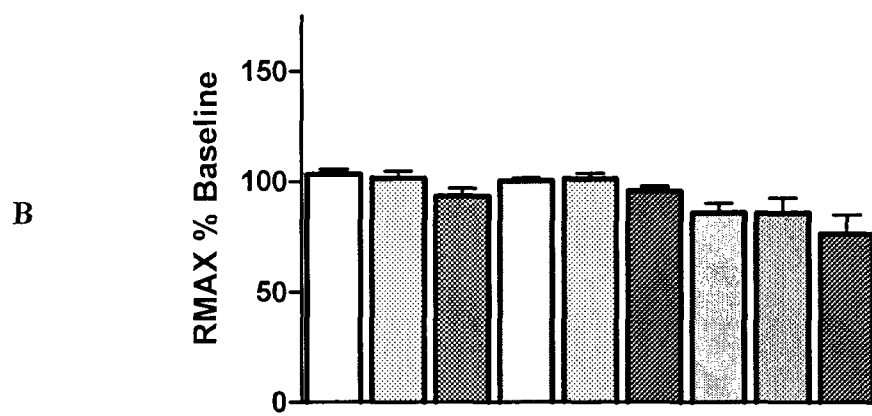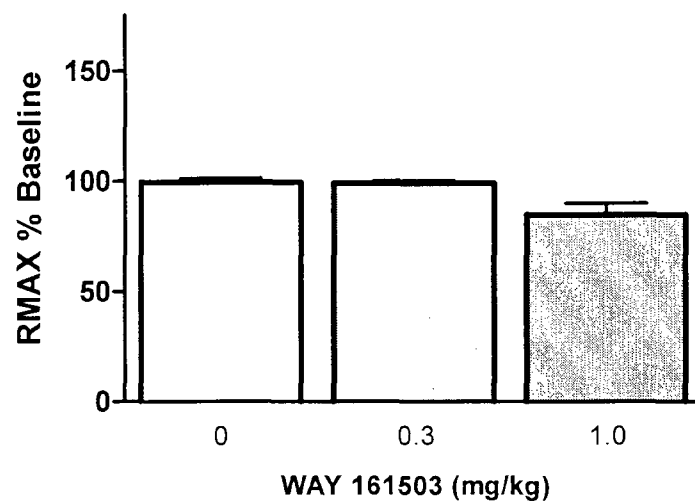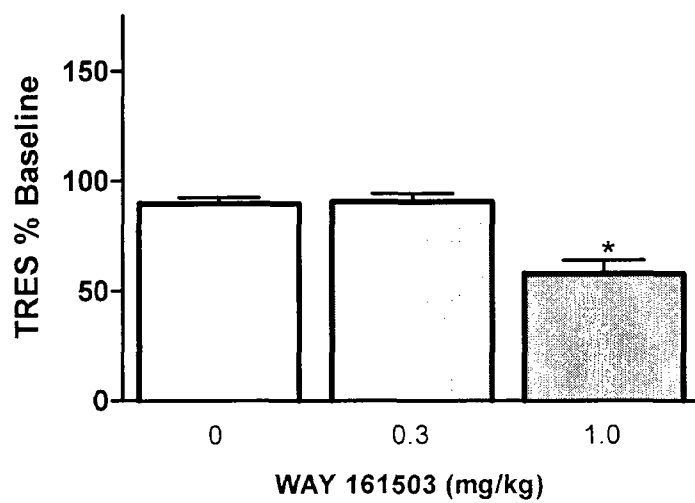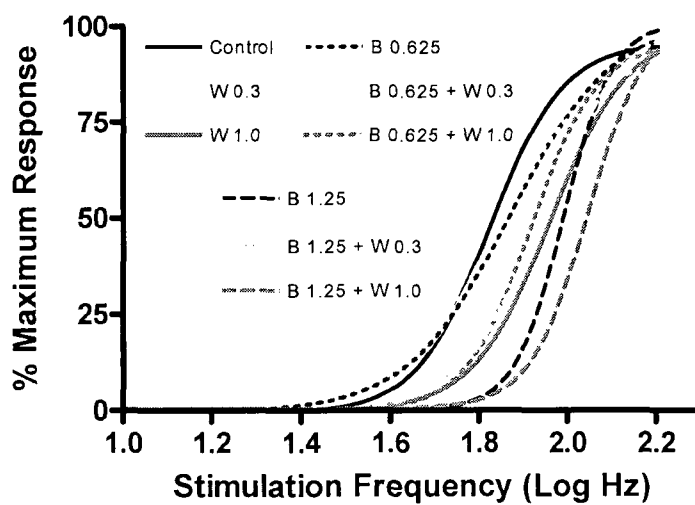
Effects of intra-NAc shell baclofen on ICSS

Intra-NAc shell baclofen (25, 75, 225 ng/side) did not significantly affect M50 thresholds [Fig. 6.4A, $F(1.38, 9.63) = 1.60$, $p > 0.05$], RMAX values [Fig. 6.4B, $F(1.20, 8.42) = 1.42$, $p > 0.05$] or TRES values [Fig. 6.4C, $F(1.22, 8.56) = 2.96$, $p > 0.05$]. This is in contrast to the positive control (+)-amphetamine (1.0 µg/side), which significantly decreased M50 values [Fig. 6.4A, $F (1, 7) = 8.41$, $p < 0.05$] without affecting RMAX or TRES values. Group-averaged rate-frequency regression curves are included to illustrate the leftward shift in M50 seen with (+)-amphetamine (indicating an increase in reward). (Fig. 6.4D).

Figure 6.4A-C Effects of intra-NAc shell (+)-amphetamine (Amph; 1.0 μg/side) and baclofen (0-225 ng/side) on **(A)** rate-frequency thresholds (M50), **(B)** maximal response rates (RMAX), and **(C)** total responses (TRES) for VTA ICSS. **(D)** Group-averaged rate-frequency regression curves are included to illustrate the leftward shift (indicating an increase in reward) in M50 seen with (+)-amphetamine. Data shown are means ± SEM expressed as a percentage of baseline performance. *Significant from control at $p < 0.05$ following Newman-Keuls post hoc tests.

C

D

294
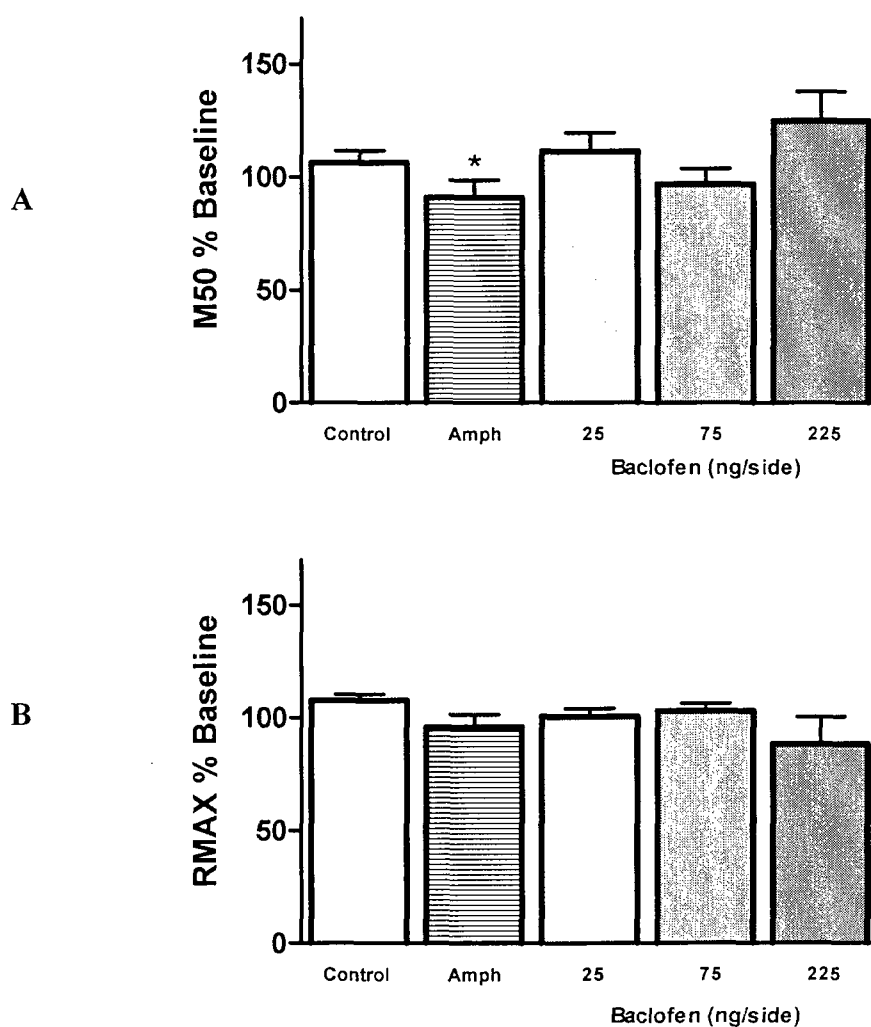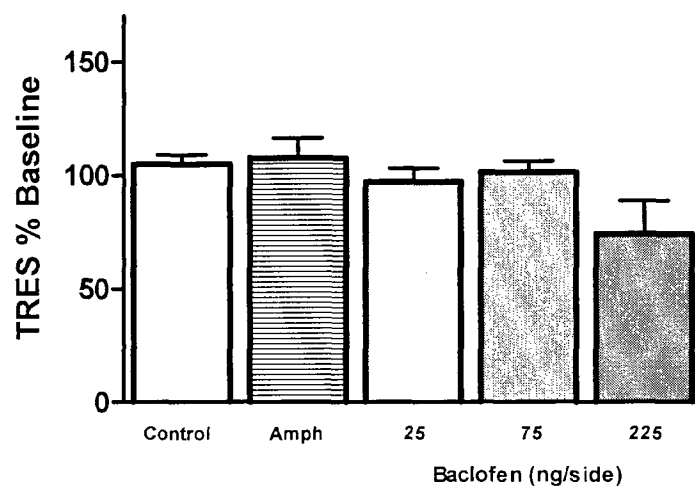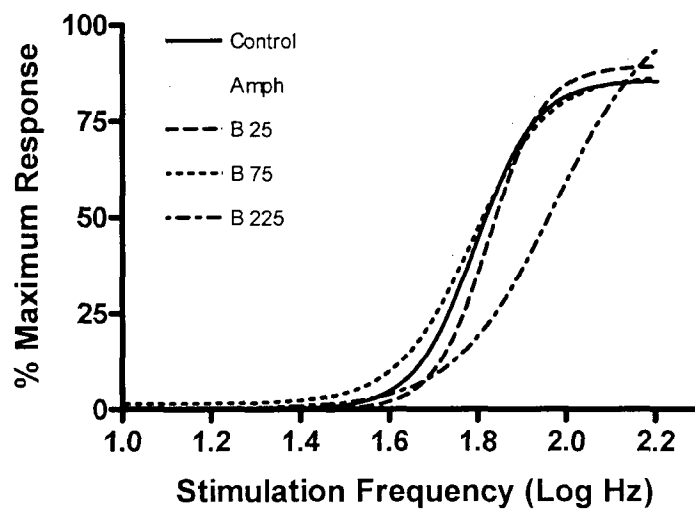
Effects of intra-NAc shell baclofen on food intake

Intra-NAc shell baclofen (25, 75, 225 ng/side) significantly increased feeding in a dose-dependent manner [Fig. 6.5., $F_{(2.45, 17.14)} = 4.16$, $p < 0.05$). Further analysis using Newman-Keuls post hoc tests ($\alpha = 0.05$) revealed that the highest dose tested (225 ng/side) significantly increased food intake.

