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Effects of Blockade of Muscarinic Receptors in the Ventral Tegmental Area: Attenuation of Cocaine Reward, but Enhancement of Locomotor Activity and Dopamine Overflow in the Nucleus Accumbens

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ABSTRACT

Effects of Blockade of Muscarinic Receptors in the Ventral Tegmental Area: Attenuation of Cocaine Reward, but Enhancement of Locomotor Activity and Dopamine Overflow in the Nucleus Accumbens

Elizabeth Munn, Ph.D.
Concordia University, 1999

The present study was designed to determine the involvement of cholinergic projections to the ventral tegmental area in the rewarding effects of cocaine. Bilateral intracranial injections of the muscarinic receptor antagonist atropine into the ventral tegmental area attenuated the rewarding effects of intravenous cocaine self-administered on either fixed ratio or progressive ratio schedules of reinforcement. Low doses of atropine sulphate, the lipophilic form of the drug, produced a compensatory increase in cocaine intake while the administration of higher doses of atropine sulphate extinguished responding for intravenous cocaine on a fixed ratio schedule of reinforcement. Bilateral microinjections of atropine methyl nitrate, the lipophobic form of the drug, into the ventral tegmental area also attenuated the rewarding effects of intravenous cocaine self-administered on a progressive ratio schedule of reinforcement. Responding following the injection of low doses of atropine resembled responding when saline was substituted for cocaine; breaking points were established rapidly and there was no post-reinforcement pause. The priming effect of the cocaine administered during "loading" was abolished by the highest dose of atropine; breaking points were reduced. Enhanced locomotion was the predominant effect on activity induced by the administration of atropine into the ventral tegmental area. Atropine sulphate initially induced a period of immobility that did not occur following the administration of atropine methyl nitrate. Microdialysis techniques were used to assess extracellular dopamine levels in the shell of the nucleus accumbens, the terminal region for mesolimbic dopamine neurons originating in the ventral

tegmental area. The injection of atropine methyl nitrate into the ventral tegmental area elevated extracellular levels of dopamine and its metabolites in the ipsilateral nucleus accumbens. Thus blockade of muscarinic receptors in the ventral tegmental area reduces cocaine reward, but increases locomotion and mesolimbic dopamine levels. As cocaine reward is believed to be dopamine-dependent, these findings suggest that separate populations of mesolimbic dopamine neurons underlie cholinergic actions on locomotion and reward.

ACKNOWLEDGEMENTS

It has been almost ten years since I turned up on the proverbial doorstep here at Concordia after a few years of gallivanting around Asia and Europe. I was considering a return to science and thought that the best step would be to find employment as a technician before undertaking another graduate degree. It wasn't long before I found myself in Phyllis Webster's office while Roy Wise sat with his legs dangling over the arm of a chair enthusiastically telling me all about the psychomotor stimulant theory of addiction and sensitization. A few days later—much to my parent's delight—I found myself with gainful employment and learning how to construct electrodes for a brain stimulation reward experiment. Apparently, since locomotor activity sensitizes following the repeated administration of amphetamine, then brain stimulation reward thresholds should do the same thing as locomotion and reward share a common neural substrate...and my job would be to demonstrate this. That was the idea anyway. I guess I've done as much or more than anyone else over the past decade to hammer a few nails into that particular notion...and I'm really looking forward to reading the next incarnation of the anhedonia hypothesis/psychomotor stimulant theory of addiction. So get writing Roy!

For the most part, I must admit that I don't really enjoy most of the things that make up my workday—surgery, perfusions, histology, number-crunching etc. etc.—and I'm allergic to rats. Yet there have been very few days during my tenure in the CSBN that I didn't wake up in the morning (please note that "morning" is a relative term) looking forward to getting into the lab. Science is an intensely social endeavour and I think one of the things that I've enjoyed most has been the friendships forged with my colleagues over the years. I greatly appreciate the freedom, independence, and financial support given to my research endeavours by my thesis advisor, Roy Wise. The assistance given to me by the internal members of my thesis committee, Jane Stewart and Zalman Amit, was indispensable. I am especially grateful for Jane's careful reading of earlier drafts of my thesis. Aileen Murray, Dany Gitnick, Richard Zereik, and Zafiro Koty taught me most of the techniques that I used to gather this data. They always brightened up my day in other ways as well. Phyllis Webster should be commended for her ability to keep the contracts straight and for being an anchor of support at all times. My fellow lab-mates—particularly the other "orphans", Pat Bauco, Cathy Marcangione, and Mark Legault, as well as Siobhan McCormick, Bill Carlezon, and Kath Bonter—played a large role in making every day entertaining. I'd like to extend my appreciation to Mark Wilkins, Graham Parker, Jim Pfaus and the rest of the guys from poker who showed great tolerance for the fact that I can never remember how to deal anything but "Texas Hold'em". Likewise, my heartfelt gratitude goes to my friends from up the hill at McGill, especially Cella Olmstead and Michaela Hynie who like reading as much as I do and excused my inability to knit, because they made the years I've spent in Montreal very satisfying and highly memorable. I guess it's time to follow you guys back to Ontario now—but I'm really going to miss this place!

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GENERAL INTRODUCTION

This thesis is concerned with the neuronal circuits underlying the rewarding effects of the drug, cocaine. Specifically, it is concerned with the role of the cholinergic inputs to the mesolimbic dopamine (DA) pathway—from the laterodorsal tegmental nucleus (LDTg) and pedunculopontine tegmental nucleus (PPTg)—on cocaine reward. The method used to study the effects of central cholinergic manipulations on cocaine reward was intravenous cocaine self-administration. Cholinergic afferents to the ventral tegmental area (VTA), the cell body region of mesolimbic DA neurons, were blocked with the non-selective muscarinic receptor and partial nicotinic receptor antagonist, atropine. The effects of VTA administration of atropine on locomotor activity and extracellular DA levels in the shell of the nucleus accumbens (Acc), the terminal region of these neurons, were also assessed.

Cocaine Reward and Dopamine

Cocaine increases the extracellular concentrations of DA and other monoamines, primarily through its actions as a reuptake blocker (Heikkila et al., 1975; and Ross and Renyi, 1969). This property of cocaine is thought to underly its rewarding effects and abuse liability (Wise, 1978; Wise and Bozarth, 1987; Koob and Bloom, 1988; and Ritz et al., 1987). DA receptor antagonists such as haloperidol and pimozide attenuate cocaine reward as inferred from intravenous self-administration (de Wit and Wise, 1977; Ettenberg et al., 1982; Roberts and Vickers, 1984; Woolverton,W.L., 1986; and Koob et al., 1987). The intake of cocaine increases after the systemic injection of a DA receptor antagonist and this is interpreted as indicating that the antagonist attenuates the

rewarding effect of cocaine. Increasing the amount of cocaine self-administered can compensate for this blunting of reward.

The Mesocorticolimbic DA System and Cocaine Reward

The mesocorticolimbic DA system has been implicated in cocaine reward in studies using selective lesions and local injections of DA antagonists (Wise, 1978; Wise and Bozarth, 1987; Koob and Bloom, 1988; Wise and Rompré, 1989; Le Moal and Simon, 1991; Koob, 1992; Bardo, 1998, and McBride et al., 1999). Central injections of DA antagonists into the region of the DA terminals in the Acc attenuate cocaine reward as evidenced by a compensatory increase in cocaine self-administration (Phillips et al., 1983; Maldonado et al., 1993; and Phillips et al., 1994). Pertussis toxin, a drug that inactivates G-proteins involved in DAergic signal transduction, also increases cocaine self-administration when it is injected into the Acc (Self et al., 1994). Cocaine self-administration is affected by 6-hydroxydopamine lesions of the mesocorticolimbic DA system that reduce presynaptic DAergic input to the Acc and prefrontal cortex from the VTA (Roberts et al., 1977; 1980; Roberts and Koob, 1982; Goeders and Smith, 1986; and Schenk et al., 1991). The lesions either disrupt responding for cocaine in a manner that is indicative of extinction or enhance responding in a manner suggestive of compensatory cocaine intake. Kainic acid lesions of the Acc also disrupt cocaine self-administration (Zito et al., 1985).

Cholinergic Projection to DA cells in VTA/SN from LDTg/PPTg

Dopaminergic cells in the VTA and the substantia nigra (SN) receive monosynaptic cholinergic projections from the pedunculopontine tegmental nucleus

(PPTg) and the laterodorsal tegmental nucleus (LDTg)(Beninato and Spencer, 1987; 1988; Bolam et al., 1991; Clarke et al., 1987; Fujimoto et al., 1990; Futami et al., 1995; Gould et al., 1989; Mesulam et al., 1983; Niijima and Yoshida, 1988; Tokuno et al., 1988; and Woolf and Butcher, 1986). Acetylcholinesterase is the enzyme that metabolizes the neurotransmitter acetylcholine after it has been released. It is contained in dopaminergic cells of the VTA and the substantia nigra pars compacta suggesting that acetylcholine is released at these sites (Butcher and Marchland, 1978; Greenfield et al., 1980; and Lehmann and Fibiger, 1978). Both nicotinic and muscarinic receptors are found in the VTA and SN (Clarke and Pert, 1985; Nastuk and Graybiel, 1991). Destruction of DA cells in the VTA or SN with 6-hydroxydopamine results in the loss of these receptors and of their mRNA showing that these cholinergic receptors are located on DA neurons (Vilaró et al., 1990; and Weiner et al., 1990).

DA Cells are Activated by Cholinergic Afferents

Most studies indicate that dopaminergic cells in the VTA or SN are directly activated by their cholinergic inputs from the PPTg and LDTg. Both nicotinic and muscarinic agonists injected into the VTA or SN excite DA neurons (Calabresi et al., 1989; Kemp et al., 1977; Lacey et al., 1990; Lichtensteiger et al., 1982; and Niijima and Yoshida, 1988). The firing rates of these neurons are increased by stimulation of the PPTg (Clarke et al., 1987; Kelland et al., 1993; and Niijima and Yoshida, 1988). Stimulation of PPTg neurons projecting to DAergic neurons in the SN in a slice preparation evokes monosynaptic excitatory postsynaptic potentials in DA neurons (Futami et al., 1995). In anaesthetized rats, single-pulse electrical stimulation of the PPTg elicits long-latency, long-duration excitations or inhibition-excitations in the majority of SN DA neurons tested and burst firing embedded in the long-duration

responses is induced by PPTg stimulation 37% of the time (Lokwan et al., 1999). Microdialysis studies show that the concentration of the DA metabolite, DOPAC, increases in the striatum following the chemical stimulation of PPTg neurons with kainic acid (Hernández-López et al., 1992) or following muscarinic stimulation of the SN (Damsma et al., 1988; Góngora-Alfaro et al., 1991; and Hernández-López et al., 1992) and that cholinergic activation of the SN facilitates DA release in the striatum (Blaha and Winn, 1993). Similarly, DA release in the ventral striatum is increased by cholinergic activation of the VTA (Blaha et al., 1996). Disinhibition of cholinergic neurons in the PPTg induced by blocking of inhibitory autoreceptors with the muscarinic antagonist, scopolamine, increases striatal DA efflux (Chapman et al., 1997).

Systemic Cholinergic Agents and Reward

Nicotine, the nicotinic cholinergic receptor agonist, is a drug that is self-administered by humans. In a test of its rewarding effects in animals it was shown that systemic administration of nicotine induces a conditioned place preference that is reversed by the nicotinic antagonist, mecamylamine (Fudala et al., 1985), but other researchers using this procedure reported negative results(Calcagnetti and Schechter, 1994; and Clarke and Fibiger). Failure to detect a conditioned place preference may be characteristic of relatively weak rewarding drugs (Bardo, 1998). There are several reports that nicotine is self-administered intravenously in rats under specific conditions (Corrigall and Coen, 1989; Donny et al., 1995; and Goldberg et al., 1981). Electrical brain stimulation reward is potentiated by the systemic administration of nicotine (Bauco and Wise, 1994). The long-lasting nicotinic antagonist, chlorisondamine, had no effect on brain stimulation reward thresholds when administered systemically although it did block the reward-enhancing effects of

systemic nicotine (Wise et al., 1998). The fact that nicotinic receptor blockade with chlorisondamine is without effect on brain stimulation reward thresholds when administered alone suggests that tonic cholinergic inputs produce effects on reward through actions at muscarinic receptors. Interestingly, however, the potentiating effects of systemic nicotine on brain stimulation reward are attenuated by the the nicotinic receptor antagonist, mecamylamine, and by the DA receptor antagonist, haloperidol, but not by the muscarinic receptor antagonist, scopolamine (Ivanova et al., 1997). These data suggest that the rewarding effects of nicotine are DA-mediated and that the rewarding effects of nicotine are independent of cholinergic actions at muscarinic receptors. Systemic injections of muscarinic drugs produce mixed effects on intracranial self-stimulation rates in rats (Murzi and Herberg, 1982; and Pradhan and Kamat, 1972) and have little effect on the threshold of electrical stimulation required for responding for brain stimulation reward (Druhan et al., 1989; Gratton and Wise, 1985; and Robertson and Laferriere, 1987). These data seem to suggest that acetylcholine acting at muscarinic receptors is not involved in reward. Nevertheless, a drug administered systemically acts on all receptors; often microinjections of the drug induces opposing effects from different sites when administered centrally and these effects may cancel each other out following systemic injections.

Central Cholinergic Manipulations and Reward

Several studies have used different methods to examine the role of the cholinergic projections from the PPTg in reward. The PPTg contains nicotine receptors (Swanson et al., 1987) and stimulation of neurons in the PPTg with nicotine produces a conditioned place preference that is reversed by the nicotinic receptor antagonist, mecamylamine (Iwamoto, 1990). Presumably, nicotine activates projection neurons in

the PPTg that activate DA neurons in the VTA. Muscarinic receptor agonists appear to inhibit PPTg neurons by acting on autoreceptors in the PPTg (Leonard and Llinas, 1994; Serafin et al., 1990; and Chapman et al., 1997). The microinjection of the cholinergic agonist, carbachol, into the PPTg raises the threshold for brain stimulation reward, whereas the antagonist, scopolamine, reduces the threshold frequencies for brain stimulation reward (Yeomans et al., 1993).

Excitotoxic lesions of the PPTg block the development of conditioned place preferences to amphetamine, morphine, and heroin (Bechara and van der Kooy, 1989; Olmstead and Franklin, 1994; and Nader et al., 1994). PPTg lesions also block the acquisition of responding for brain stimulation reward (Lepore and Franklin, 1996) and heroin self-administration (Olmstead et al., 1998). The PPTg contains cholinergic neurons and non-cholinergic neurons. The non-cholinergic neurons appear to be glutamatergic (Clements and Grant, 1990; Di Loreto et al., 1992; Lavoie and Parent, 1994b; 1994a; and Charara et al., 1996) and many of the cholinergic neurons co-localize glutamate (Clements et al., 1991; and Lavoie and Parent, 1994a). None of the lesion studies allow one to say which neurons in the PPTg (cholinergic or non-cholinergic) are responsible for mediating drug reward. In addition, these lesions rarely destroyed the entire PPTg and the LDTg, the primary source of cholinergic input to the VTA, remained intact.

Although several studies have shown that PPTg lesions disrupt the acquisition of behaviours based on rewarding events, several researchers have found that these behaviours, once they have been learned, are unaffected by the lesions. Lesions of the PPTg do not disrupt the expression of a morphine conditioned place preference (Bechara and van der Kooy, 1989). Likewise, maintenance of responding for heroin or nicotine is unaffected by PPTg lesions made after training (Nader et al., 1994; Olmstead et al., 1998; and Corrigall et al., 1994). Rats continue to respond for electrical brain

stimulation reward after PPTg lesions, but the rate is less than normal (Lepore and Franklin, 1996).

A series of studies examined the effects of cholinergic compounds injected into the DA cell body region in the VTA. VTA injections of cholinergic drugs have a much stronger effect on reward measures than would be predicted from the effects of systemic applications of these drugs. This is especially true of muscarinic drugs. Muscarinic receptor agonists injected into the VTA induce a conditioned place preference (Yeomans et al., 1985). Microinjections of the muscarinic receptor antagonist, atropine, into the VTA increases the threshold frequency required to maintain responding; the effect on peak bar-pressing rates is weak or non-existent (Kofman and Yeomans, 1989; 1990; and Yeomans et al., 1985). These data indicate that atropine injected into the VTA reduces the rewarding effects of lateral hypothalamic stimulation without inducing a non-specific performance deficit. Pretreatment with the cholinergic agonist, carbachol, blocks the increase in threshold frequency induced by atropine suggesting that the attenuation of brain stimulation reward is due to atropine's actions on cholinergic receptors (Kofman et al., 1990). The reward-attenuating effects of atropine injections into the VTA observed in the brain stimulation reward experiments do not generalize to another procedure for assessing reward, intravenous nicotine self-administration (Corrigall et al., 1994), but the effect of this manipulation on the self-administration of other drugs was not examined.

Nicotinic drugs appear to have a more limited effect on reward processes than do muscarinic agents when injected into the VTA; studies that have compared the two found that the maximal effect of nicotinic drugs is less than that produced by muscarinic drugs. Furthermore, nicotinic antagonists are effective at blocking the rewarding effects of nicotine, but have little effect on the rewarding effects of other substances. The nicotinic receptor agonist, cytisine, produces a conditioned place preference when

injected into the VTA (Museo and Wise, 1994). The nicotinic receptor antagonists, mecamylamine and dihydro-ß-erythroidine, when injected into the VTA increase the frequency required to maintain responding for brain stimulation reward in a dose-related manner (Yeomans and Baptista, 1997). The maximal effect produced by the nicotinic antagonists is considerably less than that observed when the muscarinic antagonist, atropine, is injected into the same site. Microinjection of the nicotinic antagonist, dihydro-ß-erythroidine, into the VTA decreases nicotine self-administration (Corrigall et al., 1994), but this effect is specific for nicotine reward as responding for food or cocaine reward is not altered.

These data suggest that blockade of cholinergic input to the VTA should reduce activity in DA neurons that are normally excited by endogenous acetylcholine and thus reduce DA release. It might follow then that cocaine would consequently be less able to increase extracellular DA levels through its actions as a DA reuptake blocker. Since the mesocorticolimbic DA system is believed to mediate the rewarding effects of cocaine, the administration of the muscarinic receptor antagonist, atropine, into the VTA should attenuate cocaine reward.

Is the Biological Mechanism of Positive Reinforcement Homologous with that of Psychomotor Stimulation?

The role of the mesocorticolimbic DA system has been studied in a wide variety of motivated behaviours (Mogenson, 1977). Wise and Bozarth (1987) suggested that the psychomotor stimulant and reinforcing actions of a drug like cocaine derive from a common biological substrate that includes the mesencephalic DA pathways. One of the predictions of this theory is that "the psychomotor stimulant and reinforcing actions of any addictive drug should both be disrupted by any lesion or treatment that disrupts

either one of these actions" (Wise and Bozarth, 1987). If atropine injections into the VTA attenuate the rewarding effects of cocaine by reducing the activity of the mesocorticolimbic DA system, locomotor activity should also be reduced for the same reason. A number of studies have investigated the effects of anticholinergic drugs on DA-related behaviours.

Systemic Anticholinergics have an Anticataleptic Effect and Increase Locomotor Activity

Systemically administered muscarinic receptor antagonists, such as scopolamine and atropine, can induce hyperactivity (Abood and Biel, 1962; Meyers et al., 1964; van Abeelen and Strijbosch, 1969; Pradhan and Dutta, 1971; Anisman and Cygan, 1975; Mason and Fibiger, 1979; Bushnell, 1987; and Sanberg et al., 1987) and stereotypy (Arnfred and Randrup, 1968; Morpurgo and Theobald, 1964; and Mueller and Peel, 1990). These drugs abolish catalepsy induced by cholinergic agonists (Zetler, 1968) and neuroleptics such as chlorpromazine (Morpurgo and Theobald, 1964). Scopolamine, a muscarinic receptor antagonist, induces hyperactivity and stereotypy in rats or mice when administered systemically (Anisman and Cygan, 1975; Joyce and Koob, 1981; Mueller and Peel, 1990; and Shannon and Peters, 1990). Conversely, systemic or intraventricular injections of cholinergic agonists can decrease spontaneous locomotor activity (Herman et al., 1972; Kleinrok et al., 1975; and Pradhan and Dutta, 1971) and induce catalepsy (Baez et al., 1976; Costall and Olley, 1971; Klemm, 1983a; 1983b; and Zetler, 1968). The administration of the potent and selective muscarinic receptor agonist, BM 123, reduces forward locomotion and rearing (Russell et al., 1986). In humans, elevating the extracellular levels of acetylcholine with an intravenous injection of physostigmine decreases spontaneous behaviour and causes

motor retardation, anergy, and sedation (Davis et al., 1976; Dilsaver, 1986; and Janowsky et al., 1974).

These data show that the systemic administration of cholinergic receptor agonists or the administration of drugs that increase systemic levels of acetylcholine by inhibiting acetylcholinesterase reduce general activity levels. Muscarinic receptor antagonists, such as atropine or scopolamine, enhance activity. Systemic injections might have multiple, and conflicting, actions. Nevertheless, these observations are opposite to the results that would be predicted if the effects of the systemic administration of muscarinic drugs on locomotor activity are mediated by the mesocorticolimbic DA system. Consequently, it would appear that the locomotor-stimulating effects of systemic muscarinic receptor antagonists like atropine may be mediated by some other neural mechanism. Nevertheless, a number of researchers have suggested that there is an interaction with the brain's DA circuitry.

Interaction between Cholinergic and Dopaminergic Drugs

The effects of systemic administration of a muscarinic receptor antagonist on locomotor activity are similar to those observed following systemic administration of the dopamine agonist, amphetamine. It should be kept in mind, however, that some of the procedures used to assess behaviour may not be sensitive enough to record qualitative differences in behavioural activation. Indeed, it has been reported that multi-factorial (ambulatory, rearing, stereotypic, and rotational behaviours) analysis of the locomotion induced by systemic injections of amphetamine and scopolamine differs depending on the dose and drug injected (Sanberg et al., 1987). Nevertheless, a number of pharmacological studies reported an interaction between the effects of dopaminergic drugs and muscarinic receptor antagonists. The excitatory effects of the DA agonist,

amphetamine, on activity, stereotypy, and sniffing are enhanced by antimuscarinics (Arnfred and Randrup, 1968). The inhibitory effects of systemically administered neuroleptics on activity, stereotypy, conditioned avoidance, self-stimulation, catalepsy, and rotation are attenuated by systemic muscarinic receptor antagonists (Kelly and Miller, 1975; Setler et al., 1976; Shannon and Peters, 1990; and Stephens and Herberg, 1979). The systemic administration of cholinergic agonists decreases the hyperactivity induced by intraperitoneal injections of amphetamine (Arnfred and Randrup, 1968; Kleinrok et al., 1975; Mennear, 1965; and Proctor et al., 1967). Although the neural structures mediating the systemic effects of cholinergic agents on behaviour have not been determined, these neuropharmacological studies suggest that a cholinergic system might interact with the mesolimbic or nigrostriatal DA systems to modify DA-mediated behaviours. These data are problematic, however, because of the discrepancy between the behavioural data and the neurochemical and electrophysiological data described earlier. Cholinergic agonists injected into the VTA or SN activate dopaminergic neurons and have rewarding effects—yet systemic injections of cholinergic agonists reduce activity and other DA-related behaviours. It is not uncommon for the behavioural effects of systemic manipulations to differ from those of central manipulations. Although no one has directly investigated the effects of microinjections of muscarinic receptor antagonists such as atropine into the VTA on locomotion, a number of researchers have injected cholinergic agonists into the DA cell body region of the mesencephalic DA pathways (the VTA and SN) or into the DA terminal region of the mesolimbic DA system (the Acc) and assessed locomotor activity.

Central Actions of Systemic Anticholinergics: Site of Action

A number of studies have investigated possible central sites for the stimulatory actions of systemically-administered cholinergic antagonists. Cholinergic interneurons in the ventral striatum are a possible site of action. Locomotion increases when the muscarinic receptor agonist, carbachol, is injected into the Acc, but the muscarinic receptor antagonist, atropine, has no effect on locomotion other than to block the carbachol-induced increase in activity (Jones et al., 1981). Injections of cholinergic agonists into the VTA or SN increase locomotion. Nicotinic receptor agonists injected into the VTA increase locomotion (Museo and Wise, 1990; 1995; and Reavill and Stolerman, 1990), but this effect on locomotion is not seen when the injections are into the SN (Reavill and Stolerman, 1990; and Parker et al., 1993). Activity increases when the muscarinic receptor agonist, carbachol, is injected into the VTA (Kofman, 1987; cited by Mathur et al., 1997). Higher doses of carbachol or neostigmine injected into the anterior substantia nigra increase the incidence of sniffing and rearing and ipsilateral turning (Parker et al., 1993).

These data suggest that the activating effects of systemically-administered muscarinic receptor antagonists are not mediated by the VTA or SN, but are due to actions on muscarinic receptors at some other site. Furthermore, these data reveal that cholinergic agonists stimulate locomotor activity when injected into the VTA. Presumably, cholinergic antagonists injected into this site would have the opposite effect, a reduction of locomotion. It is also possible, however, that cholinergic antagonists injected into the VTA may only block drug-induced behaviour.

It seems probable that blockade of muscarinic receptors in the VTA with the non-specific antagonist, atropine, will reduce the activity of mesolimbic DA neurons leading to an attenuation of the rewarding effects of cocaine and a reduction in locomotor activity. The following series of experiments examines the effects of atropine administration into the VTA on intravenous cocaine self-administration, locomotion, and extracellular DA concentrations in the shell of the Acc.

GENERAL METHODS

All experiments were carried out in accordance with the guidelines for the ethical use of animals in biomedical research as outlined by the Canadian Council on Animal Care and Concordia University. Methods common to the experiments conducted in this study are described in this section.

Experimental Animals, Housing, and Diet

A total of 197 adult male Long-Evans rats (Charles River, St. Constance, Quebec) were used in the three studies presented here. The animals used in the intravenous cocaine self-administration and locomotor studies were individually housed in suspended wire mesh cages (18x23x18 cm). The animals used in the microdialysis experiments were housed in pairs in plastic shoebox cages. The cages were located in a room illuminated on a reverse-cycle (lights on from 8:00 pm to 8:00 am). The rats had free access to water and rat chow. Testing was conducted during the dark phase of the light-dark cycle.

Stereotaxic Surgery

Standard stereotaxic surgical techniques were employed to place bilateral guide cannulae in the anterior VTA. The stereotaxic coordinates for the placement of chronic cannulae were: 4.7mm posterior to bregma, 2.7 mm lateral to the midline, and 7.2 mm ventral to the skull surface. The cannulae were angled toward the midline at 10 degrees from the vertical (Paxinos and Watson, 1986).

Before surgery, all rats were pretreated (10 min) with atropine sulphate (0.12 mg/kg, s.c.) to reduce bronchial secretions and were then anaesthetized with sodium pentobarbital (65 mg/kg, i.p.). Sterile penicillin G-procaine (Wyerth-Ayerst Canada, Inc.) was administered prophylactically prior to surgery (60,000 USP units in a volume of 0.2 ml i.m.). The animals were positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar 3.4 mm below the ear bars.

Chronic Cannulae Implantation

Guide cannulae, 16 mm in length, were made for intracranial implantation by modifying 23-gauge stainless steel hypodermic needles. These cannulae were chronically implanted dorsal to the VTA and guided the injection cannulae to the target sites. The inner cannulae (injection cannulae) were not chronically implanted. They were made from 30-gauge stainless steel tubing (HTX-30, Small Parts, Inc., Miami, Florida). This tubing was cut and ground to a length that exceeded the length of the guide cannulae by 2 mm. The guide cannulae were blocked when not in use with obturators made from 30-gauge stainless steel wire. The guide cannulae were secured to the skull with dental acrylic anchored by stainless steel skull screws. A minimum of 24 hr was allowed post-operatively after cannulae implantation before any animal was used in an experiment.

Cerebral Micro-injections of Atropine

Bilateral injections of the non-selective muscarinic antagonists, atropine sulphate or atropine methyl nitrate, were administered to the VTA. Atropine sulphate (15, 30, 60, or 120 µg) or atropine methyl nitrate (7.5, 15, 30, or 60 µg) was

injected into the VTA in a volume of 0.5 µl of artificial cerebrospinal fluid. Control injections consisted of an equal volume of artificial cerebrospinal fluid only. Artificial cerebrospinal fluid was composed of 2.0 mM Sorenson's phosphate buffer containing 145 mM NaCl, 2.8 mM KCl, 1.2 mM MgCl, 1.2 mM CaCl, 0.25 mM ascorbic acid, 5.4 mM glucose and adjusted to a pH of 7.2-7.4 with NaOH; this yielded concentrations of 145 mM sodium, 2.8 mM potassium, 1.2 mM magnesium, 1.2 mM calcium, 152 mM chloride, and 0.25 mM ascorbate.

Infusions of atropine or vehicle were made through an injector that extended 2 mm beyond the guide cannula. The injections were made following removal of the obturators from the guide cannulae. The injection cannulae were inserted into the guide cannulae and were connected to a 20 µl capacity syringe (Hamilton CR-700-20) via a length of polyethylene tubing (PE-10, Becton, Dickson Co.) filled with the appropriate solution. A volume of 0.5 µl was injected over a period of one minute. Injection cannulae were left in place for an additional 60 sec to reduce the possibility of reflux.

Post-Operative Testing

Following surgery, the animals were subjected to experimental procedures designed to examine the role of cholinergic afferents to the VTA in the self-administration of intravenous cocaine, locomotion, and extracellular dopamine levels in the nucleus accumbens.

Histological Verification of Cannulae Placement

At the conclusion of the experiments, rats were anaesthetized with overdoses of sodium pentobarbital and perfused intracardially with physiological saline. The brains

were removed and stored in physiological saline. They were refrigerated until they were sectioned on a freezing microtome. Coronal sections (60 μm) were cut and sections from the vicinity of the cannula tract were mounted on slides. They were stained for acetylcholinesterase (Paxinos and Watson, 1986) within 48 hr of sacrifice and perfusion. A detailed examination of the injection site was carried out using the atlas of Paxinos and Watson (1986) as a guide. Rats were selected for data analysis on the basis of histological determination of injection site. Rats with cannulae outside of the VTA were excluded as were animals with damage to the injection site due to infection or haemmorhage.

