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Intracranial Injections of a Nicotinic Agonist:

Effects on Locomotion and Reinforcement

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A Thesis

in

The Department

of Psychology

Presented in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy at

Concordia University

Montreal, Quebec, Canada

July 1991

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ABSTRACT

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Concordia University, 1991

Systemic injections of nicotine can increase locomotion. The present series of experiments was carried out in order to determine, by way of intracranial injections, which regions of the brain might mediate this effect. In Experiment 1 several doses of cytisine (0, 0.01, 0.1, 1, 10 nmol per 0.5 μL per side) were administered into the ventral tegmentum, and the three highest doses of cytisine increased locomotion; this locomotor-activating effect became progressively stronger with repeated intermittent treatments (Experiment 2). In Experiment 3, cytisine injections (1 nmol per 0.5 μL per side) were made in and around the ventral tegmentum to assess whether there is a circumscribed region into which injections of cytisine increase locomotion. The region extended from the caudal diencephalon to the ventral tegmentum. Since the mesolimbic DA cell-body region is located in the ventral tegmentum, and since it has been implicated in the locomotor-activating effects of systemic nicotine, it was also of interest to determine whether any relationship existed between the distribution of effective injection sites and the location of the mesolimbic DA cell-body region. Although

some injection sites were found in the DA cell-body region, the results neither confirmed nor disconfirmed the notion of DA involvement.

In Experiment 4, the notion that the locomotor-activating effects of a drug are associated with its effects on reinforcement was explored using the conditioned place-preference paradigm. Ventral tegmental injections of cytisine were found to be sufficient to establish a place-preference, thus providing support for the idea that a common neural substrate mediates cytisine's locomotor-activating and reinforcing effects.

Finally, in Experiment 5, the possibility that cytisine injections into DA terminal regions can also increase locomotion was explored. Cytisine (0, 0.1, 1, 10, 100 nmol per 0.5 µL per side) was administered into each of four DA-terminal fields where nicotinic receptors have been localized, namely the nucleus accumbens, caudate putamen, olfactory tubercle and medial prefrontal cortex. Whereas cytisine injections into the nucleus accumbens increased locomotion, injections into the caudate putamen, olfactory tubercle and medial prefrontal cortex were ineffective. It appears, then, that the administration of cytisine into more than one region of the brain is sufficient to increase locomotion, and that these regions may mediate some of the effects associated with systemic injections of nicotine.

ACKNOWLEDGEMENTS

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Nicotine, a naturally-occurring alkaloid found in the tobacco leaf, is believed to be the principal ingredient responsible for the habitual use of tobacco products (Clarke, 1987). Two procedures that have figured prominently in the study of the habit-forming effects of nicotine and other drugs are the intravenous drug self-administration procedure and the conditioned place-preference procedure.

In the intravenous drug self-administration procedure the administration of a drug is made contingent on the completion of a specific behavior, usually the depressing of a lever; if a drug increases the occurrence of the specific behavior, then the drug is considered to be a response reinforcer. On the basis of this definition, nicotine is reinforcing because animals learn to lever-press if the intravenous injection of nicotine is made contingent on such lever-pressing (Cox, Goldstein, & Nelson, 1984; Singer, Simpson, & Lang, 1978).

Whereas the intravenous self-administration procedure is generally thought to reflect response learning because the administration of the drug is made contingent on a particular response, the conditioned place-preference procedure is thought to reflect stimulus learning because the administration of the drug is made contiguous with specific stimuli (Beach, 1957; Rossi & Reid, 1976).[1] With regard to nicotine, while some investigators have

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[1] The effects of nicotine and other drugs that are self-administered by humans have therefore been studied in the context of two types of reinforcement. The first type of reinforcement, termed operant reinforcement (Skinner, 1938), plays a primary role in the establishment of intravenous drug self-administration, and the second type of reinforcement, termed respondent reinforcement (Skinner, 1938), is thought to play a primary role in the establishment of conditioned place-preferences (Wise, 1989).

reported that its administration in association with specific environmental stimuli will cause animals to spend more time in the proximity of these stimuli (Carboni, Acqua, Leone, Perezzani, & Di Chiara, 1988; Fudala & Iwamoto, 1986; Fudala, Teoh, & Iwamoto, 1985), other investigators have failed to find such preferences (Clarke & Fibiger, 1987; Jorenby, Steinpreis, Sherman, & Baker, 1990). It would appear, then, that while nicotine can be reinforcing in this procedure, either it is marginally reinforcing or the optimal parameters for demonstrating nicotine-induced place-preference are not yet clear.

In addition to its effects on intravenous self-administration and conditioned place-preference, nicotine has locomotor-activating effects (Clarke & Kumar, 1983; Walter & Kuschinsky, 1989). That nicotine has effects in the context of each of these procedures fits well with the suggestion that these procedures reflect the activation of a common mechanism (Wise & Bozarth, 1987).

Little is known, however, about where and how nicotine acts to produce these behavioral effects. Because nicotine binds to receptors in several brain regions and alters the release of several neurotransmitters (for a review see Balfour, 1982) any one or more of these affected brain regions or neurotransmitter systems may mediate the effects of nicotine. Of the several neurotransmitter systems, the dopaminergic system deserves special attention; the neurotransmitter dopamine (DA) appears to play an important role in the self-administration of habit-forming drugs (Lyness, Friedle, & Moore, 1979; Roberts, Corcoran, & Fibiger, 1977; Wilson & Schuster,

1972; Yokel & Wise, 1975), the establishment of conditioned place-preferences (Bozarth & Wise, 1981b; Carr & White, 1983; Spyraki, Nomikos, & Varonos, 1987), and the expression of locomotion (Costall & Naylor, 1975; Creese & Iversen, 1975; Fink & Smith, 1980). The possibility that some of the effects of nicotine are mediated by the DA system has been raised (Pert & Clarke, 1987) and will be considered later. First, however, studies of the effects of nicotine on locomotion, self-administration behavior, and the establishment of conditioned place-preferences will be presented.

NICOTINE: EFFECTS ON LOCOMOTION AND REINFORCEMENT

LOCOMOTION

In the rat, while low doses of nicotine (0.1 to 0.2 mg/kg) produce an increase in locomotion, higher doses (0.2 to 1 mg/kg) produce an initial decrease in locomotion that is followed by an increase above normal levels (Clarke & Kumar, 1983). Whereas no tolerance develops to the low-dose excitatory effects, tolerance develops fairly rapidly to the high-dose depressant effects; the initial injections of high doses cause ataxia, but with subsequent injections this ataxia decreases or disappears (Clarke & Kumar, 1983). The depressant actions of nicotine could be due, in part, to its attenuation of spinal reflexes, an effect that may involve a direct action at the level of the spinal cord (Schweitzer & Wright, 1938, cited in Clarke & Kumar, 1983). As already mentioned, whereas animals show tolerance to the locomotor-depressing

effects of nicotine, no tolerance develops to the excitatory effects of nicotine. In fact, when nicotine is administered repeatedly its locomotor-activating effects get progressively stronger (Hakan & Ksir, 1988; Ksir, Hakan, Hall, & Kellar, 1985). Some have suggested that this progressive strengthening of the locomotor response is the by-product of the tolerance that develops to nicotine's inhibitory effects (Clarke & Kumar, 1983); others, however, have suggested that it is evidence of reverse tolerance, or sensitization (Ksir et al., 1985). Although conditioning factors may play a role in the effects associated with the repetated administration of nicotine (Walter & Kuschinski, 1989), others have suggested that the contribution of such factors is, at best, minimal (Hakan & Ksir, 1988). One question that will be addressed later by some of the experiments that make up this thesis is whether the locomotor-activating effects of nicotine, and the apparent sensitization of these effects, result from the activation of a common neural mechanism.

REINFORCEMENT

In addition to studies of the locomotor-activating effects of nicotine, there have been studies of nicotine's reinforcing effects. Whereas the intravenous self-administration procedure has been used to study nicotine as a response reinforcer, the conditioned place-preference procedure has been used to study nicotine as a stimulus reinforcer.

Intravenous Drug Self-Administration

The intravenous self-administration of nicotine has been demonstrated in a variety of laboratory animals, namely rats (Cox et al., 1984; Singer et al., 1978), dogs (Risner & Goldberg, 1983), monkeys (Spealman & Goldberg, 1982), and baboons (Ator & Griffiths, 1983). Humans also self-administer nicotine intravenously (Henningfield & Goldberg, 1983b; Henningfield, Miyasato, & Jasinski, 1983). Within the range of doses that sustains reliable responding in the rat, response rates appear to be inversely proportional to the injection dose (Cox et al., 1984; Dougherty, Miller, Todd, & Kostenbauder, 1981). The intervals between injections change in a systematic fashion so that the time between successive nicotine injections becomes longer when the injection dose increases.

In order to discount the possibility that the effects of nicotine on lever-pressing are simply the result of nicotine's unconditioned effects on motor behavior, use has been made of a two-lever test-box in which presses on one lever, the active lever, are reinforced with injections of nicotine and presses on the other lever, the inactive lever, are not reinforced. Under these conditions, even though rats press the inactive lever (Cox et al., 1984), they press the active lever preferentially (Corrigall & Coen, 1989; Cox et al., 1984). That rats sometimes increase responding on the inactive lever suggests that nicotine does have activational effects, but these effects, on their own, cannot account for the effects of nicotine on operant responding.

It is evident that nicotine can act as an operant reinforcer; it is unclear, however, why it appears to be more difficult to train animals to self-administer nicotine than other drugs such as cocaine, heroin or amphetamine (for a review see Henningfield, 1984). One possibility is that intravenous nicotine is simply a relatively weak reinforcer for lower animals; alternatively, it may be that problems establishing intravenous nicotine self-administration derive from certain side-effects of the intravenous administration of nicotine. When an animal is first infused with nicotine, for example, it becomes ataxic and prostrate (Clarke & Kumar, 1983; Walter & Kuschinsky, 1989). Unless the animal is pretreated with nicotine prior to the beginning of a self-administration experiment—so that tolerance to the ataxic effects can develop prior to training—there might be some difficulty in demonstrating nicotine self-administration (Clarke, 1987). Because human subjects participating in nicotine self-administration experiments report burning sensations at the site of infusion, it is likely that in at least one of its forms, the tartrate form, nicotine produces aversive effects in other species as well (Henningfield & Goldberg, 1983a). There are, then, several possible explanations for the difficulties encountered when training animals to self-administer nicotine.

Conditioned Place-Preference

As mentioned earlier, the effects of nicotine have also been studied in the context of the conditioned place-preference procedure, a procedure that reflects Pavlovian conditioning and

stimulus reinforcement. In this procedure one portion of an environment is associated with drug injections and another is not. If animals develop a preference for the portion of the environment that is associated with the drug, then the drug is said to have established a conditioned place-preference.

Fudala et al. (1985) first reported that when injections of nicotine are paired with one of the two main compartments of a place-preference apparatus, animals will show a preference for the compartment associated with those injections. Fudala et al., (1985) obtained dose-dependent increases in place-preference with doses within the range that is associated with dose-dependent increases in locomotion (Clarke & Kumar, 1983). High doses of nicotine, on the other hand, have been reported to establish place aversions (Fudala et al., 1985; Jorenby et al., 1990). The general finding that nicotine can establish conditioned place-preferences has since been confirmed by Fudala & Iwamoto (1986) and Carboni et al. (1988), but not by Clarke & Fibiger (1987) and Jorenby et al. (1990); on the basis of these studies it is difficult to determine which conditions are essential for the establishment of conditioned place-preferences. Several factors differed between those studies in which the establishment of a place preference was reported and those in which it was not. For example, the way baseline preferences were determined, or the number of times nicotine injections were paired to the drug-compartment, or even the length of the trials during the baseline and test phases, might account for the differences in the results of some of these studies. That different methods were used

to determine whether a place-preference was established might also account for some of the discrepancies between studies (In the study of Fudala et al., (1985) a formula was used to estimate the magnitude of the place preferences. The use of such a formula could conceivably increase the sensitivity of their method.)

Intracranial injections of nicotine have also been reported to establish conditioned place-preferences. Preferences have been reported following the administration of nicotine into either the ventricular system or the pedunculopontine nucleus (Iwamoto, 1990). In the case of intracranial injectgions, a preference for the environment paired with nicotine injections can be obtained after only one drug pairing. One explanation for the difficulty in establishing conditioned place-preferences with systemic injetions of nicotine may therefore have to do with the route through which nicotine is administered. It is possible that the systemic administration of nicotine produces effects, perhaps aversive effects, that counteract the reinforcing effects apparent when the drug is administered centrally.

DOPAMINE: INVOLVEMENT IN LOCOMOTION AND REINFORCEMENT

Little is known about the brain mechanisms involved in the locomotion, drug self-administration, and conditioned place-preferences associated with nicotine. A good deal is known, however, as to the mechanisms involved in the similar effects of the habit-forming drugs amphetamine, cocaine, and morphine. What

is known about the locomotor-stimulating and reinforcing effects of the psychomotor stimulants and opiates relates most directly to dopamine-containing cells of the midbrain. Before considering the possible role of these cells in the effects of nicotine, their role in the effects of the psychomotor stimulants and opiates will be discussed.

LOCOMOTION

Systemic Injection Studies

Locomotion is generally increased by the psychomotor stimulants amphetamine (Carr & White, 1987; Segal, 1975) and cocaine (Kalivas, Duffy, DuMars, & Skinner, 1988) and the opiates morphine (Babbini & Davis, 1972) and heroin (Swerdlow, Vaccarino, & Koob, 1985). Although these compounds alter more than DA function, the effects these compounds have on DA function are thought to be sufficient to increase locomotion (Pijnenberg, Honig, Heyden, & van Rossum, 1976; Joyce & Iversen, 1979).

Locomotion appears to be increased regardless of the mechanism through which DA function is enhanced. Whereas amphetamine increases DA function by releasing DA from the terminals of DA neurons (Hunt, Raynaud, Leven, & Schacht, 1979; Raiteri, Bertollini, Angelina, & Levi, 1975), cocaine increases DA function by blocking DA re-uptake (Heikkila, Orlansky, & Cohen, 1975; Ross & Renyi, 1967), thereby prolonging the synaptic actions of DA. Morphine, unlike amphetamine and cocaine, does not appear to act directly on DA neurons; it is believed to increase DA

transmission by releasing DA neurons from a tonically inhibitory input, thus increasing their firing rate (Gysling & Wang, 1983; Hommer & Pert, 1983). Apomorphine and bromocriptine, compounds that enhance DA function by activating DA receptors directly and selectively, also increase locomotion (Dolphin, Jenner, Sawaya, Marsden, & Tests, 1977; Kelly, Seviour, & Iversen, 1975).

Drugs that interfere with DA function have behavioral effects opposite to those of drugs that enhance DA function. Spontaneous locomotion is inhibited by compounds that antagonize the actions of DA; for example, the DA-receptor antagonists alpha-flupenthixol (Ahlenius, Hillegaart, Thorell, Magnusson, & Fowler, 1987) and haloperidol (Mithani, Martin-Iverson, Phillips, & Fibiger, 1986) have inhibitory effects on spontaneous locomotion. Alpha-flupenthixol (Swerdlow, Vaccarino, Amalric, & Koob, 1986) and haloperidol (Rolinski & Scheel-Kruger, 1973) also block the locomotor-activating effects of amphetamine.

Neuroleptics do not disrupt the locomotion induced by opiates to the same extent as they disrupt the locomotion induced by the psychomotor stimulants; a dose of the DA-receptor antagonist alpha-flupenthixol that blocks the locomotion induced by the systemic administration of amphetamine has no effects on the locomotion induced by the systemic administration of heroin (Swerdlow et al., 1986). Because a disruption of DA function that is sufficient to disrupt amphetamine-induced locomotion is not sufficient to disrupt heroin-induced locomotion, some have claimed that opiates can enhance locomotion by a DA-independent mechanism

(Kalivas, Widerlov, Stanley, Breese, & Prange, 1983; Swerdlow et al., 1986). The evidence presented in subsequent sections makes it evident, however, that opiates can also increase locomotion by a DA-dependent mechanism (Broekkamp, Phillips, & Cools, 1980; Kalivas et al., 1983; Stinus, Koob, Ling, Bloom, & LeMoal, 1980).