Only rats with electrode placements in the VTA and bilateral cannulae in the rostral NAc shell were included in the analysis. Representative photomicrographs of VTA stimulation sites and NAc shell microinjection sites are seen in Fig. 6.6A & C, respectively. Histological locations of electrode terminal sites and NAc shell microinjection sites are represented in Fig. 6.6B and D, respectively.

Figure 6.5 Effects of intra-NAc shell baclofen (0-225 ng/side) on food intake (measured in grams) over 30 min. Data shown are means ± SEM. *Significant from control at $p <$ 0.05 following Newman-Keuls post hoc tests.
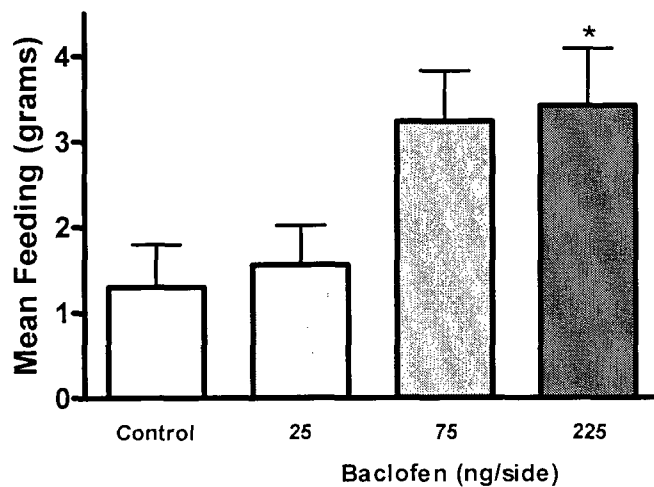
Figure 6.6A-D Histological verification of VTA and NAc shell sites. **(A)** Representative photomicrograph (circle identifies VTA electrode terminal) and **(B)** histological locations of VTA stimulation sites. **(C)** Representative photomicrograph and **(D)** histological locations of NAc shell microinjection sites. Brain diagrams from Paxinos & Watson (1998).

A

B

Interaural 3.70

Interaural 3.40

Interaural 3.20

Interaural 2.96

Interaural 2.70

C

D
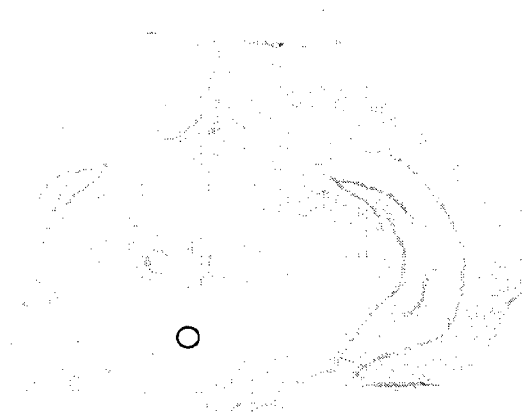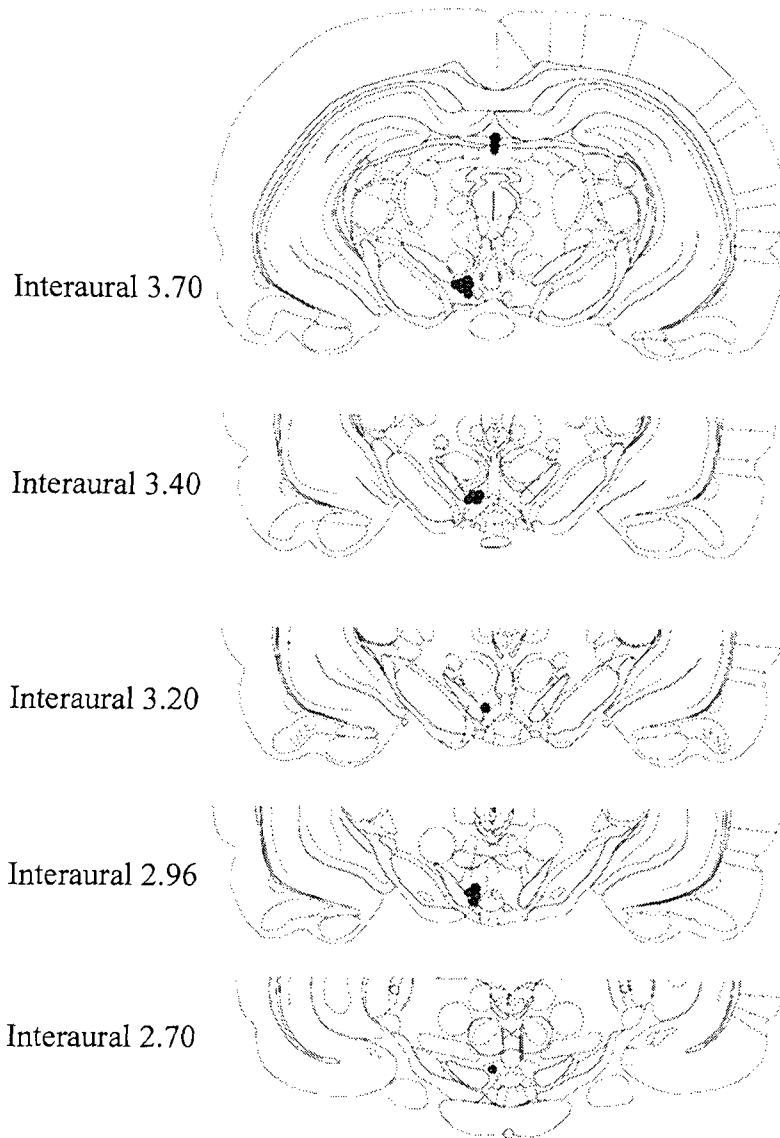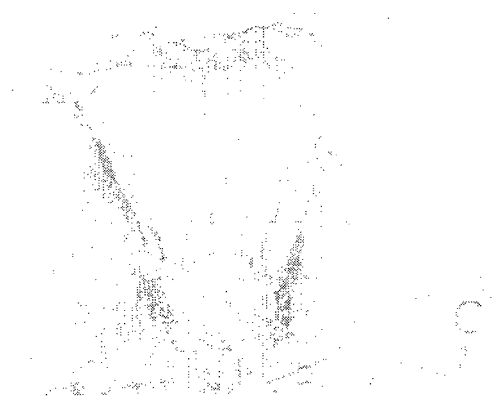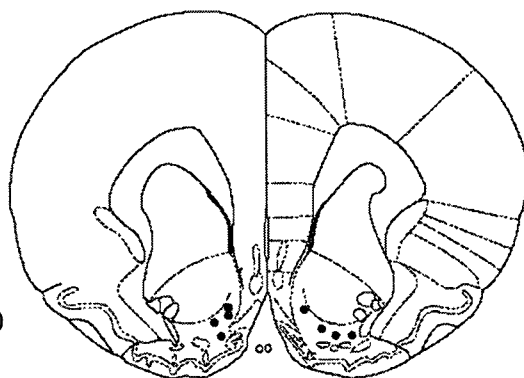
Interaural 11.20
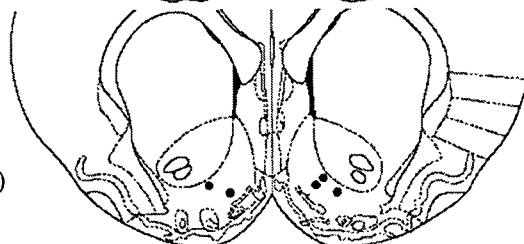
Interaural 10.70

Interaural 10.60
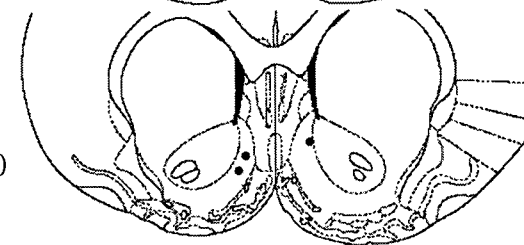
Discussion

This study investigated the role of $GABA_B$ and $5\text{-}HT_{2C}$ receptors in spontaneous locomotor activity and ICSS. Though the 1.25 mg/kg dose of the $GABA_B$ receptor agonist baclofen was chosen for its reported sub-threshold effects on locomotion (Agmo and Giordano 1985; Le Foll et al. 2008), it decreased all measures of locomotor activity in the present study (Fig. 6.1A, C, E). That systemic baclofen decreases locomotor activity, however, is consistent with a majority of studies (Agmo and Giordano 1985; Cryan et al. 2004; Gianutsos and Moore 1978; Hotsenpiller and Wolf 2003; Le Foll et al. 2008; McManus and Greenshaw 1991; Nissbrandt and Engberg 1996). Moreover, that baclofen decreased locomotor activity did not hinder the present study aimed at exploring the relationship between $GABA_B$ and $5\text{-}HT_{2C}$ receptors. Under the present conditions, the effects of the $GABA_B$ and $5\text{-}HT_{2C}$ receptor agonists were additive, suggesting that they may inhibit locomotor activity through separate mechanisms. Though this interpretation of the data would be strengthened through the use of additional doses of baclofen, it is consistent with the known location and activity of these receptors (as discussed below). The effects of the selective $5\text{-}HT_{2C}$ receptor agonist WAY 161503 (Fig. 6.1B, D, F) on locomotor activity are consistent with previous reports using this compound (Hayes et al. 2008b; Mosher et al. 2005) and other $5\text{-}HT_{2C}$ receptor ligands (Higgins et al. 2001; Kennett et al. 1997; Lucki et al. 1989; Martin et al. 2002).