Data Analysis

Standard statistical methods were used in evaluating the results of the experiments (Keppel, 1991). Results are expressed as mean ± standard error of the mean for each group. Analysis of variance was used to test for significant changes. Scheffé's F-test was applied for post-hoc analysis. For all analyses, a probability of less than 0.05 was used as the criterion for judging statistical significance.

EXPERIMENT 1: ATROPINE SULPHATE INJECTED INTO THE VENTRAL TEGMENTAL AREA REDUCES THE REWARDING EFFECTS OF INTRAVENOUS COCAINE SELF-ADMINISTERED ON A FIXED RATIO SCHEDULE OF REINFORCEMENT

Introduction

The rewarding effects of drugs of abuse depend on multiple neurotransmitter systems in the brain. A great deal of attention has focussed on the contribution of the neurotransmitter, DA, to drug reward but there is evidence indicating that other neurotransmitters, such as acetylcholine, are also involved. Experiment 1 was designed to examine the effects of bilateral injections of the muscarinic receptor antagonist, atropine sulphate, into the VTA on cocaine self-administration using a fixed ratio schedule of reinforcement. As discussed in the introduction, blockade of cholinergic input to the VTA from the LDTg and PPTg should reduce the firing of DA neurons that are normally excited by endogenous acetylcholine. As a result, the mesocorticolimbic DA neurons should release less DA, and cocaine should consequently be less able to increase extracellular DA levels through its actions as a DA reuptake blocker. Since the mesocorticolimbic DA system is believed to play an essential role in the rewarding effects of cocaine, the administration of atropine sulphate into the VTA should attenuate the rewarding effects of intravenously administered cocaine just as it does brain stimulation reward.

Partial attenuation of reward would be expected to be manifested by a compensatory increase in the rate of responding and an increase in cocaine intake. It is possible, however, that a high dose of atropine sulphate would completely block cocaine

reward. Complete blockade would lead to a pattern of responding typical of extinction conditions when a burst of lever presses is followed by a cessation of responding.

METHODS

Animal Subjects and Surgery

A total of 65 male Long-Evans rats were used in this study. Bilateral guide cannulae were implanted dorsal to the VTA as described in the General Methods section. After the cannulae implantation, the animals were implanted with chronically indwelling intravenous catheters. A silastic catheter (Dow, Montreal, Quebec, Canada; outer diameter, 1.2 mm) was inserted into the right external jugular vein and secured by sutures so that the tip reached the right atrium. The other end of the catheter was passed subcutaneously to an incision on the top of the skull. There the catheter was connected to a bent 22 ga stainless steel cannula (Plastic Products, St. Albans, VT; model C313G-5up) and attached to the skull with dental acrylic anchored by stainless steel screws. The cannula was used to connect the intravenous infusion line during self-administration sessions. The catheter was flushed with heparinized saline (200 USP units in 0.2 ml of saline) and capped daily. Behavioural testing began 24 hr after surgery.

Procedure

All animals were trained to self-administer cocaine in operant cages (26x26x28 cm) enclosed in individually ventilated chambers. Prior to the self-administration sessions, the animals were drug naive and had no experience with operant training. The rats were placed in the self-administration chambers for 5 hr daily. Initially, each lever press led to an infusion of cocaine (1.0 mg/kg mixed in physiological saline) in a volume of 0.25 ml over 28 sec. During the 28-sec infusion, a light located over the

operative lever was illuminated, and bar presses were recorded but did not lead to further infusions. The animals were tested during the dark phase of their circadian cycle. Water, but not food, was available during testing. Responding was not 'shaped' by reinforcement for successive approximations of a lever press. Acquisition of the lever pressing usually occurred within the first four or five sessions and a stable pattern of responding developed within two or three weeks.

After responding stabilized (four consecutive sessions with less than 10% variation in daily drug intake), experimental testing began. The rats were placed in the self-administration chambers and were allowed to self-administer cocaine for 1 hr. They were then removed from the chambers and received bilateral injections of either atropine sulphate (15, 30, 60, or 120 µg/0.5 ul) or vehicle (0.5 µl) into the VTA. (See General Methods for information about drugs, solutions, and injection procedures.) They were then placed back into the self-administration chambers and their self-administration of cocaine on a fixed ratio (FR)-1 schedule of reinforcement was monitored for an additional 4 hr. Drug testing was conducted on alternate days. On the intervening days, the rats self-administered cocaine but did not receive injections into the VTA.

RESULTS

Forty-three animals of the sixty-five implanted with chronic jugular catheters and guide cannulae died, lost their headcaps, or their jugular catheters lost their patency before testing with atropine sulphate was completed. Two animals were excluded from analysis because of the presence of infection at the injection site. Ten animals were excluded from analysis because one or both injection sites lay outside of the VTA. Ten animals were found to have symmetrical, bilateral cannulae placements within the VTA.

Bilateral injections of the lipophilic form of atropine, atropine sulphate, into the VTA appeared to reduce the rewarding effects of intravenous cocaine. Total cocaine intake in 4 hours decreased in a dose-dependent manner following the injections, likely because of the induction of immobility immediately after the administration of atropine sulphate. Atropine sulphate had an immediate inhibitory effect on behaviour that can be described as 'catatonia' and this effect lasted longer at higher doses. After the effects of atropine sulphate on mobility had dissipated, it appeared that the drug attenuated the rewarding effects of cocaine. In some rats atropine accelerated responding for cocaine suggesting that the rats were compensating for a reduction in the rewarding effects of cocaine by increasing their intake. Higher doses of atropine produced a burst of responding followed by extinction in some animals suggesting that the atropine injections abolished the rewarding effects of the cocaine. In some animals, however, higher doses of atropine completely disrupted cocaine self-administration.

Statistical analysis revealed that the total amount of cocaine self-administered following bilateral injections of atropine sulphate into the VTA during a 4-hr session on an FR-1 schedule of reinforcement was reduced in a dose-dependent manner ($F_{4,49}=17.044$, $p=0.0001$) (Fig. 1). Bilateral injections of 15 µg of atropine

Figure 1

Total cocaine intake following the administration of atropine sulphate bilaterally into the VTA. The mean number of cocaine injections self-administered in 4 hr (±SEM) is presented as a percentage of baseline intake.

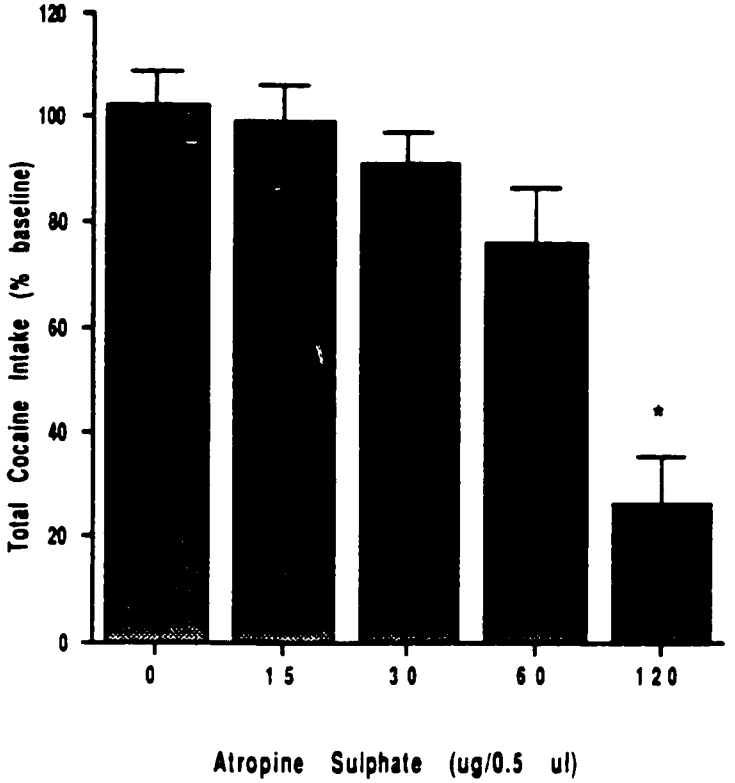
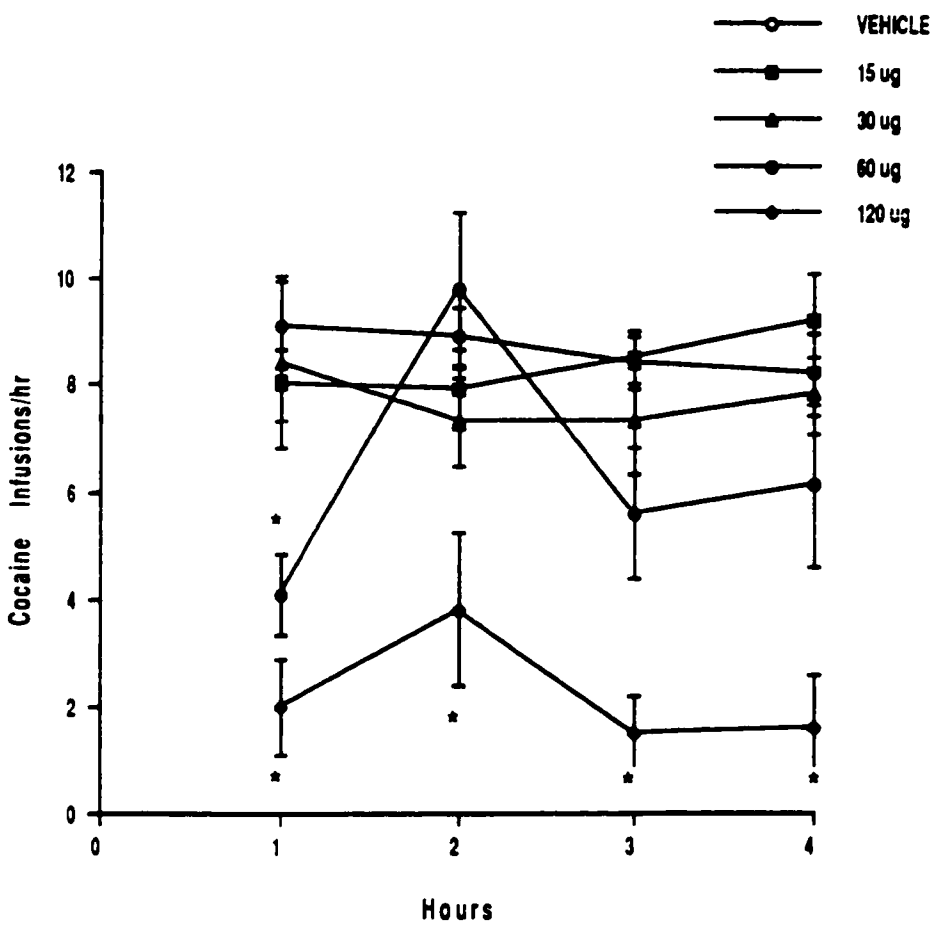

120
100
80
60
40
20
0
Total Cocaine Intake (% baseline)
*
0
15
30
60
120
Atropine Sulphate (ug/0.5 ul)

Figure 2

Time course of the effects of bilateral injections of atropine sulphate into the VTA. Points represent the mean number of injections self-administered per hour (±SEM).


VEHICLE
15 ug
30 ug
60 ug
120 ug
Cocaine Infusions/hr
12
10
8
6
4
2
0
0
1
2
3
4
Hours

sulphate had little effect on total cocaine intake (99.4±6.7% of baseline). Slight decreases in total cocaine intake occurred following the injection of 30 µg (91.1±6.2% of baseline) or 60 µg (75.7±10.9% of baseline) of atropine sulphate. A significant reduction in total cocaine intake to 26.5±9.3% of baseline levels was observed following the bilateral injection of 120 µg of atropine sulphate into the VTA (Scheffe $F=12.662$, $p<0.05$).

A dose x time analysis showed that atropine reduced cocaine intake in a dose-dependent manner ($F_{4,135}=14.756$, $p=0.0001$) and that the effect of dose was not uniform over time. A significant interaction between dose of atropine and time was observed ($F_{12,135}=2.307$, $p=0.0104$). This was most apparent when either 60 µg or 120 µg of atropine was injected. Compared to cocaine intake following vehicle injections, the rats self-administered less cocaine during the first hour of the session but increased their intake during the second hour. Their intake of cocaine dropped again during the third and fourth hours of the session (Fig. 2).

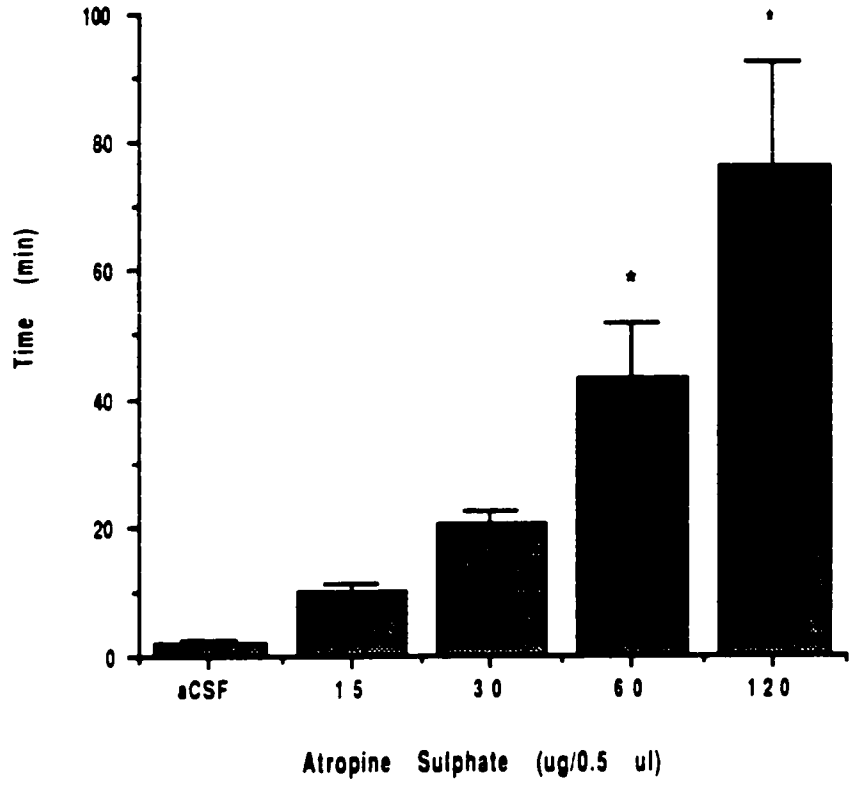
Patterns of Responding Observed Following Atropine Administration into the VTA

Latency to Respond:

In all cases, animals ceased responding immediately following the administration of atropine sulphate into the VTA. Because the rats had been self-administering cocaine for an hour before receiving atropine injections, they were very active at the time of the VTA injections. In all cases, the animals stopped moving during the atropine injections and at higher doses of atropine sulphate the animals became immobile. Once they were returned to the self-administration boxes, their behaviour was not observed directly as the operant cages were shielded by an outer wooden box. These wooden boxes were closed with a wooden door during the course of the self-administration session and the rats

Figure 3

The latency to begin lever-pressing for intravenous cocaine following the bilateral administration of atropine sulphate into the VTA. Bars represent the mean number of minutes (±SEM) between the injection of atropine and the first response.


100
80
60
40
20
0
Time (min)
*
*
aCSF
15
30
60
120
Atropine Sulphate (ug/0.5 ul)

were not visible to an observer. Nevertheless, examination of the event records indicates that higher doses of atropine resulted in longer periods of immobility. The latency to begin self-administering cocaine again significantly increased in a dose-dependent manner following the bilateral administration of atropine sulphate into the VTA ($F_{4,49}$=28.394, p=0.0001) (Fig. 3). The rats began to self-administer cocaine 2.091±0.529 min, on average, after aCSF (0.5 µl) was injected into the VTA. The mean latency to begin bar-pressing increased to 9.785±1.567 min following the injection of 15 µg of atropine sulphate and to 20.453±2.025 min after 30 µg of atropine sulphate was administered. The mean latency to begin self-administering cocaine was of significant duration following the injection of 60 µg (43.256±8.738 min; Scheffe F=6.67, p<0.05) or 120 µg of atropine (76.401±16.172 min; Scheffe F=21.736, p<0.05). Two rats did not make a single lever press after the bilateral injection of 120 µg of atropine sulphate into the VTA.

Increased Rate of Responding (Compensatory Increase in Cocaine Intake):

Following the injection of atropine sulphate, six of the ten rats significantly increased the rate at which they self-administered cocaine ($F_{3,23}$=5.533, p=0.0092) (Fig. 4). These rats self-administered a mean of 4.5±0.43 infusions of cocaine in the 30 minutes after their first response following the injection of the vehicle solution into the VTA. The mean number of infusions during this period increased to 5.7±0.76 infusions of cocaine after 15 µg of atropine sulphate was administered. Rats significantly increased their intake to a mean of 8.7±1.99 infusions after 30 µg of atropine sulphate was injected into the VTA (Scheffe F=3.397, p<0.05) and to a mean of 8.8±1.40 infusions after the administration of 60 µg of atropine sulphate (Scheffe F=3.674, p<0.05).

Fig. 5 shows the event record obtained from an animal that demonstrated this pattern of responding. In this particular animal (VTA63), the dose of 15 µg of atropine

Figure 4

The rate of cocaine intake following the period of immobility induced by atropine sulphate. Bars represent the mean number of cocaine infusions self-administered (±SEM) during the 30-min interval after the first response.

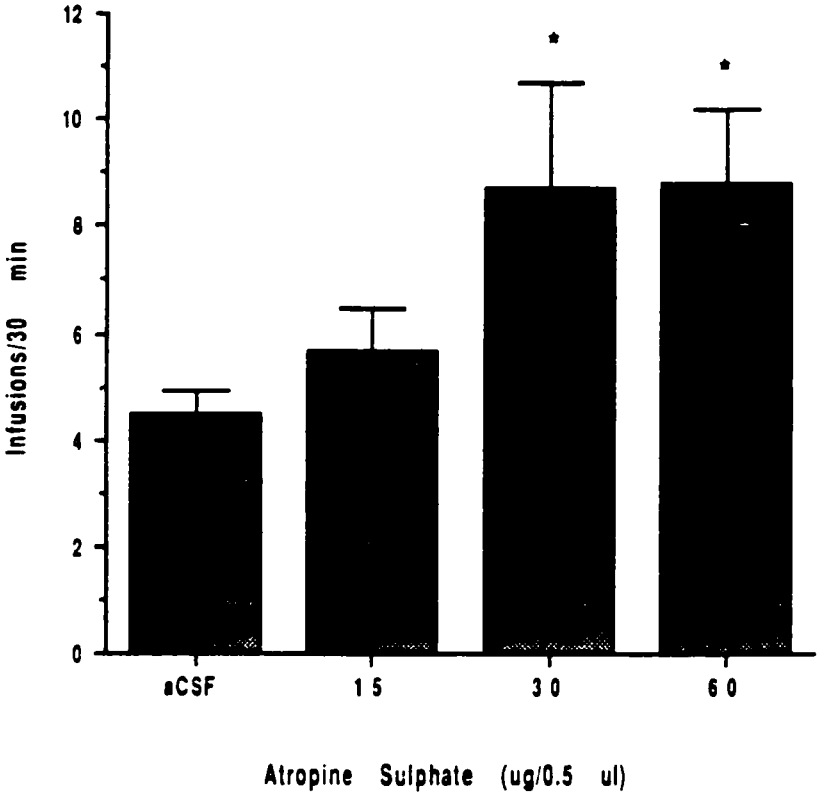
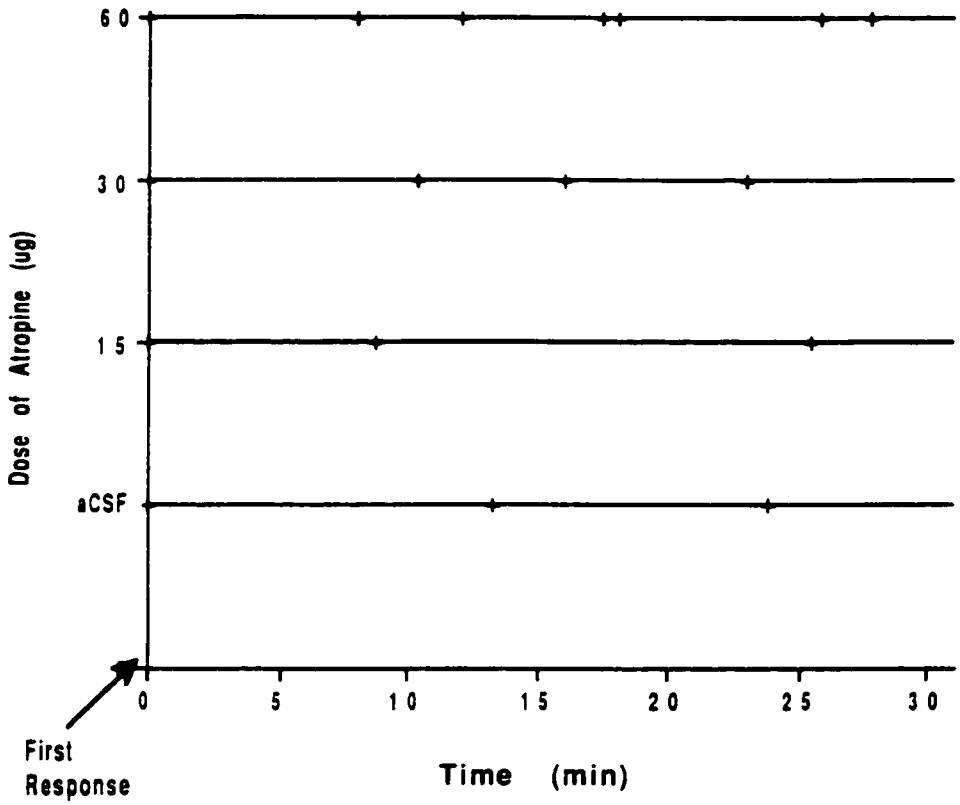

12
10
8
6
4
2
0
Infusions/30 min
*
*
aCSF
1 5
3 0
6 0
Atropine Sulphate (ug/0.5 ul)

Figure 5

Event records from a single animal after the injection of different doses of atropine sulphate into the VTA. These records are for the 30-min period following the resumption of cocaine self-administration after the immobility induced by atropine sulphate dissipates.


60
30
15
aCSF
Dose of Atropine (ug)
0
5
10
15
20
25
30
Time (min)
First
Response

sulphate had little effect on responding. The latency to begin self-administering cocaine after the injection of vehicle was 6.23 min and was 7.57 min following the administration of 15 µg of atropine sulphate. The rate of cocaine self-administration was 3-infusions/30 min in both cases. The latency to initiate responding increased to 19.98 min after 30 µg of atropine sulphate was injected into the VTA and the rate of cocaine self-administration increased to 4-infusions/30 min. Following the injection of 60 µg of atropine sulphate into the VTA, the latency to begin responding increased to 36.80 min and the rate of cocaine self-administration more than doubled, increasing to 7-infusions/30 min. Four rats did not alter their intake of cocaine dramatically, apart from the aforementioned performance deficits, when atropine sulphate was injected into the VTA.

Responding Typical of Extinction Conditions:

In some rats (five of the ten rats tested), the self-administration pattern after the administration of higher doses of atropine sulphate into the VTA was characterized by a burst of lever presses followed by complete cessation of responding. The dose of atropine that resulted in extinction varied from rat to rat. One rat stopped responding after 30 µg of atropine sulphate was administered. Responding was extinguished in three rats after 60 µg of atropine sulphate was administered. Another rat (VTA 57) did not stop responding for cocaine until the 120 µg dose of atropine sulphate was administered. The event records shown in Fig. 6 illustrate this response pattern in these five rats.

Disruption of Responding (Effects on Performance):

The highest dose of atropine sulphate administered (120 µg) completely disrupted the self-administration of intravenous cocaine in four rats. Two rats did not resume responding for cocaine at all after this dose of atropine was injected into the VTA while the other two rats made very few lever-presses. Fig. 7 illustrates the response

Figure 6

Event records for five rats (50% of the animals tested) that show a pattern of responding indicative of extinction. The records show the responses for the entire test session, including the initial 'loading' phase prior to the administration of atropine sulphate into the VTA. The vertical bar separates the record of responses prior to the injection of atropine from the record of responding after the drug is administered. The dose of atropine sulphate that produced this pattern of responding appears next to each subject's name at the left of the event record. The doses of atropine injected into the VTA that produced this pattern of responding ranged from 30 µg to 120 µg.

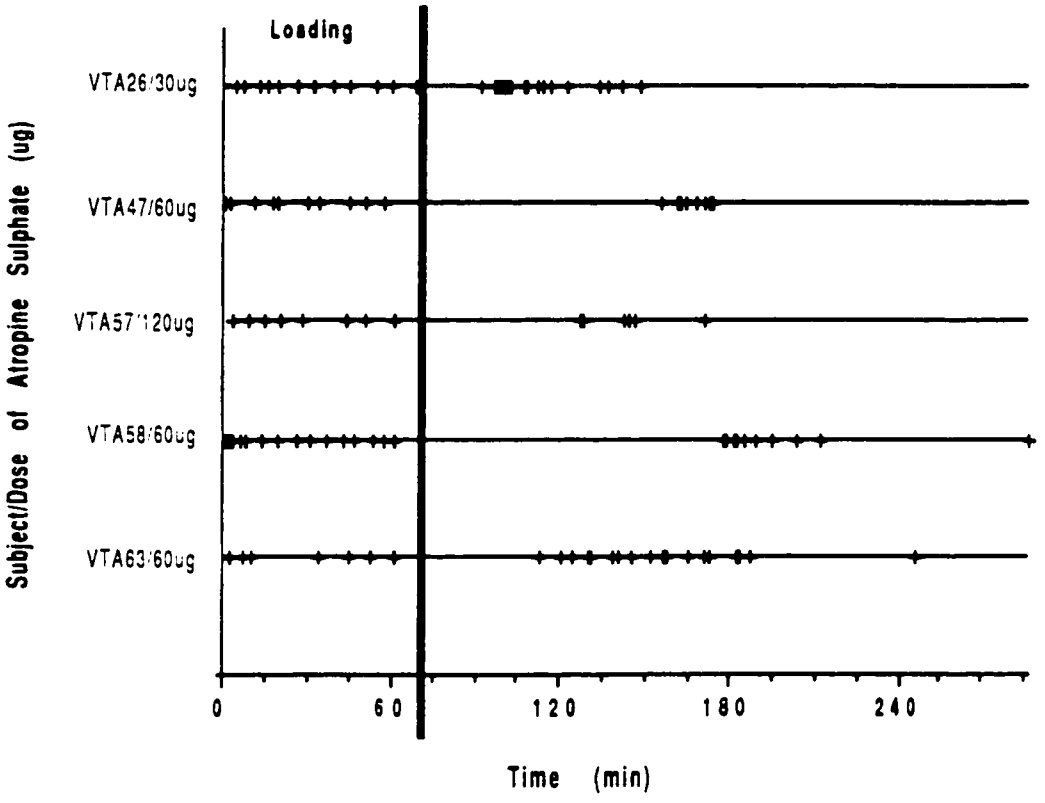
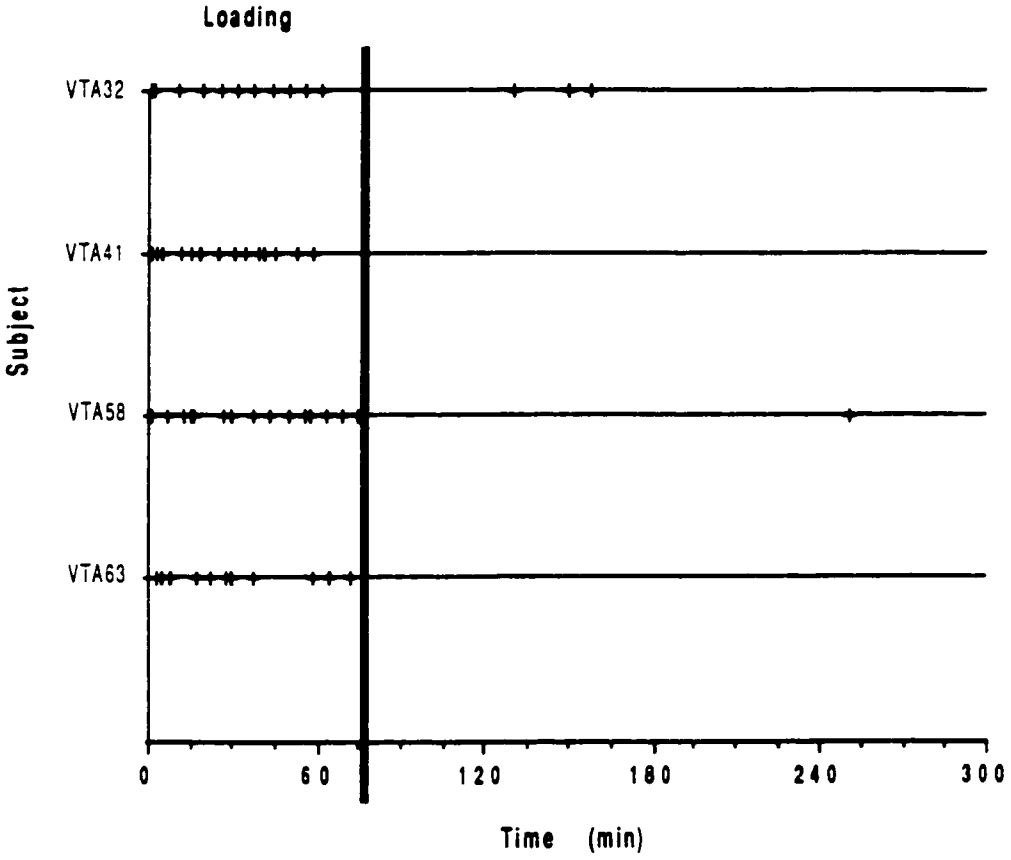

Loading
VTA26/30ug
VTA47/60ug
VTA57/120ug
VTA58/60ug
VTA63/60ug
Subject/Dose of Atropine Sulphate (ug)
0
60
120
180
240
Time (min)

Figure 7

Four of the ten rats tested did not resume normal responding for cocaine following the bilateral administration of 120 µg of atropine sulphate into the VTA. The event records for the entire session, including the initial 'loading' phase prior to atropine administration, is illustrated. The vertical bar separates the record of responses prior to the injection of atropine from the record of responding after the drug is administered.