Lesion Studies

The notion that the psychomotor stimulants and the opiates increase locomotion by enhancing DA function has also been evaluated in lesion studies. The neurotoxin six-hydroxydopamine (6-OHDA) has been especially useful since it selectively destroys neurons that utilize the catecholamine transmitters DA, noradrenaline and adrenaline (Uretsky & Iversen, 1970). If the administration of 6-OHDA is made in conjunction with the systemic administration of compounds that prevent the entry of 6-OHDA into adrenergic and noradrenergic neurons, DA projections are selectively destroyed (Creese & Iversen, 1975).[2]

The most extensively studied set of DA projections originates from a group of cells located in the mesencephalon (Lindvall & Bjorklund, 1974). The lateral component of this group of cells is identified with the substantia nigra, pars compacta (SNC), and the medial component is identified with the ventral tegmental area (VTA). The majority of SNC efferents innervate the striatum (hence the name nigrostriatal system), and, to a lesser extent, cortical

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[2] The specificity of 6-OHDA lesions is also greatest when injections are made into regions that predominantly contain DA (i.e. cell-body or terminal regions). When 6-OHDA is injected into areas that contain other catecholamines, precautions need to be taken so that toxic levels are only reached after the neurotoxin is taken up into the DA neurons (Butcher, Eastgate & Hodge, 1974).

(Berger, Thierry, Tassin & Moyni, 1976) and limbic (Beckstead, Domesick & Nauta, 1979) structures. Dopaminergic efferents of the VTA, on the other hand, terminate primarily in limbic (nucleus accumbens, olfactory tubercle, amygdala, septum and bed nucleus of the stria terminalis) as well as cortical (prefrontal, cingulate and entorhinal cortices) structures (Fallon & Moore, 1978) (hence the name mesolimbic DA system).

The locomotor-activating effects of amphetamine are disrupted by 6-OHDA-induced lesions of the VTA (Dunnett, Bunch, Gage, & Bjorklund, 1984; Herman, Choulli, Abrous, Dulluc, & LeMoal, 1988; Nadaud, Herman, Simon, & LeMoal, 1984). The locomotor-activating effects of amphetamine are also disrupted by 6-OHDA-induced lesions of the NAS (Creese & Iversen, 1975; Herman et al., 1988; Kelly & Iversen, 1976; Kelly et al., 1975). However, since the administration of 6-OHDA into the NAS nearly always results in the destruction of DA terminals in the olfactory tubercle, another DA containing region located near the NAS, it has been difficult to associate the lesion effects only to damage to the NAS (Clarke et al., 1988b). Lesions of the frontal cortex, another region that receives mesolimbic DA projections do not appear to disrupt the locomotor-activating effects of amphetamine (Clarke et al., 1988b); similarly, lesions of the caudate putamen, a region that receives a greater number of DA fibers from the substantia nigra than from the ventral tegmental area, do not disrupt the locomotor-activating effects of amphetamine (Creese & Iversen, 1975; Kelly & Iversen, 1976; Kelly et al., 1975; Koob, Stinus, & LeMoal, 1981).

The grafting of embryonic dopaminergic cells back into animals with 6-OHDA lesions reinstates the ability of amphetamine to induce locomotion. Because 6-OHDA-induced lesions of the ventral tegmentum reduce DA levels in a number of projection fields (Koob et al., 1981), the relative importance of each of these fields has been difficult to determine. Since the grafting technique enables the re-innervation of specific structures, it has been used to determine whether the reinnervation of any one particular structure is sufficient to reinstate the locomotor response to amphetamine. The locomotor-stimulating actions of amphetamine are lost in animals with total dopaminergic lesions, but are reinstated by DA cell grafts into either the NAS or medial prefrontal cortex (Dunnett et al., 1984; Herman et al., 1988); thus, the reinnervation of either structure is sufficient to reinstate the locomotor response to amphetamine. Animals with 6-OHDA-induced lesions of the NAS also regain their reponsiveness to amphetamine after DA grafts are made into the NAS (Herman et al., 1988). Generally, then, the results of experiments that make use of grafting techniques confirm that the mesolimbic DA system is involved in the expression of amphetamine-induced locomotion.

Selective lesions have also been used to study the involvement of the DA system in the locomotor-activating effects of opiates. Whereas partial 6-OHDA-induced lesions of the NAS decrease the locomotor-activating effects of amphetamine, the same lesions fail to influence the locomotor-activating effects of heroin to the same extent (Swerdlow et al., 1986). That manipulations which impair DA

function influence morphine- or heroin-induced locomotion to a smaller extent than amphetamine-induced locomotion suggests that morphine and heroin can increase locomotion in a DA-independent manner (Kalivas et al., 1983; Pert & Sivit, 1977; Swerdlow et al., 1986). As discussed below, however, it appears that the opiates can also increase locomotion by a DA-dependent mechanism (Kalivas et al., 1983).

Intracranial Injection Studies

Another method that has been used to evaluate the role of the mesolimbic DA system in locomotion is the intracranial injection method. With this method drugs can be administered into specific neural structures, thus making it possible to evaluate whether effects in these structures are sufficient to influence locomotion. The results of such manipulations serve as additional evidence implicating specific central sites of action in the locomotion ordinarily observed following systemic drug injections.

Injections of DA and DA agonists   The administration of DA into some of the DA terminal regions has been studied. On the basis of these studies alone, it appears that enhanced DA function in some structures is more closely associated with locomotor-activation than enhanced DA function in other structures. Whereas the administration of DA into the NAS increases locomotor activity (Costall & Naylor, 1975; Costall, Domeney, & Naylor, 1981; Kalivas & Miller, 1985; Pijnenburg & van Rossum, 1973), injections into the olfactory tubercle are less effective, and injections into the ventrolateral portion of the caudate-putamen or amygdala are

ineffective (Costall & Naylor, 1975). Like injections of DA, NAS injections of the direct-acting agonists apomorphine (Jackson, Anden, & Dahlstrom, 1975) and ergometrine (Pijnenburg et al., 1976) can increase locomotion. Injections of amphetamine into the NAS can also increase locomotion (Pijnenburg et al., 1976; Carr & White, 1987); injections of amphetamine into the amygdala, medial prefrontal cortex and various portions of the caudate putamen, however, do not stimulate locomotor activity (Carr & White, 1987). The NAS, then, appears to be one of the primary DA-terminal regions that translate increased DA function into locomotor activation.

Injections of DA antagonists   Experiments involving the administration of DA antagonists into specific DA terminal fields also support the view that DA is important in the mediation of locomotion. Again, although only a few DA-containing regions have been studied, it is clear that the NAS plays an important role. Whereas microinjections of the DA antagonist alpha-flupenthixol into the NAS and ventral portion of the caudate putamen inhibit spontaneous locomotion, similar injections into the dorsal caudate putamen have no effects on locomotion (Ahlenius et al., 1987). Injections of DA antagonists into the NAS also block the locomotion induced by the systemic administration of amphetamine (Pijnenburg, Honig, & van Rossum, 1975).

Injections of opiates   Injections of opiates into either the ventral tegmentum or the NAS increase locomotion. Ventral tegmental injections of morphine (Joyce & Iversen, 1979), the long-acting synthetic enkephalin analogue D-Ala^2-Met^5-enkephalinamide

(DAMA) (Broekkamp et al., 1980; Kalivas et al., 1983), and ß-endorphin (Stinus et al.,1980) increase locomotion. Treatments that interfere with DA transmission generally block the locomotion induced by ventral tegmental injections of opiates. For example, the locomotion induced by ventral tegmental injections of D-Ala^2-Leu^5-enkephalin (DALA) is blocked by systemic injections of alpha-flupenthixol (Joyce, Koob, Strecker, Iversen, & Bloom, 1981). NAS 6-OHDA lesions also block the locomotion associated with either ventral tegmental injections of DALA (Kelley, Stinus, & Iversen, 1980) or ventral tegmental injections of ß-endorphin (Stinus et al., 1980).

DALA and morphine also increase locomotion when administered directly into the NAS (Pert & Sivit, 1977). The locomotion induced by NAS DALA injections are not blocked by systemic injections of haloperidol (Pert & Sivit, 1977). Likewise, the administration of the DA antagonist fluphenazine into the NAS, or even 6-OHDA lesions of the NAS, do not attenuate the locomotor response that is associated with NAS injections of DALA (Kalivas et al., 1983).

It appears, then, that whereas the locomotion induced by ventral tegmental opioid injections is dependent on the activation of the mesolimbic DA system, the locomotion induced by NAS injections is not dependent on the activation of this system. Hence, locomotion can be increased via a DA-dependent mechanism as well as via a DA-independent mechanism. The fact that DA release in the NAS is increased by injections of opiates into the ventral tegmental

area (Leone et al., 1991), but not by injections of opiates into the NAS (Kalivas et al., 1983), is consistent with this idea.

To summarize, several experimental methods have been used to study the role of DA function in locomotion; of these, the injection method and the lesion method have been especially useful. Generally, the results of these studies are consistent with the idea that the activation of the mesolimbic DA system, from either the administration of psychomotor stimulants or the administration of opiates, is associated with locomotor activation.

REINFORCEMENT

As mentioned earlier, in addition to having locomotor-activating effects, psychomotor stimulant and opiate drugs have reinforcing effects. Two procedures, the self-administration procedure and the conditioned place-preference procedure, have been used to study these direct reinforcing effects.

Drug Self-Administration

Intravenous self-administration   Intravenous self-administration has been demonstrated using the psychomotor stimulants amphetamine (Deneau, Yanagita, & Seevers, 1969; Pickens & Harris, 1968; Wilson & Schuster, 1972) and cocaine (Balster, Macdonald, Neale, & Smith, 1973; Woods & Schuster, 1968), and the opiates morphine (Glick, Cox, & Crane, 1975; Wilson & Schuster, 1972; Woods & Schuster, 1968) and heroin (Ettenberg, Pettit, Bloom, & Koob, 1982). It was suggested earlier that the actions of the psychomotor stimulants and the opiates on the

mesolimbic DA system are sufficient to cause locomotion. A similar notion has been put forth with regard to the reinforcing actions of the psychomotor stimulants and the opiates; here too, however, it has been argued that because manipulations that affect the self-administration of psychomotor stimulants do not similarly affect the self-administration of opiates, the reinforcing effects of the opiates are not completely dependent of DA function (Ettenberg et al., 1982; Pettit, Ettenberg, Bloom, & Koob, 1984). The present section will examine the involvement of DA in psychomotor stimulant and opiate reinforcement.

Several lines of evidence serve to implicate DA in the self-administration of psychomotor stimulants and the self-administration of opiates. In the case of amphetamine and cocaine self-administration, systemic injections of DA antagonists influence lever-pressing in a dose-dependent manner; whereas low doses of DA antagonists produce increases in responding, higher doses produce an initial increase in responding that is followed by an eventual cessation in responding (Wilson & Schuster, 1972; Yokel & Wise, 1975). In the case of the increases in lever-pressing that follow the administration of DA antagonists, it has been suggested that this reflects a compensation for diminished DA activity that follows the partial blockade of DA receptors (Wilson & Schuster, 1972; Yokel & Wise, 1975). The cessation of responding that is associated with the administration of the higher doses of DA antagonists is believed to reflect the complete blockade of the drug's reinforcing effects. That 6-OHDA lesions of the NAS severely

disrupt psychomotor stimulant self-administration also implicates DA in the reinforcing effects of the psychomotor stimulants; 6-OHDA-induced lesions of the NAS disrupt the self-administration of cocaine (Roberts et al., 1977; Pettit et al., 1984) and amphetamine (Lyness et al., 1979; Robbins, et al., 1983).

Whereas injections of the DA antagonist alpha-flupenthixol effectively disrupt cocaine self-administration, they have much weaker effects on heroin self-administration (Ettenberg et al., 1982). Similarly, although lesions of the NAS disrupt cocaine self-administration to the point where lever-pressing for cocaine eventually ceases, lesions of the NAS have weaker effects on heroin self-administration; animals self-administering heroin appear to show a slight initial attenuation in lever-pressing that is followed by a significant recovery (Pettit et al., 1984). It appears, then, that although antagonist and lesion studies implicate DA in the reinforcing effects of amphetamine and cocaine, the results of similar studies with heroin self-administration suggest a less important role for DA in heroin self-administration. That DA mediates at least a portion of the reinforcing effects of opiates, however, is suggested by several lines of evidence. For example, although low doses of neuroleptics influence cocaine self-administration to a greater degree than they influence heroin self-administration (Ettenberg et al., 1982), higher doses do bring about the extinction of the lever-pressing response for heroin (Gerber & Wise, 1989; Nakajima & Wise, 1987). In addition, both cocaine and heroin self-administration are severely disrupted by kainic acid

lesions of the NAS (Zito, Vickers, & Roberts, 1985). It appears, then, that although DA transmission is not critical in certain instances of opiate reinforcement, the activation of a DA mechanism contributes to opiate reinforcement.

Intracranial self-administration   Animals will also self-administer opiates and psychomotor stimulants directly into the brain. Morphine (Bozarth & Wise, 1981a; Devine & Wise, 1990; van Ree & de Wied, 1980; Welzl, Kuhn, & Huston, 1989) and the opiates [D-Ala^2, N-Met-Phe^4-Gly^5-OL]-enkephalin (DAGO) and [D-Pen^2, D-Pen^6]-enkephalin (DPDPE) (Devine & Wise, 1990) are self-administered directly into the ventral tegmentum. The reinforcing effects of morphine injections into the ventral tegmentum appear to be receptor-mediated since the peripheral administration of the opiate antagonist naloxone reduces morphine self-administration (Bozarth & Wise, 1981a). Rats have also been reported to self-administer opiates into the NAS (Goeders, Lane, & Smith, 1984; Olds, 1982). As with ventral tegmental opiate self-administration, the reinforcing effects of opiate injections into the NAS are blocked by naloxone (Goeders et al., 1984). Animals will also self-administer morphine into the lateral hypothalamus (Olds, 1979; Olds & Williams, 1980) and hippocampus (Stevens, Shiotsu, Belluzi, & Stein, 1988). Not much is known, however, about the substrates that mediate the reinforcing effects associated with morphine self-administration into these two sites.

Psychomotor stimulants, like opiates, are self-administered into specific regions of the brain. Cocaine, for example, has been

reported to be self-administered into the medial prefrontal cortex (Goeders, Dworkin, & Smith, 1986; Goeders & Smith, 1983; 1986). Surprisingly, attempts to firmly establish cocaine self-administration into the NAS have failed (Goeders & Smith, 1983). Amphetamine, on the other hand, has been reported to be self-administered into the NAS (Hoebel et al., 1983). The findings of intracranial self-administration experiments—with the possible exception of the results of studies of cocaine self-administration into the NAS—can be used, then, to implicate the mesolimbic DA system in the reinforcing effects of the psychomotor stimulants and the opiates.

Place-Preference

<u>Systemic injections</u>   The results of conditioned place-preference studies also implicate the DA system in the reinforcing effects of the psychomotor stimulants amphetamine and cocaine and the opiates morphine and heroin. Conditioned place-preferences have been established using the psychomotor stimulants amphetamine (Mithani et al., 1986; Spyraki, Fibiger, & Phillips, 1982) and cocaine (Mucha, Bucenieks, O'Shaughnessy, & van der Kooy, 1982; Spyraki et al., 1987), and the opiates morphine (Barr, Paredes, & Bridger, 1985; Rossi & Reid, 1976; Schwartz & Marchok, 1974) and heroin (Bozarth & Wise, 1981b; Spyraki, Fibiger, & Phillips, 1983). Animals spend more time in the proximity of those stimuli that have been associated with the systemic administration of these drugs.

The ability of amphetamine and cocaine to establish conditioned place-preferences is blocked by the DA antagonist

haloperidol (Spyraki et al., 1982; 1987). Systemic injections of DA antagonists also block the place-preferences established by morphine (Schwartz & Marchok, 1974) and heroin (Bozarth & Wise, 1981b). However, because doses of DA antagonists that block the place-preferences established with injections of amphetamine do not block the place-preferences established with injections of morphine (Mackey & van der Kooy, 1985), it appears that the reinforcing effects of morphine are not dependent on DA function to the same extent that the reinforcing effects of amphetamine are. This interpretation is similar to one expressed earlier; opiates can have effects that are apparently not dependent on DA function. As with the locomotion and self-administration associated with psychomotor stimulants and opiates, however, the activation of the DA system appears to be sufficient to cause the establishment of conditioned place-preferences; this is evident when the effects of intracranial injections of psychomotor stimulants and opiates are considered.