The effects of 1.25 mg/kg baclofen on VTA ICSS (Fig. 6.2A & E) are likely reward-related as there was a significant increase in M50 thresholds without effects on RMAX values (Fig. 6.2B). This dose is also well below doses required for an inhibitory effect on the rotorod test (Lobina et al. 2005; Smith et al. 2006). In addition, it has been

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suggested that rearing or vertical locomotor activity may be an indicator of appetitive arousal (Cabeza de Vaca et al. 2007; Swanson et al. 1997); in this context, the decrease in locomotor rearing behaviour seen following systemic administration of WAY 161503 and baclofen (Fig. 6.1C & D) is consistent with the idea that 5-HT$_{2C}$ and GABA$_B$ receptor activation may inhibit reward-related behaviour. These results are also consistent with reports indicating that the GABA$_B$ receptor agonists CGP 44532 and baclofen increase rate-current ICSS thresholds (Macey et al. 2001; Slattery et al. 2005). It is interesting to note that Slattery et al. (2005) found that only the 5.0 mg/kg (and not the 2.5 mg/kg) dose of baclofen affected ICSS thresholds – a discrepancy from the current experiment which might be explained by the parameters of that study, such as route of administration (p.o.), timing (administered 1 hr prior to experiment), methodology (use of the rate-current threshold procedure), or a combination of these factors. In our hands, using rate-frequency ICSS thresholds, the 2.5 and 5.0 mg/kg doses of baclofen produced significant decreases in RMAX values (Fig. 6.2B and unpublished observations, respectively) suggesting impaired motor performance.

The investigation of sub- and supra-threshold doses of WAY 161503 (0.3, 1.0 mg/kg) and baclofen (0.625, 1.25 mg/kg) on VTA ICSS (Fig. 6.3A-C, G) replicated the effects seen with these ligands alone (Fig. 6.3D-F; Hayes and Greenshaw 2008a) and provided evidence that their combined effects were additive. These results suggest that GABA$_B$ and 5-HT$_{2C}$ receptors affect VTA ICSS through independent mechanisms. If they worked though a similar mechanism, one might expect that the combined sub-threshold doses would produce a change in M50 values and/or that the combined supra-threshold doses would result in an interaction or M50 thresholds indistinguishable from

that seen with either treatment alone. The notion that these two receptors may affect VTA ICSS through independent mechanisms is supported by anatomical, pharmacological and electrophysiological data.

Localization studies suggest that 5-HT$_{2C}$ receptors are found postsynaptically on non-DArgic cells (Clemett et al. 2000; Eberle-Wang et al. 1997; Lopez-Gimenez et al. 2001; Pasqualetti et al. 1999) – although some have been identified on tyrosine hydroxylase-containing cells in the middle of the VTA (Bubar and Cunningham 2007). 5-HT$_{2C}$ receptors have been identified on GABAergic cells of the dorsal raphe (Serrats et al., 2005), prefrontal cortex (Liu et al. 2007) and VTA (Bubar and Cunningham 2007; Di Giovanni et al. 2001). Studies have shown that 5-HT$_{2C}$ receptor activation may inhibit the release of mesolimbic DA (Di Giovanni et al. 2000; Di Matteo et al. 1999) and it has been suggested that this occurs indirectly by increasing GABAergic activity (Boothman et al. 2006; Di Giovanni et al. 2001; Serrats et al. 2005; Stanford and Lacey 1996). Electrophysiological studies have supported this notion by demonstrating that 5-HT$_{2C}$ receptor activation excites GABAergic cells in the VTA, substantia nigra and raphe nuclei (Di Giovanni et al. 2001; Invernizzi et al. 2007; Liu et al. 2000).

Alternately, GABA$_B$ receptors have been identified as having both pre- and postsynaptic locations associated with DArgic, serotonergic, cholinergic and glutamatergic cells and, to a lesser degree, GABAergic neurons (Akiyama et al. 2004; Charara et al. 2000; Margeta-Mitrovic et al. 1999; Ng and Yung 2001; Wirtshafter and Sheppard 2001). Activation of raphe nuclei GABA$_B$ receptors results in a biphasic effect on 5-HT release – which likely depends on the balance of pre- and postsynaptic receptor activity (Abellan et al. 2000a; Abellan et al. 2000b). Activation of VTA GABA$_B$

receptors reduces DA cell firing and NAc DA release which may occur via postsynaptic GABA$_B$ receptors on DA cells (Erhardt et al. 2002; Klitenick et al. 1992; Westerink et al. 1996). DA release in the NAc may be under tonic inhibition of GABA$_B$ receptors located presynaptically on DA terminals, postsynaptically on GABA output cells to the VTA or on cholinergic interneurons (Rahman and McBride 2002).

Administering drugs of abuse results in increased DA release in the nucleus accumbens (NAc) (Goodman 2008) and both GABA$_B$ and 5-HT$_{2C}$ receptors appear to play an inhibitory role in drug-induced behaviour. Systemic (Brebner et al. 2005; Brebner et al. 2000a; Chester and Cunningham 1999; Colombo et al. 2002; Di Ciano and Everitt 2003; Le Foll et al. 2008; Li et al. 2001; Markou et al. 2004; Paterson et al. 2004; Phillis et al. 2001; Zhou et al. 2004) and intra-VTA (Bechtholt and Cunningham 2005; Brebner et al. 2000b; Kalivas et al. 1990; Sahraei et al. 2008; Zhou et al. 2005) administration of GABA$_B$ receptor agonists has been shown to attenuate drug-induced place conditioning, self-administration and locomotor activity. 5-HT$_{2C}$ receptor activation via systemically (Fletcher et al. 2002; Grottick et al. 2001; Grottick et al. 2000; Hayes et al. 2008b; Tomkins et al. 2002) or intra-VTA (Fletcher et al. 2004) administered compounds is also associated with the inhibition of drug-induced behaviour, although some exceptions may exist (Filip and Cunningham 2002; Hayes et al. 2008b). In conjunction with the present data, these studies provide evidence that the inhibitory effects of GABA$_B$ and 5-HT$_{2C}$ receptor activation on reward-related behaviours may be mediated through independent mechanisms which may ultimately result in the decrease of NAc DA release.

As NAc DA release is observed following VTA ICSS (Fiorino et al. 1993), and the NAc shell has been identified as a potential site for some of the behavioural effects of

GABA$_B$ receptor activation (Lopes et al. 2007; Plaznik et al. 1990; Stratford and Kelley 1997), the hypothesis that GABA$_B$ receptors in the NAc shell are involved in regulating changes in VTA ICSS behaviour was tested using bilateral microinjections of baclofen (0-225 ng/side). Baclofen did not affect any VTA ICSS measures (Fig. 6.4A-D), in contrast to the well established reward-enhancing effects of (+)-amphetamine (Colle and Wise 1988; Schaefer and Michael 1988). This suggests that NAc shell GABA$_B$ receptor activation may not play a primary role in regulating VTA ICSS behaviour under the present experimental conditions. This result was unexpected as NAc shell GABA$_B$ receptors have been implicated in the tonic inhibition of NAc DA release (Rahman and McBride 2002) and intra-NAc GABA$_B$ receptor agonists decrease basal locomotor activity (Plaznik et al. 1990; Wachtel and Anden 1978; Wong et al. 1991) at doses comparable to those in the present study.

It is unlikely that motor performance effects played a role in the present study as there were no changes in RMAX values and the doses used were below those required to decrease muscle tone (Stefanski et al. 1990). It is also unlikely that the present results are related to misdirected cannulae as the verified rostral NAc shell cannulae placements (Fig. 6.6C & D) are similar to those in other studies (Lopes et al. 2007; Stratford and Kelley 1997). In agreement with prior studies (Lopes et al. 2007; Stratford and Kelley 1997; Ward et al. 2000; Znamensky et al. 2001) intra-NAc baclofen increased food intake (Fig. 6.5) providing further evidence of accurate placements. In addition, the rapid onset of behavioural effects, slow injection rates and small injection volumes helped to minimize the spread of drug and suggests that the current results are due to activity at the site of injection.

Contrasting the variability of the data (particularly the perceived increase in M50 thresholds at the highest dose of baclofen tested) with the stability and reliability of ICSS behaviour over time (Carlezon and Chartoff 2007), it is possible that the present data reflects a more complex role for the NAc shell $GABA_B$ receptor in VTA ICSS. It would not be unprecedented to discover biphasic effects of intra-NAc baclofen as $GABA_B$ receptors are located both pre- and postsynaptically (Rahman and McBride 2002), intra-raphe baclofen has demonstrated dual inhibitory and disinhibitory effects on 5-HT cells (Serrats et al. 2003), and intra-NAc baclofen may display time-dependent biphasic effects on locomotor activity (Wachtel and Anden 1978; Wong et al. 1991). Further complicating any interpretation of these data is the fact that most $GABA_B$ receptors may actually be extrasynaptic (Lacey et al. 2005) and there is some evidence that these receptors may be involved in signalling from astrocytes (Kozlov et al. 2006). Nonetheless, it is possible that NAc shell $GABA_B$ receptors play a less important role (e.g. compared to $GABA_A$ receptors) in regulating DA release (Rahman and McBride 2002) and that $GABA_B$ receptors in the VTA are more involved in regulating reward-related behaviours (Willick and Kokkinidis 1995; Zhou et al. 2005).

In summary, given the data regarding systemically administered compounds, this study provides support for the hypothesis that $5-HT_{2C}$ and $GABA_B$ receptor activation regulate locomotion and VTA ICSS behaviour. Under the present conditions, this study has provided evidence that these receptors may regulate VTA ICSS through separate mechanisms. Activation of $GABA_B$ receptors in the rostral NAc shell did not have an effect on VTA ICSS suggesting that the receptors in this region may not play a key role in regulating this behaviour. Further research is needed to elucidate the precise

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mechanisms involved as each of these receptors may be involved in regulating natural reward, drug addiction and many psychiatric disorders (Bowery 2006; Dremencov et al. 2005; Goodman 2008; Hill and Reynolds 2007; Kalueff and Nutt 2007; Nilsson 2006; Siuciak et al. 2007; Stratford and Kelley 1997).

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Chapter 7: General Discussion

As outlined throughout this thesis, the 5-HT_{2C} receptor has been implicated in the regulation of dopamine (DA)- and reward-related circuitry and behaviour. The main aim of this thesis was to further explore the role of the 5-HT_{2C} receptor in reward-related behaviours, such as locomotor activity, place conditioning and intracranial self-stimulation (ICSS). Emerging evidence suggests that the 5-HT_{2C} receptor may regulate reward-related behaviours through interactions with other neurotransmitters and receptors. While these interactions are probably of great complexity, there are some clear points of potential interaction such as the fact that 5-HT_{2C}, 5-HT_{1B}, $GABA_A$, $GABA_B$, and nicotinic acetylcholine receptors have all been identified on GABAergic cells and all affect DA regulation in some way. To this end, the present studies were designed to explore the role of the 5-HT_{2C} receptor, and its potential relationship to these receptors, using ICSS as the primary model for investigating reward-related behaviour.

As each respective chapter has provided a discussion of the results pertaining to each set of experiments, this general discussion will attempt to avoid reiterating the bulk of that information. Instead, this chapter begins with a general overview of the hypotheses addressed, the results obtained, and the conclusions made in each study; it continues with a general discussion of how this information has helped to elucidate the role of the 5-HT_{2C} receptor in motivation and reward-related behaviour. Finally, the chapter ends with a brief discussion of some of the limitations of the present studies and proposes some future experiments which could be undertaken to further advance our understanding in this context.