Loading
VTA32
VTA41
VTA58
VTA63
Subject
0
60
120
180
240
300
Time (min)

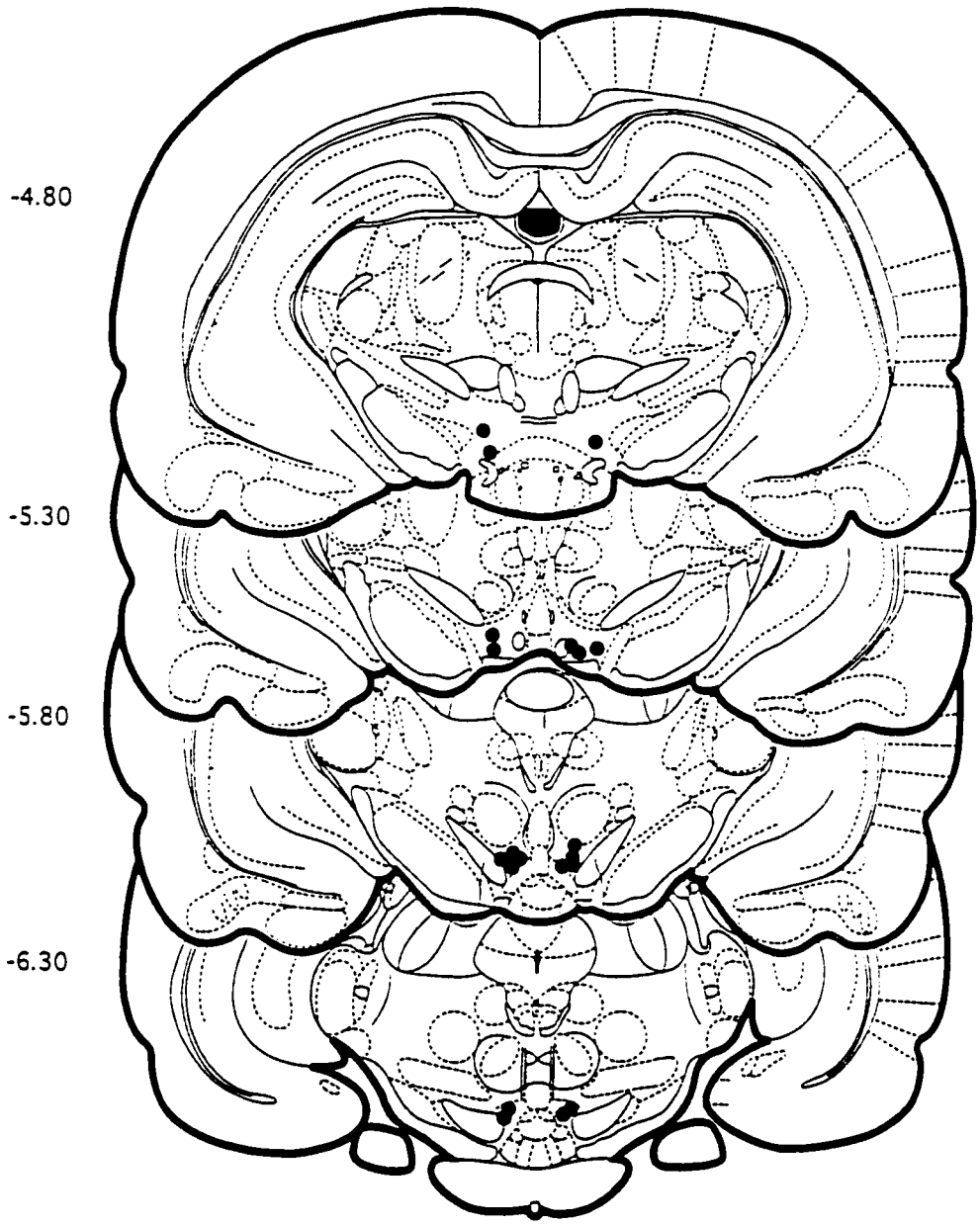
patterns observed in these animals after the administration of 120 µg of atropine sulphate.

Histological Verification of Cannulae Placement

Fig. 8 depicts the placement of the cannulae within the VTA. The placement of the cannulae within the VTA was in an area that stained for acetylcholinesterase. On the rostral-caudal plane, placements ranged from 4.8 to 6.3 mm posterior to bregma.

Figure 8

Distribution of sites where atropine sulphate was injected into the VTA (bilateral injections; n=10).


-4.80
-5.30
-5.80
-6.30

DISCUSSION

These data indicate that blockade of muscarinic cholinergic receptors in the VTA by atropine sulphate reduces the rewarding effects of intravenous cocaine. In addition, the drug induced immobility.

The rats that were very active as a result of the cocaine they had self-administered during the first hour of the session became immobile while the atropine sulphate was injected into the VTA. These rats did not move while the injector was removed, the obturators replaced, and the IVSA lead reattached. They righted themselves if necessary when placed into the operant chamber but were otherwise immobile. This effect persisted during the first hour of the test session (Figs. 2 and 3). The immobility lasted for a signicant period of time when 60 µg of atropine sulphate was injected bilaterally into the VTA (Fig. 3). Four of the ten animals tested did not resume responding normally for cocaine after 120 µg of atropine sulphate was injected into the VTA (Fig.7). Clearly, the immobility induced by the atropine sulphate injections can serve in part to explain the overall dose-related reduction in cocaine self-administration.

Ikemoto and Panksepp (1996) reported that bilateral injections of atropine sulphate into the VTA (50 µg/0.5 µl) induced immobility that precluded meaningful interpretation of data obtained during a shuttle box experiment examining sucrose consumption. The manipulation induced immobility in most of the subjects; the rats did not move within the start box as long as they were left alone. Nevertheless, they were able to right themselves normally when placed upside down and appeared to retain intact reflex responses.

Unilateral atropine sulphate injections into the VTA did not interfere with mobility in a series of intracranial self-stimulation experiments. The maximal rate of

responding for lateral hypothalamic electrical stimulation was generally unaffected by the unilateral injection of atropine sulphate into the ipsilateral VTA (Kofman and Yeomans, 1989; Kofman et al.1990; and Yeomans et al., 1985). It seems likely therefore that the immobility observed in the current study requires bilateral cholinergic blockade of the VTA.

The effects of atropine on cocaine reward outlast their effects on performance, however. Total cocaine intake during the second hour of the session increased following the injection of the highest doses of atropine sulphate (60 µg and 120 µg) into the VTA (Fig. 2). A significant increase in mean cocaine intake occurs during the 30 min period after the rats resumed responding for cocaine when 30 µg of atropine sulphate is injected bilaterally into the VTA (Fig. 4). Closer examination of the event records indicate that a dose-related increase in cocaine intake is evident during this 30 min period (Fig. 5).

Generally, an increase in intake is believed to compensate for a reduction in the rewarding properties of the drug being self-administered (Yokel and Wise, 1975). If the rewarding properties of the drug being administered are blocked then a pattern of responding indicating extinction tends to occur. Extinction is characterized by a burst of responses followed by a cessation in responding. In the present experiment, responding for cocaine extinguished after treatment with high doses of atropine sulphate in 50% of the subjects tested, indicating that cholinergic blockade of the VTA can completely block the ability of cocaine to reinforce lever pressing (Fig. 6).

These data are consistent with earlier studies of atropine's effects on reward that reported an increase in brain stimulation reward thresholds from the ipsilateral lateral hypothalamus after atropine sulphate was injected into the VTA (Kofman and Yeomans, 1989; Kofman et al., 1990; and Yeomans et al., 1985). The increase in threshold

frequency induced by atropine was blocked by pretreatment with the cholinergic agonist, carbachol (Kofman et al., 1990).

The reduction in the rewarding effects of cocaine and electrical brain stimulation produced by atropine may not generalize to the rewarding effects of another addictive drug, nicotine. Total intravenous nicotine intake during a one hour self-administration session in rats was not altered when atropine sulphate (30µg or 60 µg) was injected bilaterally into the VTA (Corrigall et al., 1994). These authors did not report that the atropine sulphate injections induced a performance deficit, but it seems likely that a significant period of immobility would have been induced by the 60 µg dose of atropine sulphate. If this was the case, then all of the nicotine would have been self-administered during the last part of the session. The animals in the present study did not resume lever-pressing for cocaine until 43.256±8.738 min after 60 µg of atropine sulphate was administered. If the same performance deficit occurred during the nicotine study, then all of the nicotine was self-administered in a 20-min period at the end of the session at three times the normal rate. This could be construed as a compensatory increase in the rate of nicotine self-administration to offset a reduction of the rewarding effects of nicotine produced by the atropine sulphate injections. Unfortunately, these authors did not report the pattern of responding during the test session.

It might be presumed that blockade of the excitatory cholinergic input to DA neurons in the VTA reduced the rewarding effects of cocaine in the current study by attenuating the release of DA from the mesocorticolimbic DA system. The compensatory increase in cocaine intake is similar to that observed after DAergic antagonists are administered into the Acc (Phillips et al., 1983; Maldonado et al., 1993; and Phillips et al., 1994).

Atropine sulphate is highly lipophilic and both the effects on reward and the effects on mobility might have been produced at locations distal from the injection site.

The following study uses the lipophobic form of atropine, atropine methyl nitrate, in order to examine the effects of cholinergic blockade with greater anatomical specificity. The lipophobic properties of atropine methyl nitrate ensure that it will not diffuse as far as atropine sulphate. Neural mechanisms close to the injection site within the VTA are thus more likely to mediate its effects.

Finally, although the observed increase in cocaine intake seems to indicate a reduction in cocaine reward, data from studies using fixed ratio schedules of reinforcement can be difficult to interpret. Typically, an increase in responding is interpreted as compensatory and indicates a reduction in reward (Yokel and Wise, 1975) whereas a decrease in intake indicates an increase in reward. On the other hand, lesions of the Acc with 6-hydroxydopamine, for example, initially decrease cocaine self-administration yet these data have also been interpreted as a reduction in reward (Roberts et al., 1980; and Roberts and Zito, 1987). (For discussion of the limitations inherent in the interpretation of data obtained using fixed ratio schedules of reinforcement, see Wise, 1987; Richardson and Roberts, 1996; and Arnold and Roberts, 1997).

An alternative schedule of reinforcement that has often been used to study cocaine reward is the progressive ratio schedule of reinforcement. Under this schedule, the response requirements to earn a drug injection escalate after the delivery of each cocaine infusion. The first injection of cocaine requires a single lever-press but the number of responses required for each subsequent injection increase exponentially. The final ratio of responses successfully completed is defined as the breaking point. A reduction in breaking point is indicative of a reduction in cocaine reward. The following study uses a progressive ratio schedule of reinforcement to determine whether the reduction of cocaine reward by cholinergic blockade seen on a fixed ratio schedule can be

produced by the anatomically specific muscarinic receptor antagonist, atropine methyl nitrate.

EXPERIMENT 2: ATROPINE METHYL NITRATE INJECTED INTO THE VENTRAL TEGMENTAL AREA REDUCES THE REWARDING EFFECTS OF INTRAVENOUS COCAINE SELF-ADMINISTERED ON A PROGRESSIVE RATIO SCHEDULE OF REINFORCEMENT

Introduction

There is no schedule of reinforcement that can quantify all aspects of drug reinforcement (Richardson and Roberts, 1996; and Arnold and Roberts, 1997). Self-administration studies often use fixed ratio schedules of reinforcement, but other schedules of reinforcement are also used to study drug reward. The progressive ratio (PR) schedule has been used in a number of cocaine self-administration studies. Rats quickly learn to self-administer cocaine on a PR schedule of reinforcement and, after only a few sessions, their responding is controlled and regular (Depoortere et al., 1993). Cocaine self-administration on a PR schedule is characterized by alternating periods of rapid, consistent responding and regular post-reinforcement pauses in responding. Response ratios are increased on a PR schedule until the animal no longer responds. The point in the series at which responding ceases is called the breaking point. The breaking point is presumed to reflect the maximum effort that an animal will expend in order to receive a cocaine infusion (Richardson and Roberts, 1996).

The administration of cocaine can reinstate or 'prime' drug-seeking behaviour (Deneau et al., 1969). Every cocaine injection influences the motivation to seek subsequent injections. 'Priming' is a standard feature of cocaine self-administration experiments and ensures that animals will respond at the beginning of a session

(Richardson and Roberts, 1996). In the current series of experiments, rats self-administer cocaine on an FR-1 schedule of reinforcement for 1 hr before atropine is injected into the VTA.

Experiment 2 was designed to examine the effects of the muscarinic antagonist, atropine methyl nitrate, bilaterally injected into the VTA on cocaine self-administration on a progressive ratio schedule of reinforcement after 'priming'. The administration of atropine methyl nitrate into the VTA is expected to attenuate the rewarding effects of intravenously administered cocaine just as atropine sulphate did in the previous experiment. As atropine methyl nitrate is not as lipophilic as atropine sulphate, its effects on cocaine reward are more likely to be due to its actions on muscarinic receptors in the vicinity of the injection site. Breaking points should be reduced and response patterns are expected to resemble the patterns obtained from animals responding for lower doses of cocaine. If the rewarding effects of cocaine are completely abolished by the injections of atropine methyl nitrate, breaking points and response patterns should resemble those obtained during extinction conditions.

METHODS

Animal Subjects and Surgery

A total of 54 male Long-Evans rats were used in this study. Bilateral guide cannulae were implanted dorsal to the VTA in 34 rats as described in the General Methods section. After the cannulae implantation, the animals were implanted with chronically indwelling intravenous catheters as described in Experiment 1. Twenty rats received only intravenous catheters during surgery.

Training

All animals were trained to self-administer cocaine as in Experiment 1.

Procedure

Thirty-four rats were used examine the effects of atropine methyl nitrate on cocaine reward. After responding had stabilized on an FR-1 schedule of reinforcement during training, a PR schedule of reinforcement was imposed. On a PR schedule of reinforcement the ratio of responses per infusion is increased after each infusion according to the following progression; 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901 etc. Eventually, the ratio requirement becomes so high that rats cease to respond. The point at which rats stop responding is referred to as the breaking point.

In the present experiment the breaking point was defined as the final ratio completed (results in an infusion) within 1 hr of the previous infusion. During a session, the rats self-administered cocaine for 1 hr on an FR-1 schedule of reinforcement. The schedule was then changed to a PR schedule of reinforcement for the remainder of the session. Training continued until responding had stabilized (the breaking points obtained over 4 consecutive sessions did not differ by more than 2 steps on the progression of ratios). Drug testing then began.

On alternate days, the rats self-administered cocaine for 1 hr on an FR-1 schedule. They were then removed from the box, anaesthetized with an intravenous injection of the fast-acting anaesthetic, methohexital sodium (10mg/ml; 0.2 ml infused i.v. and supplemented with 0.1-0.2 ml as required), and received bilateral injections of atropine methyl nitrate (7.5, 15, 30, or 60 µg/0.5 µl) or vehicle (0.5 µl) into the VTA. They were then put back into the self-administration chambers and testing continued using the PR schedule of reinforcement until a breaking point was established. On the intervening days, the rats self-administered cocaine (1 hr on FR-1 followed by PR) but the animals were not removed from the box or anaesthetized and nothing was injected into the VTA.

Extinction (Substitution of Saline for Cocaine) after 1 hr of Cocaine 'Priming'

Six of the rats from the previous experiment were used in this control experiment designed to examine the effect of cocaine 'priming' on breaking points and the pattern of responding during extinction conditions. The rats were allowed to self-administer cocaine for 1 hr on an FR-1 schedule. The syringe of cocaine was then replaced with one containing saline and the schedule was switched to PR. No injections were administered into the VTA.

Self-administration of Different Doses of Cocaine in the Absence of 'Priming'

The twenty remaining rats were used in this PR experiment designed to examine the effects of varying the dosage of self-administered cocaine on breaking points and on the pattern of responding in the absence of priming. A breaking point was determined at each of several doses of cocaine (0.25, 0.5, 1.0, 2.0, and 4.0 mg/kg). Each rat started at one of these cocaine doses and an average breaking point for that dose was determined. Subsequently, the process was repeated with a different dose of cocaine. The initial cocaine dose was counterbalanced across rats. An average breaking point was defined as the average of three breaking points obtained on three consecutive sessions that did not differ by more than two steps on the progression of ratios. These rats were not primed and did not self-administer cocaine during the hour preceding the PR session. Intracranial guide cannulae were not implanted in these rats and no injections were administered into the VTA.

RESULTS

Fourteen animals of the thirty-four implanted with chronic jugular catheters and guide cannulae died, lost their headcaps, or their jugular catheters lost their patency before testing with atropine methyl nitrate was completed. One animal was excluded from analysis because of the presence of infection at the injection site. Three animals were excluded from analysis because one or both injection sites lay in the substantia nigra pars compacta or in the retrorubral field. Four animals received unilateral VTA injections of atropine methyl nitrate. Three animals received bilateral injections outside of the VTA or the SN. Nine animals were found to have symmetrical, bilateral cannulae placements within the VTA.

The most striking and immediate effect of the atropine methyl nitrate injections was a marked induction of locomotor activity. The animals were already quite active because of the cocaine they had administered during the previous hour. The atropine injections enhanced this effect to the point that it was impossible to administer the injections properly or to reattach the self-administration leads successfully. Consequently, the rats were injected intravenously with the fast-acting anaesthetic, methohexital, before atropine methyl nitrate was injected into the VTA. The animals recovered quickly and began responding shortly after being replaced in the operant chambers. None of the animals showed any signs of catalepsy. Average breaking points were significantly reduced after the administration of the highest dose of atropine methyl nitrate (60 µg). The response patterns resembled those of animals self-administering one-quarter the concentration of cocaine (0.25 mg/kg/infusion instead of 1.0 mg/kg/infusion) without cocaine 'priming'. The 'priming' effect of the cocaine self-administered during the hour before the PR schedule of reinforcement was initiated

had a pronounced effect on breaking points. Breaking points were elevated when saline was substituted for cocaine after one hour of 'priming'. The post-reinforcement pause was reduced, however, and breaking points were reached rapidly. Low doses of atropine methyl nitrate administered into the VTA produced the same effects on breaking point and response pattern as did the substitution of saline for cocaine. In summary, low doses of atropine methyl nitrate injected into the VTA reduced the rewarding effects of cocaine to the point that responding resembled that obtained under extinction conditions; the effects of cocaine 'priming' were still apparent. A high dose of atropine methyl nitrate administered into the VTA blocked the 'priming' effect of cocaine as well as attenuating the reinforcing effects of intravenous cocaine.

Statistical analysis indicated that bilateral injections of atropine methyl nitrate into the VTA reduced the breaking point for intravenous cocaine self-administration. Unilateral injections and bilateral injections of atropine methyl nitrate placed outside of the VTA were ineffective at all doses tested (2-factor [injection site x dose] repeated measures ANOVA; significant interaction: $F_{8,52}=3.819$, $p=0.0014$)(Fig. 9). Breaking points were reduced in a dose-dependent manner by the bilateral administration of atropine methyl nitrate into the VTA (One factor [dose] repeated measures ANOVA: $F_{4,44}=5.683$, $p=0.0014$). The reduction in breaking point following the administration of 60 µg of atropine methyl nitrate was significantly different from the breaking point after the injection of vehicle into the VTA (Scheffe $F=2.525$, $p<0.05$). The average breaking point following the bilateral injection of vehicle into the VTA was 12.778±0.894 and was reduced to 9.0±1.354 when 60 µg of atropine methyl nitrate was injected (59 and 25 lever presses respectively).

The reduction in the average breaking point observed after atropine methyl nitrate was injected into the VTA is similar to the reductions in average breaking point seen when the concentration of cocaine is decreased (Fig. 10). Generally, higher doses of

Figure 9

The effect of intracranial injections of atropine methyl nitrate on intravenous cocaine self-administration using a progressive ratio schedule of reinforcement. Bars represent mean breaking points (±SEM). The effects of bilateral injections of atropine methyl nitrate into the VTA are compared to those of unilateral injections and of bilateral injections into sites outside of the VTA. The axis on the right depicts the number of responses corresponding to the breaking points.

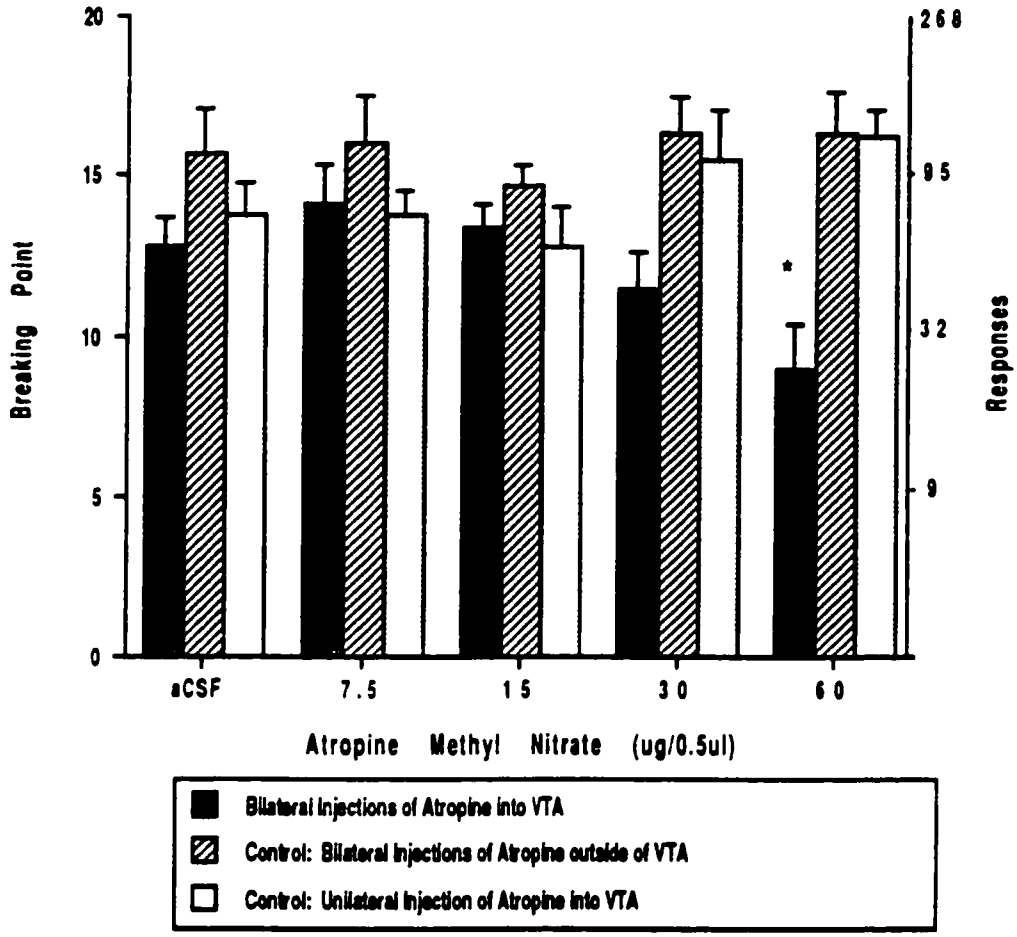
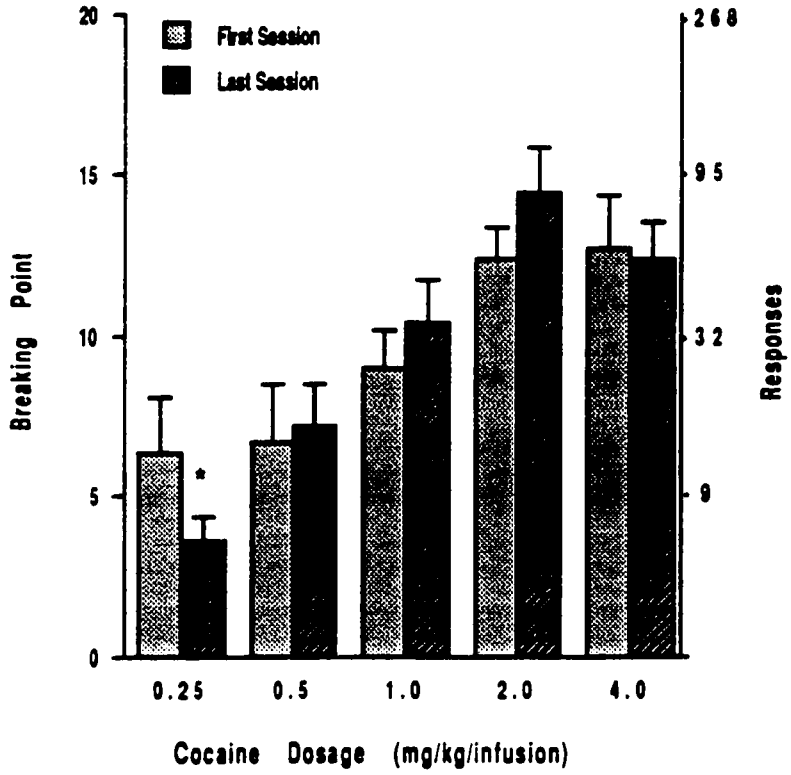

20
15
10
5
0
Breaking Point
268
95
32
9
Responses
*
aCSF
7.5
15
30
60
Atropine Methyl Nitrate (ug/0.5ul)
Bilateral injections of Atropine into VTA
Control: Bilateral injections of Atropine outside of VTA
Control: Unilateral injection of Atropine into VTA

Figure 10

The effects of concentration on intravenous cocaine self-administration using a progressive ratio schedule of reinforcement. Bars represent mean breaking points (±SEM) obtained during the first self-administration session for each concentration of cocaine compared to the mean breaking points obtained after responding had stabilized. The axis on the right depicts the number of responses corresponding to the breaking points.


First Session
Last Session
Breaking Point
Responses
20
15
10
5
0
268
95
32
9
*
0.25
0.5
1.0
2.0
4.0
Cocaine Dosage (mg/kg/infusion)

cocaine result in higher average breaking points than do low doses of cocaine (2-factor [dose x session] repeated measures ANOVA; effect of dose: $F_{4,88}=53.357$, $p=0.0001$). This effect can be seen during the first exposure to a new concentration of cocaine, but the effect of cocaine concentration on breaking point is less variable following repeated exposure to each cocaine dose (2-factor [dose x session] repeated measures ANOVA; interaction between dose and session: $F_{4,88}=3.009$, $p=0.0223$). The average breaking point for the lowest concentration of cocaine tested (0.25 mg/kg/infusion) was significantly affected by repeated testing (Scheffe $F=7.384$, $p<0.05$).

Examination of the breaking points for individual animals on the first day of testing with each concentration of cocaine revealed that the order of presentation had a strong effect on breaking point. As illustrated in Fig. 11, breaking points obtained during the initial test session were affected by the concentration of the cocaine when the concentrations were tested in ascending order. If the concentrations of cocaine were presented in descending order then breaking points remained elevated on the first exposure. After repeated testing, breaking points became stable and reflected the diminished rewarding effects of cocaine when its concentration was reduced.

The cumulative event records shown in Fig. 12A illustrate the alteration in response patterns when reward is attenuated by reducing the concentration of infused cocaine to 0.25 mg/kg/infusion from 1.0 mg/kg/infusion. Infusions were regularly spaced with consistent postreinforcement pauses for the high dose of cocaine; this was particularly evident during the early part of the session when response ratios are relatively small. In contrast to this, the low dose of cocaine is infused irregularly, the postreinforcement pause varies in duration, and very few infusions of cocaine are obtained resulting in a low breaking point.

A similar response pattern was observed when a high dose of atropine methyl nitrate (60 µg) was injected into the VTA (Fig. 12B). The response patterns for an

Figure 11

Breaking points obtained for the self-administration of different concentrations of intravenous cocaine in two animals; one animal was exposed to the different concentrations of cocaine in descending order while the second animal was presented with increasingly high concentrations of cocaine. These are the data from the first session with each concentration of cocaine. The concentration of cocaine was not changed until responding had stabilized (breaking points obtained over 4 consecutive sessions did not differ by more than 2 steps on the progression of ratios). The axis on the right depicts the number of responses corresponding to the breaking points.