Intracranial injections   Conditioned place-preferences can be established with intracranial drug injections. In the case of amphetamine, its administration into the NAS—but not its administration into the caudate putamen or amygdala—establishes conditioned place-preferences (Carr & White, 1983). Injections of morphine into several different sites also establish conditioned place-preferences. Injections of morphine into the ventral tegmentum, for example, establish conditioned place-preferences (Bozarth, 19 : Phillips & LePiane, 1980), and, importantly, the

location of the injection site correlates with the magnitude of the established place-preference; whereas injections dorsal (Phillips & LePiane, 1980), anterior, or posterior (Bozarth, 1987) to the DA cell group fail to establish place-preferences, injections within the group of cells establish place-preferences (Bozarth, 1987; Phillips & LePiane, 1980). The boundaries of the zone from which place-preferences are established, then, correlate well with the boundaries of the mesolimbic DA cell-body region. Similar correlations have yet to be established with regard to other regions. Hence, although rats have been reported to show preferences for places that have been associated with the injection of opiates into the lateral hypothalamus, periaqueductal gray, or NAS (van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982), it remains uncertain whether the reinforcing effects of morphine are solely due to its actions at each of these sites; only if injections inside a given structure are reinforcing—and injections outside the same structure are not—can it be concluded with a any degree of certainty that it is actions in the structure in question that are reinforcing.

Taken together the findings of studies involving the locomotion and reinforcement procedures make clear that psychomotor stimulants and opiate drugs have locomotor-activating and reinforcing effects. Although DA function is critical for the effects of the psychomotor stimulants, DA function is not critical for the effects of opiates. Nevertheless, the activation of a DA mechanism by opiates is sufficient to increase locomotion and reinforce behavior.

NICOTINE

Nicotine, like the psychomotor stimulants and the opiates, has locomotor-activating and reinforcing effects. Since the effects the psychomotor stimulants and the opiates have on DA function appear to be sufficient to cause locomotion and reinforcement, the possibility exists that nicotine also produces its effects via the DA system. A variety of methods have been used to determine whether or not nicotine influences DA activity. Whereas some of the methods have been used to determine the location of the receptors that may mediate the effects of nicotine, others have been used to determine the effects of nicotine on various measures of DA function.

NICOTINE BINDING STUDIES

Radioactive-ligand binding techniques have been used to quantify characteristics of nicotine binding in brain tissue and it appears that nicotine binds, in a reversible manner, to high-affinity and saturable sites (Martin & Aceto, 1981). Radioactively labelled ligands have been used to determine the density as well as the distribution of these central nicotinic binding sites (Clarke, Pert, & Pert, 1984). The central nervous system contains a variety of putative nicotinic receptor subtypes; for example, although both ^{3}H-nicotine and ^{125}I-alpha-bungarotoxin bind to nicotinic receptors, the binding patterns of each ligand do not appear to overlap (Clarke, Schwartz, Paul, Pert, & Pert, 1985). On the other hand, in the rat

brain, the distribution of binding sites labelled by ^{3}H-nicotine overlaps the disribution of sites labelled by ^{3}H-acetylcholine (Clarke et al., 1985). This pattern of binding, in turn, resembles the pattern of glucose utilization produced by systemic injections of nicotine (London, Connoly, Szirksay, & Wamsley, 1985) thereby suggesting that nicotine acts at brain cholinergic receptors to increase cell activity.

Although nicotinic binding sites are present in a large number of brain regions, the fact that there are binding sites in regions that contain dopamine is of specific interest (Clarke et al., 1984). In the rat brain there are binding sites in the VTA and SNC as well as in structures that receive VTA and SNC projections (Clarke et al., 1984). Lesions made with the administration of 6-OHDA into the medial forebrain bundle—a tract of fibers that contains, among others, DA projections that originate in the SNC and VTA and that terminate principally in the caudate putamen and NAS, respectively—reduce DA levels in the caudate putamen and NAS and reduce the density of radioactively labelled nicotinic binding sites in the VTA, SNC, NAS, and caudate putamen (Clarke and Pert, 1985). While this is evidence that there are nicotinic binding sites on DA neurons, transsynaptic degeneration does accompany 6-OHDA-induced degeneration of nigrostriatal neurons; hence, the disappearance of nerve terminals in the caudate putamen also brings about the disappearance of non-DA neural elements (Hattori & Fibiger, 1982). As Clarke & Pert (1985) point out, however, it is unlikely that the process of transsynaptic degeneration could, on its

own, completely account for the degree of loss in receptor binding that was reported. Thus, it appears that there are high-affinity sites to which nicotine can bind, and the actions of nicotine at these sites may very well account for some of the effects nicotine has on DA function.

At least in the case of the systemic effects of nicotine on locomotion, there is evidence that nicotinic sites located on DA neurons may play a role. Consistent with this is the finding that ventral tegmental injections of the nicotinic agonist cytisine increase locomotion (Pert & Chiueh, 1986; Pert & Clarke, 1987; Reavill & Stolerman, 1990). This effect appears to have been receptor-mediated since the systemic administration of the nicotinic agonist mecamylamine blocked the effects of cytisine (Pert & Clarke, 1987). Because injections of cytisine into other DA-containing regions such as the substantia nigra and the caudate putamen failed to have any appreciable effects on locomotion, it appears that not all DA systems are equally implicated in the locomotor-activating effects of nicotine. Importantly, ventral tegmental injections of cytisine increase levels of DA in the NAS (Pert & Chiueh, 1986); also consistent with the idea of DA involvement in the locomotor effects associated with systemic nicotine is the finding that the locomotor-activating effects associated with either ventral tegmental injections of cytisine or systemic injections of nicotine are not apparent in animals that have previously been injected with 6-OHDA into the NAS (Clarke et al., 1988a; Pert & Clarke, 1987).

NICOTINE: ACUTE AND CHRONIC EFFECTS ON DA ACTIVITY

The effects of nicotine on various aspects of DA function, have been studied using *in vitro* and *in vivo* preparations. The acute and chronic effects of nicotine have been examined with each type of preparation.

Acute Effects

Nicotine increases the release of DA from striatal (Giorguieff-Chesselet, Kemel, Wandscheer, & Glowinski, 1979; Westfall, 1974) as well as NAS (Rowell, Carr, & Garner, 1987) slices. In low concentrations (1 μM - 100 μM) nicotine apppears to release DA via a receptor-mediated process (for a review see Rowell, 1987 and Westfall, Perry, & Vickery, 1987). This is supported by the observation that nicotinic antagonists block nicotine's effects on DA release (Giorguieff-Chesselet, et al., 1979). There is also evidence that at low concentrations the effects of nicotine on DA release are calcium-dependent (Giorguieff-Chesselet, LeFloch, Glowinski, & Besson, 1977; Giorguieff-Chesselet et al., 1979; Westfall, 1974) and are attenuated when neural conduction is blocked with tetrodotoxin, a sodium-channel blocker (Westfall et al., 1987).

In addition to increasing DA release from DA terminals via a receptor-mediated process, nicotine *in vitro* is also thought to increase DA release from DA terminals via a non-receptor-mediated process.[3] Because lethal injections of nicotine would be required to

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[3] The other mechanism through which DA appears to be released from terminals apparently comes into play when DA-containing tissue is exposed to high concentrations of nicotine (100 μM - 1000 μM). The facilitation of DA release persists in the absence of calcium or when neural conduction is blocked. Because the effects of nicotine at such

increase DA release via this non-receptor-mediated process, it appears that only the receptor-mediated actions are behaviorally relevant (Westfall et al., 1987).

Nicotine also appears to act at the level of the DA cell body. As mentioned earlier, nicotinic receptors have been localized in the ventral tegmentum (Clarke et al., 1984; 1985), and lesions of DA neurons decrease the number of receptors in this region (Clarke & Pert, 1985). The systemic administration of nicotine (Clarke, Hommer, Pert, & Skirboll, 1985; Grenhoff, Aston-Jones, & Svensson, 1986; Lichtensteiger, Felix, Lienhart, & Hefti, 1976; Mereu et al., 1987) or its iontophoretic application onto DA neurons in the SNC (Lichtensteiger et al, 1982) increases the firing rates of these DA neurons. Systemic injections of nicotine also increase the activity of DA neurons in the VTA (Grenhoff et al., 1986; Mereu et al., 1987).

With the aid of a histological technique that permits the identification of catecholaminergic cells on the basis of the fluorescence they emit in the presence of ultraviolet light (Falck, Hillarp, Thieme, & Torp, 1962), a strong positive correlation has been established between the effects of systemic nicotine on the firing rate of SNC DA cells and the intensity of the fluorescence these cells emit (Lichtensteiger et al., 1976; 1982). The effects of nicotine on DA cell-firing in the SNC are also associated with changes in DA metabolite levels in the striatum; for example, the systemic administration of nicotine increases levels of homovanillic acid in the striatum (Lichtensteiger et al., 1982).

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high concentrations are not antagonized by nicotinic antagonists, this release is unlikely to be receptor-mediated (for a review see Rowell, 1987 and Westfall et al., 1987).

Although nicotine acts on both nigrostriatal and mesolimbic DA neurons, mesolimbic DA neurons appear to be more sensitive to nicotine. Mesolimbic DA neurons show greater increases in firing than nigrostriatal neurons (Mereu et al., 1987; Yoon, Curfman, & Westfall, 1989). In a study by Andersson, Fuxe, Agnati, & Eneroth (1981), in which the effects of nicotine on DA stores in mesolimbic and nigrostriatal terminal regions were measured, whereas injections of nicotine reduced DA stores in regions of the NAS and caudate putamen, low doses that had an effect on the NAS had no effect on the caudate putamen. In a study with similar aims, Grenhoff and Sveisson (1988) reported no changes in DA content in the striatum and NAS in response to nicotine, but they did note a significant enhancement of DA synthesis and metabolism in the NAS.

The effects of nicotine on DA release have also been studied *in vivo* using the technique of microdialysis; the systemic administration of nicotine increases DA release in the NAS and the caudate putamen (Imperato, Mulas, & Di Chiara, 1986). Systemic injections of nicotine have a greater effect on NAS DA release than on caudate putamen DA release (Imperato et al., 1986). With regard to the effects of nicotine on NAS DA release, when a microdialysis probe is used to administer nicotine directly into the NAS and to simultaneously monitor DA levels in this structure, levels of DA are found to increase (Misfud, Hernandez, & Hoebel, 1989).

On the basis of the pharmacological, electrophysiological and biochemical evidence presented thus far, it is evident that nicotine has excitatory effects on DA function (with apparently greater

effects on mesolimbic than nigrostriatal DA function). The studies discussed thus far, however, have served to demonstrate the acute effects of nicotine on DA activity. Interest has also been expressed in determining the effects that accompany chronic nicotine administration, a condition that is more likely to mirror the effects of prolonged use that accompany the chronic self-administration of nicotine observed in humans.

Chronic Effects

The effects of chronic nicotine on DA synthesis, turnover and release have been studied, as have the effects of chronic nicotine on the firing rates of DA neurons and DA-receptor binding. In these studies nicotine is administered in one of two ways: the first way involves repeated systemic injections (i.e. one injection per day for a number of days), and the second way involves the subcutaneous implantation of drug reservoirs that release predetermined concentrations of nicotine at constant rates.

As mentioned earlier, single injections of nicotine increase DA turnover in the NAS (Andersson et al., 1981; Grenhoff & Svensson, 1988). If nicotine is injected five times (one injection of 0.8 mg/kg/day), however, its effects on DA turnover begin to diminish (Vezina, Blanc, Glowinski, & Tassin, 1989). An even larger number of injections (one injection of 0.8 mg/kg/day, 5 days per week, for 2 weeks) (Lapin, Maker, Sershen, & Lajtha, 1989), or the chronic infusion of nicotine (1.5 mg/kg/day for two weeks) (Fung & Lau, 1989), also reduces DA turnover. And although acute challenges with nicotine injections still increase DA turnover in chronically

treated animals, some have reported that this effect is smaller than that observed in naive animals (Lapin et al., 1989). With regards to the effects of nicotine on DA synthesis (as determined by the accumulation of the dopamine precursor DOPA, following the inhibition of DOPA-decarboxylase), whereas some have reported that chronic nicotine decreases basal DA synthesis (1 mg/kg/day for two weeks; Holt & Westfall, 1989) others have failed to find an effect (Mitchell, Brazell, Joseph, Alavijeh, & Gray (1989). Under certain circumstances, then, nicotine can decrease the basal activity of the mesolimbic DA system. Surprisingly, however, the chronic administration of nicotine does not appear to reduce the release of DA. An acute injection of nicotine (0.35 mg/kg) still enhances DA release in animals that have previously been repeatedly injected with nicotine (once daily injections of 0.35 mg/kg for fifteen days); basal DA release in these chronically treated animals, although somewhat higher, was not significantly different from basal DA release in naive animals (Damsma, Day, & Fibiger, 1989). Chronic infusions of nicotine (1 mg/kg/day for two weeks), however, increase basal DA release in the NAS, and acute challenges in this preparation increase DA release in a manner similar to that seen in animals never previously exposed to nicotine (Westfall & Vickery, 1989). There are reports, then, that in chronically treated animals, there is no tolerance to the acute effects nicotine has on basal DA release.

It appears, then, that although chronic exposure to nicotine can reduce DA synthesis and turnover, the responsiveness of the

mesolimbic DA system to acute nicotine challenges is not generally diminished. This is also apparent if the effects of chronic nicotine (1 mg/kg/day for two weeks) on the firing rate of mesolimbic DA neurons are considered. The basal firing rate of mesolimbic DA neurons decreases when animals are chronically treated with nicotine, but in the event of an acute nicotine challenge the average firing-rate of these neurons increases four-fold (Yoon et al., 1989). Interestingly, nigrostriatal neurons do not show changes in their basal firing-rate following chronic treatment; and in the event of an acute challenge there is only a small increase in the firing-rate, similar to that seen prior to the chronic administration of nicotine (Yoon et al., 1989). These findings confirm that the effects nicotine has on the mesolimbic DA system are more potent than the effects it has on the nigrostriatal system. These findings also demonstrate that while nicotine can decrease the basal firing rates in the mesolimbic DA cells (an effect that may be directly related to the effects of nicotine on DA synthesis and turnover), nicotine can still have powerful effects on the activity of these cells.

In addition to influencing DA release, DA turnover and DA-cell firing, the chronic administration of nicotine is associated with changes in the number of binding sites in the striatum. Fung & Lau (1989) examined the effects of nicotine on nicotine binding as well as DA binding in tissue samples obtained from three groups of animals: one group had never been exposed to nicotine, a second group had been exposed to nicotine continuously for 5 days (1.5 mg/kg/day), and a third group had been exposed to nicotine

continuously for 14 days (1.5 mg/kg/day). Although nicotine binding was higher than normal in the 5-day group, nicotine binding was normal in the fourteen-day group. And although DA binding was normal in the 5-day group, it was higher in animals exposed to nicotine for 14 days. Repeated injections of nicotine (once daily injections of 0.8 mg/kg, five days per week for six weeks) also increase the number of DA binding sites in the NAS (Reilly, Lapin, Maker, & Lajtha, 1987).

The mechanisms that account for the changes in the number of nicotine or DA binding sites are not known. Some researchers have hypothesized that the chronic administration of nicotine desensitizes nicotinic receptors and because of this desensitization there is a subsequent proliferation of nicotine binding sites (Marks, Burch, & Collins, 1983). The desensitization of nicotinic receptors in response to the chronic effects of nicotine is likely to play an important role in the bahavioral effects of nicotine and could account for those differences observed between the effects of acute and chronic treatments.