Overview of hypotheses, experimental results, and conclusions

Chapter 2: Effects of 5-HT$_{2C}$ receptor ligands on nicotine-induced locomotor activity and place conditioning

Nicotine, the potent nicotinic acetylcholine receptor agonist, may facilitate some mesolimbic DA-related behaviours (Di Chiara 2000; Ivanova and Greenshaw 1997; Wonnacott et al. 2005), and activation of the 5-HT$_{2C}$ receptor may inhibit the release of basal (Di Giovanni et al. 2000; Di Matteo et al. 1999) and nicotine-induced DA release (Di Matteo et al. 2004; Pierucci et al. 2004). In addition, 5-HT$_{2C}$ receptor activation may inhibit some DA-related behaviours (Fletcher et al. 2004; Grottick et al. 2000; Ji et al. 2006; Mosher et al. 2005; Wilson et al. 1998), including nicotine-induced locomotor activity and self-administration (Batman et al. 2005; Fletcher et al. 2006; Grottick et al. 2001). However, while 5-HT$_{2C}$ receptor stimulation alone inhibits spontaneous locomotor activity, it does not affect place conditioning (Mosher et al. 2005) – although it may induce a state-dependent conditioned place aversion (Mosher et al. 2006).

Nicotine-sensitized locomotor activity and nicotine-induced conditioned place preference (CPP) were used in this thesis as DA-related behavioural models to test the hypotheses that:

- 5-HT$_{2C}$ receptor activation, via systemic administration of the selective agonist WAY 161503 (0-3.0 mg/kg), would attenuate nicotine-induced (0.6 mg/kg) increases in locomotor activity; this effect would be blocked by the selective 5-HT$_{2C}$ receptor antagonist SB 242084 (1.0 mg/kg).

- 5-HT$_{2C}$ receptor activation, using systemically administered WAY 161503 (0-3.0 mg/kg), would attenuate nicotine-induced (0.6 mg/kg) CPP without inducing place conditioning on its own.

Consistent with prior data (Grottick et al. 2001; Mosher et al. 2005), 5-HT$_{2C}$ receptor activation, via WAY 161503 (1.0 mg/kg), decreased locomotion when administered alone and in combination with nicotine and these effects were attenuated by the selective 5-HT$_{2C}$ receptor antagonist SB 242084 (1.0 mg/kg). These data further support an inhibitory role for the 5-HT$_{2C}$ receptor in basal and nicotine-induced locomotor activity.

Consistent with prior data (Mosher et al. 2005), 5-HT$_{2C}$ receptor activation, via WAY 161503 (1.0, 3.0 mg/kg), did not induce place conditioning. Contrary to the second hypothesis noted above, however, WAY 161503 (1.0, 3.0 mg/kg) did not attenuate nicotine-induce CPP. These data support studies demonstrating that 5-HT$_{2C}$ receptor stimulation does not induce place conditioning, but questions the notion that 5-HT$_{2C}$ receptor activation may inhibit all nicotine-induced behaviours.

Chapter 3: Effects of 5-HT$_{2C}$ receptor ligands on intracranial self-stimulation (ICSS)

As noted above, 5-HT$_{2C}$ receptor activation may inhibit DA release and some reward-related behaviour. In addition, there is evidence that the nucleus accumbens (NAc) shell may be a potential site for the inhibition of 5-HT$_{2C}$ receptor-related dopamine efflux (Navailles et al. 2006).

ICSS was used in this thesis – as electrical stimulation of the ventral tegmental area (VTA) drives self-stimulation behaviour in rats and results in dopamine release in

the nucleus accumbens (NAc) (Blaha and Phillips 1990; Fiorino et al. 1993) – to test the hypotheses that:

- 5-HT$_{2C}$ receptor activation, via systemic administration of the selective agonist WAY 161503 (0-1.0 mg/kg) and the mixed 5-HT$_{1A/1B/2C}$ receptor agonist TFMPP (0.3 mg/kg), would attenuate ICSS behaviour, as indicated by increases in frequency thresholds (M50); without affecting changes in motor performance, as indicated by maximal response rates (RMAX).

- As TFMPP's effects on behaviour are believed to be 5-HT$_{2C}$ receptor-related, its effects on ICSS thresholds would be blocked by the selective 5-HT$_{2C}$ receptor antagonist SB 242084 (1.0 mg/kg).

- 5-HT$_{2C}$ receptor activation within the NAc shell, via bilateral microinjections of WAY 161503 (0-1.5 μg/side), would result in increases in M50, similar to those seen following systemic administration of the 5-HT$_{2C}$ receptor agonists.

Consistent with the majority of studies investigating the role of the 5-HT$_{2C}$ receptor in reward-related behaviour, systemically administered WAY 161503 (1.0 mg/kg) and TFMPP (0.3 mg/kg) produced increases in M50 thresholds without affecting RMAX values. The effects of the mixed agonist TFMPP were blocked by the selective 5-HT$_{2C}$ receptor agonist SB 242084 (1.0 mg/kg). These data provide the first evidence for an inhibitory role of the 5-HT$_{2C}$ receptor in ICSS behaviour and further support an inhibitory role for the 5-HT$_{2C}$ receptor in reward-related behaviours.

Contrary to the third hypothesis above, intra-NAc shell administration of WAY 161503 (0-1.5 μg/side) did not affect VTA ICSS, although the positive control (+)-amphetamine (1.0 μg/side) did decrease M50 thresholds (indicating an increase in

reward) without affecting RMAX values. These data suggest that 5-HT$_{2C}$ receptors in the NAc shell do not play a primary role in VTA ICSS behaviour – although the possibility exists that these receptors may modulate the effects of other drugs of abuse such as cocaine (Filip and Cunningham 2002; Navailles et al. 2008).

Chapter 4: Effects of 5-HT$_{2C}$ and 5-HT$_{1B}$ receptor ligands on ICSS

As noted above, 5-HT$_{2C}$ receptor activation may inhibit DA release and some reward-related behaviour. 5-HT$_{1B}$ receptor activation may increase mesocorticolimbic DA release (Boulenguez et al. 1998; Iyer and Bradberry 1996; O'Dell and Parsons 2004; Yan and Yan 2001a; Yan et al. 2004). Interestingly, behavioural studies have indicated that although 5-HT$_{1B}$ receptor stimulation may enhance the rewarding effects of cocaine (Cervo et al. 2002; Parsons et al. 1998; Przegalinski et al. 2007), it may have the opposite effect on other drugs of abuse, such as (+)-amphetamine or ethanol (Fletcher et al. 2002; Fletcher and Korth 1999; Silvestre et al. 1998; Tomkins and O'Neill 2000); these effects may be explained by the activation of 5-HT$_{1B}$ receptors within different anatomical locations. In addition, 5-HT$_{1B}$ receptor stimulation alone may inhibit reward-related behaviours (Cervo et al. 2002; Harrison et al. 1999). Furthermore, both the 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors may be found on GABA cells (Bubar and Cunningham 2007; Liu et al. 2007; Sari et al. 1999; Serrats et al. 2005), although they are believed to have opposite effects on GABAergic function (Bankson and Yamamoto 2004; Boothman et al. 2006; Johnson et al. 1992; Parsons et al. 1999; Stanford and Lacey 1996; Yan and Yan 2001b; Yan et al. 2004).

The systemic administration of 5-HT$_{1B}$ and 5-HT$_{2C}$ receptor ligands was used, in conjunction with VTA ICSS, to test the hypotheses that:

- 5-HT_{1B} receptor activation, via systemic administration of the selective agonist CP 94253 (0-5.0 mg/kg), would potentiate ICSS behaviour, as indicated by decreases in M50 thresholds without affecting changes in RMAX values; these effects would be attenuated by the $5\text{-HT}_{1B/1D}$ receptor antagonist GR 127935 (10.0 mg/kg).

- 5-HT_{2C} receptor stimulation, via WAY 161503 (0-1.0 mg/kg), would increase M50 thresholds, and this effect would be potentiated by simultaneous 5-HT_{1B} receptor antagonism, via GR 127935 (10.0 mg/kg).

Contrary to the initial hypothesis, CP 94253 (5.0 mg/kg) produced an increase in M50 thresholds without changes in RMAX values; this effect was attenuated by the $5\text{-HT}_{1B/1D}$ receptor antagonist GR 127935 (10.0 mg/kg). In addition, administration of GR 127935 had no effect on VTA ICSS alone and did not alter increases in M50 thresholds induced by WAY 161503 (1.0 mg/kg). These data provide the first evidence for an inhibitory role for the 5-HT_{1B} receptor in VTA ICSS, using a selective 5-HT_{1B} receptor agonist; they replicate the previous finding of an inhibitory role for the 5-HT_{2C} receptor in ICSS behaviour. The possibility that 5-HT_{2C} receptor activation may inhibit DA- and reward-related behaviour through an excitatory action on GABA cells is not refuted by these data; while the possibility that CP 94253 acts via inhibitory presynaptic 5-HT_{1B} receptors on GABA cells is questioned.

Chapter 5: Effects of 5-HT_{2C} and $GABA_A$ receptor ligands on locomotor activity and ICSS

As noted above, 5-HT_{2C} receptors may affect reward-related behaviour through their stimulation of GABA cells. While both 5-HT_{2C} and $GABA_A$ receptor activation may inhibit DA release (Di Giovanni et al. 2000; Di Matteo et al. 1999; Ikemoto et al. 1997;

Rahman and McBride 2002; Westerink et al. 1996; Yan 1999), the role of the $GABA_A$ receptor in this regard is less clear (Aono et al. 2008; Klitenick et al. 1992; Oakley et al. 1991; Xi and Stein 1998; Yoshida et al. 1997). In locomotor activity studies, $5-HT_{2C}$ receptor activation has been shown to decrease locomotor activity (Gleason et al. 2001; Hayes et al. 2008b; Higgins et al. 2001; Kennett et al. 2000; Kennett et al. 1997; Lucki et al. 1989; Martin et al. 2002; Mosher et al. 2005). $GABA_A$ receptor agonists and antagonists decreased locomotion when administered systemically (Mukhopadhyay and Poddar 1995; Sienkiewicz-Jarosz et al. 2003); increased locomotion following intra-VTA administration (Kalivas et al. 1990; Oakley et al. 1991; Schwienbacher et al. 2002); and decreased and increased locomotor activity, respectively, following intra-NAc injections (Austin and Kalivas 1989; Morgenstern et al. 1984; Plaznik et al. 1990; Pycock and Horton 1979). In ICSS studies, $5-HT_{2C}$ receptor activation has been shown to increase current or frequency thresholds (Borisenko et al. 1996; Hayes et al. 2008a). Systemically administered $GABA_A$ receptor agonists may enhance, while antagonists inhibit, ICSS reward (Bossert and Franklin 2003; Gomita et al. 2003; Ichimaru et al. 1983; Porrino and Coons 1980; Zarevics and Setler 1981), although studies using intra-VTA injections of GABAergic compounds are more variable (Panagis and Kastellakis 2002; Singh et al. 1997; Willick and Kokkinidis 1995). In addition, there is evidence that the NAc shell may be a potential site for the behavioural effects of $GABA_A$ receptor activation (Lopes et al. 2007; Reynolds and Berridge 2002; Stratford and Kelley 1997).