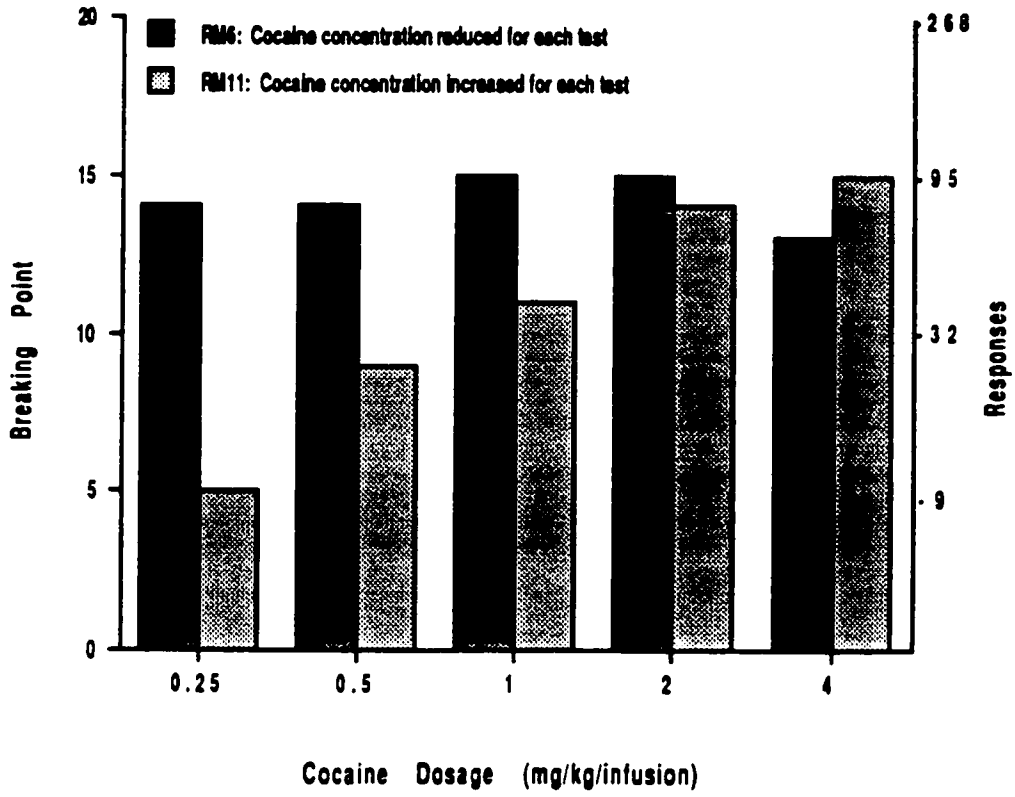
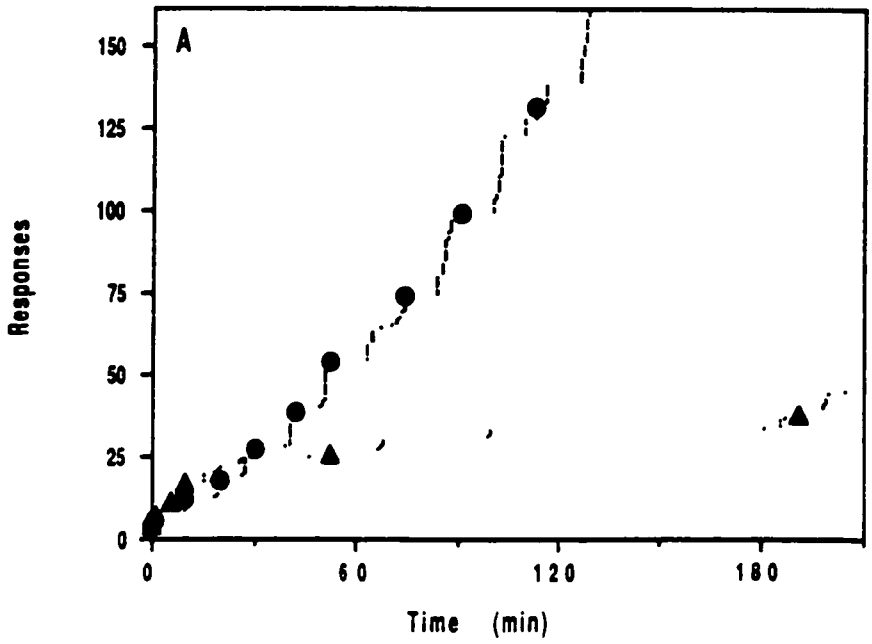
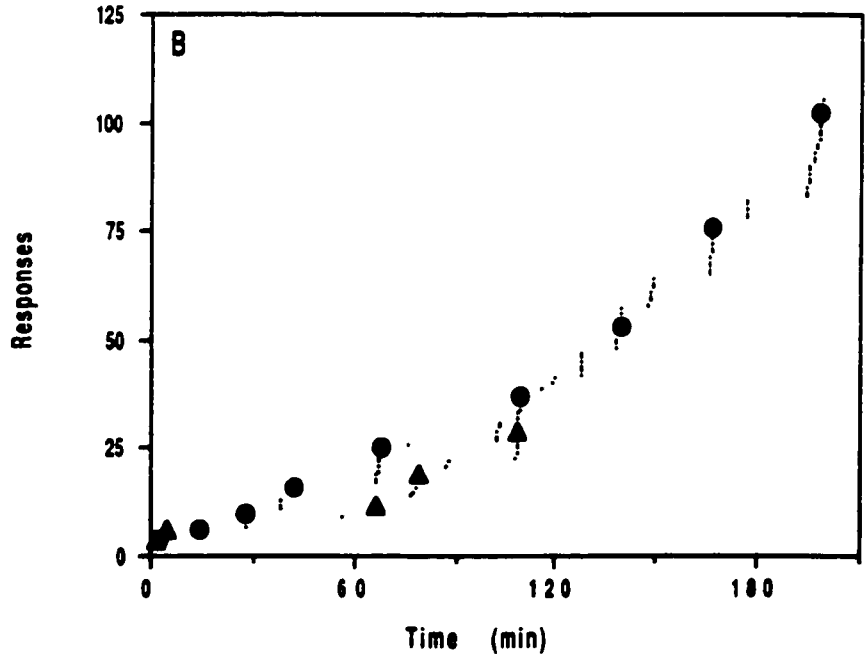

RM6: Cocaine concentration reduced for each test
RM11: Cocaine concentration increased for each test
Breaking Point
Responses
20
15
10
5
0
268
95
32
9
0.25
0.5
1
2
4
Cocaine Dosage (mg/kg/infusion)

Figure 12

Cumulative response records for individual animals self-administering intravenous cocaine on a progressive ratio schedule of reinforcement. Top panel: the effect of concentration of cocaine on self-administration (circles-1.0 mg/kg/infusion; triangles-0.25 mg/kg/infusion). Bottom panel: the effect of bilateral intra-VTA injections of the highest dose of atropine methyl nitrate tested on intravenous cocaine self-administration (circles-vehicle (0.5 µl); triangles-60 µg/0.5 µl of atropine methyl nitrate).


A
150
125
100
75
50
25
0
Responses
0
60
120
180
Time (min)


B
125
100
75
50
25
0
Responses
0
60
120
180
Time (min)

animal self-administering the high dose of cocaine (1.0 mg/kg/infusion) after injections of either vehicle or atropine methyl nitrate are illustrated. When atropine methyl nitrate was injected into the VTA, very few infusions were obtained and they were irregularly spaced; the duration of the postreinforcement pause was inconsistent.

Responding was unaffected by the administration of vehicle into the VTA. The response pattern following vehicle injections (Fig. 12B) resembled the response pattern for the same dose of cocaine (1.0 mg/kg/infusion) shown in Fig. 12A. It should be noted that the animals infusing different doses of cocaine (Fig. 12A) were not 'primed' while the animals receiving VTA injections (Fig. 12B) self-administered cocaine for one hour before the PR session started.

The 'priming' effect of the cocaine self-administered before the PR session had a marked effect on responding. Breaking points remained elevated after 'priming' even during extinction. (Fig. 13). After one hour of self-administering cocaine, the syringe of cocaine was replaced with one containing saline. There was no significant difference in the breaking points obtained for saline and cocaine; the breaking point for saline was 11.33±0.76 compared to 12.0±0.931 for cocaine.

Although the breaking points were unaffected by saline substitution, the response patterns were markedly different when rats were self-administering saline instead of cocaine. Breaking points were reached rapidly and there was no pause in responding after an infusion of saline. By comparison, responding was regular and postreinforcement pauses were consistent when these rats self-administered cocaine (1.0 mg/kg/infusion). Fig. 14 shows the cumulative event records for six rats self-administering cocaine or saline after one hour of cocaine 'priming'.

The injection of low doses of atropine methyl nitrate into the VTA also resulted in a pattern of responding indicative of extinction in six of the nine rats tested (Fig. 15). Breaking points, although elevated, were reached quickly and there were no

Figure 13

Self-administration of either saline or cocaine on a progressive ratio schedule of reinforcement after one hour of cocaine self-administration on an FR-1 schedule of reinforcement. Bars represent the mean breaking points (±SEM) obtained when cocaine (1.0 mg/kg/infusion) was self-administered and when the syringe of cocaine was replaced with a syringe containing isotonic saline. The axis on the right depicts the number of responses corresponding to the breaking points.

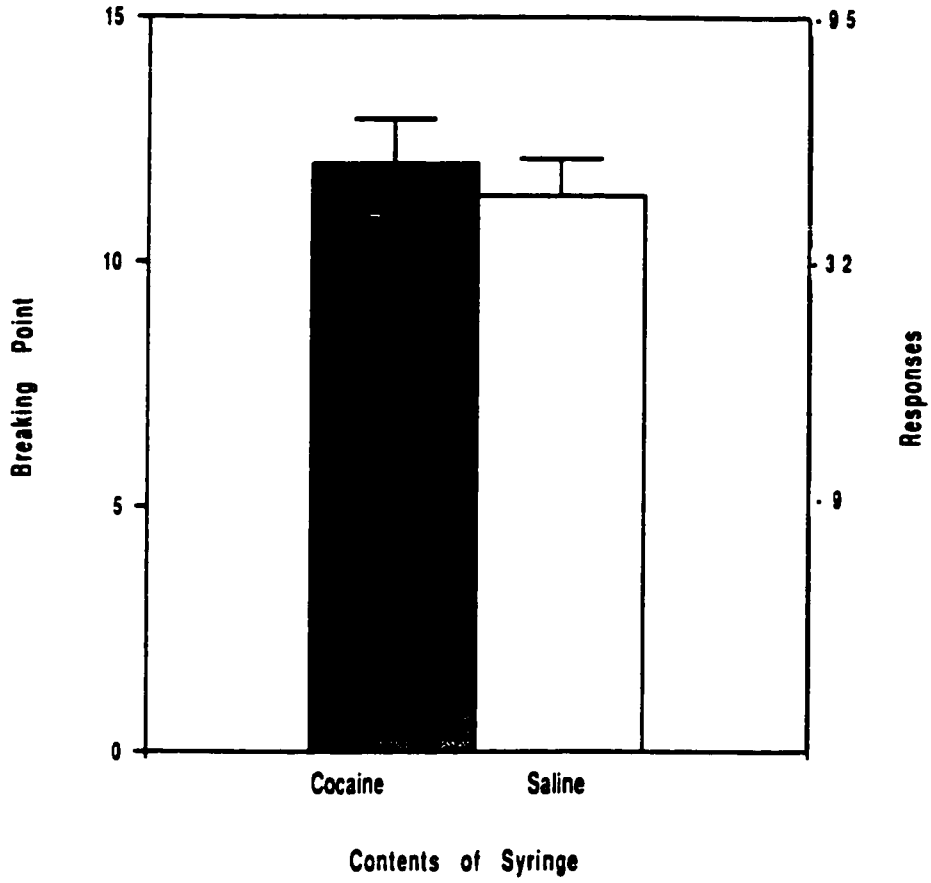
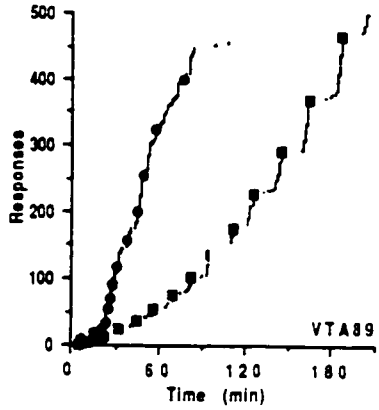
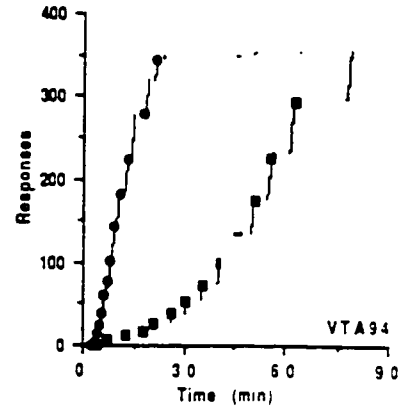
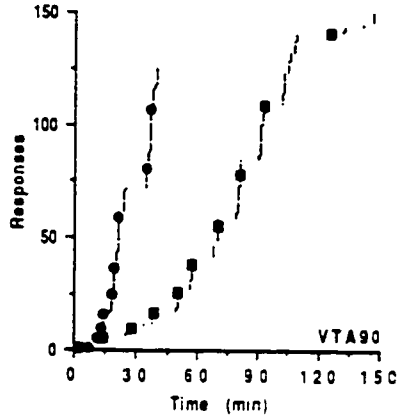

15
10
5
0
Breaking Point
95
32
9
Responses
Cocaine
Saline
Contents of Syringe

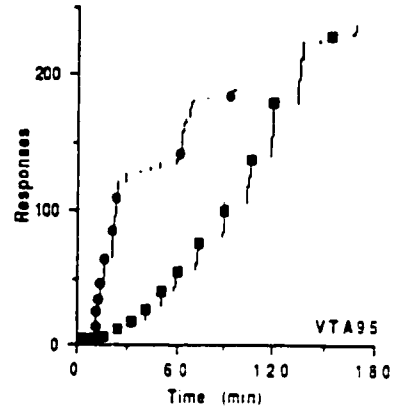
Figure 14

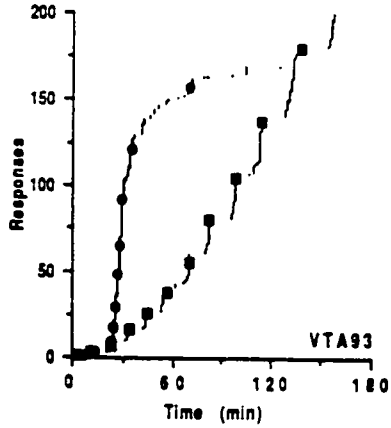
Cumulative response records for six animals depicting the pattern of responding for either intravenous cocaine or saline on a progressive ratio schedule of reinforcement following one hour of cocaine 'loading' (one hour of cocaine self-administration on an FR-1 schedule of reinforcement). Each dot represents a lever-press; squares represent infusions of cocaine (1.0 mg/kg/infusion); circles represent infusions of isotonic saline.


Responses
500
400
300
200
100
0
VTA89
0
60
120
180
Time (min)


Responses
400
300
200
100
0
VTA94
0
30
60
90
Time (min)


Responses
150
100
50
0
VTA90
0
30
60
90
120
150
Time (min)


Responses
200
100
0
VTA95
0
60
120
180
Time (min)


Responses
200
150
100
50
0
VTA93
0
60
120
180
Time (min)

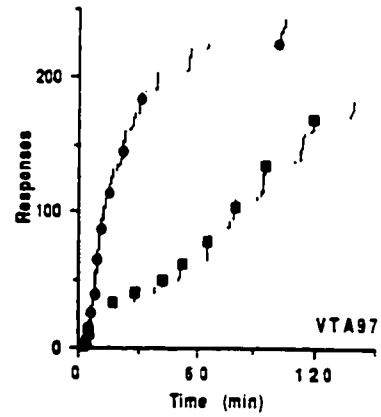
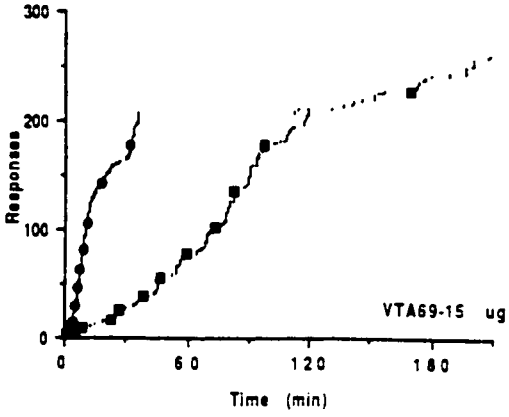
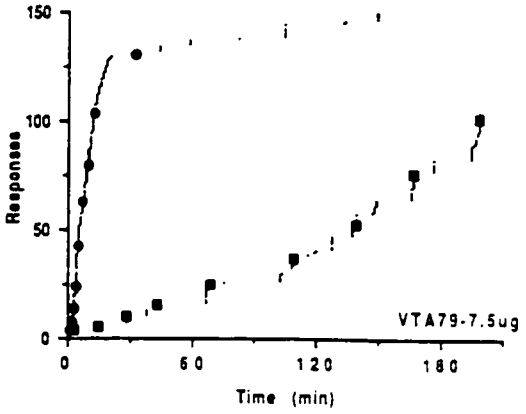
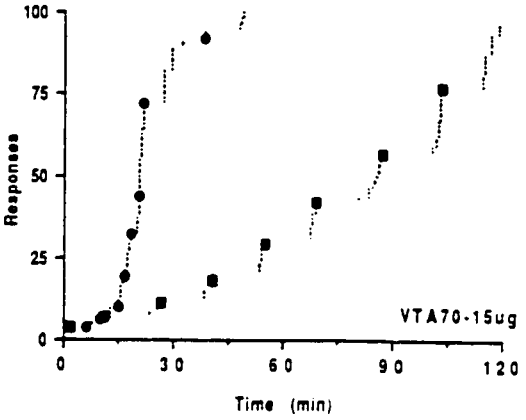

Responses
200
100
0
VTA97
0
60
120
Time (min)

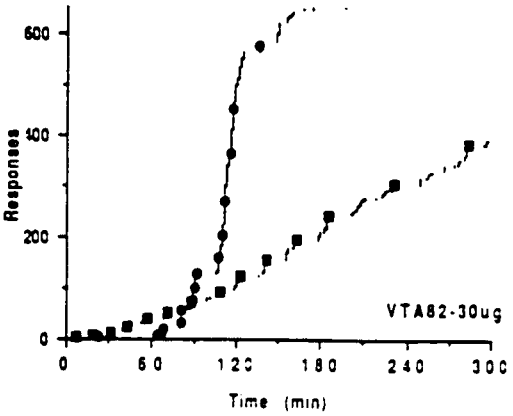
Figure 15

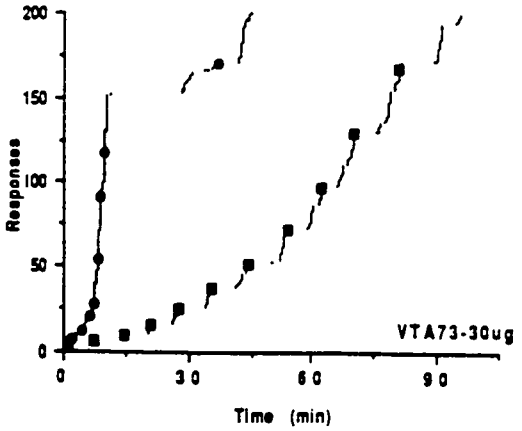
The effects of bilateral administration of atropine methyl nitrate into the VTA on response patterns: cumulative event records for six animals depicting the pattern of responding for intravenous cocaine (1.0 mg/kg/infusion) on a progressive ratio schedule of reinforcement following one hour of cocaine 'loading' (one hour of cocaine self-administration on an FR-1 schedule of reinforcement). Each dot represents a lever-press; squares represent infusions of cocaine following the injection of vehicle; circles represent infusions of cocaine following the injection of atropine methyl nitrate. The dose of atropine that produced this effect is noted in the bottom right corner of each panel.

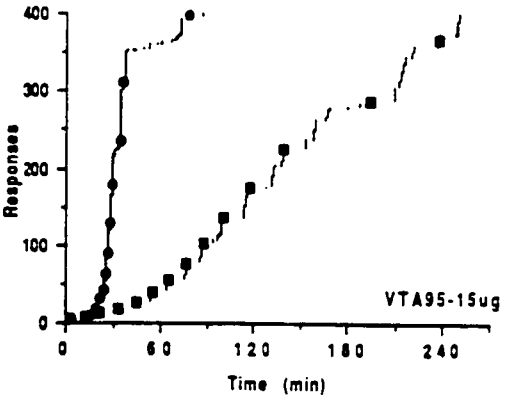

Responses
300
200
100
0
VTA69-15 ug
0
60
120
180
Time (min)


Responses
150
100
50
0
VTA79-7.5ug
0
60
120
180
Time (min)


Responses
100
75
50
25
0
VTA70-15ug
0
30
60
90
120
Time (min)


Responses
600
400
200
0
VTA82-30ug
0
60
120
180
240
300
Time (min)


Responses
200
150
100
50
0
VTA73-30ug
0
30
60
90
Time (min)


Responses
400
300
200
100
0
VTA95-15ug
0
60
120
180
240
Time (min)

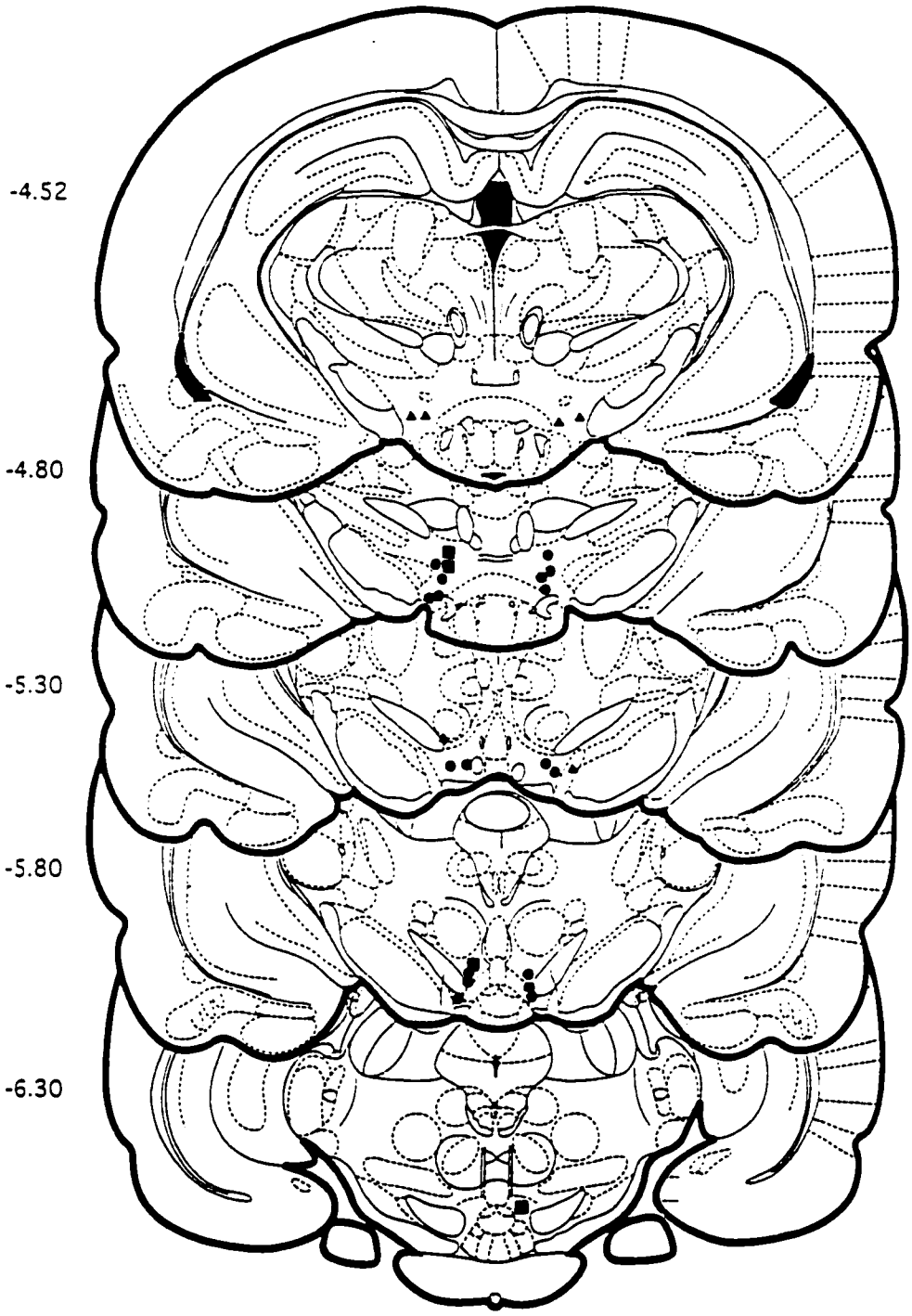
postreinforcement pauses during the early part of the session. The dose of atropine methyl nitrate that produced this effect varied between rats, but ranged from as low as 7.5 µg up to 30 µg. The 'priming' effect of previously self-administered cocaine was still evident, however. In two rats (VTA70 and VTA73), there was some indication that regular responding and pausing after reinforcement resumed during the latter part of the session after the atropine methyl nitrate was metabolized.

Histological Verification of Cannulae Placement

The injection sites for atropine methyl nitrate are illustrated in Fig. 16. Only bilateral placements within the VTA in an area showing staining for acetylcholinesterase were effective at reducing the rewarding effects of intravenous cocaine. On the rostral/caudal plane, effective injection sites were located 4.8 to 5.8 mm posterior to bregma. The location of unilateral injection sites and of bilateral injection sites outside of the VTA that failed to attenuate cocaine reward are also shown in Fig. 16.

Figure 16

Distribution of sites where atropine methyl nitrate was injected. Circles represent bilateral injections of atropine into the VTA (n=9); squares represent unilateral injections of atropine into the VTA (n=4); triangles represent bilateral injections of atropine outside of the VTA (n=3).


-4.52
-4.80
-5.30
-5.80
-6.30

DISCUSSION

These data indicate that blockade of muscarinic cholinergic receptors in the VTA by atropine methyl nitrate reduces the rewarding effects of intravenous cocaine, as assessed by responding on a progressive ratio schedule of cocaine reinforcement. In contrast to the effects of atropine sulphate, however, catalepsy is not induced by the lipophobic form of atropine. Locomotor activity is induced by the injections of atropine methyl nitrate.

The activity induced by the cocaine self-administered during the hour before the progressive ratio session was started was greatly enhanced when atropine methyl nitrate was injected into the VTA. This suggests that the immobility induced by atropine sulphate is due to muscarinic cholinergic blockade of some other site, perhaps the caudal substantia nigra. Injections of the cholinergic agonist, carbachol, into the caudal substantia nigra induced activity (contralateral circling) and was associated with a release of DA in the neostriatum in a study by Hernández-López et al. (1994). The opposite effect, a reduction of activity and striatal DA release, might be elicited if the cholinergic antagonist, atropine, was injected into the caudal substantia nigra pars compacta.

The breaking point, or final ratio of responses successfully completed under this progressive ratio schedule of reinforcement, presumably reflects the motivation of an animal to self-administer a drug such as cocaine. Generally, the breaking point is reduced when the rewarding effects of cocaine are attenuated. This result was obtained in the present experiment where it was found that breaking point was lower when lower dosages of cocaine were self-administered (see Fig. 10). Following bilateral administration of a high dose of atropine methyl nitrate (60 µg) into the VTA, the average breaking point was reduced and the response pattern was similar to that

observed when cocaine concentration is reduced to one-quarter of the concentration used during training (0.25 mg/kg/infusion versus 1.0 mg/kg/infusion) (Fig. 12). The similarity in the patterns of responding suggests that VTA injections of atropine effectively reduce the rewarding effects of cocaine.

On average, the breaking points obtained for different concentrations of cocaine were indicative of their reinforcing efficacy on the first test session. Examination of the results for individual animals, however, revealed that the order of presentation of the different cocaine concentrations could have a marked effect on breaking point. If the animal had previously received higher concentrations of cocaine then the breaking point remained elevated on the first session with a lower concentration of cocaine. Presumably, the animal's expectation of a higher concentration of cocaine affected its responding.

In Experiment 2, the rats self-administered cocaine (1.0 mg/kg/infusion) for one hour on an FR-1 schedule before a PR schedule of reinforcement was initiated. The 'priming' effect of the cocaine self-administered during this hour also had a strong effect on responding for cocaine on the subsequent PR schedule. If saline was substituted for cocaine after the drug had been self-administered for one hour, breaking points remained elevated (Fig. 13). Although breaking points were high, the response patterns were markedly different during extinction conditions than they were when cocaine was self-administered (Fig. 14). Breaking points were reached rapidly and there were no postreinforcement pauses after an infusion of saline.

Similar results were obtained when low doses of atropine methyl nitrate were bilaterally injected into the VTA. Although breaking points were unaffected by the manipulation, the response patterns were identical to those observed during saline substitution; breaking points were reached rapidly and there were no postreinforcement pauses after an infusion of cocaine (Fig. 15.). Evidently, low doses of atropine methyl

nitrate can completely abolish the rewarding effects of cocaine so that the animals respond as though they are self-administering saline. The 'priming' effect of the high levels of cocaine that have already been injected, however, continues to affect responding. The highest dose of atropine methyl nitrate tested is capable of blocking the 'priming' effect of cocaine as well as attenuating its rewarding effects.

In conclusion, it appears that muscarinic cholinergic blockade with atropine reduces cocaine reward. Atropine sulphate and atropine methyl nitrate attenuate the reinforcing efficacy of cocaine and also block the drug's 'priming' effects through actions in the VTA. The induction of catalepsy by atropine sulphate seems to be mediated at some other site. Blockade of muscarinic receptors at the same site in the VTA unexpectedly induced locomotor activity. Experiment 3 examined the effects of VTA injections of atropine sulphate and atropine methyl nitrate on locomotion.

EXPERIMENT 3: THE EFFECTS OF MUSCARINIC RECEPTOR BLOCKADE OF THE VENTRAL TEGMENTAL AREA WITH ATROPINE SULPHATE OR ATROPINE METHYL NITRATE ON LOCOMOTION

Introduction

As discussed in the General Introduction, systemic injections of antimuscarinic drugs such as atropine or scopolamine induce locomotor activity. The neuronal site that mediates this effect has not been determined. Cholinergic stimulation of the DA cell body regions, the VTA or SN, activates DA neurons. Consequently, blockade of muscarinic receptors in the VTA with atropine would be expected to reduce the activity of these cells with a concommitant reduction in locomotor activity and other DA-related behaviours. As seen in Experiments 1 and 2, both atropine sulphate and atropine methyl nitrate injected into the VTA attenuated the rewarding effects of intravenous cocaine.

Locomotor activity was not measured directly during Experiments 1 and 2, however. Furthermore, the animals were not visually monitored after the atropine was injected and they were returned to the intravenous self-administration operant cages. Nevertheless, marked effects on locomotion were observed while the atropine was injected into the VTA. Atropine sulphate injections into the VTA induced catalepsy. The cataleptic effects of atropine sulphate administered into the VTA were reported by Ikemoto and Panksepp (1996) and were also observed in Experiment 1 of this thesis. In Experiment 2, however, the administration of atropine methyl nitrate into the VTA appeared to increase locomotor activity. The animals became so active when the atropine methyl nitrate was injected that it was impossible to finish the injections properly or to reattach the self-administration leads unless the animals were anaesthetized. The

contrasting effects of the two forms of atropine on locomotion are likely due to the difference in their lipid solubility. The lipophilic form of atropine, atropine sulphate, is more likely to block muscarinic receptors at sites distal to the injection site while the actions of the lipophobic form of the drug, atropine methyl nitrate, are more likely to be confined to muscarinic receptors near the injector tip in the VTA. This series of experiments investigated the effects of unilateral and bilateral VTA injections of atropine sulphate or atropine methyl nitrate on locomotor activity.