With regards to DA binding, it may perhaps be important to note that similar increases in DA binding typically result when large lesions of mesolimbic DA projections are made (Creese, Burt, & Snyder, 1977); in such cases the proliferation of DA receptors, sometimes referred to as DA-receptor supersensitivity, is thought to compensate for the reduced availability of DA (Creese et al., 1977). The increase in receptor number in animals with large lesions of the mesolimbic DA system is believed to explain how it is

that these animals are, on the one hand, insensitive to the locomotor-activating effects of amphetamine, and, on the other hand, very sensitive to the locomotor-activating effects of apomorphine, a direct-acting DA agonist (Kelly et al., 1975). It appears that while the severely reduced number of DA terminals in the NAS accounts for amphetamine's lack of effect, the increased number of receptors that presumably results from the loss of the DA terminals, accounts for apomorphine's strong locomotor-activating effects (Kelly et al., 1975).

Like 6-OHDA-lesioned animals, animals chronically treated with nicotine show an enhanced response to the locomotor-activating effects of apomorphine, but, unlike 6-OHDA-lesioned animals, chronically treated animals (1.5 mg/kg/day for two weeks) also show an exaggerated response to the locomotor-activating effects of amphetamine (Fung & Lau, 1988; 1989). That apomorphine increases locomotion in animals that have been chronically treated with nicotine is perhaps explained by the fact that apomorphine is acting on a greater number of DA receptors. Similarly, the exaggerated response to the locomotor-activating effects of amphetamine is perhaps best accounted for by the possibility that the DA released by amphetamine is also acting on a larger number of DA receptors; in agreement with this is a report that NAS tissue slices obtained from animals chronically-treated with nicotine are more sensitive to amphetamine's effects on DA release than slices obtained from saline-treated controls (Fung, 1989).

Although the results of studies of chronic nicotine's effects on locomotion easily lead to speculation as to how the chronic administration of nicotine influences DA transmission—and how this contributes to changes in behavior—any interpretation of the results of these studies is complicated by apparently inconsistent findings. For example, although the reports of the effects of nicotine on DA turnover and synthesis are consistent with the idea that chronic nicotine reduces DA transmission, there are other changes, most notably the up-regulation in the number of DA binding sites, that could result in a net increase in DA transmission.

Although there remain numerous questions with regard to the precise dynamics of nicotine's actions on DA neurons—and the behavioral effects associated with such actions—on the basis of what has been presented thus far, it is evident that nicotine has effects on DA activity. Since the mesolimbic DA system has been implicated in some of the locomotor-activating and reinforcing effects of psychomotor-stimulant and opiate drugs, the possibility exists that nicotine's actions on the mesolimbic DA system are sufficient to account for nicotine's locomotor-activating and reinforcing effects. The present experiments were designed to explore this possibility.

THE PRESENT EXPERIMENTS

Systemic injections of nicotine can increase locomotion. The present series of experiments was carried out in order to determine, by way of intracranial injections, which regions of the brain might mediate this effect. Experiment 1 was carried out to confirm that injections of the nicotinic agonist cytisine into the ventral tegmentum increase locomotion; the degree and the time course of the locomotor effects associated with the administration of each of several doses of cytisine were of interest. Experiment 2 was designed to determine whether repeated injections of cytisine into the ventral tegmentum produce progressively greater effects on locomotion; such changes in locomotion have been associated with the repeated activation of the mesolimbic DA system. In Experiment 3 cytisine injections were made in and around the ventral tegmentum to assess whether there is a circumscribed region into which injections of cytisine increase locomotion; it was also of interest to determine whether the boundaries of the region within which injections of cytisine increased locomotion bore some relationship with the boundaries of the DA cell-body region.

In addition to determining whether ventral tegmental injections of cytisine affect locomotion, the notion that the effects of a drug on locomotion are associated with its effects on reinforcement was explored. In Experiment 4 the conditioned place-preference procedure was used to determine whether ventral

tegmental injections of cytisine are sufficient to establish a preference for the environment with which they are paired.

Although the possibility exists that the locomotor-activating and reinforcing effects of nicotine involve actions on DA cell bodies, the actions of nicotine on DA terminals may also be sufficient to produce behavioral effects. Experiment 5 was designed to determine the extent to which actions at each of several mesolimbic DA-terminal regions might contribute to the effects of nicotine on locomotion.

EXPERIMENT 1 :

CYTISINE MICROINJECTIONS INTO THE VENTRAL TEGMENTUM : EFFECTS ON LOCOMOTION

It is well established that systemic injections of nicotine increase locomotion (Clarke & Kumar, 1983; Walter & Kuschinski, 1989). The possibility exists that this effect is in part due to the actions of nicotine on DA cell bodies located in the ventral tegmentum; autoradiographic (Clarke et al., 1984; 1985; Clarke & Pert, 1985), electrophysiological (Grenhoff et al., 1986; Mereu et al., 1987) and biochemical (DiChiara et al., 1987; Imperato et al., 1986) findings are consistent with this idea. A more direct evaluation of the role of the ventral tegmentum, however, could be provided by experiments that involve the localized injection of nicotine directly into this region. Unfortunately, nicotine's lipophilic nature makes it a poor candidate for intracranial administration; because it diffuses rapidly from its site of injection, it is difficult to accurately determine nicotine's locus of pharmacological action. This source of complication is believed to be minimized if the nicotinic agonist cytisine, a less lipophilic structural analogue of nicotine, is injected in the place of nicotine.[4]

The effects of ventral tegmental injections of cytisine have been examined previously, in which case they were reported to

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[4] In vitro, cytisine competes very effectively against nicotine for binding sites (Clarke et al., 1985). The distribution of ^{3}H-cytisine sites also overlaps the distribution of ^{3}H-nicotine binding sites (Pabreza, Dhawan & Kellar, 1991). On the basis of behavioral experiments there is also evidence that cytisine acts at the same sites as nicotine. When administered cytisine, rats trained to discriminate the effects of nicotine from the effects of saline, respond in a manner that suggests that cytisine is producing effects similar to those produced by nicotine (Stolerman, Garcha, Pratt, & Kumar, 1984).

increase locomotion (Pert & Chiueh, 1986). The present experiment was an attempt to replicate the findings of Pert & Chiueh (1986); it was also of interest to establish a suitable experimental protocol for subsequent studies of the locomotor effects of cytisine. Several doses of cytisine were tested and the magnitude and duration of the locomotor effects of each dose were measured. The effects of several antagonists on the locomotor actions of cytisine were also examined.

METHOD

Subjects

Twenty-one male Long-Evans rats (Charles River Inc.), weighing between 350 and 400 g at the time of surgery, were housed individually in stainless-steel wire cages. The animals had access to food and water, and the lights in the animal room were turned on at 8 a.m. and off at 8 p.m..

Cannulae: Their Use and Their Construction

Three types of cannulae were used; guide, injector, and dummy cannulae. It was through a chronically implanted guide cannula that the injector cannula was lowered to a particular brain site. In order to prevent the occlusion of the guide cannulae, a third type of cannula, the dummy cannula, was fitted into each guide cannula. The dummy cannula remained in place at all times except for the duration of the intracranial brain injections.

Each guide cannula was constructed from 22 ga stainless-steel hypodermic tubing that was cut and filed to a length of 15 mm. The

injector cannulae were constructed from 30 ga stainless-steel hypodermic tubing. When inserted into a guide cannula, the tip of an injector protruded 1.5 mm beyond the tip of the guide cannula. This distance was maintained by a section of 22 ga tubing (approximately 3 mm in length) that was bonded at one end of the injector cannula; by serving as a mechanical "stop" it set the depth of the intracranial injection. The dummy cannulae were made from 30 ga wire, and they were cut and filed so as to protrude approximately 0.5 mm beyond the end of the guide cannulae.

Surgical Procedure

Prior to the bilateral implantation of the guide cannulae, each animal was anaesthetized with pentobarbitol (60 mg/kg, i.p., supplemented with 0.1 mg/Kg, s.c., of atropine sulphate) and placed in a stereotaxic instrument. A midline scalp incision was made to expose the skull, and holes were drilled through the skull to accomodate guide cannulae and stainless steel screws (see below). With the incisor bar set 5 mm above the interaural line, the guide cannulae were implanted at a 10-degree angle, off the vertical (the angling of the left and right cannulae toward bregma formed a 'v' shape). The angle of penetration ensured that the cerebral aqueduct was not punctured, an e\ ,it that would facilitate the quick diffusion of any injected compound to other areas of the brain. The tips of the guide cannulae were lowered stereotaxically to a location 1.5 mm short of the final injection sites. The stereotaxic coordinates for the injection sites in the ventral tegmentum were as follows: 2.5 mm behind bregma, 2.0 mm lateral to bregma and 8.6

mm ventral to the surface of the skull. A dental acrylic cement was used to maintain the position of the guide cannulae, and stainless steel screws that had previously been threaded into the skull anchored the cement. At the back of each animal's head, a piece of plastic (1.5 X 1.5 cm) was partially embedded in the acrylic cement. This prevented the guide and injector cannulae from being bent and minimized the loss of dummy cannulae.

At the end of the surgical procedure, the wound was covered with an antibacterial agent and each animal was placed under a heating lamp until post-surgical locomotion was observed. One week was given for recovery.

Apparatus

Twelve activity boxes were used to quantify locomotor activity. Each box (20 X 41 X 25 cm) was constructed of wood—except for a Plexiglas front and a wire grid floor. Two photocells, each positioned 4 cm above the floor, separated the compartment along its longest side into three equal areas; the photocells were connected via an electrical interface to a computer located in an adjoining room. While the animals were in the activity boxes, the room was dark (except for the dim illumination provided by the photobeams) and white noise was continuously present.

Intracranial-injection procedure

Bilateral injections were made while animals were free to move in a small (20 X 40 X 16 cm), open-topped, plastic container. The injections were made using two 1-μL glass syringes, each of which was connected, by way of polyethylene tubing (PE-20), to a

separate injector cannula. Before the start of each testing session, the microsyringes were filled with alcohol (70%); the inside of each piece of PE tubing was then filled with alcohol before it was thoroughly flushed and again filled with sterile, physiological saline. A small amount of air (approximately 0.05 μL) separated the alcohol in the syringe from the saline in the tubing; a similar amount of air separated the saline in the tubing from whatever solution was taken up into the injector cannula.

Drug or vehicle (sterile 0.9 % physiological saline) solutions were injected in a volume of 0.5 μL per side over a period of 100 sec with the aid of an electric pump. Injections into the left and right parts of the brain were made concurrently, and, to maximize drug diffusion, the injector cannulae were left in place for 60 sec following the injections.

One day prior to the beginning of the experiment, animals were placed in the activity boxes for 60 min. On each test day thereafter animals were placed in the boxes for 30 min before they were given injections into the ventral tegmentum. The habituation and testing sessions took place during the light part of the animals' light-dark cycle (between 8 a.m. and 8 p.m.).

Cytisine treatments

Every animal was tested with each of two doses of cytisine (1 and 10 nanomoles (nmol)) and the vehicle solution (0.9 % saline). Following the testing of these doses, two additional doses (0.01 and 0.1 nmol) were tested in an attempt to determine a threshold dose below which locomotion was not facilitated. Locomotion was

recorded for 60 min, beginning immediately after the animal was injected. Forty-eight hours separated the administration of each dose, and the order of the injections was counterbalanced. Animals were grouped into sets of three or four animals. Each set was administered every treatment, and no two sets received the same treatment on the same test day (the order of treatments was therefore counterbalanced with regards to sets of animals and not with regards to individual animals).

Antagonist pretreatment

In a second experiment, animals were randomly assigned to one of three groups. One group (n=6) was given an injection of the nicotinic antagonist mecamylamine (2 mg/kg, i.p.) 20 minutes prior to the ventral tegmental administration of cytisine (10 nmol/side). A second group (n=7) was administered the nicotinic antagonist hexamethonium (2 mg/kg, i.p.) 20 minutes prior to the administration of cytisine. A third group (n=7) was given an injection of pimozide (0.3 mg/kg, i.p.) five hours prior to ventral tegmental injections of cytisine. The dose of each antagonist, and the amount of time that separated its administration and the administation of cytisine, were selected on the basis of reports in the literature.

Test drugs

Cytisine, hexamethonium bromide, and mecamylamine (Sigma) were each dissolved in sterile physiological saline; pimozide (Janssen Pharmaceutica) was dissolved in a 0.1 molar solution of

tartaric acid (Sigma). The pH of drugs injected intracranially varied between 6 (saline) and 9 (10 nmol cytisine).

Confirmation of cannulae placements

Following the completion of the experiment, animals were anaesthetized (chloral hydrate: 400 mg/kg) and perfused transcardially with 50 mL of saline followed by 50 mL of 10% formalin. Each brain was then frozen with dry ice and sliced with a microtome to obtain 40-μ-thick coronal sections that were collected on glass slides. The location of the tip of each injection cannula was determined during a visual inspection of a magnified projection of the brain section.

Statistical Analysis

The data were analyzed using a two-way analysis of variance, and post-hoc comparisons were made using Tukey's Honestly Significant Difference test. Only animals with both injector tips located in the ventral tegmentum were included in the data analyses (Figure 1). Of the 21 animals implanted with guide cannulae, three were excluded on the basis of this criterion.

RESULTS

Ventral tegmental injections of cytisine increased locomotion (F[4,68] = 4.32, P<.003); however, whereas each of the three highest doses were effective (P<.01 in each case), the lowest dose was not (Figure 2). The locomotor-activating effects of cytisine were attenuated by systemic injections of the nicotinic antagonist mecamylamine (t[4] = 2.8, P<.05: Figure 3a). Hexamethonium, a

Figure 1.   (see page 46).   Anatomical localization of injector

cannulae tips in animals that were given injections into

the ventral tegmentum. The number on the side of each

section designates the location behind bregma of the

coronal section (in millimeters). (Adapted from

Pellegrino et al., 1979.)