The systemic, and intra-NAc, administration of $GABA_A$ ligands was used, in conjunction with the systemic administration of a $5-HT_{2C}$ receptor agonist, in locomotor activity and VTA ICSS to test the hypotheses that:

- GABA$_A$ receptor stimulation or antagonism, via systemic administration of the GABA$_A$ receptor agonist muscimol (0-0.75 mg/kg) and GABA$_A$ receptor antagonist picrotoxin (0-1.0 mg/kg), would result in decreases in locomotor activity; the effects of muscimol would be attenuated by picrotoxin.

- 5-HT$_{2C}$ receptor-related decreases in locomotor activity, via WAY 161503 (0-3.0 mg/kg), would be attenuated by picrotoxin (0.25 mg/kg).

- GABA$_A$ receptor stimulation, via systemic administration of muscimol (0-4.0 mg/kg), would increase M50 thresholds; GABA$_A$ receptor antagonism, via systemic administration of picrotoxin (0-1.0 mg/kg), would decrease M50 thresholds; the effects of muscimol would be attenuated by picrotoxin.

- GABA$_A$ receptor activation within the NAc shell, via bilateral microinjections of muscimol (0-225 ng/side), would result in increases in M50 thresholds, similar to those seen following systemic administration of GABA$_A$ or 5-HT$_{2C}$ receptor agonists; these effects would be unaltered or potentiated by systemic administration of the 5-HT$_{2C}$ receptor agonist WAY 161503 (1.0 mg/kg).

- GABA$_A$ receptor antagonism within the NAc shell, via bilateral microinjections of picrotoxin (125 ng/side), would result in decreases in M50 thresholds, similar to those seen following systemic administration of the GABA$_A$ receptor antagonist; these effects would be attenuated by systemic administration of the 5-HT$_{2C}$ receptor agonist WAY 161503 (1.0 mg/kg).

Consistent with the literature, systemic administration of the GABA$_A$ receptor agonist muscimol (0.10-0.75 mg/kg) and the antagonist picrotoxin (0.25-1.0 mg/kg) resulted in a decrease in locomotor activity; these effects were attenuated via co-

administration of muscimol (0.10 mg/kg) and picrotoxin (0.25, 0.50 mg/kg). Also consistent with the literature, 5-HT_{2C} receptor activation, via WAY 161503 (0.3-3.0 mg/kg), produced decreases in locomotor activity. Contrary to the second hypothesis noted above, however, the 5-HT_{2C} receptor-related effects were not affected by picrotoxin (0.25 mg/kg). These data suggest that the $GABA_A$ receptor may play a role in the regulation of locomotor behaviour, but that decreases in locomotor activity related to stimulation of the 5-HT_{2C} receptor may be independent of $GABA_A$ receptor-related effects.

Somewhat consistent with the ICSS literature, $GABA_A$ receptor activation, via muscimol (4.0 mg/kg), produced increases in M50 thresholds. However, these effects appear to be non-specific for reward and the $GABA_A$ receptor, as a notable decrease in RMAX values was noted and picrotoxin (0.25-1.0 mg/kg) failed to attenuate these effects. Contrary to the literature, picrotoxin (1.0 mg/kg) increased M50 thresholds; this effect was not coupled with changes in RMAX values, but may be related to the anxiogenic properties of picrotoxin. These data suggest that the $GABA_A$ receptor may be involved in VTA ICSS behaviour (although it is difficult to ascertain their impact on reward), and that data related to the systemic administration of GABAergic compounds must be interpreted with caution.

Consistent with the self-administration data (Gavello-Baudy et al. 2008), but in opposition to data involving feeding and place conditioning (Lopes et al. 2007; Reynolds and Berridge 2002; Stratford and Kelley 1997), intra-NAc shell administration of muscimol decreased reward, as indicated by increased M50 thresholds without changes in RMAX values. In addition, these effects were comparable to those seen following

systemic administration of WAY 161503 (1.0 mg/kg); WAY 161503 (1.0 mg/kg) and muscimol (225 ng/side) together did not produce results different from either treatment alone. These results suggest an inhibitory role for NAc shell $GABA_A$ receptors and are consistent with the hypothesis that $5\text{-}HT_{2C}$ and $GABA_A$ receptors act through a similar mechanism to regulate VTA ICSS behaviour. Further support of this hypothesis was seen with the data showing that intra-NAc injections of picrotoxin (125 ng/side) produced decreases in M50 thresholds, without changes in RMAX values; the threshold-increasing effect of WAY 161503 (1.0 mg/kg) was attenuated by intra-NAc picrotoxin.

Chapter 6: Effects of $5\text{-}HT_{2C}$ and $GABA_B$ receptor ligands on locomotor activity and ICSS

As noted above, $5\text{-}HT_{2C}$ receptor activation may affect reward-related behaviour by stimulating GABA cells; in particular, $5\text{-}HT_{2C}$ receptors may interact with the $GABA_A$ receptor to regulate VTA ICSS behaviour. In addition, activation of either $5\text{-}HT_{2C}$ or $GABA_B$ receptors may inhibit DA release (Di Giovanni et al. 2000; Di Matteo et al. 1999; Erhardt et al. 2002; Fadda et al. 2003; Klitenick et al. 1992; Rahman and McBride 2002; Westerink et al. 1996). As noted above, $5\text{-}HT_{2C}$ receptor activation has been shown to decrease locomotor activity. $GABA_B$ receptor activation has been shown to decrease basal locomotor activity in most studies (Agmo and Giordano 1985; Cryan et al. 2004; Gianutsos and Moore 1978; McManus and Greenshaw 1991; Nissbrandt and Engberg 1996; Plaznik et al. 1990; Wachtel and Anden 1978; Wong et al. 1991), although some studies have reported biphasic (Hotsenpiller and Wolf 2003; Le Foll et al. 2008) or no (Chester and Cunningham 1999; Phillis et al. 2001) effects. ICSS studies have shown that $5\text{-}HT_{2C}$ receptor activation may produce decreases in reward, while $GABA_B$ receptor

stimulation may decrease (Fenton and Liebman 1982; Liebeman and Prowse 1980; Macey et al. 2001; Panagis and Kastellakis 2002; Willick and Kokkinidis 1995), or have no effect on (Dobrovitsky et al. 2002; Slattery et al. 2005), reward-related changes in ICSS behaviour. In addition, there is evidence that the NAc shell may be a potential site for the behavioural effects of $GABA_B$ receptor activation (Lopes et al. 2007; Plaznik et al. 1990; Stratford and Kelley 1997).

The systemic, and intra-NAc, administration of a $GABA_B$ agonist was used, in conjunction with the systemic administration of a $5\text{-}HT_{2C}$ receptor agonist, in locomotor activity and VTA ICSS to test the hypotheses that:

- $GABA_B$ receptor stimulation, via systemic administration of the $GABA_B$ receptor agonist baclofen (1.25 mg/kg), would result in a decrease in locomotor activity; an interaction would be noted following systemic administration of the $5\text{-}HT_{2C}$ receptor agonist WAY 161503 (0-1.0 mg/kg) and baclofen.

- $GABA_B$ receptor stimulation, via systemic administration of baclofen (0-2.5 mg/kg), would increase M50 thresholds without effects on RMAX, comparable to systemically administered WAY 161503 (1.0 mg/kg); an interaction would be noted following co-administration of baclofen (0.625, 1.25 mg/kg) and WAY 161503 (0.3, 1.0 mg/kg).

- $GABA_B$ receptor activation within the NAc shell, via bilateral microinjections of baclofen (0-225 ng/side), would result in increases in M50 thresholds, similar to those seen following systemic administration of $GABA_B$ or $5\text{-}HT_{2C}$ receptor agonists; these effects would be unaltered or potentiated by systemic administration of the $5\text{-}HT_{2C}$ receptor agonist WAY 161503 (1.0 mg/kg).

Consistent with the literature, 5-HT$_{2C}$ receptor activation via WAY 161503 (0.3, 1.0 mg/kg) produced decreases in locomotor activity. Although the 1.25 mg/kg dose of the GABA$_B$ receptor agonist baclofen was chosen for its reported sub-threshold effects on locomotion (Agmo and Giordano 1985; Le Foll et al. 2008), this drug decreased all measures of locomotor activity. Contrary to the second hypothesis noted above, the 5-HT$_{2C}$ receptor-related effects were additive with baclofen; no statistical interaction was noted. These data suggest that the GABA$_B$ receptor may play a role in the regulation of locomotor behaviour, but that decreases in locomotor activity related to stimulation of the 5-HT$_{2C}$ receptor may be independent of GABA$_B$ receptor-related effects.

Consistent with the ICSS literature, GABA$_B$ receptor activation, via baclofen (1.25, 2.5 mg/kg), produced increases in M50 thresholds, although the effects of the highest dose may be non-specific as a notable decrease in RMAX values was noted. In addition, sub- and supra-threshold doses of WAY 161503 (0.3, 1.0 mg/kg) and baclofen (0.625, 1.25 mg/kg) on VTA ICSS replicated the effects seen with these ligands alone and provided evidence that their combined effects were additive. These data suggest that GABA$_B$ and 5-HT$_{2C}$ receptors may both be involved in the regulation of VTA ICSS behaviour, although increases in M50 thresholds related to stimulation of the 5-HT$_{2C}$ receptor may be independent of GABA$_B$ receptor-related effects.

Contrary to data indicating that NAc shell GABA$_B$ receptors may be involved in inhibiting DA- and reward-related behaviours (Brebner et al. 2002; Brebner et al. 2000; Plaznik et al. 1990; Shoaib et al. 1998; Wachtel and Anden 1978; Wong et al. 1991), intra-NAc shell administration of baclofen did not affect any VTA ICSS measures, although the positive control (+)-amphetamine (1.0 µg/side) did decrease M50 thresholds

(indicating an increase in reward) without affecting RMAX values. These data suggest that $GABA_B$ receptors in the NAc shell do not play a primary role in VTA ICSS behaviour. However, these data should be interpreted with caution. The variability of the data (particularly the perceived increase in M50 thresholds at the highest dose of baclofen tested) suggests the possibility of a more complex role for NAc shell $GABA_B$ receptors in VTA ICSS. For example, this variability may reflect biphasic effects due to a functional balance between activation at pre-, post-, and extrasynaptic receptors.

What these results suggest about the role of the 5-HT$_{2C}$ receptor in motivation and reward-related behaviour

By exploring the selective activation of this receptor, and its potential relationship to other receptors, using locomotor activity, place conditioning, and VTA ICSS, the experimental results presented in this thesis have helped to elucidate the role of the 5-HT$_{2C}$ receptor in reward-related behaviour.