Methods

Atroprine Injections into the VTA

Thirty-five animals were used in this study. Bilateral guide cannulae were implanted 2 mm dorsal to the VTA as described in the General Methods section. The drug and vehicle solutions and injection and histological procedures are also described in the General Methods.

The locomotor activity of the rats was measured following either unilateral or bilateral injections of atropine methyl nitrate, atropine sulfate, or vehicle into the VTA. The activity boxes (20x41x25 cm) were constructed of wood with a clear plastic front and a wire grid floor. Two photocells were positioned 4 cm above the floor and separated the compartment into three equal areas along its length. The photocells were connected via an electrical interface to a computer in an adjoining room. Red lights were used to activate the photocells, and beam crossings were recorded at 15-min intervals. During testing, the room was dark and sounds were masked with white noise. Activity was measured for 3 hr; recording began immediately following the placement of the animals into the activity boxes. Data from seven animals with both cannulae placed outside of the VTA and from four animals in which histological analysis indicated tissue damage due to haemmorhage or infection were excluded from the statistical analysis. One animal was also excluded from the study because the cerebral ventricles were abnormally enlarged.

Dorsal Controls

Six animals were used in this study. In this case, the injection cannulae extended only 1 mm (rather than 2 mm) beyond the guide cannulae. Bilateral injections of atropine methyl nitrate (7.5 µg/0.5 µl) were administered. The animals were immediately placed into the activity boxes and their behaviour was observed by the experimenter until any drug-induced behavioural effects had dissipated (generally within 30 min). A unilateral injection of atropine methyl nitrate was tested in one animal and higher doses of atropine methyl nitrate were injected into another subject. After histological examination, one animal was excluded from the study because the injectors were located more than one mm dorsal to the VTA.

Results

Atropine Injections into the VTA

An increase in locomotor activity was the predominant effect following the administration of both atropine sulphate and atropine methyl nitrate into the VTA. An initial decrease in activity (due to the induction of catalepsy) was observed when atropine sulphate was injected into the VTA. This effect dissipated within 15 to 30 minutes and an increase in locomotion was then evident. Atropine methyl nitrate induced locomotor activity immediately after it was injected. At lower doses, the activity resembled amphetamine-induced locomotion. The animals were very active and engaged in exploratory behaviour (sniffing and forward locomotion). Strong, driven locomotion was induced by higher doses of atropine methyl nitrate in some rats; tight circling was observed.

Bilateral injections of atropine sulphate caused a dose-dependent increase in locomotor activity (Fig. 17) (2-factor [dose x time] repeated measures ANOVA; $F_{4,605}=4.553$, $p=0.003$). Nevertheless, a reduction in activity was apparent immediately after the atropine sulphate was administered (2-factor [dose x time] repeated measures ANOVA; interaction: $F_{44,605}=3.051$, $p=0.001$). This initial immobility was due to the catalepsy-inducing effects of atropine sulphate. The subsequent increase in locomotion persisted throughout most of the 3-hour test session for all doses tested.

Bilateral injections of atropine methyl nitrate immediately induced locomotor activity. The activating effects of the drug were of shorter duration and were less intense than the effects of atropine sulphate (Fig. 18). The activating effects of 7.5 µg or 15 µg of atropine methyl nitrate lasted for only 15 min (Scheffe F=5.426 and 7.953

Figure 17

Time course of the effects of bilateral injections of atropine sulphate into the VTA on locomotion. Points represent the mean number of beam interruptions (±SEM): open circles-vehicle; closed circles-atropine sulphate; n=12. Dose of atropine sulphate administered is noted in the top left corner of each panel.

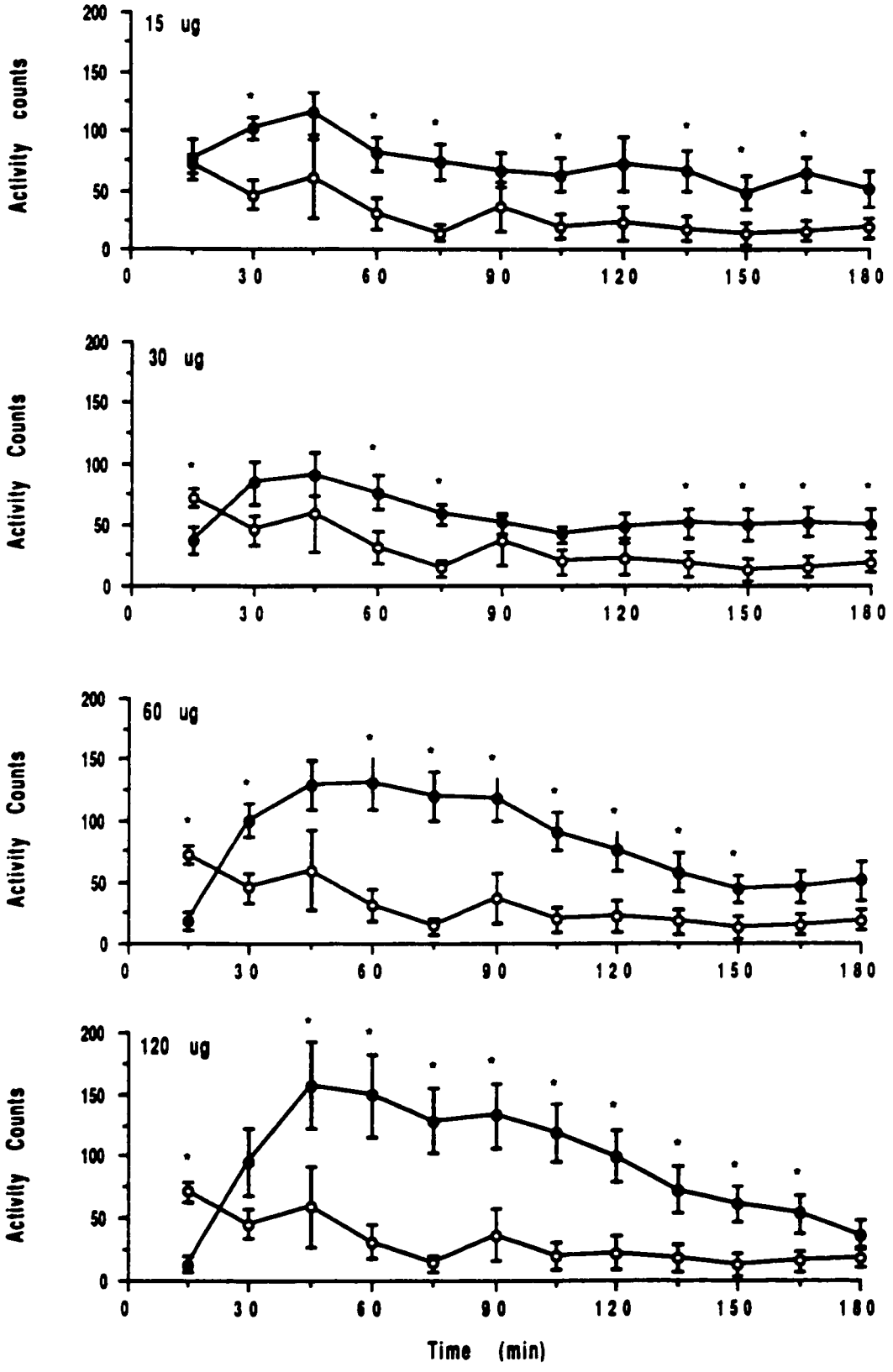
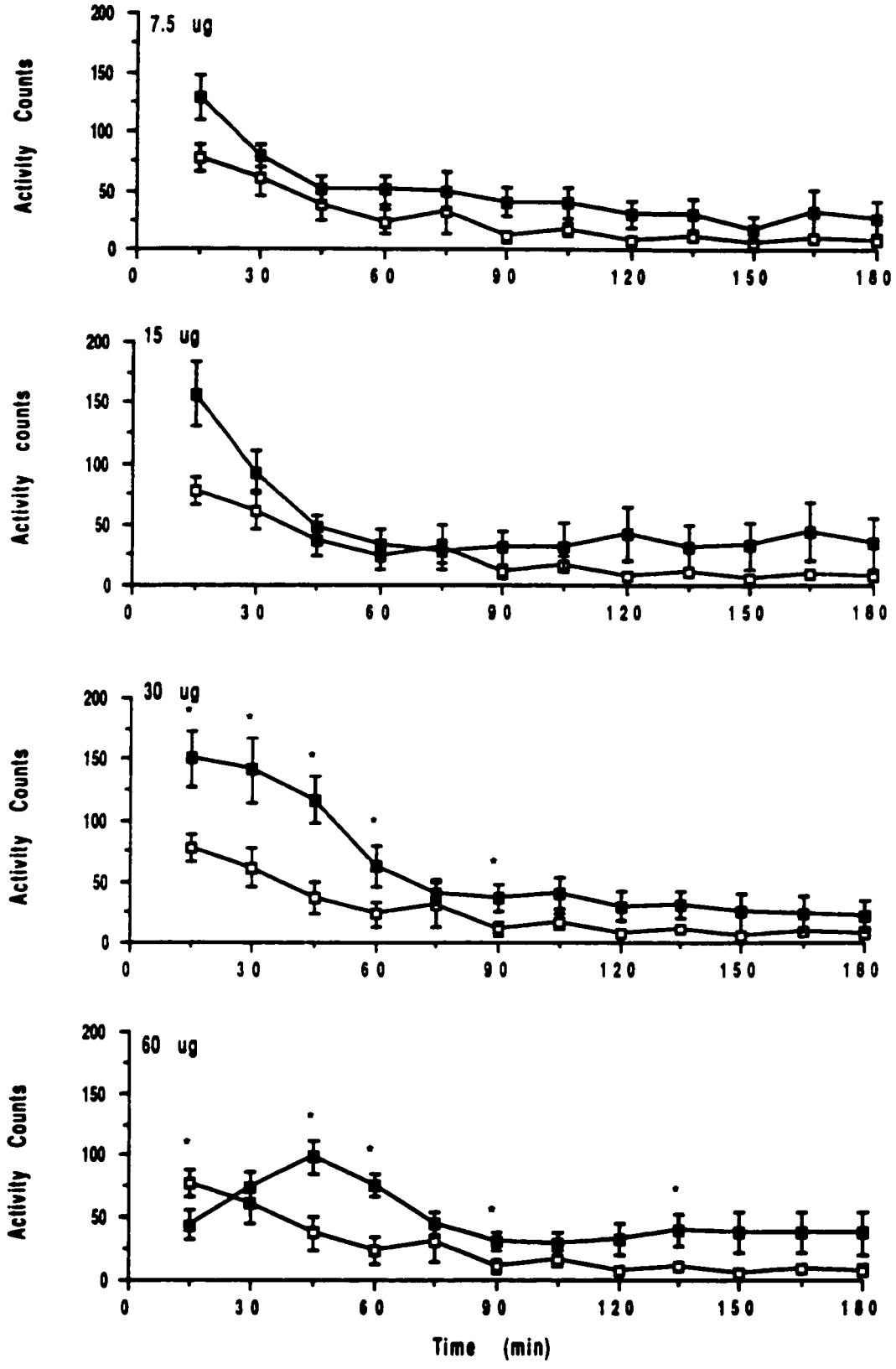

15 ug
30 ug
60 ug
120 ug
Activity counts
Activity Counts
Time (min)
0
50
100
150
200
30
60
90
120
150
180

Figure 18

Time course of the effects of bilateral injections of atropine methyl nitrate into the VTA on locomotion. Points represent the mean number of beam interruptions (±SEM): open circles-vehicle; closed circles-atropine sulphate; n=13. Dose of atropine methyl nitrate administered is noted in the top left corner of each panel.


7.5 ug
15 ug
30 ug
60 ug
Activity Counts
Activity counts
200
150
100
50
0
0
30
60
90
120
150
180
Time (min)

respectively, $p<0.05$). Locomotion was stimulated for one hour following the bilateral administration of 30 µg of atropine methyl nitrate into the VTA. The highest dose of atropine methyl nitrate (60 µg) initially reduced the number of activity counts (2-factor [dose x time] repeated measures ANOVA; interaction: $F_{44, 660}=2.814$, $p=0.001$). This effect was not due to immobility or catalepsy, however. Generally, the animals were engaged in locomotor behaviours (tight circling, rearing) that did not result in photobeam interruptions.

Unilateral injections of atropine sulphate were also effective at inducing locomotion, although higher doses of the drug were required and the effect was of shorter duration (Fig. 19) (2-factor [dose x time] repeated measures ANOVA; main effect of dose: $F_{4,715}=8.093$, $p=0.001$). An initial reduction in activity was apparent during the first 15 min of the session although not to the same degree as was the immobility produced by bilateral injections (2-factor [dose x time] repeated measures ANOVA; interaction: $F_{44,715}=4.175$, $p=0.001$). The locomotor activation following the unilateral administration of the highest dose of atropine sulphate tested (120 µg) lasted for 120 min compared to 165 min after bilateral injections of this drug (Fig. 17).

Unilateral injections of atropine methyl nitrate increased locomotor activity (2-factor [dose x time] repeated measures ANOVA; main effect of dose: $F4,1265=3.911$, $p=0.0051$), but the duration of the effect was less than that for bilateral injections (Fig. 20). The locomotor-stimulating effects of the drug were not apparent unless 30 µg of atropine methyl nitrate was injected; activity levels following 7.5 µg or 15 µg of atropine methyl nitrate did not differ from activity levels after injections of vehicle into the VTA. Unlike bilateral injections, unilateral injections of the highest dose of atropine methyl nitrate tested (60 µg) did not induce behaviours that interfered with locomotion. An enhancement of activity was apparent immediately after the injection of atropine methyl nitrate and persisted for 75 min.

Figure 19

Time course of the effects of unilateral injections of atropine sulphate into the VTA on locomotion. Points represent the mean number of beam interruptions (±SEM): open circles-vehicle; closed circles-atropine sulphate; n=14. Dose of atropine sulphate administered is noted in the top left corner of each panel.

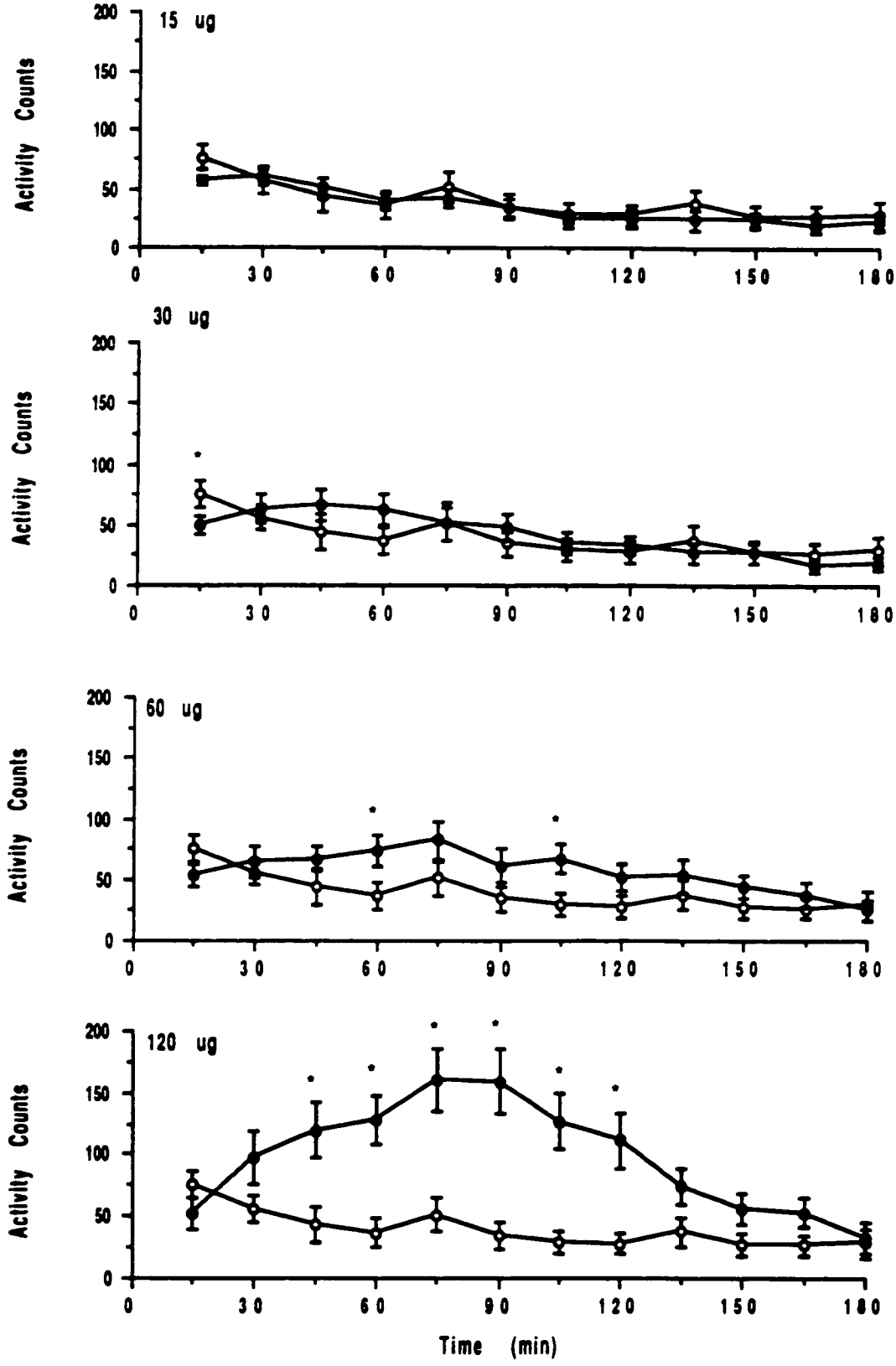
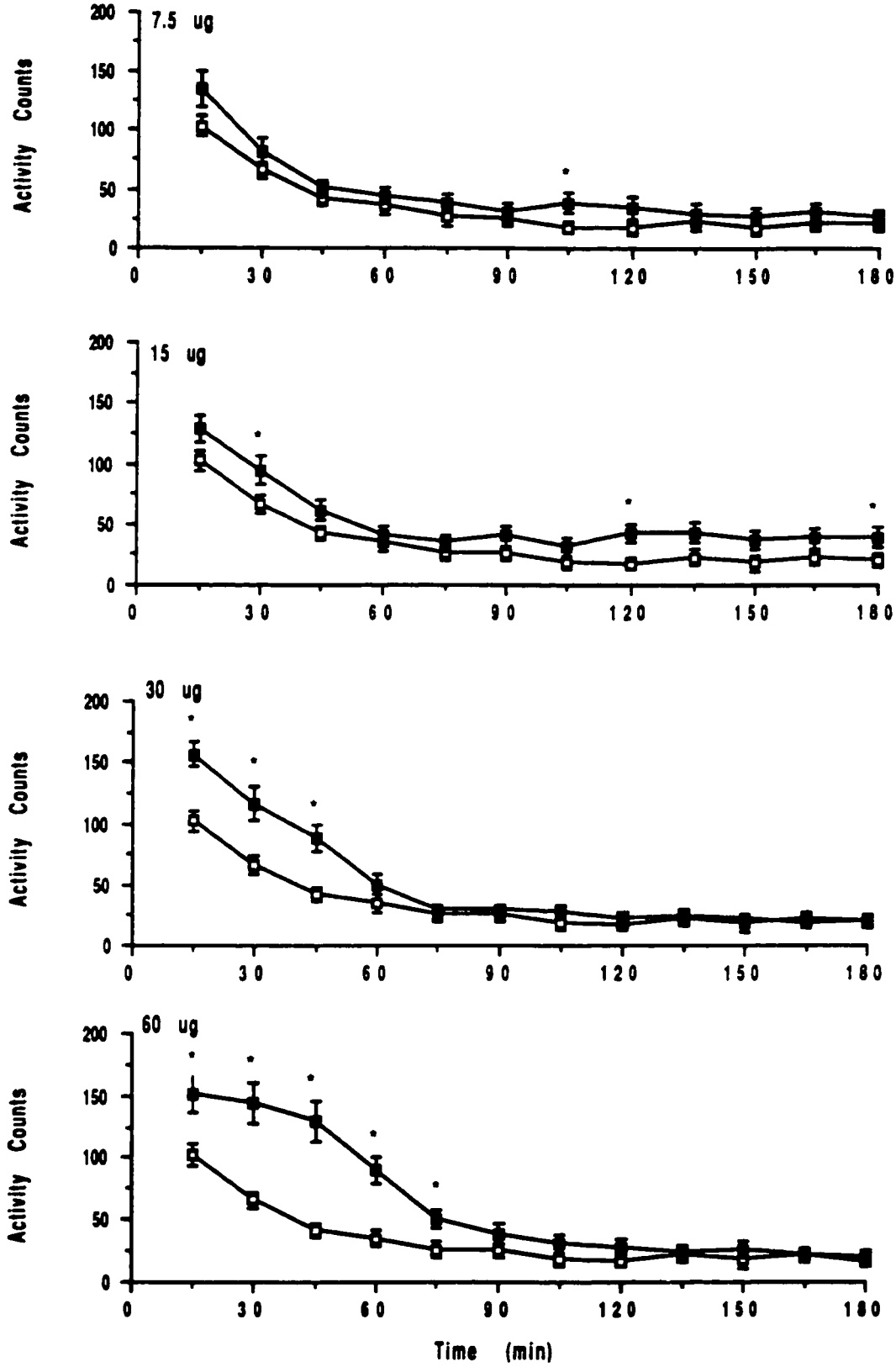

15 ug
30 ug
60 ug
120 ug
Activity Counts
Time (min)
0
50
100
150
200
30
60
90
120
150
180

Figure 20

Time course of the effects of unilateral injections of atropine methyl nitrate into the VTA on locomotion. Points represent the mean number of beam interruptions (±SEM): open circles-vehicle; closed circles-atropine methyl nitrate; n=24. Dose of atropine methyl nitrate administered is noted in the top left corner of each panel.


7.5 ug
15 ug
30 ug
60 ug
Activity Counts
Time (min)
0
50
100
150
200
30
60
90
120
150
180

Dorsal Controls

Diffusion of the drug from the place where it was injected to more distal sites is a problem when trying to determine the locus of a drug's actions. Often the drug will diffuse up the cannula shaft to more dorsal sites. Atropine methyl nitrate was injected one mm dorsal to the VTA in several animals and the ensuing behavioural effects were markedly different from those observed when the drug was injected into the VTA. Whereas low doses of atropine methyl nitrate injected into the VTA induce sniffing and exploratory locomotion, dorsal bilateral injections of 30 µg of atropine methyl nitrate were clearly aversive in the single animal tested. The animal began squealing, ran backwards, rearing and flipping over, and eventually died, presumably from the induction of seizures.

The rest of the animals were injected with the lowest dose of atropine methyl nitrate used in these experiments (7.5 µg). During the injections, the predominant behaviour was defensive digging into the wood chips in the bottom of the bucket. One animal had a postural deficit and was tilted over on one side while in the injection bucket. This postural deficit was not apparent once the rat was placed into the activity box. Heavy sniffing was the predominant behaviour but the animal also squealed and backed up while the drug was active. These effects dissipated within 30 min. A second animal also began digging in the wood chips (predominant behaviour) during the injections although it also did some sniffing, circling, and rearing. When picked up, the animal's tail flailed and the legs pumped. Once it was placed in the activity box, the animal began gnawing on the bars in the floor (predominant behaviour) although it also engaged in rearing and heavy sniffing. A slight postural deficit was observed and the animal was tilted a bit to one side. These effects dissipated within 30 min. The third

animal also dug in the wood chips during the injection. The digging was followed by strong, driven locomotion (tight circling). The tail flailed and limbs continued pumping when the animal was picked up. A postural deficit was apparent when the animal was placed in the activity box; it lay on its side except for explosive bursts of circling. Once the drug began to wear off, the animal appeared to be pushing itself backwards during the bursts of locomotion. These effects dissipated after 30 min and the animal lay prone across the front of the cage. The final animal received a unilateral injection of 7.5 µg of atropine methyl nitrate dorsal to the VTA. This injection induced tight circling that is sometimes termed 'barrel-rolling' behaviour.

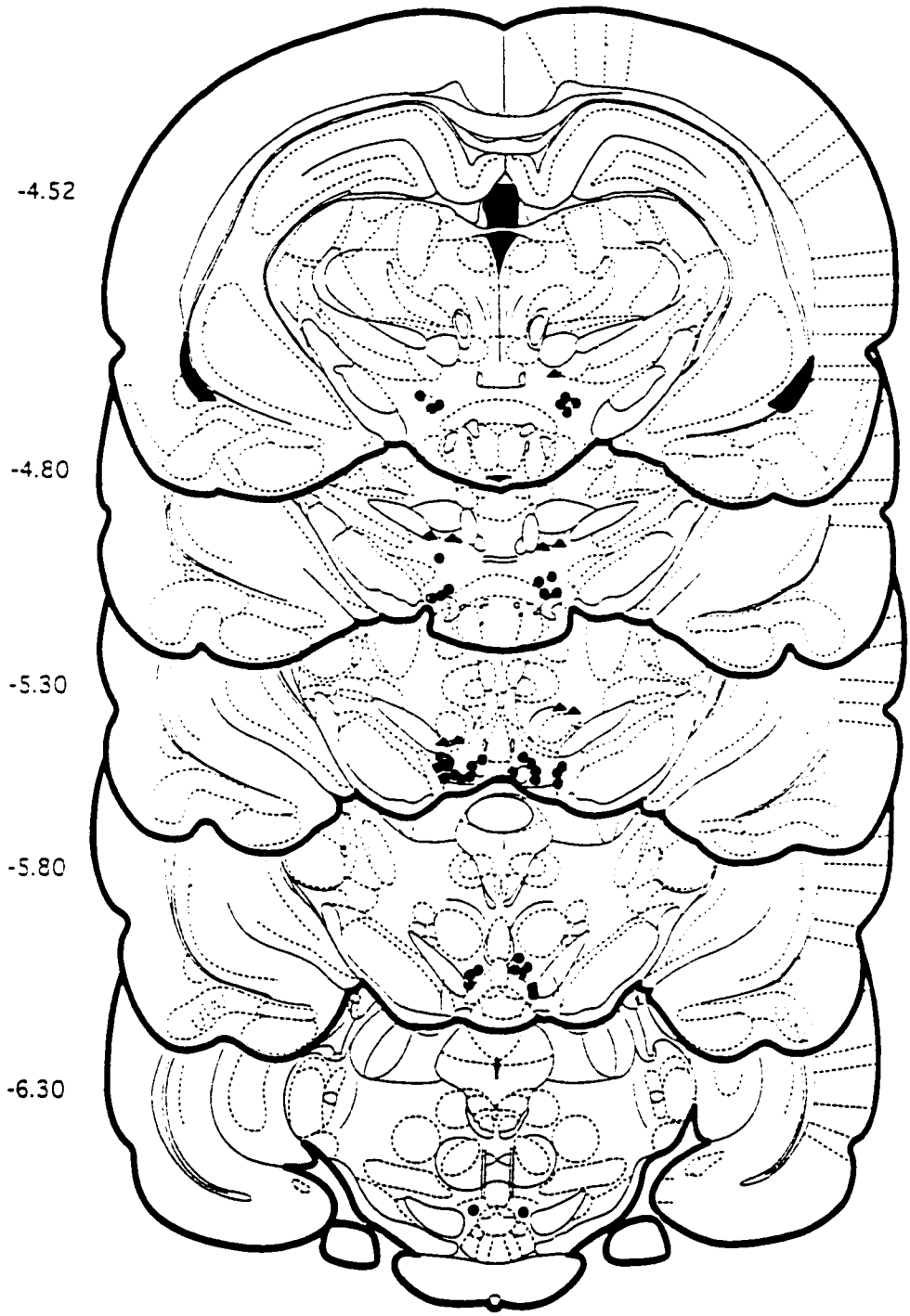
These behaviours (digging, squealing, backwards locomotion, seizures, gnawing, heavy sniffing, explosive bursts of circling, 'barrel-rolling') were not observed when the atropine methyl nitrate was injected directly into the VTA.

Histological Verification of Cannulae Placement

Fig. 21 depicts the placement of the cannulae within the VTA as well as the placements of the dorsal injections. The VTA cannulae were located in an area of acetylcholinesterase staining. The dorsal cannulae were located within 1 mm of the area stained for acetylcholinesterase. Placements ranged from 4.52 to 6.04 mm posterior to bregma.

Figure 21

Distribution of sites in the vicinity of the VTA where atropine was injected. Circles represent injections of atropine into the VTA; triangles represent injections of atropine dorsal to the VTA.


-4.52
-4.80
-5.30
-5.80
-6.30

Discussion

Thus blockade of muscarinic receptors in the VTA with either atropine sulphate or atropine methyl nitrate increases locomotor activity. The locomotor-stimulating effects of atropine are apparent after unilateral injections into the VTA although bilateral injections produce a stronger effect of longer duration. The activating effects of atropine methyl nitrate on locomotion appear to be specific to its actions in the VTA as injections of this drug dorsal to the VTA produce locomotion of a markedly different character. The initial reduction in locomotor activity seen following the administration of atropine sulphate is mediated by some site other than the VTA as this effect is not produced by atropine methyl nitrate.

Contrary to expectation, locomotor activity was stimulated when muscarinic receptors in the VTA were blocked by atropine. This behavioural activation was opposite to the effects of similar injections on cocaine reward. The locomotion induced by atropine resembled the locomotion induced by amphetamine. Exploratory locomotion and sniffing were enhanced following the administration of atropine into the VTA. As reviewed in the General Introduction, electrophysiological and neurochemical studies indicated that cholinergic afferents excite the mesolimbic DA system. It was predicted, therefore, that a cholinergic antagonist like atropine would reduce DAergic activity in the mesolimbic system and DA-mediated behaviours such as exploratory locomotion and cocaine reward would be reduced.