2.4

2.6

2.8

3.0

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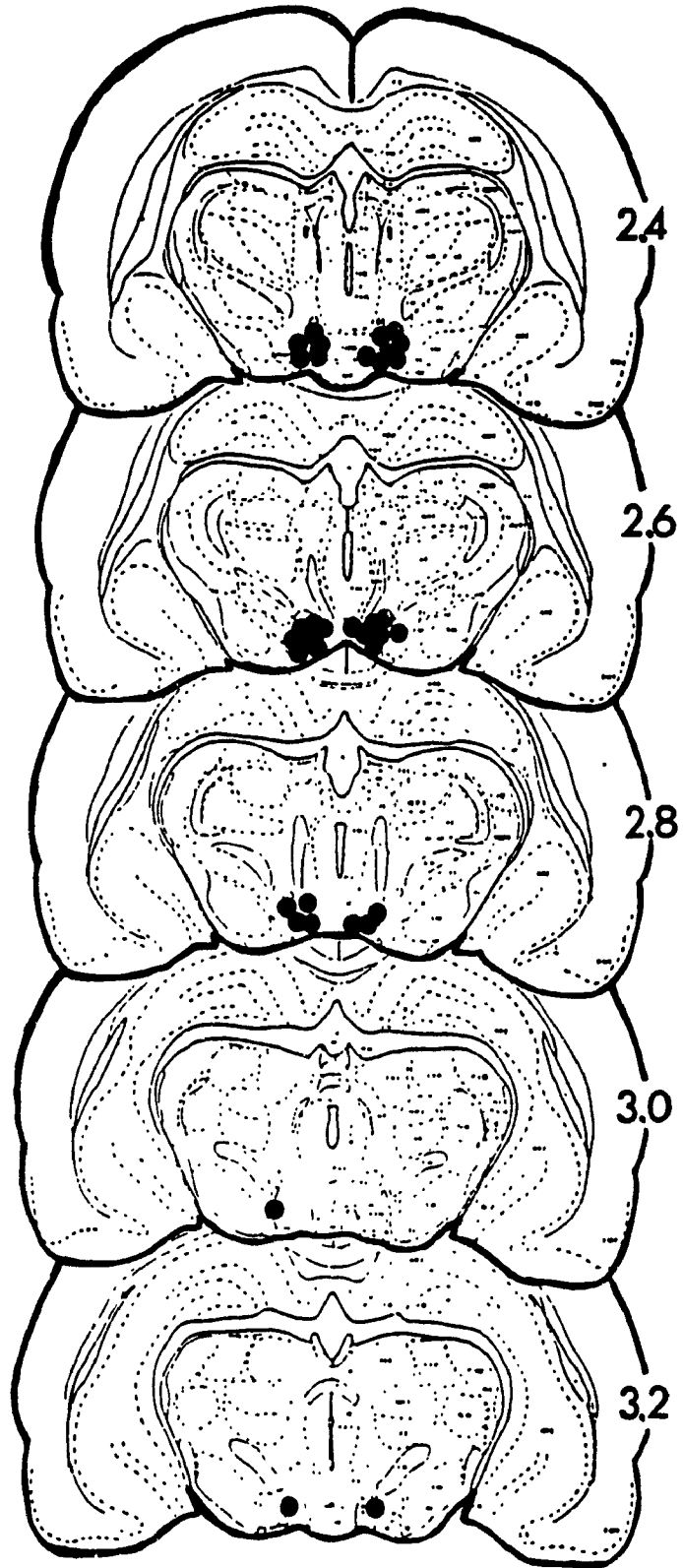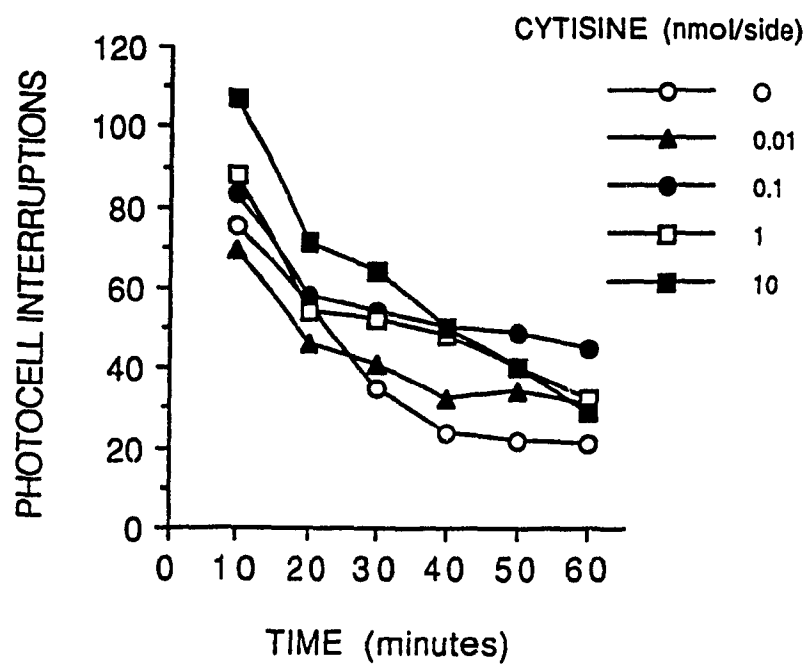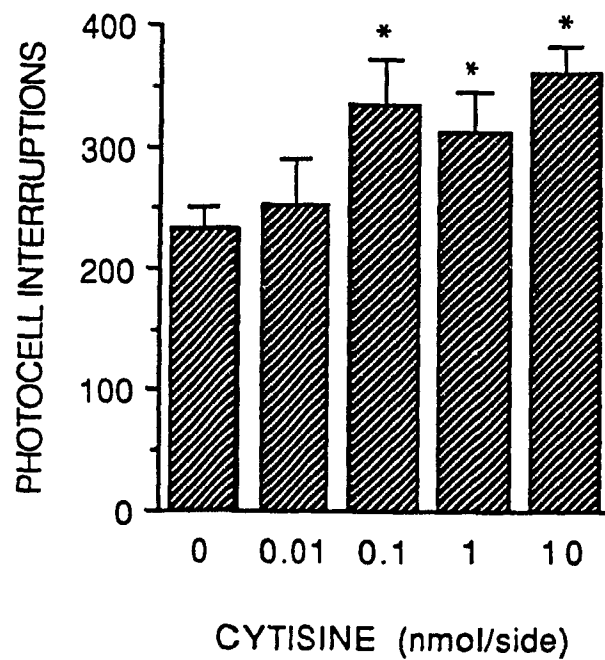Figure 2.   (see page 48).   Locomotor effects associated with
ventral tegmental injections of cytisine as a function of
dose (nmol/0.5 μL/side) and time after injection: (A) the
number of photocell interruptions associated with each
cytisine treatment plotted at 10-min intervals, and (B)
the total number of photocell interruptions for the 60-
min sessions.

A

B

nicotinic antagonist that does not easily cross the blood-brain barrier, did not attenuate the effects of cytisine (t[5] = 0.6, P>.05: Figure 3b). Systemic injections of the DA antagonist pimozide attenuated the locomotor activation associated with injections of cytisine (t[6] = 6.8, P<.01: Figure 3c).

In order to ensure that the attenuation of cytisine-induced locomotion by mecamylamine or pimozide was not due to a general inhibitory effect, four additional groups of eight animals each were tested to determine the effects of each antagonist pretreatment and saline on spontaneous locomotion. Whereas mecamylamine tended to increase locomotion, and pimozide tended to decrease it, an analysis of variance conducted on the locomotor counts for each of the treatments revealed that these trends were not statistically significant (F[3,28] = 2.46, P > 0.05).

DISCUSSION

The systemic administration of nicotine is known to increase locomotor activity in rats (Clarke & Kumar, 1983). The present study confirms an earlier report that ventral tegmental injections of the nicotinic agonist cytisine can have this behavioral effect (Pert & Chiueh, 1986). These data are in agreement with the notion that the mesolimbic DA system plays a role in mediating increases in locomotion produced by systemic nicotine. The locomotor-activating effects of ventral tegmental cytisine fit with autoradiographic and electrophysiological reports that show, respectively, the presence of nicotinic receptors in the ventral

Figure 3.   (see page 51).   Effects of mecamylamine (A): MEC, 2 mg/kg, i.p.), hexamethonium (B): HEX, 2 mg/kg, i.p., and pimozide (C): PIM: 0.3 mg/kg, i.p., on the locomotor-activating effects associated with ventral tegmental injections of cytisine (10 nmol/0.5 μL/side).
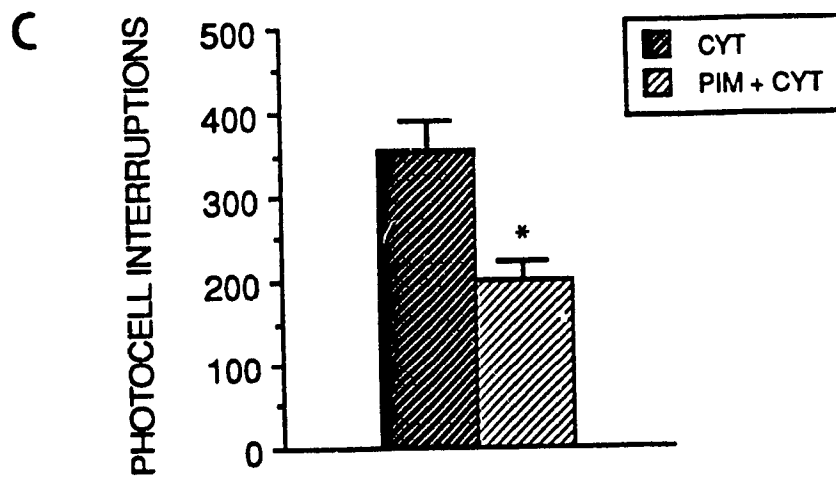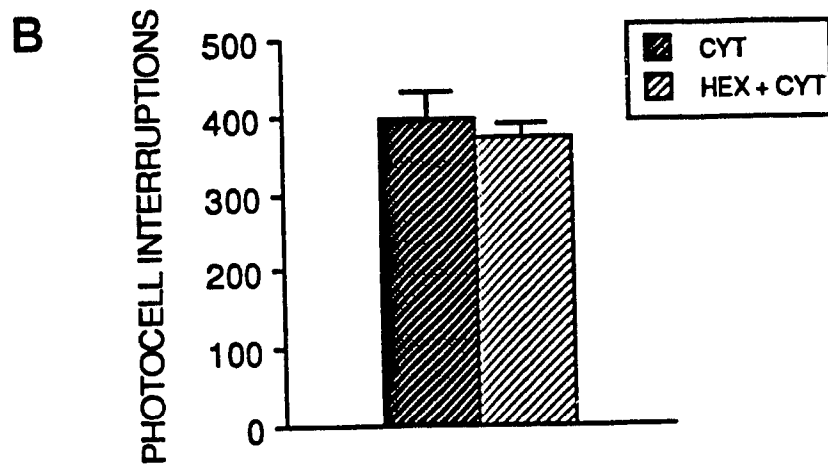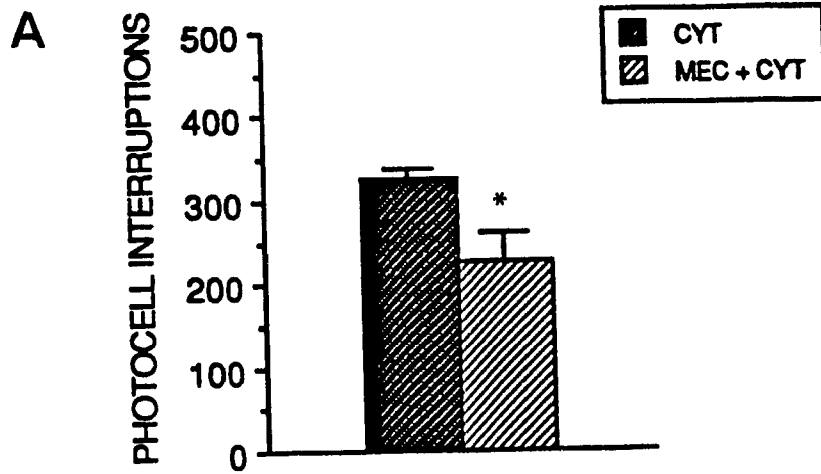
tegmentum (Clarke & Pert, 1985; Clarke et al., 1984) and increased DA cell firing following the iontophoretic application of nicotine (Grenhoff et al.,1986). In addition, evidence based on the use of the in vivo microdialysis technique shows that systemic nicotine increases the release of DA in the NAS (Di Chiara et al., 1987) and that the release of DA in the NAS correlates with locomotor activity (Freed & Yamamoto, 1985). Since nicotine increases the firing of mesolimbic DA cells, and since increased activity of the mesolimbic DA system is associated with hyperactivity, it would be expected that the administration of a nicotinic agonist into the ventral tegmentum would produce hyperactivity.

The results of the present experiment confirm that the administration of the nicotinic agonist cytisine into the ventral tegmentum increases locomotor activity and that this effect on locomotion is seen across a large range of doses. Because the three highest doses increased locomotion to the same extent it may be that each of these doses brought about a maximal occupation of the relevant receptors.

In addition, the nicotinic antagonist mecamylamine antagonized ventral tegmental cytisine's facilitation of locomotion, whereas the nicotinic antagonist hexamethonium did not. Although these results are preliminary, they suggest that the central actions of mecamylamine accounted for the attenuation of cytisine-induced locomotion: both mecamylamine and hexamethonium block peripheral nicotinic receptors, but only mecamylamine has easy access to the central nervous system. On the basis of the tests with these drugs

alone, the decrease in cytisine-induced locomotor activity seen in animals pretreated with mecamylamine or pimozide is interpreted as reflecting the attenuation by these drugs of cytisine's excitatory effect, and is not likely to be due to a general inhibitory effect on loccomotion, especially in the case of mecamylamine. Taken together, the data reported here support the notion that nicotinic and dopaminergic substrates interact at the level of the ventral tegmentum to influence locomotor activity.

EXPERIMENT 2:
REPEATED VENTRAL TEGMENTAL INJECTIONS OF CYTISINE:
EFFECTS ON LOCOMOTION

The locomotor-activating effects associated with the sytemic administration of nicotine become preogressively stronger with repeated testing (Clarke & Kumar, 1983; Ksir et al., 1985). This behavioral response, sometimes referred to as behavioral sensitization, has been reported with the repeated systemic administration of other compounds such as amphetamine (Segal, 1975), cocaine (Kalivas et al., 1988) and morphine (Babbini & Davis, 1972; Bartoletti, Gaiardi, Gubellini, & Bacchi, 1983). The present experiment was designed to determine whether the locomotor-activating effects of ventral tegmental injections of cytisine also become more pronounced with repeated testing. Because the mesolimbic DA system has been implicated in the process of sensitization (Kelley et al., 1980; Kalivas et al.,1983; Vezina & Stewart, 1984), the possibility exists that the progressively stronger locomotor-activating effects associated with the systemic administration of nicotine also reflect the repeated activation of the mesolimbic DA system. To explore this possibility, four groups of animals were tested. Two groups of animals were repeatedly injected with cytisine, and two other groups were repeatedly injected with the drug vehicle, saline. Of the two groups that received repeated injections of cytisine, one group received injections into the ventral tegmentum, and the other group received injections into sites dorsal to the ventral tegmentum; this latter

group of animals was used in order to guard against the possibility that the diffusion of drug to sites dorsal to the ventral tegmentum was responsible for any of the effects associated with ventral tegmental injections. The two other groups of animals received repeated injections of saline; one group received injections into the ventral tegmentum, and the other group received injections into sites dorsal to the ventral tegmentum.

METHOD

Subjects

Thirty-four male Long-Evans rats, weighing between 400 and 500 g at the time of surgery, were used for this experiment. The animal supplier and the housing conditions were the same as in Experiment 1.

Surgical Procedure

The general surgical procedure was the same as in Experiment 1. The guide cannulae were implanted bilaterally into either the ventral tegmentum or an area dorsal to the ventral tegmentum. The tips of the guide cannulae were lowered stereotaxically to a location 1.5 mm short of the final injection sites. The stereotaxic coordinates for the injection sites located in the ventral tegmentum were as follows: 2.8 mm posterior to bregma, 2.0 mm lateral to bregma, and 8.6 mm below the skull surface. The stereotaxic coordinates for sites dorsal to the ventral tegmentum were the same as those for the ventral tegmentum, with the exception that

the cannulae were lowered 7.1 mm instead of 8.6 mm. Animals were allowed at least one week to recover from the surgical procedure.

Testing Procedure

Two days prior to the beginning of the experiment, the animals were habituated to the activity boxes for 60 min. Four groups of animals were then tested. Two groups were injected with cytisine (10 nmol per 0.5 µL per side) and two other groups were injected with saline (0.5 µL of saline per side). Of the two groups that were repeatedly injected with cytisine, one group had cannulae aimed at the ventral tegmentum and the other group had cannulae aimed at sites dorsal to the ventral tegmentum. As mentioned earlier, this latter group (here referred to as a dorsal, or anatomical, control group) was used in order to guard against the possibility that the diffusion of drug to sites dorsal to the ventral tegmentum might account for the locomotor effects associated with the administration of cytisine into the ventral tegmentum. Two other groups were repeatedly injected with saline; one group had cannulae aimed at the ventral tegmentum and the other had cannulae aimed at sites dorsal to it. These two groups were used as vehicle control groups, and helped determine whether the repeated administration of the vehicle alone could account for any of the behavioral effects noted with repeated injections of cytisine.

The injection procedure was the same as in Experiment 1. Immediately after the injection procedure was over, each animal was placed in an activity cage and locomotor activity was measured

for 60 min. In all, animals were injected six times at the rate of once every 48 hours.

Statistical Analysis

Two-way analyses of variance with repeated measures on one factor (day) were carried out on the locomotor activity scores. One analysis involved the scores of animals receiving repeated injections of cytisine, and the other analysis involved the scores of animals receiving repeated injections of saline.

Only animals with both injector tips located in the ventral tegmentum, or with both injector tips located in sites dorsal to the ventral tegmentum, were included in the statistical analyses. Seven animals failed to meet either one of these criteria; the data relating to these animals were not included in the statistical analyses. The location of the injector cannulae tips of the animals included in the statistical analyses are shown in Figure 4 (cytisine condition) and Figure 5 (saline condition)

RESULTS

Repeated Cytisine Injections

Injections of cytisine into the ventral tegmentum produced more locomotion than injections of cytisine into more dorsal sites, $F(1, 13) = 15.00$, $P< .002$. Overall activity increased across days, $F(5, 65) = 4.60$, $P< .001$, and only in the ventral tegmentum group was there a progressive increase (Figure 6), $F(5, 65) = 7.55$, $P< .001$.