As discussed above, increases in mesocorticolimbic DA release are broadly associated with increases in reward-related behaviours. As the 5-HT$_{2C}$ receptor appears to play an inhibitory role in the regulation of DA release in this system, the main prediction in this thesis was that 5-HT$_{2C}$ receptor stimulation would inhibit reward-related behaviours. Because these receptors are found largely postsynaptically on non-dopaminergic cells, including cholinergic (Lopez-Gimenez et al. 2001; Pasqualetti et al. 1999; Pompeiano et al. 1994), glutamatergic (Stein et al. 2000) and GABAergic cells (Bubar and Cunningham 2007; Eberle-Wang et al. 1997; Liu et al. 2007; Serrats et al.

2005), potential interactions between the 5-HT$_{2C}$ receptor and other receptors were also investigated.

Experiments from chapter 2 extended data investigating the role of the 5-HT$_{2C}$ receptor in locomotor activity and place conditioning. Locomotor activity is sensitive to changes in DA and can be used to compare the potential motor effects of drugs (Mogenson et al. 1980; Pijnenburg et al. 1975). Nicotine administration results in increased mesocorticolimbic DA release and may underlie its ability to increase locomotor activity (Balfour et al. 2000; Clarke et al. 1988; Imperato et al. 1986; Nisell et al. 1997; Nisell et al. 1996). Inhibition of basal and nicotine-induced locomotor activity following stimulation of the 5-HT$_{2C}$ receptor is consistent with the literature and supports a general inhibitory role for the 5-HT$_{2C}$ receptor in locomotor activity.

The inability of 5-HT$_{2C}$ receptor stimulation to inhibit nicotine-induced CPP was unexpected. There are a few possible explanations for the differential results between the locomotor and place conditioning data. For instance, as discussed above, the nigrostriatal and mesolimbic pathways are broadly associated with regulating motivated and sensorimotor aspects of behaviour, respectively. 5-HT$_{2C}$ receptor activation has been shown to attenuate increases in DA release induced by acute nicotine administration in the dorsal striatum, but not in the NAc or VTA (Di Matteo et al. 2004; Pierucci et al. 2004). In this context, 5-HT$_{2C}$ receptor-related inhibition of nicotine self-administration may take place because nicotine is administered repeatedly during the training phase; over a longer period of time compared to the present CPP experiments (i.e. four treatments) (Grottick et al. 2001). Another possibility involves the notion that nicotine-induced CPP may be dopamine-independent (Laviolette and van der Kooy 2003); this is

supported by data demonstrating that nicotinic receptor-stimulated increases in NAc DA levels and locomotor activity do not always correspond with the establishment of CPP (Janhunen and Ahtee 2004; Janhunen et al. 2005) and some direct manipulations of DA signalling may differentially affect nicotine-induced CPP and locomotor activity (Le Foll et al. 2005).

It is important to note, however, that nicotine's rewarding effects may be related to direct activation of DA cells in the VTA as well as its desensitizing effects on GABA cells (Ferrari et al. 2002; Mansvelder et al. 2002; Nisell et al. 1994; Pontieri et al. 1996; Sziraki et al. 2002); 5-HT$_{2C}$ receptors have been identified on GABAergic cells in the VTA, prefrontal cortex and raphe nuclei (Bubar and Cunningham 2007; Liu et al. 2007; Serrats et al. 2005) and their activation has been associated with increased GABA cell activity (Bankson and Yamamoto 2004; Boothman et al. 2006; Stanford and Lacey 1996). While the relationship between nicotine's rewarding effects and GABAergic activity was not addressed, the possibility that the 5-HT$_{2C}$ receptor may act through a GABAergic mechanism to indirectly inhibit DA-related behaviours was addressed in chapters 5 and 6.

Experiments from chapter 3 provided the first clear evidence of a role for the 5-HT$_{2C}$ receptor in ICSS behaviour, and suggested that 5-HT$_{2C}$ receptors in the NAc shell may not be primarily involved. As noted above, VTA ICSS provides an excellent animal model for the investigation of reward-related behaviour as it directly stimulates brain regions thought to be involved in motivation and reward and results in increased mesolimbic DA release, believed to be involved in mediating reward signals (Fouriezos and Wise 1976; Gallistel and Freyd 1987; Gallistel and Karras 1984; Wise 1996). That 5-

HT$_{2C}$ receptor stimulation increased rate-frequency (M50) thresholds without affecting maximum response rates (RMAX), indicative of performance effects, is consistent with the literature suggesting an inhibitory role for this receptor in reward-related behaviours.

The inability of intra-NAc 5-HT$_{2C}$ receptor stimulation to increase M50 thresholds was unexpected, although the positive control (+)-amphetamine did decrease M50 thresholds. Although systemic, intra-NAc, and intra-VTA, administration of 5-HT$_{2C}$ receptor antagonists attenuates decreases in DA release following systemic administration of a 5-HT$_{2C}$ receptor agonist (Di Giovanni et al. 2000; Di Matteo et al. 1999; Navailles et al. 2006), and intra-NAc 5-HT$_{2C}$ receptor activation produces biphasic effects on cocaine-induced DA release (Navailles et al. 2008), no studies to date have reported effects on basal DA efflux or behaviour following intra-NAc 5-HT$_{2C}$ receptor stimulation (Filip and Cunningham 2002; Navailles et al. 2008). These data suggest that NAc shell 5-HT$_{2C}$ receptor stimulation may not affect VTA ICSS behaviour, but do not rule out a role for 5-HT$_{2C}$ receptor blockade, as the 5-HT$_{2C}$ receptor is known to have constitutive activity (Berg et al. 2005; Teitler et al. 2002; Westphal et al. 1995). Nonetheless, these data suggest that activation of these receptors in other brain areas, such as the VTA, dorsal raphe, or prefrontal cortex may be important in the regulation of VTA ICSS; interactions with other neurotransmitter systems may be important for relaying reward-related signals in the NAc.

Experiments from chapter 4 investigated the potential interaction between 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors and provided the first clear evidence of a role for the 5-HT$_{1B}$ receptor in ICSS behaviour. 5-HT$_{1B}$ receptor stimulation increased M50 thresholds without affecting RMAX values. As noted above, and discussed in greater detail in

chapter 4, an interpretation of these data is complicated by the literature suggesting that 5-HT$_{1B}$ receptor stimulation may increase mesolimbic DA release and enhance cocaine reinforcement. Nonetheless, these data are consistent with the literature suggesting an inhibitory role for this receptor in other reward-related behaviours, such as place conditioning, ICSS, and (+)-amphetamine and ethanol self-administration. These data do not support the hypothesis that 5-HT$_{1B}$ receptors alter VTA ICSS behaviour through their activity as GABAergic heteroceptors (Hurley et al. 2008; Johnson et al. 1992; Parsons et al. 1999; Yan and Yan 2001b; Yan et al. 2004) whose activation leads to an increase in DA efflux (Boulenguez et al. 1998; Boulenguez et al. 1996; Iyer and Bradberry 1996; O'Dell and Parsons 2004; Yan and Yan 2001a; Yan et al. 2004). Indeed, these results may underscore the fact that DA release can not be simply correlated to reward-related behaviour (Alcaro et al. 2007; Guarraci and Kapp 1999; Salamone 1994).

That 5-HT$_{1B}$ receptor antagonism did not affect 5-HT$_{2C}$ receptor-related increases in M50 thresholds is consistent with the literature indicating that reward-related behaviours do not appear to be under tonic control of the 5-HT$_{1B}$ receptor (Cervo et al. 2002; Fletcher et al. 2002; Fletcher and Korth 1999; Parsons et al. 1998; Tomkins and O'Neill 2000). Nonetheless, if 5-HT$_{2C}$ receptors act to increase GABAergic tone, and 5-HT$_{1B}$ heteroceptors on GABAergic terminals act to inhibit GABA release, one would expect to uncover an interaction between these receptors under conditions in which 5-HT transmission may be increased – as is the case with ICSS (Fiorino et al. 1993; Ishida et al. 2001; Nakahara et al. 2000). In addition, it seems unlikely that stimulation of 5-HT$_{1B}$ autoreceptors is involved in increasing M50 thresholds, as stimulation of presynaptic 5-HT$_{1A}$ autoreceptors induces a CPP (Fletcher et al. 1993; Papp and Willner 1991;

Shippenberg 1991) and decreases M50 thresholds in VTA ICSS (Ahn et al. 2005; Fletcher et al. 1995).

Experiments from chapter 5 investigated the potential interaction between $GABA_A$ and $5-HT_{2C}$ receptors and provided evidence of a role for the $GABA_A$ receptor in locomotor activity and ICSS behaviour. Consistent with the literature, systemic administration of a $GABA_A$ receptor agonist and antagonist, respectively, produced decreases in locomotor activity; these data may be due to a functional balance between activation at pre- and postsynaptic receptors. The inability of $GABA_A$ receptor antagonism to affect $5-HT_{2C}$ receptor-related decreases in locomotor activity suggests that these receptors affect locomotor activity through independent mechanisms.

Systemic administration of the $GABA_A$ receptor compounds resulted in ICSS data that was difficult to interpret, but did not exclude a role for the $GABA_A$ receptor in this context. Intra-NAc shell $GABA_A$ receptor stimulation resulted in increased M50 thresholds, comparable to those seen following $5-HT_{2C}$ receptor stimulation, while intra-NAc shell $GABA_A$ receptor antagonism resulted in decreased M50 thresholds. These data are consistent with the notion that GABAergic transmission in the NAc may inhibit reward signalling (Rahman and McBride 2002; Yan 1999). In addition, the effects of $5-HT_{2C}$ receptor stimulation were unaltered by NAc shell $GABA_A$ receptor stimulation and were blocked by $GABA_A$ receptor antagonism. These data are consistent with idea that $5-HT_{2C}$ receptor activation decreases mesolimbic DA efflux indirectly by increasing GABAergic activity (Boothman et al. 2006; Di Giovanni et al. 2001; Serrats et al. 2005; Stanford and Lacey 1996). These results support the hypothesis that $5-HT_{2C}$ receptor stimulation increases M50 thresholds by increasing NAc shell GABA transmission.

Experiments from chapter 6 investigated the potential interaction between GABA$_B$ and 5-HT$_{2C}$ receptors and provided evidence of a role for the GABA$_B$ receptor in locomotor activity and ICSS behaviour. Consistent with the literature, systemic administration of a GABA$_B$ receptor agonist produced decreases in locomotor activity. GABA$_B$ receptor stimulation produced additive effects when combined with 5-HT$_{2C}$ receptor stimulation, suggesting that these receptors affect locomotor activity through independent mechanisms.