Unilateral injections of atropine methyl nitrate into the VTA did not affect the average breaking points for intravenous cocaine self-administration (Fig. 9). Examination of the cumulative event records for the four animals that received unilateral atropine injections revealed that the injection of 60 µg of atropine methyl nitrate disrupted the post-reinforcement pause and caused the breaking point to be

reached more quickly in one animal. The responding of the remaining three animals was unaffected by unilateral injections of atropine methyl nitrate. Generally, bilateral injections of atropine into the VTA were necessary for the attenuation of cocaine reward. On the other hand, BSR thresholds are reported to be increased significantly by ipsilateral injections of atropine (Kofman and Yeomans, 1989; Kofman et al., 1990; and Yeomans et al., 1985). The intravenous self-administration procedure does not seem to be sensitive enough to detect the attenuation of reward produced by unilateral muscarinic blockade of the VTA. The procedure used to assess locomotor activity did detect changes in behaviour produced by the unilateral administration of atropine.

Atropine sulphate is highly lipophilic and likely to diffuse throughout the brain. The cataleptic effect induced by the administration of atropine sulphate into the VTA probably was mediated by blockade of muscarinic receptors outside of the VTA as atropine methyl nitrate did not induce catalepsy when it was injected into the VTA. Atropine methyl nitrate is lipophobic and the effects produced by this drug were more likely to be mediated by receptors in the proximity of the injection site. The common effect of both atropine sulphate and atropine methyl nitrate on locomotion was an increase in the level of activity. One possible site for the induction of catalepsy by atropine sulphate is the caudal substantia nigra pars compacta. Injections of the cholinergic agonist, carbachol, into this site have been reported to increase activity (Hernández-López et al., 1994). Perhaps muscarinic blockade of receptors in the caudal substantia nigra would induce the opposite effect, immobility. Injections of atropine methyl nitrate into sites dorsal to the VTA were clearly aversive and induced behaviours (digging, gnawing, backwards locomotion, seizures) that did not resemble in any way the exploratory locomotion observed after similar injections into the VTA. Comparison of the histology figures for the cocaine self-administration experiment (Figs.16) and the locomotion experiment (Fig. 21) indicates that the atropine methyl

nitrate injections producing an attenuation of cocaine reward and those that stimulated locomotor activity were similarly located in the VTA.

Exploratory locomotion and cocaine reward have been believed to be DA-mediated. Manipulations that reduce the extracellular level of DA in the nucleus accumbens generally reduce locomotor activity and attenuate reward (Wise and Bozarth, 1987). The current series of experiments, however, demonstrates a clear dissocation between the effects of muscarinic blockade in the VTA on reward processes and locomotor activation; cocaine reward is attenuated while locomotor activity is enhanced. Blaha et al. (1996) demonstrated that injections of the cholinesterase inhibitor, neostigmine, into the VTA increased DA efflux in the Acc. The VTA placements used by Blaha et al. (1996) were similar to those employed in the current series of experiments (5.0 to 6.5 mm posterior to bregma). These data suggest that cholinergic blockade of the VTA with a muscarinic antagonist like atropine should reduce DA levels in the Acc. The following experiment was designed to investigate the effects of atropine methyl nitrate administration into the VTA on extracellular levels of DA within the ipsilateral Acc. The relationship between DA levels in the Acc and locomotor activity following the unilateral administration of atropine methyl nitrate into the VTA was also examined.

EXPERIMENT 4: BLOCKADE OF MUSCARINIC RECEPTORS IN THE VENTRAL TEGMENTAL AREA WITH ATROPINE METHYL NITRATE INCREASES LOCOMOTION AND DOPAMINE RELEASE IN THE IPSILATERAL SHELL OF THE NUCLEUS ACCUMBENS AS MEASURED BY MICRODIALYSIS

Introduction

As reviewed in the General Introduction, neurochemical, electrophysiological, and behavioural studies indicate that cholinergic stimulation of DA cells in the VTA or SN enhances their activity, increases DA release in the ventral striatum or striatum, and stimulates locomotor activity. One would predict, therefore, that blockade of muscarinic receptors in the VTA would reduce the activity of mesolimbic DA cells and inhibit DA-related behaviours. It is known that following the systemic administration of the muscarinic receptor antagonists, scopolamine or atropine, locomotor activity is enhanced, and that following the systemic administration of cholinergic agonists, it is attenuated. This would suggest that like many other drugs, those that act at cholinergic receptors do so at many different sites in the brain, having in some cases apparently opposite effects on general activity.

Furthermore, the self-administration of intravenous cocaine is believed to be dependent on the activity of DA neurons in the mesocorticolimbic system, and it was shown in Experiments 1 and 2 that blockade of muscarinic receptors in the VTA with atropine sulphate or atropine methyl nitrate abolished the rewarding effects of intravenous cocaine. These results are consistent with the finding that injections of atropine sulphate into the VTA increase brain stimulation reward thresholds (Kofman

and Yeomans, 1989). This attenuation of reward was anticipated because of the neurochemical and electrophysiological data indicating that acetylcholine stimulates dopaminergic neurons in the VTA. It was expected, therefore, that blockade of the cholinergic muscarinic receptors in the VTA with the muscarinic antagonist, atropine, would inhibit these dopaminergic neurons and reduce reward in a manner similar to that seen after the administration of DA antagonists.

A corresponding decrease in locomotor activity was expected to follow the administration of atropine into the VTA for the same reason; atropine would reduce the activity of mesolimbic DA neurons. Locomotion would thus be predicted to decrease as it does following the administration of dopaminergic antagonists. The increased locomotion seen following systemic injections of a muscarinic antagonist was, therefore, believed to arise from a site other than the VTA. Nevertheless, as the data obtained in Experiment 3 indicate, this is not the case. Injections of atropine into the VTA increase locomotor activity just as systemic injections do.

The striking dissociation between locomotion and reward that occured in this series of experiments was unexpected and difficult to explain as both reward and locomotion are believed to be DA-mediated behaviours (Wise and Bozarth, 1987; and Wise and Rompré, 1989). The next experiment examined the effects of VTA injections of atropine methyl nitrate on extracellular DA levels in the shell of the ipsilateral Acc. The effects of these injections on locomotor activity were also monitored.

Methods

Animal Subjects and Surgery

Thirty-seven rats were used in this study. Bilateral guide cannulae were implanted dorsal to the VTA as described in the General Methods section. Bilateral stainless steel guide cannulae (18-gauge; Plastic Products, St. Albans, VT; model C309GA) were also implanted 5.0 mm dorsal to the nucleus accumbens. The coordinates were 1.5 mm anterior to bregma, 2.5 mm lateral to the midline, and 3.3 mm ventral to the skull surface (Paxinos and Watson, 1986). The cannulae were angled 10 degrees to the midline.

Experimental Procedure

Concentric microdialysis probes were constructed according to the design of Robinson and Whishaw (1988). Each probe consisted of a fluid inlet constructed of polyethylene tubing (PE20) attached to a 24-gauge stainless steel outer cannula. This cannula was cemented to a 4 mm length of cellulose dialysis fiber (Brain Research Institute, i.d.=215 µm; o.d.=251 µm; 13,000 MW cut-off) that was sealed at the end with epoxy cement. This cement covered 1 mm of cellulose fiber leaving a working surface of 3 mm. The fluid outlet consisted of an inner length of silica tubing (Polymicro Technologies, Phoenix, AZ, i.d.=75 µm, o.d.=150 µm) that stopped 1 mm from the plugged end of the cellulose dialysis fiber. The silica tubing exited the polyethylene tubing through a hole that was sealed with epoxy cement.

The rat was anaesthetized lightly with 30 mg/kg i.p. of sodium pentobarbital for the insertion of a dialysis probe into the nucleus accumbens. A probe was inserted into the guide cannula, threaded through a metal spring attached to a brass dual-channel swivel, and cemented into place with acrylic dental cement. The animal recovered from anaesthesia after approximately one hour. Following implantation, the probe was continuously perfused at a rate of 1 µl/min with artificial cerebrospinal fluid through one channel of the swivel.

Microdialysis sampling began twenty hours after probe implantation. Baseline dialysate samples were collected every 15 min (15 µl) for 90 min. Next, the obturator was removed from the ipsilateral guide cannula. An injector extending 2 mm beyond the tip of the guide cannula was inserted into the VTA and 0.5 µl of the vehicle (artificial cerebrospinal fluid) was injected through the second channel of the dual-channel swivel. The injector was removed after five minutes. In all other respects, the injection procedure was identical to that described in the General Methods. Dialysate samples were then collected for 60 minutes. Finally, the injector was inserted into the guide cannula a second time and atropine methyl nitrate (7.5, 15, 30, or 60 µg/0.5 µl) was injected into the VTA through the swivel. The injector was removed after five minutes and dialysate samples were collected for 180 minutes.

Analytical Procedure

Dialysate samples were assayed for DA, DOPAC, HVA, and 5-HIAA using isocratic, reverse-phase, high-performance liquid chromatography (HPLC) with electrochemical detection. Each dialysate sample was injected through a reverse-phase column (supelcosil, 3 µm, LC-18, Supelco) via a Rheodyne injection valve. DA, DOPAC, HVA, and 5-HIAA were quantified on an ESA Coulochem II detector (model 5200) with an

analytical cell (ESA, model 5011) with two electrodes in series: an oxidizing electrode (+340 mV; 500 nA) and a reducing electrode (-270 mV; 5 nA). The mobile phase for this system consisted of 60 mM NaH_2PO_4, 3.0 mM ascorbate, 0.035 mM sodium dodecyl sulphate (SDS), and 0.1 mM ethylenediaminetetraacetic acid (EDTA) in 15% HPLC grade methanol in nanopure water adjusted to pH 3.3-3.5 with NaOH. A Waters 510 HPLC pump pumped the recycled mobile phase at a rate of 1.4 ml/min. A detection limit of 5 fmoles was routinely achieved.

Locomotor Activity during Microdialysis

Microdialysis experiments were conducted in octagonal chambers constructed of opaque plastic wall panels and a wire grid floor. The boxes were 38 cm in diameter and 33 cm in height. Each wall panel was 15.5 cm in width. Four photocells (one on alternating wall panels) were positioned 5 cm above the floor. The photocells were connected via an electrical interface to a computer. Red lights were used to activate the photocells and beam crossings were recorded at 15-min intervals. Activity was measured for the duration of the microdialysis experiment; recording began when the collection of baseline dialysate samples was initiated.

Data Analysis

Standard statistical methods were used in evaluating the results of the experiments (Keppel, 1991). Results are expressed as mean ± standard error of the mean for each group. The concentration of DA and its metabolites are described as a percentage of baseline levels while the measure of activity is the number of counts registered in a 15-min period. Analysis of variance was used to test for significant

changes. Fisher's least significant difference test was applied for post-hoc analysis. The relationship between locomotor activity and DA concentration in the nucleus accumbens was evaluated by determining the correlation coefficient. A probability of less than 0.05 was used as the criterion for judging statistical significance.

Results

Two animals of the thirty-seven implanted with microdialysis probes and guide cannulae were excluded from analysis because of the presence of infection or haemmorhage at the injection site. One animal was excluded from the study because the cerebral ventricles were abnormally enlarged. Four animals were excluded from analysis because injection sites lay outside of the VTA. Animals with cannulae placements within the VTA, but with non-functional microdialysis probes or with microdialysis probes located outside of the Acc received VTA injections of atropine and their locomotor activity was assessed.

The unilateral administration of the cholinergic antagonist, atropine methyl nitrate, into the VTA increased both locomotor activity and the concentration of DA in the ipsilateral nucleus accumbens. Although the relationship between locomotion and DA concentration was not strong, it was statistically significant. Generally, activity and DA concentration returned to baseline levels within 15 min of each other at all doses tested. The metabolites of DA, DOPAC and HVA, were also elevated after atropine was injected into the VTA but the serotoninergic metabolite, HIAA, was not.

DA Concentration in the Nucleus Accumbens

Injection of vehicle into the VTA produced a transient increase in DA levels in the Acc in a few animals, but this was not a general effect. Consequently, there was no significant increase in extracellular DA concentration because of handling or other non-pharmacological effects of an intracranial injection into the VTA.

All of the doses of atropine methyl nitrate injected into the VTA produced a similar elevation in the concentration of extracellular DA in the nucleus accumbens although the duration of the effect was greater for higher doses of atropine (Fig. 22)[2-factor (dose x time) repeated measures ANOVA; significant effect for time, $F_{12,360}=8.415$, $p=0.0001$]. There was no significant difference in DA levels because of the dose of atropine administered. The maximal concentration of DA observed for each dose of atropine injected was as follows: 157.72±25.21% (60 µg), 151.92±25.36% (30 µg), 150.77±34.72% (15 µg), and 129.18±14.96% (7.5 µg).

Post-hoc analysis indicates that the concentration of dopamine was significantly elevated within 15 min of the atropine injection. The duration of the effect was greater for higher concentrations of atropine methyl nitrate. DA remained significantly elevated for 90 min following the injection of 60 µg of atropine methyl nitrate [One-factor repeated measures ANOVA; $F_{12,116}=2.658$, $p=0.004$; Fisher LSD=37.965]. The duration of the effect was only 45 min following 30 µg [One-factor repeated measures ANOVA; $F_{12,90}=2.418$, $p=0.0107$; Fisher LSD=29.563] or 15 µg of atropine [One-factor repeated measures ANOVA; $F_{12,103}=2.748$, $p=0.0034$; Fisher LSD=30.336]. The effect lasted for 30 min following the injection of 7.5 µg of atropine [One-factor repeated measures ANOVA; $F_{12,129}=4.251$, $p=0.0001$; Fisher LSD=16.344].

Locomotion

An increase in locomotor activity was observed following the injection of vehicle. The locomotor activation was transient, lasting only 15 min [2-factor (group x time) repeated measures ANOVA; significant effect for time, $F_{3,189}=20.068$, $p=0.0001$]. The effect was not general, occurring in some animals but not others, and as a result was

Figure 22

The effects of unilateral injections of atropine methyl nitrate on extracellular DA concentrations in the shell of the ipsilateral Acc. Each point represents a 15-min sample and is presented as a percentage of mean baseline concentration (±SEM). Dose of atropine administered and sample size is noted in the top left corner of each panel.

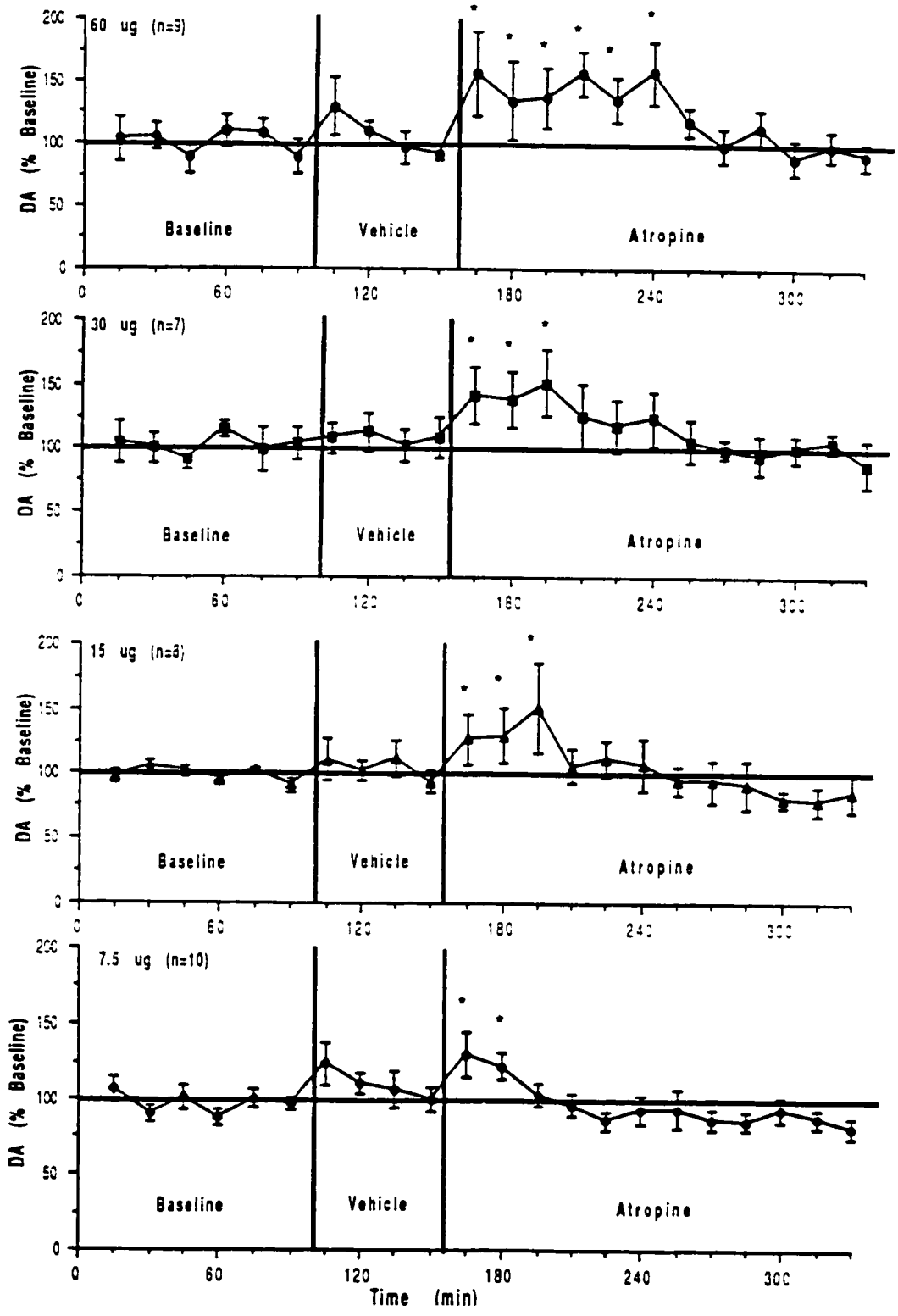
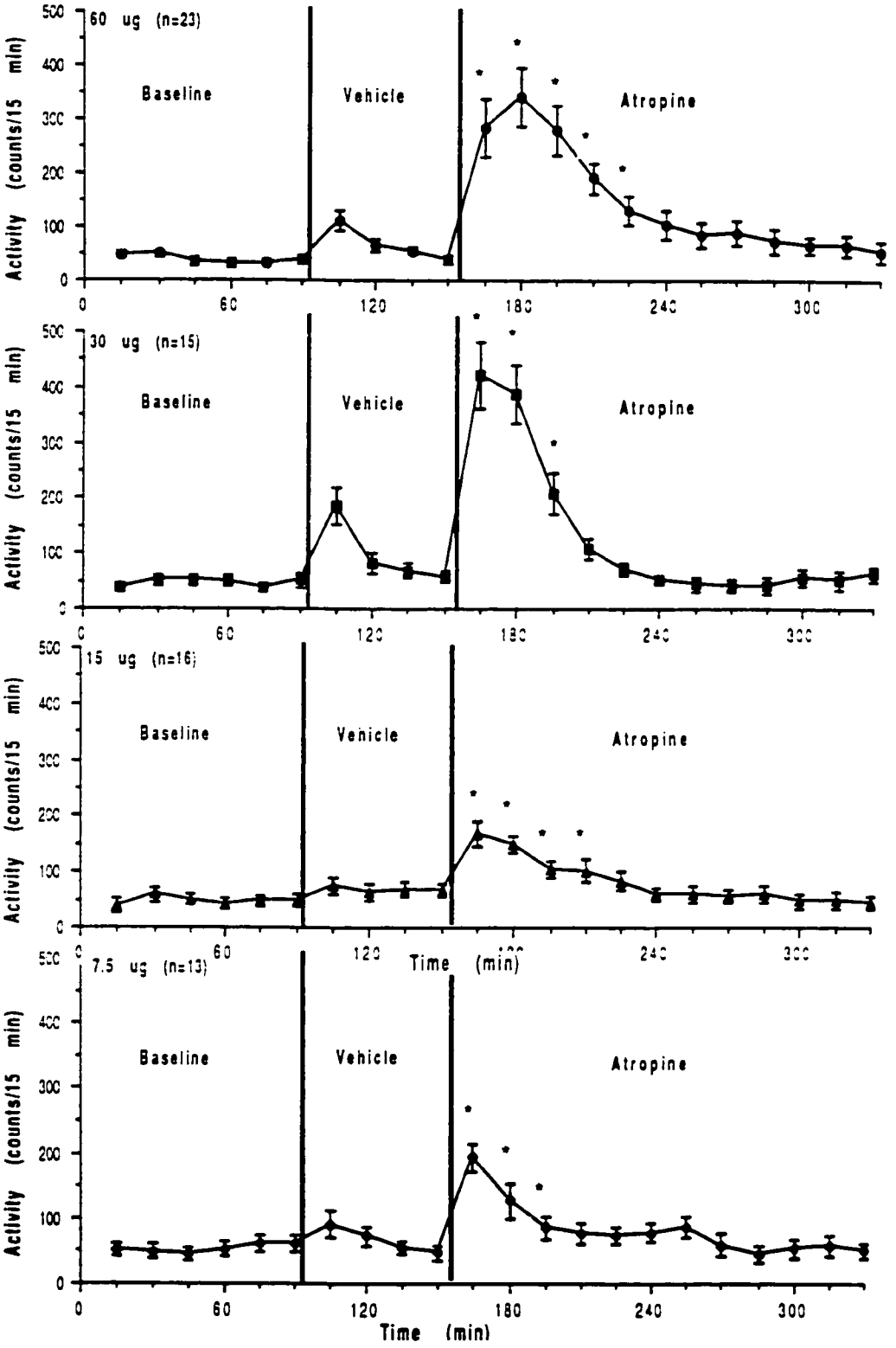

60 ug (n=9)
30 ug (n=7)
15 ug (n=8)
7.5 ug (n=10)
DA (% Baseline)
Baseline
Vehicle
Atropine
Time (min)

Figure 23

The effects of unilateral injections of atropine methyl nitrate on locomotor activity. Each point represents the mean number of beam interruptions in 15 min (±SEM). Dose of atropine administered and sample size is noted in the top left corner of each panel.


60 ug (n=23)
30 ug (n=15)
15 ug (n=16)
7.5 ug (n=13)
Baseline
Vehicle
Atropine
Activity (counts/15 min)
Time (min)
0
60
120
180
240
300
100
200
400
500

only significant in two of the four groups tested [2-factor (group x time) repeated measures ANOVA; significant interaction, $F_{9,189}=3.33$, $p=0.008$].

The increase in locomotor activity that occurred following the injection of atropine methyl nitrate into the VTA was more pronounced and longer lasting than the activation caused by the injection of vehicle (Fig. 23). Different doses of atropine resulted in different degrees of locomotor activation [2-factor (dose x time) repeated measures ANOVA; significant effect for dose, $F_{3,660}=7.843$, $p=0.0002$]. The elevation in locomotor activity occurred within 15 min of the injection of atropine and dissipated over time [2-factor (dose x time) repeated measures ANOVA; significant effect for time, $F_{12,660}=5.365$, $p=0.0001$].

The maximal effect on locomotor activity was observed at different times following the injection of different doses of atropine. The maximal effect was observed after 30 min following 60 µg of atropine and after 15 min following the injection of lower doses of atropine. The strongest effect on locomotion was observed after the injection of the second highest dose of atropine, 30 µg (422.67±59.11 compared to 341.35±53.71 after 60 µg, 168.63±21.35 after 15 µg, and 194.15±22.00 after 7.5 µg of atropine). These results indicate an interaction between the dose of atropine injected and the time elapsed since the injection [2-factor (dose x time) repeated measures ANOVA; significant interaction between dose and time, $F_{36,660}=5.365$, $p=0.0001$].

Post-hoc analysis indicates that locomotor activity was significantly elevated within 15 min of the atropine injection. The duration of the effect tended to be greater for higher concentrations of atropine methyl nitrate. Locomotion remained significantly elevated for 75 min following the injection of 60 µg of atropine methyl nitrate [One-factor repeated measures ANOVA; $F_{12,220}=10.615$, $p=0.0001$; Fisher LSD=98.981]. The duration of the effect was 45 min following 30 µg [One-factor repeated measures

ANOVA; $F_{12,168}=30.968$, $p=0.0001$; Fisher LSD=61.296] or 7.5 µg of atropine [One-factor repeated measures ANOVA; $F_{12,168}=9.061$, $p=0.0001$; Fisher LSD=31.809]. The effect lasted for 60 min following the injection of 15 µg of atropine [One-factor repeated measures ANOVA; $F_{12,207}=9.451$, $p=0.0001$; Fisher LSD=30.229].

Relationship between Locomotion and DA Concentration in the Nucleus Accumbens

As shown by comparison of Figs. 22 and 23, the increases in DA concentration and locomotor activity following atropine injections into the VTA tend to return to baseline levels within 15 min of each other. The maximal concentration of DA measured is the same regardless of the dose of atropine administered while the maximal level of locomotion is affected by the dose of atropine. There is a great deal of variability, but the positive relationship between locomotor activity and extracellular DA levels is significant ($r_{calc}=0.276$, $n=544$; $r_{crit}=0.115$, $df=500$, $p<0.01$; $R_2=0.076$). Locomotor activity increases as DA levels increase.

Metabolites

The extracellular concentrations of the metabolites of DA, DOPAC and HVA, increased in the Acc following the injection of atropine into the VTA while the serotoninergic metabolite, HIAA, was unaffected.

DOPAC

A small but insignificant increase in DOPAC levels occurred after the vehicle solution was injected into the VTA. DOPAC concentration in the Acc increased significantly after atropine was injected into the VTA. This increase lagged behind the increase in DA levels and first became significant 30 min after the atropine was injected (Fig. 24). A dose-dependent effect was evident [2-factor (dose x time) repeated measures ANOVA; significant for dose, $F_{3,360}=3.194$, $p=0.0376$]. The duration of the increase also depended on the dose of atropine. DOPAC remained significantly elevated for 1 hr following the administration of 7.5 µg of atropine and for over 2 hr following the injection of 60 µg of atropine [2-factor (dose x time) repeated measures ANOVA; significant for time, $F_{12,360}=24.627$, $p=0.0001$]. Nevertheless, the rate at which the enhanced DOPAC levels dissipated after reaching their peak varied from group to group resulting in a significant interaction between the dose of atropine administered and time after injection [2-factor (dose x time) repeated measures ANOVA; significant interaction, $F_{36,360}=1.587$, $p=0.02$].

HVA

A small increase in HVA levels occurred after the vehicle solution was injected into the VTA. HVA concentration in the Acc increased markedly after atropine was injected into the VTA. This increase lagged behind the increase in DA and DOPAC levels and first became significant 45 min after the atropine was injected (Fig. 25). The effect of dose was less evident and HVA levels remained elevated throughout the test session for all but the lowest dose of atropine administered [2-factor (dose x time) repeated measures ANOVA; significant for time, $F_{12,360}=24.775$, $p=0.0001$; significant

Figure 24

The effects of unilateral injections of atropine methyl nitrate on extracellular DOPAC concentrations in the shell of the ipsilateral Acc. Each point represents a 15-min sample and is presented as a percentage of mean baseline concentration (±SEM).