Figure 4.   (see page 59).   Anatomical localization of injector
cannulae tips in animals that were administered cytisine
repeatedly into either the ventral tegmentum (●) or more
dorsal sites (▼).   The number on the side of each
section designates the location behind breyma of the
coronal section (in millimeters). (Adapted from
Pellegrino et al., 1979.)

2.4

2.6

2.8

3.0

3.2

Figure 5. (see page 61). Locomotor effects associated with repeated injections of cytisine into either the ventral tegmentum (●) or more dorsal sites (O).
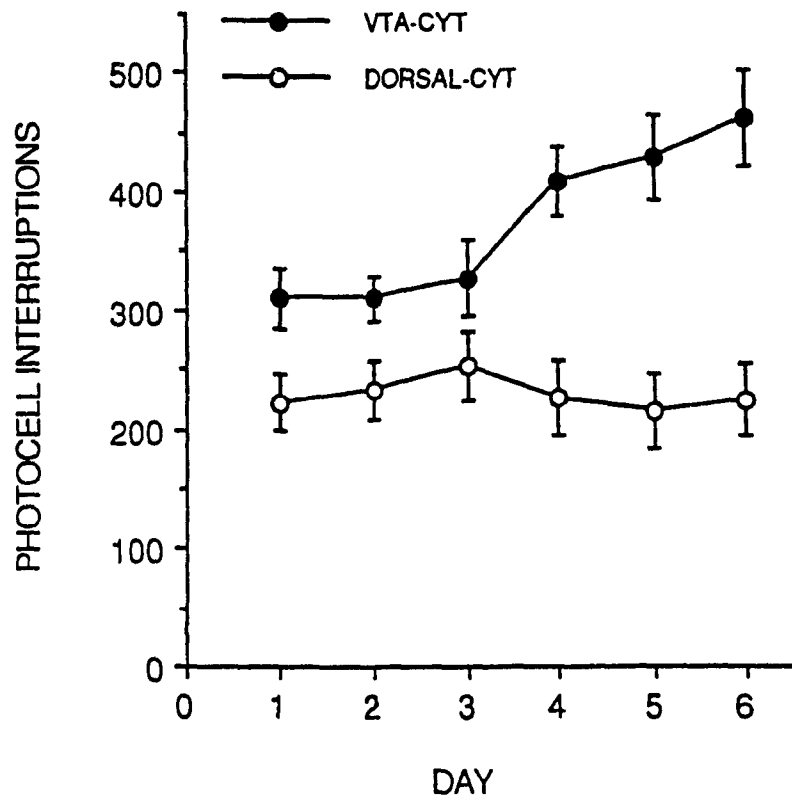
Repeated Saline Injections

Repeated saline injections into the ventral tegmentum produced no more locomotion than did similar injections into sites dorsal to the ventral tegmentum, $F(1, 10) = 0.24$, $P > .05$. The animals in both groups locomoted less and less with repeated testing, $F(5, 50) = 2.75$, $P < .025$ (Figure 7).

DISCUSSION

The locomotor-activating effects associated with ventral tegmental injections of cytisine became progressively stronger with repeated testing. A sensitized locomotor response was not apparent, however, in animals that were repeatedly injected with cytisine into sites dorsal to the ventral tegmentum. It appears, then, that the sensitized response resulted from the actions of cytisine in the ventral tegmentum and not from the actions of cytisine in a region dorsal to the ventral tegmentum. In addition to being associated with injections into a specific anatomical region, the sensitized response was associated with a specific pharmacological treatment; whereas repeated injections of cytisine brought about a sensitization of the locomotor response, repeated injections of saline did not.

As mentioned earlier, although the locomotor-activating effects of nicotine apparently sensitize, other factors may just as well account for the effects of nicotine. Some researchers have suggested that the progressively stronger locomotor-activating effects associated with repeated systemic injections of nicotine

Figure 6.   (see page 64).   Anatomical localization of injector

cannulae tips in animals that were administered saline

repeatedly into either the ventral tegmentum (●) or more

dorsal sites (▼).   The number on the side of each

section designates the location behind bregma of the

coronal section (in millimeters). (Adapted from
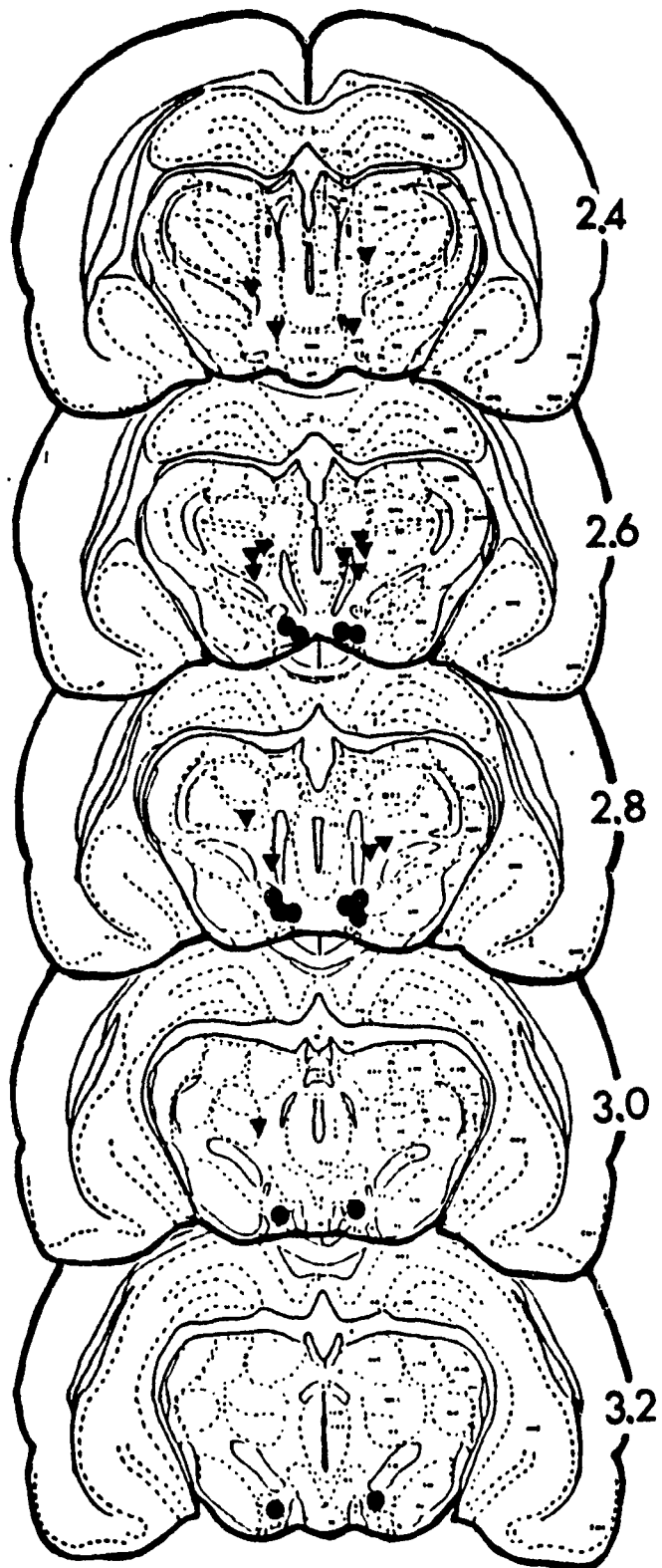
Pellegrino et al., 1979.)

2.4

2.6

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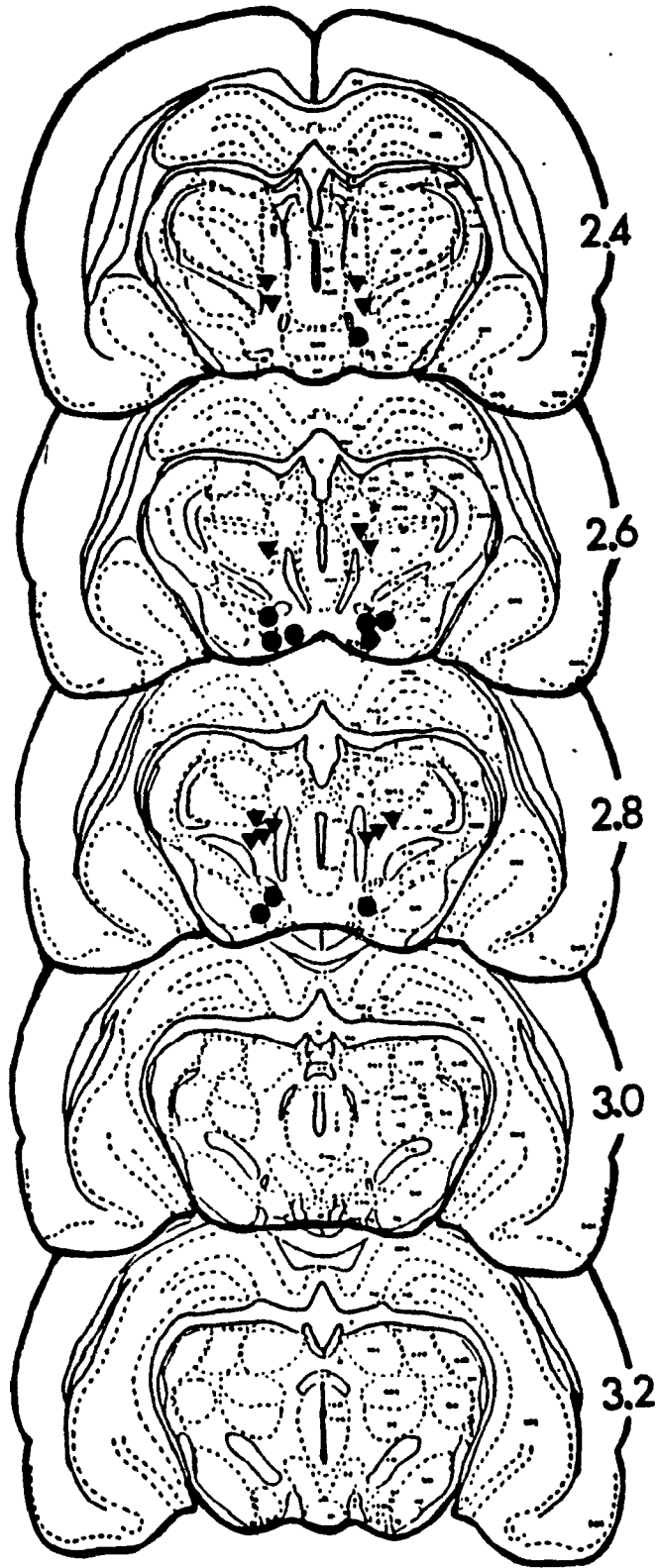
Figure 7.   (see page 66).   Locomotor effects associated with repeated injections of saline into either the ventral tegmentum (●) or more dorsal sites (O).
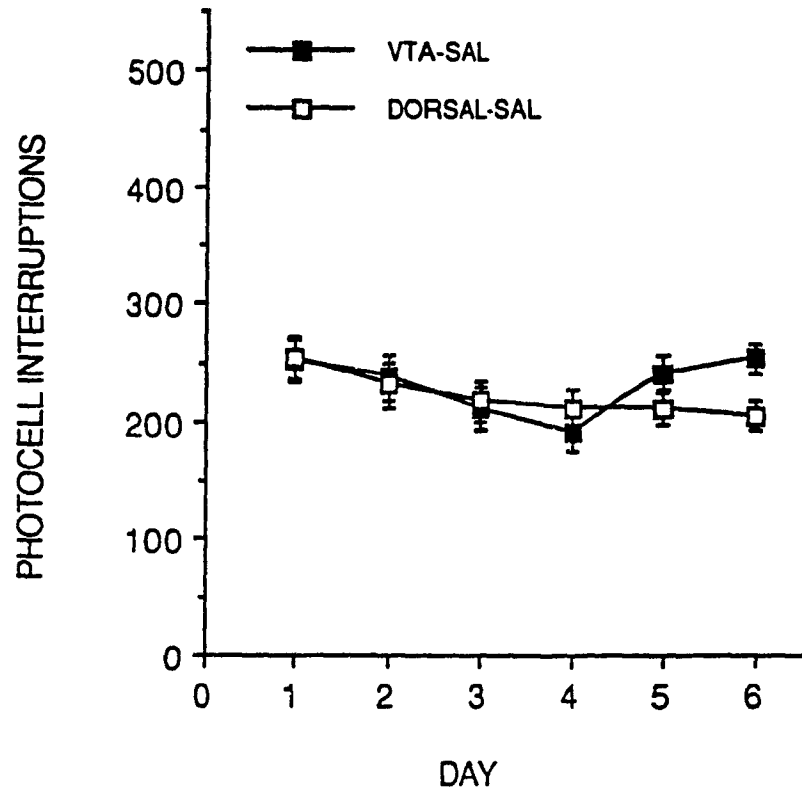
are not the result of a sensitizing action, but rather, are the by-product of the tolerance that develops to the inhibitory effects of nicotine (Clarke & Kumar, 1983); the present findings, although not inconsistent with the view that tolerance to the inhibitory effects can partly account for the locomotor effects of repeated injections of nicotine, suggest that a second mechanism is possible. Essentially, since the administration of cytisine into the ventral tegmentum did not appear to have any depressant effects on locomotion, it is unlikely that tolerance to any inhibitory effect accounted for the sensitized response associated with the repeated administration of cytisine; rather, it is likely that some action at the level of the ventral tegmentum was, by itself, sufficient to bring about a progressive increase in locomotion, irrespective of any tolerance to an inhibitory action.

The effects reported in the present experiment mirror the effects observed when morphine is administered into the ventral tegmentum; as with repeated morphine injections into the ventral tegmentum (Joyce & Iversen, 1979; Vezina & Stewart, 1984), repeated injections of cytisine produced progressively stronger effects on locomotion. In addition, as with repeated morphine injections at sites dorsal to the ventral tegmentum (Vezina & Stewart, 1984), cytisine injections at sites dorsal to the ventral tegmentum failed to induce locomotion in the present experiment.

The sensitization of the locomotor-activating effects associated with ventral tegmental injections of morphine and other opiates are believed to be mediated by the mesolimbic DA system

(Joyce & Iversen, 1979; Kalivas, 1985; Vezina & Stewart, 1984). Opiates bind to sites in the ventral tegmentum (Goodman, Snyder, Kuhar, & Young, 1980), and, when administered iontophoretically, morphine increases the firing of mesolimbic DA cells (Gysling & Wang, 1983), an effect that is thought to account for the facilitation of NAS DA release that accompanies systemic (Di Chiara et al., 1987) and ventral tegmental (Leone, Pocock, & Wise, 1991) injections of morphine. Ventral tegmental injections of enkephalin also increase DA turnover in the NAS, and, importantly, both the increase in turnover and the associated locomotor-activating effects sensitize with repeated injections (Kalivas, 1985).

A parsimonious explanation of the present findings is that cytisine acts on a neural substrate in the ventral tegmentum to increase locomotion and that its repeated actions on this substrate also cause a strengthening of the locomotor response. The parallels between the effects of nicotine and the effects of morphine on the mesolimbic DA system suggest that the sensitizing effects on locomotion of systemic nicotine or ventral tegmental cytisine may also be mediated by the mesolimbic DA system.[5] Nicotine (Clarke et

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[5] Unlike the sensitization found with compounds such as amphetamine, cocaine and morphine, the basis of the sensitization found with cytisine is unknown. The possibility exists that at some level, chronic nicotine injections produce sensitization through its actions on the mesolimbic DA system, but perhaps not in exactly the same manner that amphetamine and cocaine do. The sensitization found with amphetamine, cocaine, and morphine is accompanied by increased release of DA from terminals; in the case of chronic nicotine DA turnover appears to be reduced rather than enhanced. One must keep in mind, however, that there are other changes such as the increases in DA receptors that might result in a net increase in DA transmission (Fung & Lau, 1989; Reilly et al., 1987). It has been reported that infusions of nicotine can sensitize animals to later injections of amphetamine and apomorphine (Fung & Lau, 1988; 1989), giving some credence to the idea that these drugs share a common mechanism of action; on the other hand, a different group of investigators failed to find cross-

al., 1984) and cytisine (Pabreza et al., 1991) bind to sites in the ventral tegmentum and, when administered peripherally, nicotine increases the firing of mesolimbic DA cells (Grenhoff et al., 1986; Mereu et al., 1987) and the release of DA in the NAS (Di Chiara et al., 1987; Imperato et al., 1986). The present findings raise the possibility that repeated ventral tegmental injections of cytisine— and perhaps also systemic injections of nicotine—bring about a sensitization of locomotion by acting on this system.