Systemic administration of a GABA$_B$ receptor agonist resulted in increases in M50 thresholds, without effects on RMAX values, in ICSS behaviour. Co-administration with a 5-HT$_{2C}$ receptor agonist revealed that these two receptors may affect VTA ICSS behaviour through separate mechanisms. If they worked though a similar mechanism, one might expect to observe: a statistically significant interaction; a change in M50 values following the combination of sub-threshold doses; M50 thresholds indistinguishable from that seen with either treatment alone. The inability of intra-NAc GABA$_B$ receptor stimulation to affect ICSS measures was unexpected. Although intra-NAc administration of a GABA$_B$ receptor antagonist resulted in increased NAc DA efflux, without changes in VTA DA release (Rahman and McBride 2002), only a few studies have investigated the effects of intra-NAc GABA$_B$ receptor ligands on locomotor activity (Plaznik et al. 1990), feeding (Lopes et al. 2007; Stratford and Kelley 1997), and self-administration (Brebner et al. 2000; Shoaib et al. 1998; Xi and Stein 1999).

GABA$_B$ receptors have been identified as having both pre- and postsynaptic locations associated with dopaminergic, serotonergic, cholinergic and glutamatergic cells and, to a lesser degree, GABAergic neurons (Akiyama et al. 2004; Charara et al. 2000;

Margeta-Mitrovic et al. 1999; Ng and Yung 2001; Wirtshafter and Sheppard 2001); biphasic effects have been noted in a number of studies (Abellan et al. 2000a; Abellan et al. 2000b; Hotsenpiller and Wolf 2003; Le Foll et al. 2008; Serrats et al. 2003; Tao et al. 1996; Wachtel and Anden 1978; Wong et al. 1991). In addition, as discussed above, $GABA_B$ receptors may be located largely at extrasynaptic sites, suggesting that, unlike most $GABA_A$ receptors, they may require higher synaptic concentrations of GABA to be activated. These facts suggest the possibility that the present results may reflect a more complex role for $GABA_B$ receptors in the NAc shell and underscore the need for future studies. In this context, it is possible that $5\text{-}HT_{2C}$ receptor-mediated effects may partially rely on $GABA_B$ receptors in the NAc shell – this experiment was not undertaken in the present thesis as the effects of intra-NAc shell $GABA_B$ receptor activation were not clear – however, a partial reliance on $GABA_B$ receptors seems unlikely given the systemic data noted above and the data suggesting that $GABA_A$ receptors in the NAc shell may be required for $5\text{-}HT_{2C}$ receptor-mediated ICSS effects.

In summary, $5\text{-}HT_{2C}$ receptors appear to play an inhibitory role in regulating motivation and reward-related behaviours. Although $5\text{-}HT_{2C}$ receptor activity may also inhibit conditioned responding associated with some drugs of abuse (Filip 2005; Ji et al. 2006; Wilson et al. 2000; Wilson et al. 1998), their inability to affect nicotine-induced place conditioning in the present studies suggests that these effects may be drug-specific. While $5\text{-}HT_{2C}$ receptor activation has been shown to inhibit other nicotine-induced behaviours, the present results call into question the potential usefulness of $5\text{-}HT_{2C}$ receptor agonists as effective treatments for nicotine addiction (Fletcher et al. 2008). The present studies also provided evidence that the inhibitory effects of $5\text{-}HT_{1B}$ and $GABA_B$

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receptor activation on reward-related behaviour may be independent from the effects of 5-HT_{2C} receptor activation; the effects of 5-HT_{2C} receptor activation may be mediated through a $GABA_A$ receptor-related mechanism. In addition, 5-HT_{2C} receptors in the NAc shell do not, while $GABA_A$ receptors do, appear to play a key role in the primary rewarding effects of VTA ICSS. Together, these results may help us to better understand the reward-related circuitry which may underlie many psychiatric disorders such as depression, schizophrenia, and drug addiction. This information may also assist in the development of new pharmacotherapies aimed to alleviate these disorders through targeted and selective interactions with appropriate receptors.

Limitations

There are several methodological limitations which should be kept in mind when interpreting data from the present, and related, studies. Major limitations of the present work are related to pharmacological selectivity, microinjections, interpretation of place conditioning data, and interpretation of ICSS data.

The selectivity of a ligand for its target receptor is an important factor in the correct interpretation of experimental data. Only in recent decades have ligands which are highly selective for 5-HT receptor subtypes been developed; studies employing the use of non-selective ligands must always be interpreted with caution. Therefore, it is important to note that many of the ligands in the present thesis were chosen for their high receptor selectivity.

WAY 161503 and SB 242084 were chosen for their high selectivity at the 5-HT_{2C} receptor (Kennett et al. 1997; Rosenzweig-Lipson et al. 2006; Schlag et al. 2004). WAY

161503 acts as full 5-HT$_{2C}$ receptor agonist in stimulating 5-HT$_{2C}$ receptor-coupled inositol phosphate formation and calcium mobilization (Rosenzweig-Lipson et al. 2006) and its effects in behavioural studies are believed to be 5-HT$_{2C}$ receptor-mediated (Cryan and Lucki 2000; Egashira et al. 2007; Hayes et al. 2008b; Mosher et al. 2005). TFMPP was chosen as many studies have suggested its behavioural effects to be 5-HT$_{2C}$ receptor-mediated (Kennett and Curzon 1988b; Lucki et al. 1989; Mora et al. 1997), although some studies have identified behavioural effects for TFMPP that appear to be 5-HT$_{1B}$ receptor-mediated (Kennett and Curzon 1988a; Rodriguez-Manzo et al. 2002; Schechter 1988). CP 94253 is highly selective for the 5-HT$_{1B}$ receptor (Koe 1992); GR 127935 is a highly selective 5-HT$_{1B/1D}$ receptor antagonist (Skingle et al. 1996; Skingle et al. 1995), although as noted above, 5-HT$_{1D}$ receptor expression is believed to be relatively low in the brain (Bonaventure et al. 1998). It is also important to note that there is some evidence that GR 127935 may act as a partial agonist in some instances (Pauwels 1997). Muscimol is highly selective for the GABA$_A$ receptor (Chan-Palay 1978; Chebib and Johnston 1999; Jansen et al. 2008); picrotoxin is a highly selective non-competitive antagonist for the GABA$_A$ receptor (Newland and Cull-Candy 1992; Quast and Brenner 1983). Another highly selective, but competitive, antagonist at the GABA$_A$ receptor, bicuculline, was not chosen for use in the present experiments. This was because studies have suggested that it may be unstable at physiological pH levels, it is not very soluble in aqueous solutions, and it may have effects on ion channels other than the GABA$_A$ receptor (Heyer et al. 1981; Olsen et al. 1976; Olsen et al. 1975; Zhang and Feltz 1991). The use of bicuculline salts may avoid the two former issues, however, their non-GABA$_A$ receptor related effects are still prominent (Debarbieux et al. 1998; Johnson and Seutin

1997; Seutin et al. 1997). Baclofen is highly selective for the GABA$_B$ receptor (Kaupmann et al. 1997; Kerr et al. 1987).

Intracranial microinjections are commonly used to investigate the role of specific brain regions in various behaviours. However, this technique does have limitations which should warrant a careful interpretation of results (Malpeli 1999; McBride et al. 1999). For instance, it is difficult to accurately target the site of interest because, while injections with low volumes generally diffuse in uniform circular gradients, most brain structures are not circular in shape; this may result in drug effects on surrounding brain areas or incomplete effects on the site of interest. In the present studies, slow injection rates (0.2 μl/min), small injection volumes (0.5 μl), and histological verification of microinjection sites, helped to accurately target the site of interest.

A number of factors may affect experimental outcomes in the place conditioning method, such as: the number and length of conditioning sessions, the type of environmental (i.e. conditioned) stimuli paired with the natural or drug (i.e. unconditioned) stimuli, the use of natural vs. drug stimuli, animal strain and age, timing and route of administration of unconditioned stimuli, state dependency (i.e. some stimuli only induce place conditioning when administered on the post-conditioning test day), novelty seeking (i.e. some reports have shown that low, or high, novelty seeking animals are more, or less, likely to display place conditioning), and the use of the biased design procedure vs. a biased apparatus. This latter issue was discussed in chapter 2 of this thesis, and has been a source of great confusion throughout the literature. For a more in-depth discussion of methodological issues, see reviews by Bardo and Bevins (2000), Schechter and Calcagnetti (1998), and Tzchentke (2007).

As reviewed extensively by Ranck (1975) and Yeomans (1990), many factors may influence experimental outcomes using the ICSS method. As discussed in chapter 1, many methods of ICSS use parameters that can not differentiate between reward-related and performance-related effects. The rate-frequency and rate-current threshold methods are best for differentiating these effects. Changes in frequency (i.e. rate-frequency threshold analysis) are used within the present thesis, compared to changes in current, as changes in frequency have been shown to alter the firing rates of neurons without affecting the population of cells activated while increases in current are positively correlated to the number of stimulated cells. Analogous to the issues noted above with microinjections, electrical stimulation can 'diffuse' to nearby brain areas in a manner that is proportional to current intensity. In this regard, the use of low currents (usually below 200 µA) helped to accurately target the VTA. Other issues include: the use of cathodal (negative) over anodal stimulation – as anodal stimulation results in iron deposits at the site of stimulation and requires higher currents for comparable effects; the use of a resistor between pulses to cancel any effects of electrode polarisation; the use of a constant-current (not a constant-voltage) stimulator, as the stimulation pulses will remain constant.

Future research

The studies in this thesis have revealed many results and raised a number of interesting questions. An increasing number of behavioural studies investigating motivation and reward, and the development and availability of highly selective ligands for 5-HT receptor subtypes, make it possible to devise many interesting experiments. Some

potential experiments may include obvious extensions of the work outlined in this thesis, such as exploring the effects of: 5-HT$_{2C}$ receptor stimulation on place conditioning induced by other drugs of abuse; 5-HT$_{2C}$ receptor stimulation on nicotine self-administration; chronic administration of 5-HT$_{2C}$ receptor ligands on ICSS; intra-VTA, intra-raphe, intra-prefrontal cortex, or intra-ventral pallidum administration of 5-HT$_{2C}$ receptor ligands in ICSS; more doses of intra-NAc shell GABA$_A$ receptor ligands in conjunction with 5-HT$_{2C}$ receptor stimulation in ICSS; more doses of intra-NAc shell GABA$_B$ receptor ligands.

In addition to further work investigating the potential role of 5-HT$_{2C}$ receptor interactions with the GABAergic and cholinergic systems, interactions with the glutamatergic system should also be explored. Glutamatergic inputs to the VTA and NAc have been implicated in mediating DA- and reward-related behaviours (Biondo et al. 2005; Choi et al. 2005; Clements and Greenshaw 2005; Gerdjikov and Beninger 2006; Shabat-Simon et al. 2008). There is evidence that 5-HT$_{2C}$ receptors may be located on glutamatergic cells in the prefrontal cortex, amygdala, and hippocampus (Clemett et al. 2000; Liu et al. 2007; Stein et al. 2000). To date, no studies have been published regarding 5-HT$_{2C}$ receptor-glutamate interactions.