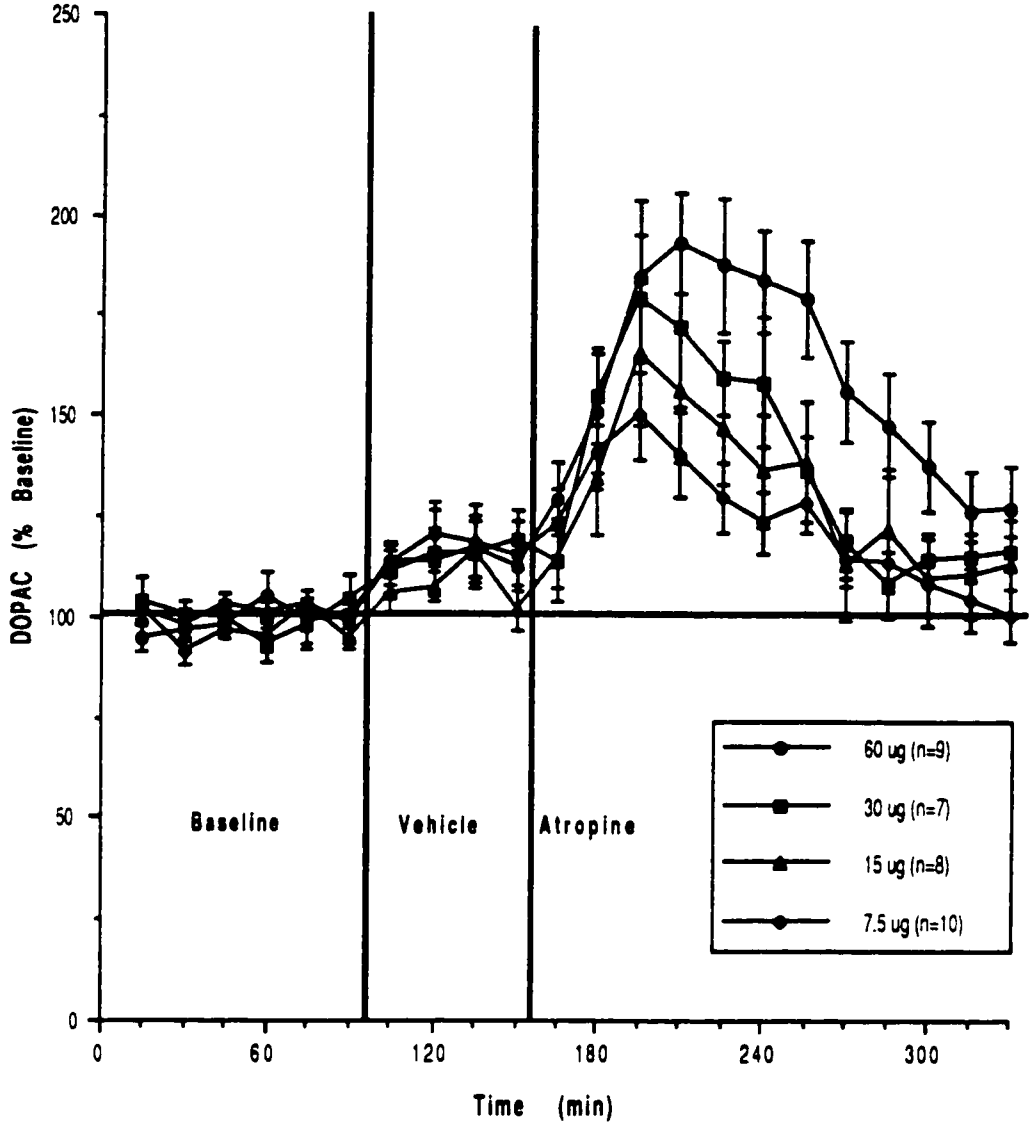
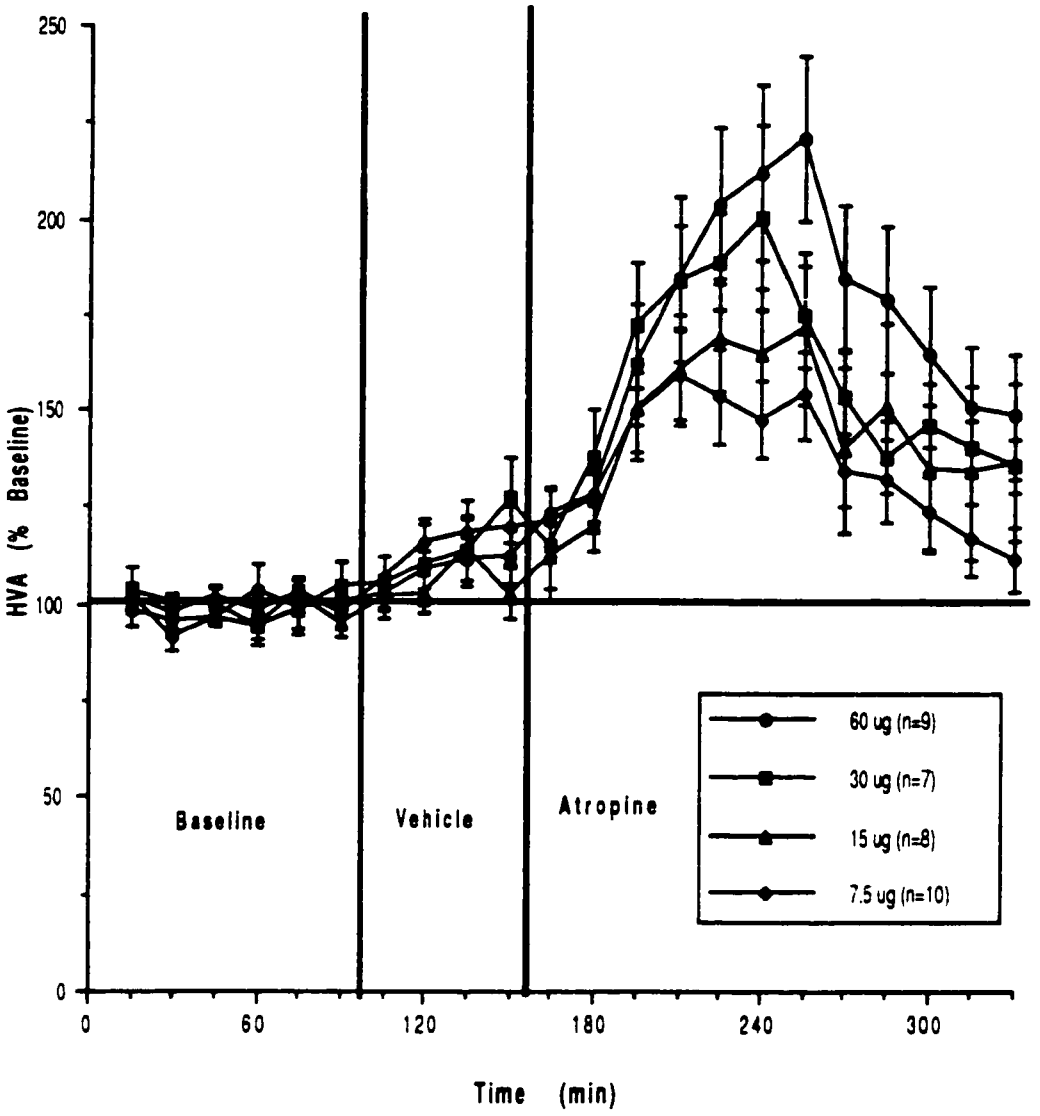

250
200
150
100
50
0
DOPAC (% Baseline)
Baseline
Vehicle
Atropine
60 ug (n=9)
30 ug (n=7)
15 ug (n=8)
7.5 ug (n=10)
0
60
120
180
240
300
Time (min)

Figure 25

The effects of unilateral injections of atropine methyl nitrate on extracellular HVA concentrations in the shell of the ipsilateral Acc. Each point represents a 15-min sample and is presented as a percentage of mean baseline concentration (±SEM).


250
200
150
100
50
0
HVA (% Baseline)
0
60
120
180
240
300
Time (min)
Baseline
Vehicle
Atropine
60 ug (n=9)
30 ug (n=7)
15 ug (n=8)
7.5 ug (n=10)

interaction between dose of atropine and time after injection, $F_{36,360}=1.739$, $p=0.0067$].

HIAA

As Fig. 26 illustrates, levels of the serotoninergic metabolite, HIAA, rose minimally over the course of the experiment and the dose of atropine injected had no impact on the levels of HIAA measured at any time [2-factor (dose x time) repeated measures ANOVA; significant effect for time, $F_{12,360}=5.58$, $p=0.0001$].

Histology

The photomicrograph in Fig. 27 illustrates the placement of the microdialysis probes within the Acc. All probes were located in the shell of the Acc. The placement of the injections in the VTA for rats in which DA was measured is illustrated in the schematic diagram shown in Fig. 28. The VTA injection sites for rats that were monitored for locomotor activation are illustrated in Fig. 29. All injections were in the VTA in an area of acetylcholinesterase staining. Injection cannulae were located 4.52 to 5.60 mm posterior to bregma.

Figure 26

The effects of unilateral injections of atropine methyl nitrate on extracellular HIAA concentrations in the shell of the ipsilateral Acc. Each point represents a 15-min sample and is presented as a percentage of mean baseline concentration (±SEM).

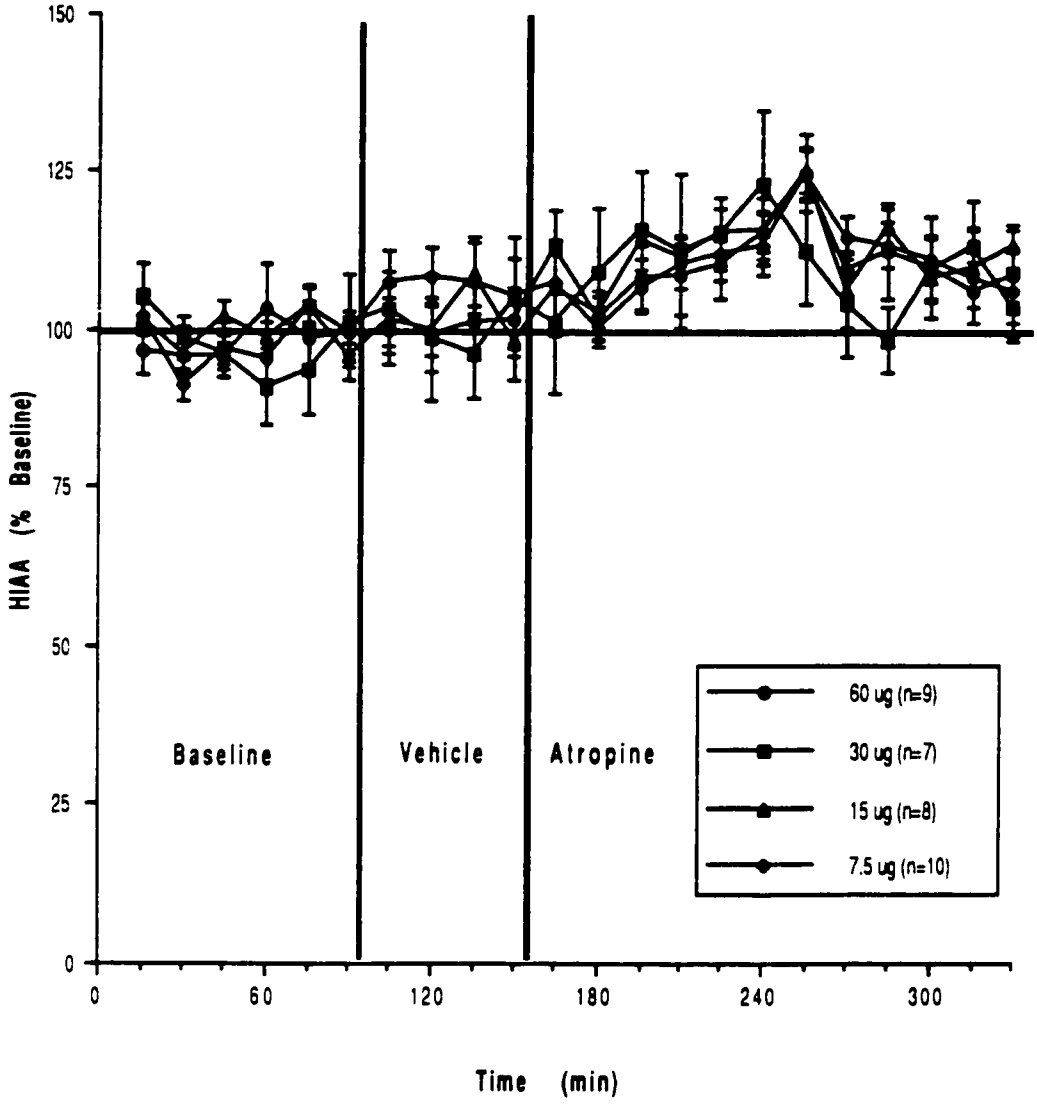

150
125
100
75
50
25
0
HIAA (% Baseline)
Baseline
Vehicle
Atropine
60 ug (n=9)
30 ug (n=7)
15 ug (n=8)
7.5 ug (n=10)
0
60
120
180
240
300
Time (min)

Figure 27

Photomicrograph showing the placement of a microdialysis probe in the shell of the Acc. Scale bar=200 μm; ac: anterior commissure.


ac

Figure 28

Microdialysis: distribution of sites in the VTA where unilateral injections of atropine methyl nitrate were administered.

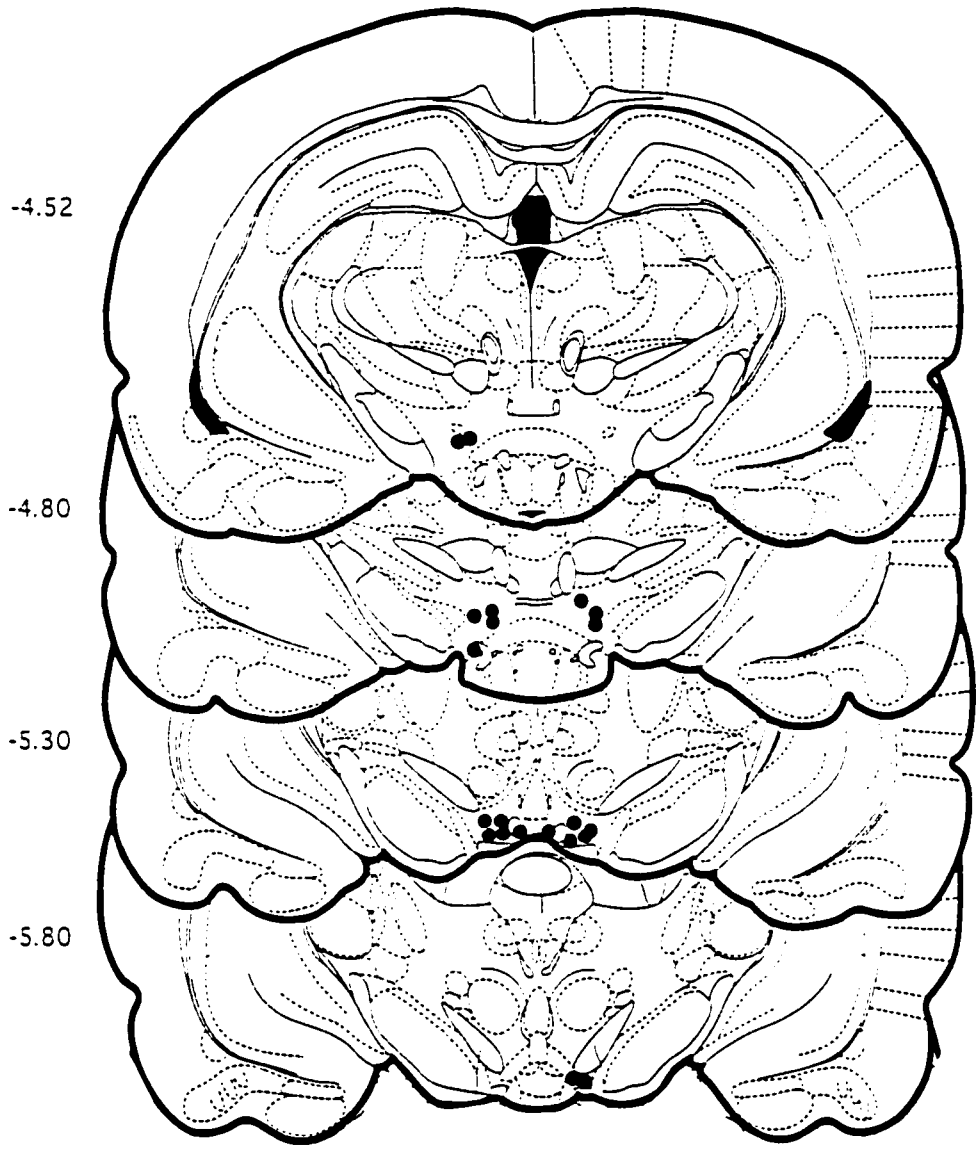
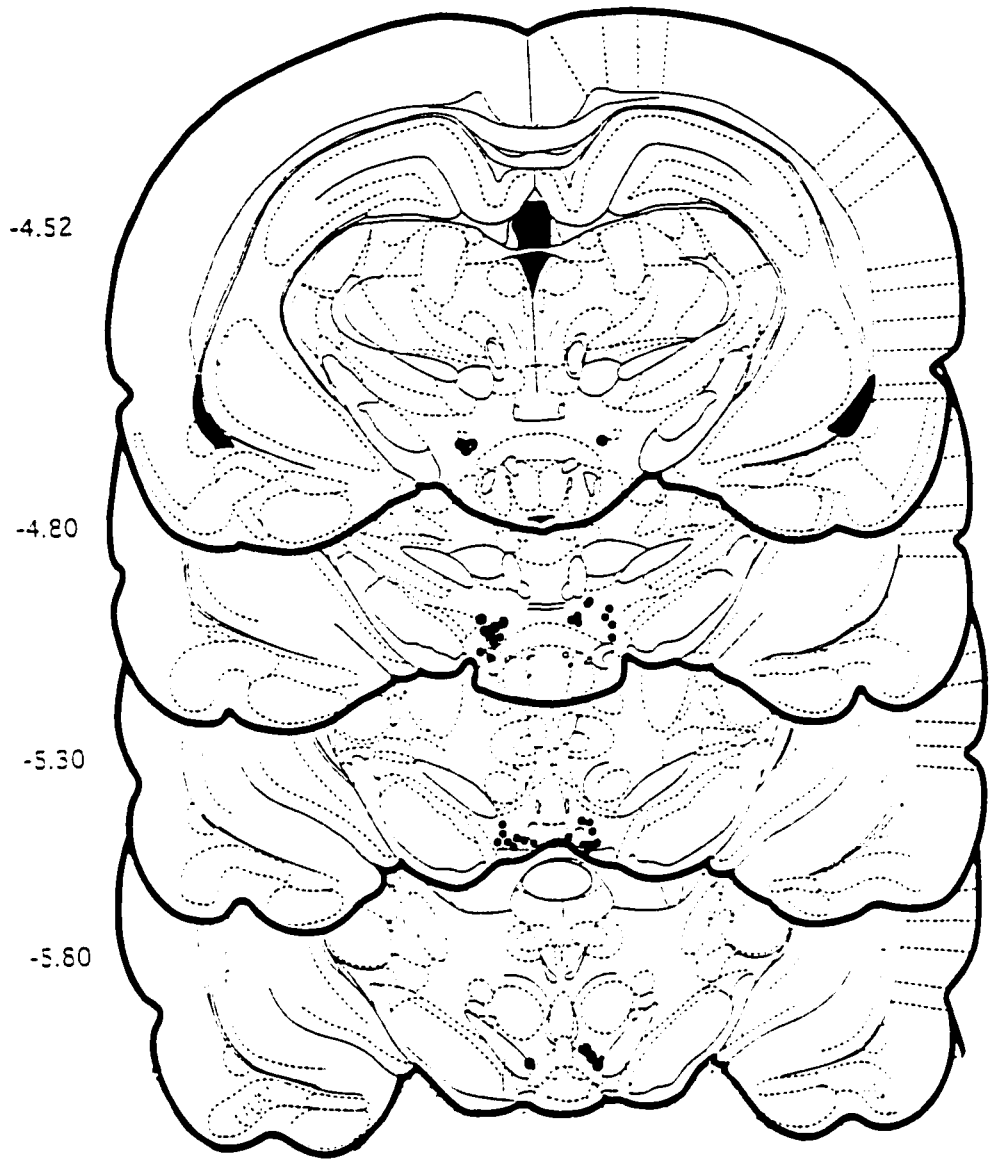

-4.52
-4.80
-5.30
-5.80

Figure 29

Locomotion: distribution of sites in the VTA where unilateral injections of atropine methyl nitrate were administered.


-4.52
-4.80
-5.30
-5.80

DISCUSSION

Unilateral injections of atropine methyl nitrate into the VTA increased the concentrations of extracellular DA and its metabolites, DOPAC and HVA, in the ipsilateral nucleus accumbens and induced locomotor activity. Our finding that blockade of the cholinergic input to the VTA from the PPTg and LDTg with the muscarinic antagonist, atropine, activates the mesolimbic DA system is consistent with previous evidence obtained with systemic injections of cholinergic antagonists (Arnfred and Randrup, 1968; Kelly and Miller, 1975; Setler et al., 1976; Shannon and Peters, 1990; and Stephens and Herberg, 1979). It is contrary to the evidence obtained with central injections of cholinergic agonists into the VTA, however. Cholinergic agonists injected into the VTA also activate the mesolimbic DA system (Blaha et al., 1996) and increase activity (Museo and Wise, 1990; 1995; Reavill and Stolerman, 1990; and Kofman, 1987). Two possible explanations for these inconsistent findings are presented here. The first is based on the topography of the VTA and suggests that cannulae placement differences along the rostral/caudal plane might account for the observation that cholinergic agonists and antagonists produce similar effects on DA release and locomotor activity when injected into the VTA. The second examines a mechanism which would allow muscarinic blockade to activate DA neurons in the VTA; namely, that atropine disinhibits DA cells by blocking an excitatory muscarinic input to GABA neurons which normally inhibit DA neurons in the VTA.

It is difficult to imagine how cholinergic agonists and antagonists could produce the same effects on activity and DA release in the ventral striatum. The VTA is not a homogeneous structure, however, and one possible explanation is that different parts of the VTA may react to cholinergic drugs in opposing ways. This certainly seems to be the case in the SN.

A number of studies have shown that unilateral cholinergic stimulation of the SN can elicit ipsilateral or contralateral circling (Arnt and Scheel-Krüger, 1979a; Costall et al., 1972; De Montis et al., 1979; James and Massey, 1978; Nashold et al., 1965; and Wolfarth et al., 1978; 1979). Generally, ipsilateral circling is observed when the cholinergic agonists are placed in the substantia nigra pars reticulata (De Montis et al., 1979; James and Massey, 1978; and Wolfarth et al., 1978). Several studies reported that the direction of the circling elicited by carbachol was dependent on the injection site within the substantia nigra pars compacta—either rostral or caudal in rats (Arnt and Scheel-Krüger, 1979b; and Hernández-López et al., 1994) and medial or lateral in cats (Nashold et al., 1965).

Distinct functional compartments may exist within the substantia nigra. Electrophysiological studies indicate that there are several sub-populations of nigral DAergic neurons that are segregated following a rostral-caudal gradient in the substantia nigra pars compacta of rats and guinea-pigs (Shepard and German, 1988; Hajós and Greenfield, 1993; Hounsgaard et al., 1992; and Nedergaard and Greenfield, 1992). Hernández-López et al. (1994) found that cholinergic stimulation of the substantia nigra pars compacta with the mixed muscarinic-nicotinic agonist, carbachol, modulated striatal dopamine release and this effect was accompanied by circling and stereotypy. Caudal injections induced contralateral circling associated with a release of DA in the

neostriatum. In contrast to this, ipsilateral circling and reduction of striatal DA release was elicited when the same dose of carbachol was injected into the rostral substantia nigra pars compacta.

Arnt and Scheel-Krüger (1979b) noted differential regional effects on locomotion, aggression, and food intake following the microinjection of GABAergic agonists and antagonists into either the anterior or posterior VTA. Electrolytic lesions of the Acc reduced glutamate decarboxylase activity in the anterior VTA, but not in the posterior VTA, indicating that Acc GABA neurons project only to the anterior VTA (Walaas and Fonnum, 1980). In a study of the afferent and efferent connections of the LDTg, Cornwall et al. (1990) reported differential labelling of regions within the anterior and posterior VTA following the injection of anterograde or retrograde tracers into the LDTg.

Thus it is certainly possible that a topographical pattern similar to that seen in the substantia nigra would emerge following the injection of cholinergic drugs into the VTA. If a similar topographical arrangement exists in the VTA then injections of the cholinergic agonist, carbachol, into the caudal VTA would be associated with a release of DA in the ventral striatum while the opposite effect (a decrease in DA levels) would occur if the injections into the VTA were rostral to the interpeduncular nucleus. Conversely, injections of the cholinergic antagonist, atropine, into the rostral VTA should increase extracellular dopamine levels in the ventral striatum. There does not appear to be any difference in the cannulae placements used by Blaha et al. (1996) to inject carbachol, however, and the cannulae placements used in the present study to inject atropine. Most of the cannulae are positioned anterior to the interpeduncular nucleus. Systematic investigation of regional differences in the responsiveness of VTA neurons to cholinergic drugs would be of interest.

Disinhibition of DA cells in the VTA by Atropine acting on GABA Neurons

VTA dopaminergic neurons fire spontaneously in a slow, random pattern that is punctuated by short bursts of action potentials *in vivo.* Unlike the situation *in vivo*, VTA DA neurons do not exhibit a burst-firing pattern *in vitro*; they fire in a pacemaker fashion (Grace and Onn, 1989; Johnson et al., 1992; Rayport et al., 1992; Wang and French, 1993; and White, 1996). The lack of burst firing *in vitro* suggests that afferent inputs modulate the activity, particularly the firing pattern, of these neurons.

It seems likely, on the basis of the electrophysiological and neurochemical evidence described earlier, that a cholinergic agonist like carbachol directly excites DAergic neurons in the VTA. The activation of the mesolimbic DA system following the administration of the cholinergic antagonist, atropine, into the VTA could occur via an indirect neural mechanism—through disinhibition of DAergic cells. The following section examines the possibility that acetylcholine tonically stimulates the release of GABA as well as DA in the VTA. In turn, GABA inhibits VTA DA neurons. Thus, blockade of muscarinic cholinergic receptors in the VTA with atropine could reduce the activity of GABAergic neurons and lead to disinhibition of mesolimbic DA neurons. The result would be an increase in DA overflow in the Acc and an enhancement of DA-related behaviours.

Evidence supporting this possibility will be discussed as follows. First, the effects of acetylcholine and cholinergic drugs on GABA release in the SN and VTA will be presented. Secondly, GABAergic influences on the activity of VTA DA neurons will be examined. Thirdly, the effects of GABAergic drugs on behaviour that has been associated with mesolimbic DA activity will be discussed; in particular, the role of GABA in the VTA on locomotor activity will be examined. Finally, in light of the dissociation between the effects of muscarinic receptor blockade on reward and locomotion observed in the

current series of experiments, experiments investigating the role of GABA in the VTA on reward will be discussed.

Acetylcholine and GABA in the VTA/SN

Cholinergic neurons form synaptic connections with non-dopaminergic as well as dopaminergic cells in the VTA and SN. Rubin et al. (unpublished observations) injected the anterograde tracing compound *Phaseolus vulgaris*-leucoagglutinin (PHA-L), which allows for the identification of synaptic connections, into the PPTg and processed midbrain sections for PHA-L and tyrosine hydroxylase (TH) immunochemistry. PHA-L-labelled fibers were observed throughout the VTA and SN. Axonal swellings indicative of synaptic connections were visible on both TH-positive and TH-negative neurons (Kelland et al., 1993).

The administration of acetylcholine in the presence of eserine, a cholinesterase inhibitor, significantly increased spontaneous [^{3}H]GABA release in nigral slices (Kayadjanian et al., 1994a). Nicotine also increased the spontaneous release of [^{3}H]GABA from SN slices *in vitro* (Kayadjanian et al., 1994b). The addition of carbachol to a slice preparation induced a maximal effect on [^{3}H]GABA release. Atropine did not modify spontaneous [^{3}H]GABA release by itself, but it did abolish the effect of carbachol, indicating that muscarinic receptors are involved in the response (Kayadjanian et al., 1994a). The nicotine-induced [^{3}H]GABA release appears to be mediated by DA neurons while the carbachol-induced [^{3}H]GABA release appears to be directly mediated by m4 muscarinic receptors localized on GABAergic terminals in the SN (Kayadjanian et al.1994a; 1994b).

If a similar neural mechanism exists in the VTA then acetylcholine can stimulate the release of GABA from a number of sources including collaterals from GABAergic

projection neurons, GABAergic afferents, and interneurons. Spontaneous GABA-mediated membrane polarizations occur in VTA tissue slices (Johnson and North, 1992a and Sugita et al., 1992). Molecular anatomical studies suggest that there are both extrinsic and intrinsic sources of GABA within the VTA (Kalivas, 1993). GABAergic neurons in the VTA have been demonstrated using immunocytochemistry for both GABA and the GABA synthetic enzyme, glutamic acid decarboxylase (GAD) (Nagai et al., 1983; Oertel et al., 1982; and Smith and Bolam, 1990). *In situ* hybridization for GAD mRNA indicates that 20% of the cells within the VTA are GABAergic(Kalivas et al., 1992). Following immunohistochemical staining for glutamic acid decarboxylase (GAD), small GAD-positive neurons were observed throughout the VTA codistributed with larger, dopaminergic neurons (Ford et al., 1995). GABA-containing neurons synapse on DAergic neurons as well as project outside of the ventral mesencephalon (Bayer and Pickel, 1991; and Tepper et al., 1995). The inhibitory neurotransmitter, GABA, is contained within VTA cells that project to the nucleus accumbens (Van Bockstaele and Pickel, 1995).

Neurons in the VTA and substantia nigra receive GABAergic afferents from the neostriatum (Grofová, 1975; Kataoka et al., 1974; Kim et al., 1971; Fonnum et al., 1974) and ventral striatum (Walaas and Fonnum, 1980; Yim and Mogenson, 1980; and Kalivas et al., 1993). GABAergic projections from the globus pallidus and ventral pallidum to the ventral mesencephalon have also been established (Fonnum et al., 1978; Ribak et al., 1980; and Zahm, 1989). The shell of the Acc and ventromedial ventral pallidum project to the VTA while the core of the Acc and the dorsolateral ventral pallidum project to the substantia nigra (Heimer et al., 1991; and Zahm, 1989). In summary, there are many sources of GABA within the VTA—both extrinsic and intrinsic—some of which might be stimulated by acetylcholine. The next section examines the effects of GABA on DA neurons in the VTA and SN.

Descending GABAergic projections to the VTA and substantia nigra synapse on both DAergic and non-DAergic neurons (Smith and Bolam, 1990; Smith and Bolam, 1991; Sugita et al., 1992; and Van Den Pol et al., 1985). Electron microscopy indicates that more than half of all synapses on DAergic neurons in the ventral mesencephalon are GABAergic (Smith and Bolam, 1990).

The administration of GABA into the VTA inhibits the discharge rate of DAergic neurons (Yim and Mogenson, 1980; and Suaud-Chagny et al., 1992). Electrophysiological studies indicate that $GABA_B$ receptors are located on midbrain DAergic neurons. DAergic neurons in a VTA slice preparation are hyperpolarized and inhibited by the selective $GABA_B$ receptor agonist, baclofen (Mueller and Brodie, 1989). Stimulation of $GABA_B$ receptors results in membrane hyperpolarization mediated by an increase in the same potassium conductance that is regulated by DA D_2 autoreceptors (Grace and Bunney, 1984; Innis and Aghajanian, 1987; and Lacey et al., 1988). Intracellular recordings demonstrate that $GABA_A$ agonists may also directly hyperpolarize DAergic neurons in the VTA (Johnson and North, 1992a; Olpe et al., 1977; and Seabrook et al., 1990). The synapse between intrinsic GABAergic neurons and DA cells utilizes $GABA_A$ receptors that regulate chloride channels (Johnson and North, 1992a; and Sugita et al., 1992). Application of the $GABA_A$ antagonist, picrotoxin, increasesthe discharge rate of DA neurons *in vivo* (Yim and Mogenson, 1980). Application of the $GABA_A$ antagonist, bicuculline, activates DAergic neurons *in vitro* (Johnson and North, 1992b). Both $GABA_A$ and $GABA_B$ receptor-mediated components are evident in the inhibitory postsynaptic potentials elicited in VTA DAergic neurons by focal stimulation of the slice (Johnson and North, 1992a; and Sugita et al., 1992). Synaptic inputs to $GABA_A$ and $GABA_B$ receptors may originate from discrete

afferent neurons (Sugita et al., 1992). In any case, it is clear that GABA can inhibit DA neurons in the VTA. If acetylcholine normally stimulates the release of GABA within the VTA, then a concommitant inhibition of DA neurons should occur.