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sensitization between the locomotor-activating effects of nicotine and cocaine (Schenk, Snow, & Horger, 1991).

EXPERIMENT 3:

DETERMINATION OF BEHAVIORALLY-RELEVANT ANATOMICAL BOUNDARIES INVOLVED IN CYTISINE-INDUCED LOCOMOTION

Experiments 1 and 2 confirmed that ventral tegmental injections of cytisine increase locomotion. In the present Experiment, injections of cytisine were made in and around the ventral tegmentum to determine whether the region from which cytisine injections increase locomotion has anatomical boundaries. Because the cell bodies of the mesolimbic DA system are located in the ventral tegmentum (Fallon & Moore, 1978; Lindvall & Bjorklund, 1974) and because the mesolimbic DA system has been implicated in locomotion (Costall & Naylor, 1975; Dunnett et al., 1984; Fink & Smith, 1980; Herman et al., 1988), a secondary aim of the present experiment was to explore the possibility that this system mediates the locomotor-activating effects associated with ventral tegmental injections of cytisine; if ventral tegmental injections of cytisine increase locomotion by activating mesolimbic DA cell-bodies, then the region where injections are effective should bear some resemblance to the mesolimbic DA cell-body region.

METHOD

Subjects

Male Long-Evans rats, weighing between 400-500 g at the time of surgery, were subjects in this experiment. The animal supplier and housing conditions were the same as in Experiment 1.

Surgical implantation of guide cannulae

The guide cannulae were implanted bilaterally and at a 10-degree angle using the general method and surgical procedure described in Experiment 1. Tips of the guide cannulae were lowered stereotaxically to a location 1.5 mm short of the final injection sites. The stereotaxic coordinates for the injection sites varied within the following limits: between 0.8 and 4.6 mm posterior to bregma, between 0.8 and 2.5 mm lateral to bregma, and between 5.5 and 9.0 mm ventral to the skull surface. Animals were allowed at least one week to recover from the surgical procedure.

Procedure

Three days before the beginning of the experiment, animals were habituated to the photocell activity cages for one hour. The next day, animals received bilateral injections of saline (0.5 μL per side), and they were then placed in the activity cages for 60 min. These injections were made to familiarize the animals to the injection procedure; the injections were also made in order to minimize the disruptive effects on locomotion that appear to follow the first of any series of injections into the ventral tegmentum (personal observation). On each of the two subsequent test days animals were injected with either saline (0.5 μL saline per side) or cytisine (1 nmol per 0.5 μL per side). The order of treatments was counterbalanced so that for each group of animals with the same stereotaxic coordinates half of the group received saline as the first of the two treatments and the other half received cytisine as the first of the two treatments. Before each treatment, animals

were habituated to the activity cages for 30 min, and, immediately following the treatment, locomotion was recorded for 60 min. Forty-eight hours separated the two treatments.

Confirmation of cannulae placements

Following the experiment, and while under deep chloral hydrate anaesthesia, animals were perfused transcardially with 50 mL of saline followed by 50 mL of 10% formalin. Cannula placements were determined by examining 40 μ coronal sections obtained from each animal. The most ventral penetration of an injector cannula was used as a marker of the site of injection, and to increase the reliability of the estimate, the location of each injection site was estimated on two separate occasions. The records from each occasion were compared, and, in the few instances where there were discrepancies, the relevant brain sections were viewed a third time so that a final decision could be made with regard to the location of the injection site.

Of the animals that were implanted, 17 animals were not included in the statistical analyses; in 5 animals the tips of one or both injector cannulae penetrated the bottom of the brain, and, in each of the other 12 animals, the injector cannulae tips were not symmetrically positioned (there was a large difference between the location of the left and right cannulae, particularly along the dorsal-ventral plane). The boundaries of the mesolimbic DA cell-body region were determined by consulting the work of Fallon & Moore (1978) and Lindvall & Bjorklund (1974), and by observations of brains processed with the glyoxylic-acid method.

Statistical Analysis

To determine whether the effects of cytisine varied in relation to the location of the injection sites, the whole range of placements was divided into 5 regions, and in each region the effects of cytisine on locomotion were compared to the effects of saline. The injection sites were distributed over a region that extended almost 4-mm along the anterior-posterior plane (from 0.8 to 4.6 mm behind bregma) (Pellegrino et al., 1979). This 4 mm region was divided into 5 subregions, each spanning 0.8 mm: 2 subregions coincided with the DA cell-body region, 2 were anterior and the other was posterior to the cell-body region. For each of the 5 regions the mean number of photocell interruptions associated with the saline treatment was compared to the number of photocell interruptions associated with the cytisine treatment; the locomotor scores that were used for the statistical analyses were obtained by subtracting the the number of photocell interruptions associated with injections of saline from the number of photocell interruptions associated with injections of cytisine. The t-test was used for these comparisons. (To hold the alpha level constant at P < .05 across the several tests, the alpha level for each test was adjusted by dividing the .05 value by 5, the number of tests that were carried out; an alpha value of 0.01 was therefore used (Kirk, 1982).)

RESULTS

The effects of cytisine varied on the basis of the location of the injector tips along the anterior-posterior plane; whereas injections of cytisine facilitated locomotion in those groups of animals that had the injector tips located between 1.6 and 2.2 mm behind bregma, $t(22) = 4.99$, $P < .0001$, or between 2.4 and 3.0 mm behind bregma $t(26) = 3.70$, $P < .001$, injections of cytisine did not facilitate locomotion in those groups of animals that had the injector tips located between 0.8 and 1.4 mm behind bregma, $t(13) = 1.06$, $P > .007$, between 3.2 and 3.8 mm behind bregma, $t(14) = 1.87$, $P > .007$, or between 4.0 and 4.6 mm behind bregma, $t(8) = 1.40$, $P > .007$. The effects associated with each of the five regions are shown in Figure 8.

DISCUSSION

The present data identify a region of the ventral tegmentum and caudal diencephalon in which nicotinic actions can increase locomotion. The region was not readily identified with any well-defined substrate, however; there was no simple relationship between the anatomical location of the injection sites and the amount of locomotion that was associated with these sites (Figure 9). Cytisine injections at the most anterior sites (Figure 9A, B, C, D) usually had only weak effects on locomotion. Injections of cytisine into the dorsal or ventral premamillary nuclei, for example, failed to increase locomotion. Injections into the arcuate nucleus of the hypothalamus also had minimal effects on locomotion. Amongst

Figure 8.   (see page 76).   Mean locomotor scores associated with
cytisine injections into the ventral tegmentum as a
function of the location of the injector tips behind
bregma.   Injection sites were divided into five groupings,
each spanning 0.8 mm.   The locomotor scores were
obtained by subtracting the number of photocell
interruptions associated with injections of saline from
the number of photocell interruptions associated with
injections of cytisine.   (These scores were from the first
10 min following the intracranial injections.)

PHOTOCELL INTERRUPTIONS
cytisine-saline

MILLIMETERS POSTERIOR TO BREGMA
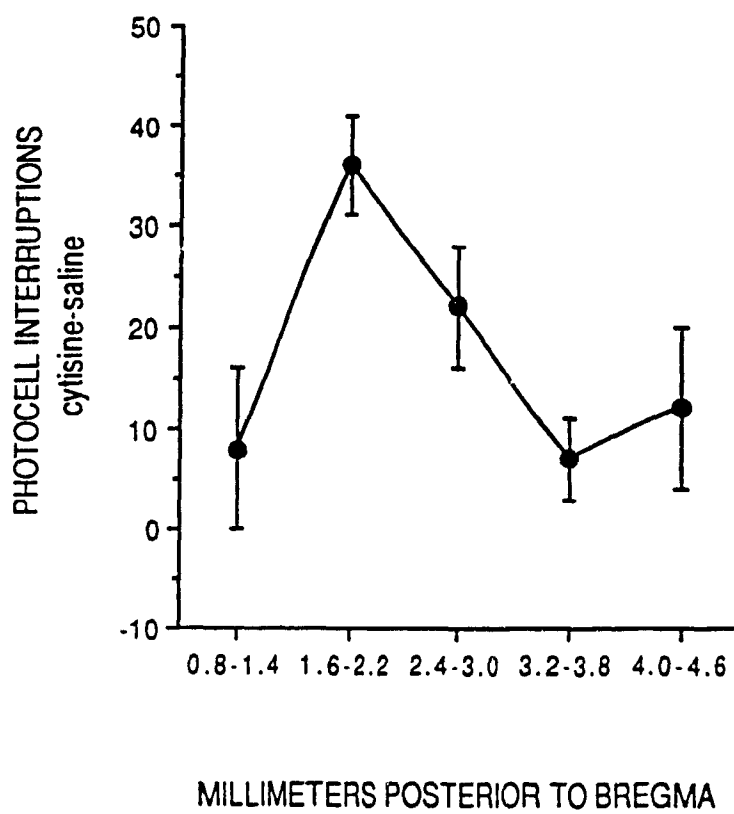
these anterior sites a few positive sites were located near the fornix (F) and mamillothalamic tract (MT).

The number of positive sites increased significantly at somewhat more posterior levels (Figure 9E, F and G), as did the amount of locomotion associated with the injections. Whereas the most ventral of these positive sites were located just above the mamillary peduncle (MP), the most dorsal of these sites were located close to the medial lemniscus (ML); the most lateral of the sites were in the medial zona incerta. The majority of the sites in this group, then, fell within the confines of the medial forebrain bundle at the level of the posterior hypothalamus.

At the level of the posterior mamillary nucleus (PM), whereas some injection sites were associated with weak locomotor activation (Figure 9H), others (Figure 9I) were associated with strong locomotor activation. Further back, just posterior to the level of the mamillary nucleus (PM) (Figure 9J), ventral tegmental injections again only produced weak effects on locomotion; in fact, within this region injections of cytisine often decreased locomotion. The negative injection sites were located in or around the ventral tegmental nucleus; they were located above the mamillary peduncle but below the mamillotegmental tract (TT).

At a more posterior level (Figure 9K, L, M) injections around the interpeduncular nucleus (IP) and into the ventral tegmental nucleus were associated with weak to moderate locomotor activation (with the exception of a few sites in the ventral tegmental nucleus from which strong effects on locomotion were

Figure 9. (see pages 79 to 83). The degree of locomotor activation associated with cytisine injections into the ventral tegmentum as a function of the location of the injector tips behind bregma. Locomotor scores were obtained by subtracting the number of photocell interruptions associated with injections of saline from the number of photocell interruptions associated with injections of cytisine. The degree of locomotor activation associated with each injection site is designated by one of four symbols: ⬤, strong locomotor effect (the cytisine score was more than 30 counts larger than the saline score); ⊘, moderate locomotor effect (the cytisine score was more than 10—but less than 30—counts greater than the saline score); O, weak locomotor effect (the cytisine score was 10 or less counts larger than the cytisine score (and vice versa); ▢ , depression of locomotor activity (the saline score was more than 10 counts larger than the cytisine score). List of abbreviations: F, fornix; IP, interpeduncular nucleus; ML, medial lemniscus; MP, mamillary peducle; MT, mamillothalamic tract; P, pons; PM, posterior mamillary nucleus; TT, mamillategmental tract; V, ventricle. (Adapted from Pellegrino et al., 1979).