Future experiments in this field will aim to further clarify the role of the various receptor subtypes in motivation and reward-related behaviour. With an increase in the development and availability of ligands selective for receptor subtypes, future studies will be able to further differentiate the effects of each subtype. Studies will also be needed to help connect the significance of these results to the human brain; this work has already begun (de Greck et al. 2007; O'Doherty 2004; Volkow et al. 2008). Together, these

studies may ultimately lead to a better understanding of the complex interactions between neurotransmitter systems; may result in the elucidation of the brain's emotional circuitry and lead to the development of more effective therapies for the treatment of many psychiatric disorders, such as depression, schizophrenia, and substance abuse.

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Appendix A: Drug preparation

All drug doses are expressed as free-base. The formula, true molecular weight, and actual molecular weight needed to calculate free-base concentrations are in Table A.1:

Drug	Formula	Free base molecular weight	Salt molecular weight
WAY 161503 hydrochloride	$C_{11}H_{11}Cl_2N_3O \cdot HCl$	272.13	308.59
SB 242084	$C_{21}H_{19}ClN_4O_2 \cdot 2HCl$	394.86	467.78
TFMPP hydrochloride	$C_{11}H_{13}F_3N_2 \cdot HCl$	193.77	230.23
CP 94253 hydrochloride	$C_{15}H_{19}N_3O \cdot HCl$	257.34	293.80
GR 127935 hydrochloride	$C_{29}H_{31}N_5O_3 \cdot HCl$	497.60	534.06
(+) α-Methylphenethylamine sulphate	$C_9H_{13}N \cdot {}^1/_2H_2SO_4$	135.21	184.25
(–)-Nicotine hydrogen tartrate salt	$C_{10}H_{14}N_2 \cdot 2C_4H_6O_6$	162.22	462.40

Muscimol, picrotoxin, and baclofen are not salts; i.e. no conversion to free-base was required. All drugs came in the form of solids, were stable at room temperature, and were stored with desiccant if necessary. All solutions were made daily in a volume of 1 ml/kg and determined to be between pH levels of 6.0–7.0.

Appendix B: Artificial cerebrospinal fluid preparation

Artificial cerebrospinal fluid (CSF) was freshly prepared according to Elliot and Lewis (1950). Three solutions (A, B, C) are required to prepare the final CSF solution (D).

Solution A: alkaline-salt stock solution

Dissolve the following reagents in 1 liter of double distilled water: sodium chloride (236 g), sodium carbonate (92 g), potassium chloride (12 g), disodium hydrogen phosphate dodecahydrate (8 g).

Solution B: acid-salt stock solution

Dissolve the following reagents, with gentle warming, in 300 ml concentrated hydrochloric acid: calcium chloride (24 g), magnesium chloride hexahydrate (12 g).

Solution C: glucose solution (made fresh each day)

Dissolve dextrose anhydrous (0.4 g) in 25 ml double distilled water.

Solution D: artificial CSF

1. In a 100 ml flask, dilute 2.5 ml solution A to 100 ml double distilled water.

2. In a 250 ml flask, combine the entire solution from step 1 with 5 ml of solution C.

3. Titrate to pH ~7.0 with solution B.

4. Suction filter solution from step 3 into a 250 ml suction flask through a 0.2 μm nylon membrane filter

5. De-gas using the separator filter on same apparatus for at least 10 min.

6. Aliquot the solution into 1.5 ml microfuge tubes for in vivo injection. Solution should be used within hours as the pH will change over time.

Appendix C: Standard operating procedure (SOP) for central implantation and microinjection

The following SOP was adapted from Clements (2005).

In vivo implantation of electrodes and cannulae in rats

The care and use of animals were in accordance with guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care. Animals that undergo in vivo implantation of cannulae and/or electrodes (i.e. probes) must recover completely in order to perform behavioural testing over the course of many months. Aseptic technique is required to prevent infection and maximize the lifetime of the implant. The surgeon should wear a facemask, back-fastening gown with tight arm cuffs, and latex gloves disinfected frequently with washes involving antiseptic hand cleanser. The surgical field and surrounding areas are cleaned with bleach. The following serves as a general list of surgical supplies required for implantation:

Bead sterilizer, Dremel drill, dental burr tip, drill bit and pin vise, flathead jeweler's screwdriver, cordless hair clippers, heat lamp, narrow-wire cage lids without food/water separator, paper towels, cotton-tipped applicators, Kimwipe tissues, weigh boats (for saline, keeping surgical parts in 70% ethanol, and dental cement), saline, 70% ethanol, 3-4 jeweler's screws, scalpel with #10 blade, spatula (large), spatula (small), forceps (with teeth), forceps (fine), 4 hemostats, for 100 ml beaker (for Germex solution), Stereotaxic frame, cauterizer with 2 AA batteries, suture with curved needle, isoflurane, Germex, orthodontic resin and cement, electrode, cannulae, pedestal, ground, Xylocaine.

All instruments are cleaned and immersed in Germex solution overnight before surgery; they are sterilized in the bead sterilizer for at least 10 min at 200-300°C and subsequently kept in Germex throughout the procedure. Surgical parts to be implanted (e.g. screws, pedestal, ground electrode) should be placed in 70% ethanol throughout the procedure.

All components to be implanted are aligned and zeroed using the stereotaxic apparatus. Stereotaxic coordinates are determined using Paxinos and Watson (1998); coordinates were interpolated from the target site (Greenshaw 1997). To zero each component, the tip (of the electrode or cannula) is centered in three dimensions in the gap produced by the each ear bar when it is set to 0.2 mm (for the electrode centering) or 0.5 mm (for cannulae centering). Animals (weighing 200-250 g upon arrival to the colony) undergo surgery following a minimum of two days in the colony and when their weight is greater than 250 g. The animal is initially anesthetized using inhalation of isoflurane (5%); maintenance of the anesthetized state is achieved with isoflurane (1-2%) over the course of the procedure, using the periodic foot-pinch reflex as a measure of the anesthetic plane. The animal's head is shaved and the animal is secured to the stereotaxic apparatus via the ear bars. The head is cleaned thoroughly with Germex.

An incision (~2 cm) is made beginning just posterior of the eyes and proceeding rostrally. The large spatula is used to remove all connective tissue from the surgical area (SA); the area is dried with cotton applicators. Hemostats are clamped to the perineum and used to maximize the SA. All bleeding must be stopped at this point and minimized for the remainder of the surgery; the cauterizer may be used. The SA is cleaned with saline and dried. The zeroed probes are placed above the skull to estimate the point of

entry. The handheld drill is used to make holes for the screws and the ground electrode; the placement of the screws should be as far apart as possible, and must not interfere with the future placement of probes. A hatched pattern of grooves should be made on the skull using a blunt tool (e.g. the opposite side of the scalpel blade) to assist in dental acrylic adherence. Dental acrylic is prepared (by mixing the cement powder and resin) and is applied to the SA. The acrylic should be of sufficient consistency such that it readily moulds into the grooves and around the screws without touching the surrounding perineum. It is essential that the SA be dry. The acrylic is allowed to dry for 15 min; hardness is subsequently tested with a blunt tool. Once again, the zeroed probes are placed above the skull to estimate the point of entry; the drill is used to make appropriate holes for entry. The probe is lowered into position and secured with acrylic followed by a 10-15 min drying period. This procedure is repeated for each additional probe; bilateral cannulae can be set in place simultaneously. When set, carefully remove the stereotaxic arm holding the probe in place. The electrode and ground may then be carefully manipulated to fit into the pedestal; this is cemented in place by building upon layers of cement. Minimizing the vertical distance of the pedestal from the skull, and any sharp edges, are believed to assist in maximizing the lifetime of the entire implant (as animals will be less likely to bump it or get it caught in their cage wires etc.). Again, the cement should not come into contact with the tissue surrounding the SA, but should be maximally attached to the skull. When the implant is set, the wound should be closed using suture. Lastly, the topical anesthetic Xylocaine is applied around the wound to reduce post-surgical discomfort and the animal is placed it a clean cage, on its side, and under a heat lamp to assist in thermoregultion. The animal must be monitored for

appropriate signs of discomfort and adequate respiration. An inverted cage lid is used (and kept in place using twist ties and binder clips), to minimize implant loss. Food is placed in a dish within the cage. During recovery, animals should be monitored (e.g. appropriate weight gain, no signs of distress, good implant integrity) each day for a week. Signs of distress or loss of implant integrity must result in immediate killing.

Microinjection procedure

Briefly, prior to injection, the tubing, injector cannulae, and Hamilton microsyringes (10 µl) must be cleaned with 70 % ethanol and double distilled water and tested for proper function. The drug solution of interest should be taken up in tubes that contain double distilled water followed by an air bubble (movement of the air bubble can be used to ensure the proper injection of solution). Each animal is held loosely in a towel with its head exposed. To minimize stress, the animal is habituated to this by using the same towel daily during the ICSS training period to transfer the animal to and from the ICSS apparatus and its home cage. The dummy cannulae are removed and placed into 70% ethanol; the injector cannulae are fully inserted into the implanted guide cannulae. The drug is administered in a total volume of 0.5 µl at a rate of 0.2 µl/min over a period of 2.5 min; they are left in place for an additional minute following injection to ensure proper absorption. During the injection period, the dummy cannulae are removed from the ethanol and allowed to dry. They are replaced at the end of the injection process and the animal is subsequently tested.

Appendix D: Histology protocol

Probe placements were verified at the end of the experiment by microscopic inspection of flash-frozen coronal brain sections (40 μm). Flash-freezing was achieved using isopentane cooled on dry ice. Sections were made using a cryostat at a temperature of approximately -20°C; slices were mounted on slides. Some slides were chosen for Nissl staining, using cresyl violet, in order to obtain representative photomicrographs of electrode or cannulae placements.

Cresyl violet stock solution preparation

Two solutions (A, B) are required to make the final cresyl violet solution (C).

Solution A: Sodium acetate (1 M): Dissolve sodium acetate (68 g) in 500 ml double distilled water. *Solution B: Acetic acid (1 M)*: Dissolve acetic acid (60 ml) in 940 ml double distilled water. *Solution C: 0.5% cresyl violet (pH ~3.9)*: Dissolve cresyl violet (5 g) in 600 ml double distilled water along with 60 ml of solution A and 340 ml of solution B. Cover and mix for approximately 5 days on magnetic stirrer. Filter the solution, using Whatman #4 filter and a Buchner funnel, over 3-5 days.

Cresyl violet (Nissl) staining protocol

This protocol is adapted from a protocol developed in Katherine Todd's laboratory. Place each basket of slides in the following solutions (for a given amount of time): 70% ethanol (2 min), 95% ethanol (2 min), 100 % ethanol (2 min), xylene (2 min), xylene (2 min), 100% ethanol (2 min), 95% ethanol (2 min), 70% ethanol (2 min), double distilled water (2 washes), 0.5% cresyl violet solution (~60-90 sec), wash with running tap water, 70%

ethanol (2 min), 95% ethanol (2 min), 100% ethanol (2 min), xylene (2 min), xylene (2 min). Finally, coverslip slides with Permount.

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