Of course, the situation is not this straightforward because GABAergic afferents to the VTA also synapse on GABAergic neurons intrinsic to the VTA (Smith and Bolam, 1990). Both $GABA_A$ and $GABA_B$ receptor-mediated membrane polarizations can be produced in these cells (Kalivas, 1993). Systemic or iontophoretic administration of $GABA_A$ agonists reduces the firing rate of non-DA cells (Grace and Bunney, 1979; Waszczak and Walters, 1980). This inhibition of local GABAergic neurons may result in disinhibition of DAergic neurons in the VTA. *In vivo* electrophysiological studies found that low doses of $GABA_A$ agonists increase the firing rate of DAergic neurons (Grace and Bunney, 1979; MacNeil et al., 1978; Waszczak and Walters, 1980; and O'Brien and White, 1987). Low intensity electrical stimulation of GABAergic afferents decrease non-DA cell firing and disinhibit DA neurons, while higher intensity stimulation directly inhibits DA cells (Grace and Bunney, 1985).

To summarize, GABAergic influences on the activity of DAergic neurons in the VTA may be either excitatory or inhibitory. Direct GABAergic projections from forebrain structures inhibit VTA DAergic neurons while forebrain GABAergic influences on local GABAergic neurons excite (or disinhibit) VTA DAergic neurons. It has been suggested that these two sources of GABA may act through separate receptor populations, $GABA_A$ receptors for local (intrinsic) neurons and $GABA_B$ receptors for forebrain (extrinsic) sources of GABA (Sugita et al., 1992). It would be interesting to know if muscarinic receptors are differentially localized on intrinsic versus extrinsic sources of GABA within the anterior and posterior VTA.

Consistent with the electrophysiological studies showing that GABA inhibits the activity of DA neurons within the VTA, the administration of GABA into the VTA decreases

extracellular levels of DA in the Acc (Suaud-Chagny et al., 1992). Stimulation of $GABA_B$ receptors with the agonist, baclofen, reduces somatodendritic release of DA in the VTA when infused through the microdialysis probe (Klitenick et al., 1992). The microinjection of baclofen into the VTA decreases basal and induced dopamine transmission in the Acc (Kalivas et al., 1990).

Some microdialysis studies indicate that blockade of $GABA_A$ receptors within the anterior VTA with the antagonists, picrotoxin or bicuculline, increases extracellular levels of DA and its metabolites in the Acc (Ikemoto et al., 1997a; and Westerink et al., 1996). These data are consistent with a direct action of the drugs on $GABA_A$ receptors located on DAergic neurons. Other studies, however, suggest that the activation of $GABA_A$ receptors in the VTA inhibits inhibitory inputs to DAergic neurons so that VTA DAergic neurons are disinhibited by $GABA_A$ agonists (Kalivas, 1993). The microinjection of the $GABA_A$ agonist, muscimol, into the VTA increased extracellular levels of DA, DOPAC, and HVA in the Acc (Kalivas et al., 1990). Perfusion of muscimol through a microdialysis probe placed in the VTA increased somatodendritic release of DA detected by the same probe (Klitenick et al., 1992). An *in vitro* study found that the application of muscimol to slices containing the VTA produced an efflux of DA (Beart and McDonald, 1980). Similar results were obtained with voltammetry; administration of muscimol into the VTA significantly increased DA release in the Acc in 70% of the animals tested. Pretreatment with the $GABA_A$ antagonist, bicuculline, blocked the effect of muscimol. DA levels in the Acc were reduced, however, following the application of muscimol in the remaining animals (Xi and Stein, 1998). As was the case with the electrophysiological studies, GABA can either excite or inhibit DA neurons in the VTA.

The microinjection of GABA agents into the VTA produces similarly mixed effects on behaviour. Some studies reported that the administration of $GABA_A$ antagonists into the VTA increased locomotion (Mogenson et al., 1979; 1980; Stinus et al., 1982; Ikemoto et al., 1997a). Microinjection of GABA into the VTA decreased locomotor activity (Ikemoto and Panksepp, 1996) while administration of the $GABA_A$ agonist, muscimol, had no effect of locomotion (Ikemoto et al., 1997a). Conversely, other studies reported that the injection of GABA or the $GABA_A$ agonist, muscimol, into the VTA increased locomotor activity (Tanner, 1979; Kalivas et al., 1990; and Klitenick et al., 1992). Xi and Stein (1998) reported that locomotor activity levels increased or decreased concomitantly with changes in extracellular Acc DA following the injection of muscimol; increases in DA were accompanied with increases in locomotion and vice versa.

The heterogeneous organization of GABAergic systems within the VTA might account for these discrepancies. GABAergic drugs produce differential effects on locomotion when injected into either the anterior or posterior VTA. Locomotor activity is increased by GABAergic antagonists injected into the anterior VTA or by GABAergic agonists injected into the posterior VTA (Arnt and Scheel-Krüger, 1979b). The VTA placements used by Klitenick et al. (1992) were more posterior than those of Westerink et al. (1996) or Ikemoto et al. (1997a).

Another possibility was raised by the results of the electrophysiological and neurochemical studies discussed earlier; $GABA_A$ receptors are located on both DAergic neurons and GABAergic neurons in the VTA. Activating them separately may produce opposite effects on locomotor activity as a result of either direct inhibition or by indirect disinhibition of DAergic neurons. There might be rostral-caudal differences in

the ratio of DA and GABA contacts that could account for the topographical differences in drug actions. Likewise, there might be topographical differences along a rostral-caudal gradient in the localization of muscarinic receptors on DA neurons and on local GABA neurons and GABAergic afferents. Under normal conditions, when both direct and indirect cholinergic influences on the activity of DAergic neurons may be present, the behavioural outcome likely depends upon the balance between these mechanisms. In the current study, the indirect effect appears to predominate with the result that administration of the muscarinic antagonist, atropine methyl nitrate, into the VTA induces locomotor activity and increases DA release in the ipsilateral Acc shell.

GABA in the VTA and Reward

If the neural mechanisms underlying reward and locomotion are homologous, then disinhibition of mesolimbic DA neurons because of a reduction in GABA release would be expected to enhance reward processes as well as facilitating locomotion. If the rewarding actions of morphine injected into the VTA are due to inhibition of GABA interneurons, thereby disinhibiting DA neurons (Johnson and North, 1992b; and Klitenick et al., 1992), then local administration of GABA receptor antagonists should be reinforcing for the same reason.

$GABA_A$ agonists and antagonists are both self-administered into the VTA of rats (Ikemoto et al., 1997b) and mice (David et al., 1997). Ikemoto et al. (1997b) demonstrated that Wistar rats will self-administer the $GABA_A$ antagonists, picrotoxin and bicucculline methiodide, into the anterior VTA. The animals will not self-administer picrotoxin into the posterior VTA or SN. Co-infusion of the $GABA_A$ agonist, muscimol, reduces the self-administration of picrotoxin into the anterior VTA. Using *in vivo* microdialysis, Ikemoto et al. (1997a) showed that microinjection of either picrotoxin

or bicuculline into the anterior VTA increases the release of DA in the Acc. These results suggest that blocking tonic $GABA_A$- mediated inhibition within the anterior VTA is reinforcing and that blocking $GABA_A$ receptors results in an enhancement of the activity of DA neurons in the VTA.

Similar results were obtained in BALB/c mice by David et al. (1997). The mice chose the arm of a Y-maze that enabled the microinjection of the $GABA_A$ antagonist, bicuculline, into the VTA. Systemic injection of the D_2 receptor antagonist, sulpiride, 30 min before the test prevented the acquisition of bicuculline self-administration. If the animals were trained to self-administer bicuculline into the VTA before testing with sulpiride, the D_2 receptor antagonist reduced bicuculline self-administration and eventually extinguished self-administration behaviour. These results indicate that blockade of $GABA_A$ receptors in the VTA is rewarding and that the rewarding effects of bicuculline may be mediated through activation of postsynaptic D_2 receptors.

Although the $GABA_A$ antagonist, picrotoxin, was not self-administered into the posterior VTA (Ikemoto et al., 1997b), the $GABA_A$ agonist, muscimol was self-administered into this site. Ikemoto et al. (1998) showed that, in contrast to $GABA_A$ antagonists, muscimol was self-administered into the posterior VTA, but not into the anterior VTA. Co-infusion with picrotoxin into the posterior VTA reduced responding. These data suggest that different $GABA_A$-mediated reward circuitry may be operating in the anterior and posterior VTA. Similar regional differences were obtained in a study assessing locomotor activity following the administration of $GABA_A$ agonists or antagonists into the VTA (Arnt and Scheel-Krüger, 1979b). The $GABA_A$ antagonist, picrotoxin, reduced activity when injected into the posterior VTA, but increased locomotion when it was injected into the anterior VTA. Conversely, the $GABA_A$ agonist, muscimol, increased locomotor activity when administered into the posterior VTA, but reduced spontaneous activity in the anterior VTA. Perhaps $GABA_A$ receptors are located

on DA neurons in the anterior VTA so that $GABA_A$ agonists would inhibit DA neurons and reduce locomotor activity and reward, but are located on GABA neurons in the posterior VTA so that $GABA_A$ agonists would disinhibit DA neurons, increase locomotion and enhance reward.

There are no reports of the intracranial self-administration of $GABA_B$ drugs, but Shoaib et al. (1998) found that injections of the $GABA_B$ agonist, baclofen, into the VTA reduced responding for intravenous cocaine. Cocaine self-administration was also attenuated when baclofen was injected into the Acc. These data suggest that $GABA_B$ receptors are located on DA neurons in this region of the VTA and directly inhibit the activity of these neurons with a corresponding reduction in cocaine reward.

Implications for the Current Study

Unfortunately, none of these findings help to explain the results obtained from the current series of experiments. The injection sites used in Experiments 1 and 2 are much the same as those used in Experiments 3 and 4 so the dissociation between atropine's effects on cocaine reward and its effects on locomotion and DA activity are unlikely to be due to the different circuity within the anterior and posterior VTA. Furthermore, the studies discussed above did not report a dissociation between the reinforcing effects of a drug and its effects on locomotion and DA release.

It appears that the neural circuitry underlying reward and locomotion is not homologous and that muscarinic receptors within the VTA do not play similar roles in reward and behavioural activation. Perhaps atropine blocks muscarinic receptors located on reward-relevant neurons that are endogenously activated by acetylcholine and thus leads to a reduction in cocaine reward. Presumably a different set of mesolimbic DA

neurons is disinhibited by blockade of muscarinic receptors located on GABA neurons that tonically inhibit DA activity; locomotor activity and DA release in the Acc are thus enhanced following the administration of atropine into the VTA.

Pharmacological experiments that utilize the co-infusion of GABAergic drugs with atropine into the VTA might clarify the possible involvement of GABA neurons in the induction of locomotor activity and DA release following the blockade of muscarinic receptors within the VTA. The effects of DA receptor antagonists injected into the Acc on both the induction of locomotion and on the attenuation of cocaine reward following atropine administration into the VTA would be interesting to investigate as mesolimbic DA neurons are believed to be involved in both of these processes.

The findings from the four experiments described in this thesis suggest that: 1) the neural substrates underlying locomotion and reward are not completely homologous; and 2) that long-lasting elevations in extracellular DA concentration in the Acc may be related to the psychomotor stimulant effects of a drug rather than to its effects on reward. These issues will be discussed more thoroughly in the next section.

GENERAL DISCUSSION

Blockade of muscarinic receptors in the VTA with the cholinergic antagonist, atropine, produced disparate effects on reward and locomotion. Experiments 1 and 2 revealed that (a) the ability of cocaine to maintain a lever-pressing response as well as (b) the priming effects of the drug were attenuated following the administration of atropine into the VTA. Locomotor activity, on the other hand, was induced by atropine injections into the VTA in Experiment 3. Experiment 4 showed that the extracellular concentration of the neurotransmitter, DA, in the shell of the nucleus accumbens was increased by the injection of atropine into the VTA; this increase was positively correlated with enhanced locomotor activity. As both cocaine reward and locomotion are believed to be DA-mediated behaviours, the data presented here raise two issues.

In the first place, the dissociation between the effects of atropine on reward and its effects on locomotion suggest that the neural substrates underlying reward and psychomotor stimulation are not completely homologous. Related to this point is the belief that the mesolimbic DA system is a common neural substrate for reward and psychomotor stimulation (Wise and Bozarth, 1987). Extracellular levels of DA in the shell of the Acc are increased following the administration of atropine into the VTA. Does this imply that the activity of mesolimbic DA neurons in the VTA is related to locomotion but unrelated to reward?

Reward and Locomotion are not Homologous

Typically, experimental manipulations that enhance reward also stimulate locomotor activity. Schneirla (1959) suggested that the most basic functional division of the brain consists of the different brain mechanisms underlying approach and

withdrawal. Glickman and Schiff (1967) suggested that there was a common neural mechanism for all approach behaviour and that this was the basis of reinforcement by brain stimulation and natural rewards. In their view, elicitation of approach responses and the induction of positive reinforcement derives from a common neural substrate. Wise and Bozarth (1987) extended this thinking to positive reinforcement induced by drug reinforcers such as cocaine. They made three principal assertions: "(a) that all addictive drugs have psychomotor stimulant actions, (b) that the stimulant actions of these different drugs have a shared biological mechanism, and (c) that the biological mechanism of these stimulant actions is homologous with the biological mechanism of positive reinforcement" (Wise and Bozarth, 1987). One of the predictions of this theory is "that the psychomotor stimulant and reinforcing actions of any addictive drug should both be disrupted by any lesion or treatment that disrupts either one of these actions" (Wise and Bozarth, 1987).

In the case of the experiments presented in this thesis, blockade of muscarinic receptors in the VTA with atropine was expected to attenuate intravenous cocaine reward because it increases brain stimulation reward thresholds when injected into the VTA (Kofman and Yeomans, 1989; Kofman et al., 1990; and Yeomans et al., 1985) and because cholinergic activation of the VTA stimulates neurons believed to mediate cocaine reward, the mesocorticolimbic DA neurons (Blaha et al.1996). This proved to be the case; both the reinforcing and priming actions of cocaine were abolished when atropine was bilaterally administered to the VTA (Experiments 1 and 2). On the basis of Wise and Bozarth's (1987) psychomotor stimulant theory of addiction, it was predicted that atropine injections into the VTA would inhibit locomotor activity just as it inhibits cocaine reward. This did not happen; in fact, atropine injections into the VTA stimulated locomotor activity (Experiment 3). Although atropine sulphate induced catalepsy, an obvious reduction in locomotor activity, its reward-attenuating (Experiment 1) and

locomotor-stimulating (Experiment 3) effects were apparent as soon as the cataleptic effect had dissipated. Atropine methyl nitrate injections reduced cocaine reward (Experiment 2) and stimulated activity (Experiments 3 and 4) as soon as the drug was administered to the VTA.

Strictly speaking, however, Wise and Bozarth's (1987) theory predicts that an experimental manipulation should affect drug-induced reward and drug-induced locomotion in the same way. The series of experiments presented here did not examine cocaine-induced locomotion. It is possible that the actions of atropine interacted with the actions of cocaine in such as way as to reduce reward. Perhaps an interaction between the drugs, atropine and cocaine, would also inhibit locomotor activity. This seems unlikely, however, as the activity levels of the animals self-administering cocaine in Experiment 2 were increased markedly during the injection procedure, so much so that the animals had to be anaesthetized in order to make the injections properly and to reconnect the self-administration leads. Nevertheless, a detailed investigation of the effects of intra-VTA atropine administration on cocaine-induced locomotion should be conducted.

This is not the first reported instance of a dissociation between the effects of a drug on reward and on locomotion; sensitization of drug-induced locomotion and sensitization of the rewarding effects of drugs both seem to occur, but the time course for the development of sensitization can differ suggesting that separate neural adaptations underlie the two processes. Repeated intermittent systemic injections of drugs of abuse such as amphetamine sensitize locomotor activity; repeated treatment progressively enhances the locomotor activating effects of the drug (behavioural sensitization) (Segal, 1975; and Robinson and Becker, 1986). Behavioural sensitization is produced by the repeated administration of many drugs including amphetamine (Kuczenski and Segal, 1988; and Robinson and Becker, 1986), cocaine

(Post and Contel, 1983), opioids (Babbini and Davis, 1972; Joyce and Iversen, 1979; and Shuster et al., 1975), nicotine (Clarke, 1990; and Ksir et al., 1985), and phencyclidine (Greenberg and Segal, 1986; Iwamoto, 1986; and Nabeshima et al., 1987).

A number of experiments, using either self-administration or conditioned place preference procedures, showed that prior exposure to amphetamine, cocaine, or morphine results in sensitization to the rewarding effects of these drugs. Woolverton et al. (1984) found that the threshold dose necessary to sustain the self-administration of methamphetamine is lowered following methamphetamine pretreatment. Pretreatment with d-amphetamine or cocaine facilitates the acquisition of responding for the self-administration of the same drug (Piazza et al., 1989; 1990; and Horger et al., 1990). Likewise, the acquisition of a preference for the place where a drug is experienced is enhanced by pre-exposure to amphetamine, cocaine, or morphine (Lett, 1989; Gaiardi et al., 1991; and Shippenberg and Rea, 1997). Although exposure to amphetamine, cocaine or morphine facilitates the acquisition of a place preference or of self-administration responding, repeated exposure to these drugs does not typically potentiate the synergism of drug reward with brain stimulation reward. Brain stimulation reward thresholds are reduced by the administration of many drugs of abuse including cocaine, amphetamine, morphine, opioids, nicotine, and phencyclidine (Wise, 1996). The reward-potentiating effects of cocaine (Frank et al., 1988; 1992; and Bauco and Wise, 1997), amphetamine (Wise and Munn, 1993), morphine (Schenk et al., 1981; and Bauco et al., 1993), nicotine (Bauco and Wise, 1994), and phencyclidine (Carlezon and Wise, 1993) on electrical stimulation of the lateral hypothalamus were not enhanced by repeated exposure to the drug. Predy and Kokkinidis (1984) reported, however, that the brain stimulation reward-potentiating effects of amphetamine were sensitized when the electrical stimulation was administered to the SN or Acc. The rate of

lever-pressing increased—particularly for lower intensities of stimulation—with repeated administration of amphetamine over 10-20 days. The data from this study suggest that it is possible to sensitize reward mechanisms.

Repeated electrical stimulation of the VTA is reported to sensitize the locomotor response to amphetamine (Ben-Shahar and Ettenberg, 1994) suggesting that repeated electrical stimulation can induce behavioural sensitization just as drugs do. Furthermore, this finding suggests that the rewarding effects of brain stimulation may have been fully sensitized during training in some studies. As discussed above, pretreatment with drugs like amphetamine, cocaine, and morphine facilitate the acquisition of a place preference or responding for drug reward. Perhaps pretreatment with these drugs would also facilitate the acquisition of responding for brain stimulation reward. Nevertheless, although the reward-enhancing effects of amphetamine failed to show sensitization in animals with a great deal of experience with brain stimulation reward, sensitization to the locomotor-stimulating effects of amphetamine were demonstrated in the same animals. Furthermore, the behavioural sensitization occurred in response to the same injections of amphetamine that potentiated reward thresholds without sensitizing the drug's effects on reward (Wise and Munn, 1993). These findings suggest that the reward system was fully sensitized during brain stimulation reward training and that repeated exposure to amphetamine was unable to produce any greater effect on reward processes. The fact that it was possible to induce behavioural sensitization in these animals suggests that the sensitization of reward processes and of locomotor processes can occur at different times indicating that independent neural circuitry underlies or at least contributes to reward and locomotion.

Further support for the suggestion of the anatomical dissociation of the neural substrates mediating reward and locomotion comes from studies investigating the properties of brain stimulation reward. It had been noted that exploratory activity is a

frequent correlate of intracranial self-stimulation of the medial forebrain bundle. Exploratory behaviour induced through self-stimulation electrodes could be elicited by current intensities too low to maintain self-stimulation (Christopher and Butter, 1968; and Miliaressis and Le Moal, 1976). The two behaviours could not be dissociated on the basis of their refractory periods (Rompré and Miliaressis, 1980), but it was possible to obtain a collision effect between electrodes placed at two points along the medial forebrain bundle, the lateral hypothalamus and the VTA, in one behaviour at the exclusion of the other (Durivage and Miliaressis, 1987). The collision test is based on the fact that antidromic and orthodromic action potentials, triggered at different points on the same axon, will collide if the time interval between the stimulating pulses is shorter than the sum of the conduction time between the two electrodes and the refractory period (Lucas, 1913). In a behavioural collision test, trains of twin pulses are delivered through the two electrodes. The interval between the two pulses is systematically varied. At long inter-pulse intervals, no collision will occur between the two action potentials, and both pulses will contribute to the behavioural response. If a collision occurs when the interval between pulses is shortened, then only one of the orthodromic action potentials will reach the synapse and affect the behavioural response. An increase in the number of twin pulses per train required to maintain the behavioural response at a set level is evidence that a collision has occurred. Durivage and Miliaressis (1987) found that a collision effect could be obtained only for self-stimulation in some animals and only for exploratory locomotion in others. These data are consistent with the results obtained in the pharmacological studies discussed above and indicate that the neural substrates within the medial forebrain bundle for locomotion and brain stimulation reward can be dissociated.

Blockade of muscarinic receptors in the VTA with atropine increased DA release in the Acc. This increase in the extracellular concentration of DA in the shell of the Acc was correlated with an increase in locomotor activity. Atropine injections into the VTA, however, reduced cocaine reward. Do these findings imply that the activity of mesolimbic DA neurons in the VTA is related to locomotion, but unrelated to reward? The following section examines some of the evidence supporting a role for DA in reward processes. Reward is a rather broad term that has been suggested to include hedonia, reinforcement, priming, arousal, and attention to significant environmental stimuli. Under normal conditions, a positive reinforcer encompasses all of these aspects of reward. Nevertheless, it is possible experimentally to dissociate some of these components of reward. It will be argued that the method used to measure the effects of VTA injections of atropine on DA function in Experiment 4 is best suited for assessing the tonic elevations in DA concentration that may underlie arousal and attention. Phasic changes in DA function that were not detected in this study might contribute to atropine's attenuation of cocaine reward. This possibility would have to be ruled out before concluding that mesolimbic DA neurons are not involved in reward processes. The possiblity that separate populations of DA neurons within the VTA might contribute to attention and reinforcement is also discussed.

Dopamine is believed to play a critical role in motivation and reward. The anhedonia hypothesis of reward proposed by Wise (1982) holds that dopamine receptor antagonists block the positive reinforcement and positive affect associated with a rewarding event. Currently, many researchers no longer believe that DA mediates its effects on reward by producing feelings of pleasure or euphoria (Robinson and Berridge, 1993; and Wise, 1994) although DA antagonists are believed to attenuate both the

reinforcing and priming effects of drugs, food, and other rewards (Wise, 1989; 1994). DA has long been considered to make an important contribution to the incentive motivational properties of reinforcers (Stewart and de Wit, 1987; Wise and Bozarth, 1987; and Wise, 1989). Speculation about the role of DA in facilitating learning by heightening attention to external events and enhancing the salience of stimuli that are significant has increased recently (Robinson and Berridge, 1993; Wickelgren, 1997; and Schultz, 1998).

It is arguable that the increase in exploratory activity induced by DA agonists is indicative of arousal and a concommitant heightened attention to external events. If this is the case, then the tonic elevation of extracellular DA concentration in the Acc, after manipulations that increase locomotor activity, might be related to attention rather than reinforcement. It is possible that data from some studies indicating that reinforcement is reduced by the administration of DA receptor antagonists are actually the result of reduced attention to salient stimuli. This is most likely to be the case in studies of the acquisition of responding for natural rewards, drug reward, brain stimulation reward, or a preference for a place associated with a reward. Cholinergic projections from the PPTg (that include projections to DA neurons in the SN and VTA) have been implicated in attentional processes (Steckler et al., 1994) and, as reviewed in the General Introduction, lesions of the PPTg block the acquisition of responding for brain stimulation reward and intravenous heroin and prevent the development of conditioned place preferences to amphetamine, morphine, and heroin (Lepore and Franklin, 1996; Olmstead et al., 1998; Bechara and van der Kooy, 1989; Olmstead and Franklin, 1994; and Nader et al., 1994).

On the other hand, it seems less likely that an attention deficit could account for the reduction in reward following the administration of DA receptor antagonists reported in studies of brain stimulation reward that used the rate-frequency curve shift

paradigm. Administration of the DA antagonist, pimozide, caused dose-dependent shifts of the rate-frequency curve to the right (i.e., greater frequencies of electrical stimulation are required to maintain responding). It is possible to increase the threshold frequency of brain stimulation reward without inducing performance deficits with lower doses of pimozide. The animals are obviously attentive to the cues signifying the availability of brain stimulation reward as they respond vigourously for the highest frequencies of electrical stimulation (Gallistel and Karras, 1984; and Gallistel and Freyd, 1987). Similarly, pretreatment with a DA antagonist results in compensatory increases in responding for intravenous amphetamine or cocaine over a wide range of neuroleptic doses. Eventually, following an initial period of compensatory increase in drug self-administration, responding extinguishes (Yokel and Wise, 1975; de Wit and Wise, 1977; and Ettenberg et al., 1982). Again, it is difficult to envisage how an inability to attend to the cues signalling that drug reward is available could account for this increase in responding. These studies indicate that DA neurons do play a role in reinforcement.

Furthermore, the method used to assess extracellular DA levels in Experiment 4 (microdialysis samples collected over 15-min periods) would not be sensitive to short-term alterations in the activity of DA neurons. Electrophysiological experiments have demonstrated that stimulation of PPTg projections to DA neurons in the SN induces burst firing (Lokwan et al., 1999). An alteration in firing pattern of this sort would not necessarily be detected using microdialysis. Similarly, the microdialysis technique might not be sensitive to changes in the activity patterns of DA neurons like those observed by Schultz and his colleagues when recording from cells in the SN that increased their responding upon presentation of a reward or upon presentation of stimuli that predict a reward (Schultz et al., 1997; 1998). VTA neurons do not comprise a homogeneous population and several subsets of neurons have been identified

(Cameron et al., 1997; and Kiyatkin and Rebec, 1998). The microdialysis technique used in Experiment 4 would not distinguish subpopulations of DA neurons if they differed in their responses to atropine treatment.

Recent in vivo neurochemical studies with greater temporal resolution (voltammetry or microdialysis with 1-min samples) showed that tonic elevations in the extracellular DA concentration in the Acc occur in response to cocaine or heroin self-administration. Phasic fluctuations in the concentration of DA in the Acc against a background of elevated DA levels are detectable with these techniques; the phasic fluctuations in DA levels are time-locked to the lever presses made in order to obtain drug reward. It is these phasic fluctuations in DA levels that are correlated to drug-seeking behaviour rather than the large, tonic elevations in extracellular DA levels induced by cocaine or heroin (Wise, 1993; Kiyatkin et al., 1993; Gratton and Wise, 1994; and Wise et al., 1995). It is possible that a subset of DA neurons in the VTA is responsible for the phasic fluctuations in DA that signal drug-seeking behaviour while another population of DA neurons is responsible for the tonic elevation in DA levels in the Acc following cocaine or heroin. The tonic drug-induced elevation in DA concentration might be related to increased arousal, hyperactivity, and heightened attention.

Microdialysis studies that have attempted to correlate DA levels in the Acc with both the appetitive and consummatory aspects of motivated behaviour are consistent with the notion that different responses in DA neurons in the VTA can occur during the anticipation of reward and during reward. In a study of DA efflux in the Acc during the Coolidge effect in male rats, Fiorino et al., (1997) found that DA levels rose when male rats were exposed to a female who was behind a screen. The levels rose even higher when they were allowed to copulate with the female rat, but dropped back to baseline levels during sexual satiety. The presentation of a novel female induced another small

increase in DA release that increased further during copulation. Similar results were obtained with a study of food reward. In this case, a highly palatable liquid meal was presented behind a screen (appetitive phase) to food-deprived or non-deprived rats. The screen was then removed and the animals were allowed to consume the liquid (consummatory phase). During the appetitive phase, DA levels in the Acc were significantly increased in animals that were food-deprived, but were not altered in non-deprived animals. Both the food-deprived and non-deprived animals consumed the palatable liquid meal. DA concentration increased further in the hungry rats and DA release in the Acc was significantly enhanced in both groups during this time (Wilson et al., 1995). It is possible that the increase in DA concentration in the Acc observed during the appetitive phase is related to arousal and attentional processes while the increase in DA release seen during the consummatory phase is related to reinforcement. Presumably hungry animals are more aroused and attentive than non-deprived animals during the appetitive phase. Although active exploratory behaviour was observed in both deprived and non-deprived animals during the appetitive phase, it was not quantified; it is not clear whether the amount of exploratory activity and the amount of DA in the Acc are related in this experiment.

In any case, it is possible that in these microdialysis experiments, DA release from different subpopulations of neurons within the VTA is measured during the appetive and consummatory phases of sexual and feeding behaviour. One population of neurons might be responsible for the reinforcing effects of sex or food while the other set of neurons is involved in the arousal and heightened attention to external events that allows the animal to anticipate the coming reward. Of course, it is also possible that the activity of the same population of DA neurons differs during the appetitive and consummatory phases of sexual and feeding behaviour. Perhaps an increase in tonic

activity underlies arousal while a phasic change in firing pattern—such as an increase in burst firing—occurs during reinforcement.

There might be a similar explanation for the effects of VTA atropine injections on cocaine reinforcement and locomotion. Perhaps two different populations of DA neurons in the VTA may be affected by atropine pretreatment. One population of neurons that mediates reinforcement and priming might normally be activated by acetylcholine acting on muscarinic receptors. Blockade of these receptors with atropine attenuates the reinforcing and priming effects of cocaine. A second population of DA neurons in the VTA appears to be inhibited by GABA under normal conditions. Acetylcholine acting on muscarinic receptors on these GABAergic cells inhibits the activity of this group of DA neurons. Blockade of these receptors with atropine disinhibits this group of DA neurons and induces locomotion and DA release in the Acc; this population of DA neurons may be involved in arousal and attention. An alternative explanation is that atropine differentially affects the tonic and phasic activity of mesolimbic DA neurons. In this case, an increase in tonic activity induced by atropine leads to behavioural activation and increased DA overflow in the Acc. The attenuation of the reinforcing and priming effects of cocaine might be due to a phasic alteration in the firing pattern of these DA neurons.

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