E 1.6

ML

F 1.8

TT

F

G 2.0

V

MP

H 2.2

M

V

3.2

N

ML

3.4

O

IP

3.6

P

3.8

P
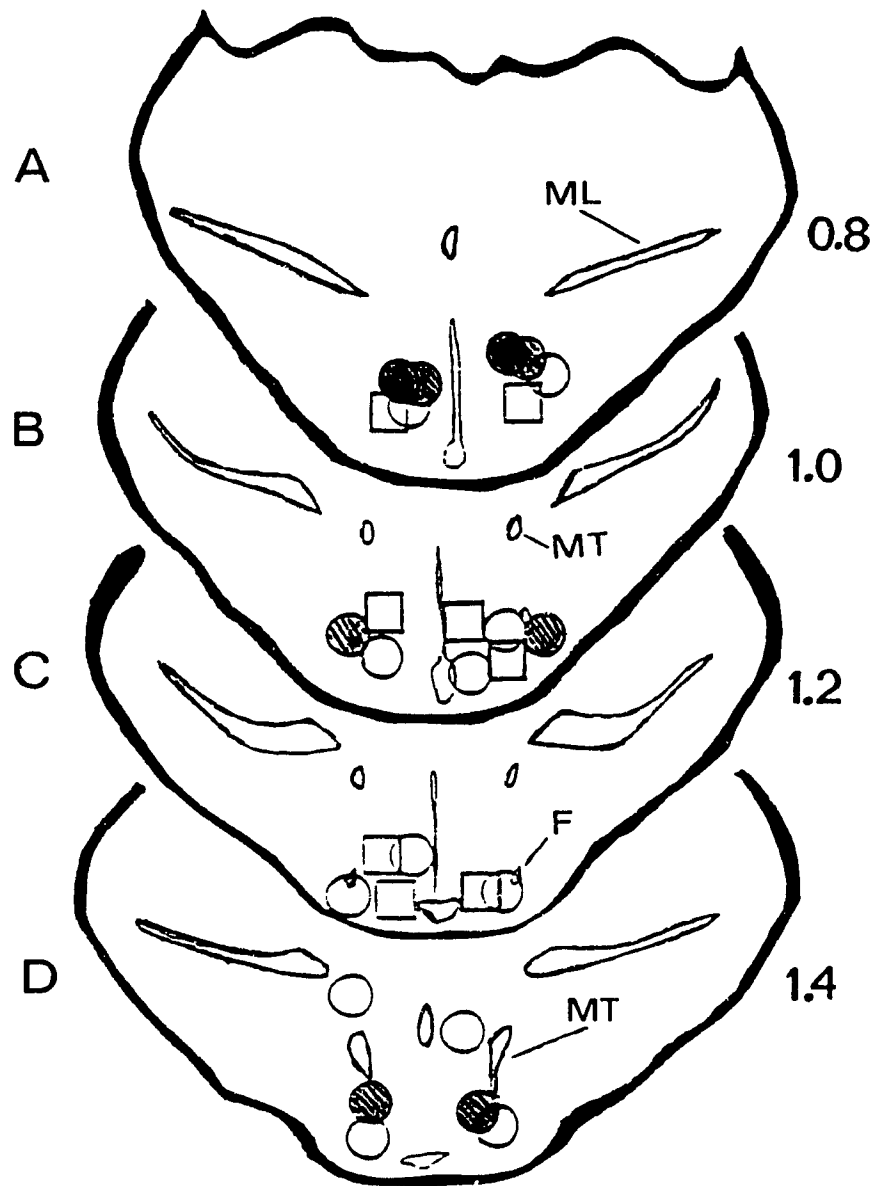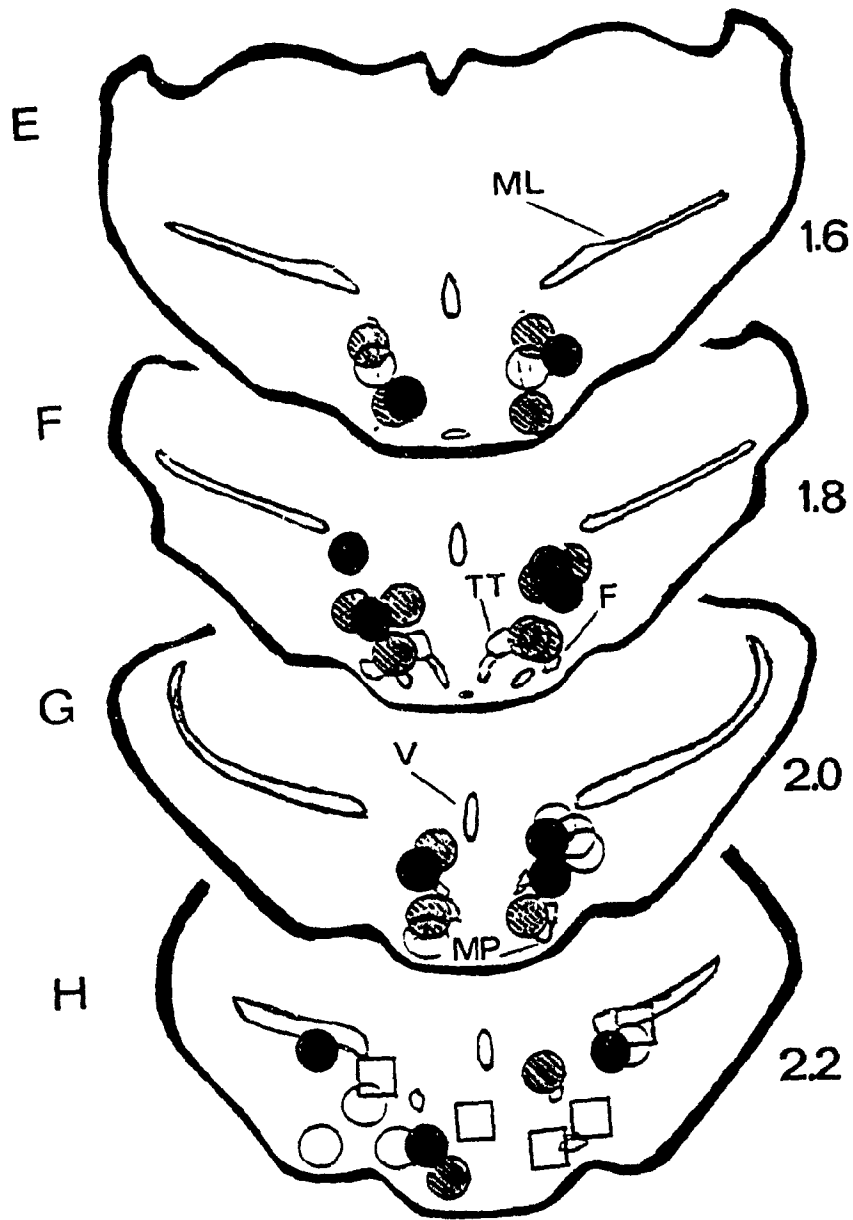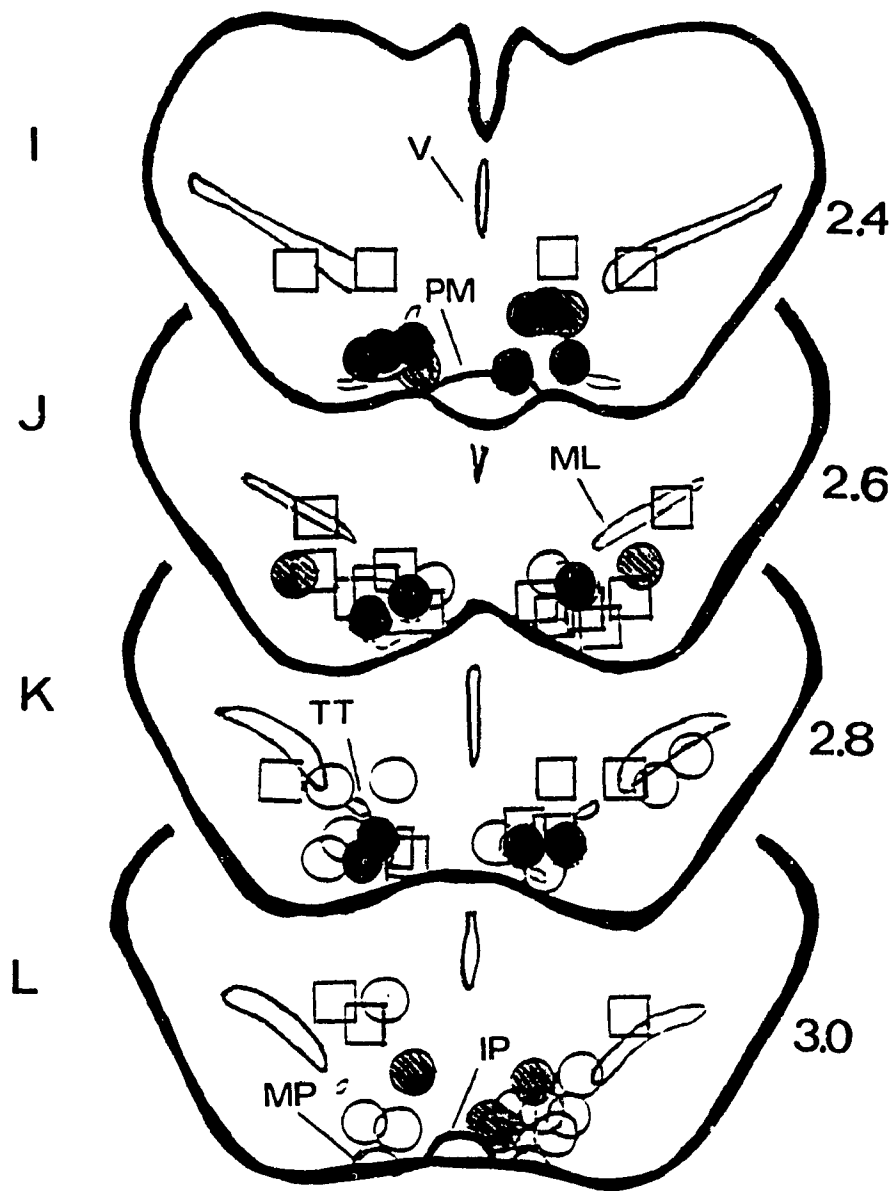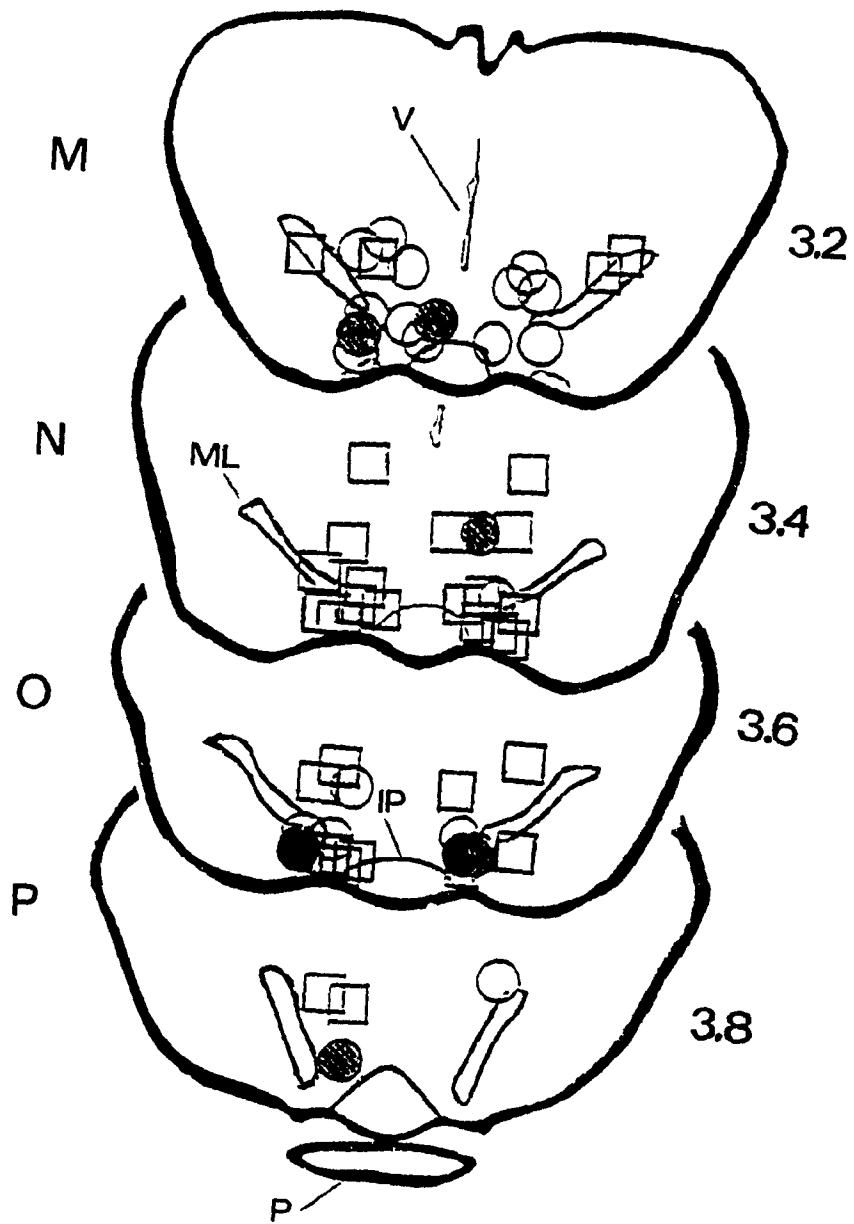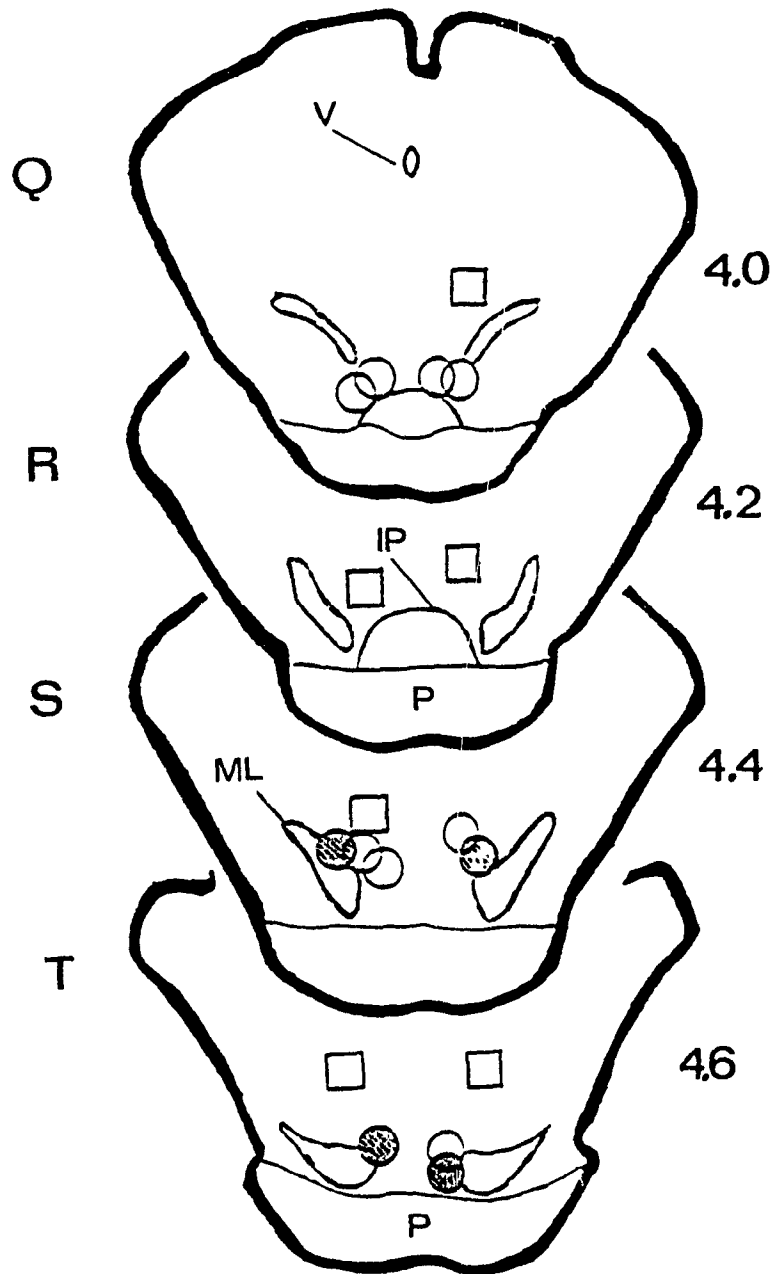
obtained). Injections dorsal to this region, like most other injections near or dorsal to the medial lemniscus, did not increase locomotion. Just anterior to the pons (P) (Figure 9N, O), injections were also associated with decreases in locomotion. Here again, the sites were in the ventral tegmental nucleus and around the interpeduncular nucleus.

Finally, a few positive sites were obtained at more posterior levels. These sites (Figure 9Q, R, S, T), from which slight to moderate locomotor-stimulant effects were obtained, were located medial or just dorsal to the medial lemniscus. Injections at more dorsal sites, in or near the red nucleus (Figure 9P, Q, R, S, T), did not increase locomotion.

To summarize, then, a region was found within which injections of cytisine increased locomotion. This region appeared to have a reasonably well-defined anterior boundary and a less well-defined posterior boundary; to the extent that the more dorsally-placed injection sites were not associated with increased locomotion, the region also had a dorsal boundary (because the present study did not include injection sites that were dorsal to the effective sites in the anterior regions (Figure 9A-F) the dorsal boundary in this region cannot be inferred from the present findings). Within the anterior, posterior and dorsal boundaries, the majority of injection sites were associated with locomotor activation. In several instances within this region, however, injections of cytisine either failed to increase locomotion or actually decreased locomotion. Thus, the boundaries of the region of

positive injection sites did not correspond to the boundaries of any obvious anatomical unit.

Because the mesolimbic DA cell-body region is located in the ventral tegmentum (Fallon, 1988; Lindvall & Bjorklund, 1974), and because the mesolimbic DA system has been implicated in locomotion (Costall & Naylor, 1975; Fink & Smith, 1980; Herman et al., 1988; Pijnenburg & van Rossum, 1973), the possibility of particular interest is that the mesolimbic DA system mediates the locomotor-activating effects associated with ventral tegmental injections of cytisine (Pert & Chiueh, 1986) and systemic injections of nicotine (Pert & Clarke, 1986; Westfall et al., 1986). While a portion of the region where injections of cytisine were effective overlapped the cell-body region of the mesolimbic DA system, the boundaries of the effective zone did not correspond precisely to the boundaries of the DA cell group. The present data, then, neither confirm nor disconfirm the DA hypothesis.

The effective sites that coincided with the DA cell-body region were located in the anterior portion of the DA cell-body region (Figure 9I, J, K and L). There are nicotinic receptors in this region (Clarke et al., 1984), and the number of receptors decreases dramatically following the destruction of mesolimbic DA neurons (Clarke & Pert, 1985). The activation of these receptors may mediate the increases in DA levels in the NAS following either ventral tegmental injections of cytisine (Pert & Chiueh, 1986) or systemic injections of nicotine (Di Chiara et al., 1987; Imperato et al., 1987). In turn, the increases in DA levels may account for the

locomotor-activating effects associated with ventral tegmental injections of cytisine (Pert & Clarke, 1987; Pert & Chiueh, 1986; Reavill & Stolerman, 1990) and systemic injections of nicotine (Clarke et al., 1988a).

Although the finding that injections of cytisine into the anterior pole of the mesolimbic DA cell group increased locomotion fits with the idea that the mesolimbic DA system mediates the locomotor-activating effects associated with ventral tegmental injections of cytisine, other findings from the present experiment are not easily reconciled with this interpretation. First, injections in the posterior half of the DA cell group were frequently ineffective (Figure 1J and N). Second, injections anterior to the DA cell group were frequently very effective (Figure 9E, F, G and H), as were some injections posterior to the DA cell group (Figure 9S and T). While drug diffusion to the DA cell region might explain the effectiveness of injections into sites anterior and posterior to the DA cell group, how might the ineffectiveness of more proximal sites in the DA region (Figure 9J and N) be explained? The difficult finding for the DA hypothesis, then, is that some injections directly into the DA cell group were ineffective; indeed, in some cases, these injections appeared to suppress locomotion. If DA cells were the target of cytisine actions, then injections into the DA cell group would have had either (1) to damage the system or (2) to debilitate it functionally. Each is a possibility, and since no alternative substrate for cytisine-induced locomotion has, thus far, been suggested, both possibilities merit consideration.

-

With regard to the issue of tissue damage, it is possible that the location of the injections at the heart of the cell-body region caused significant damage to DA cells and fibers. Injections into this region, like injections into all other regions, are known to produce damage, and because the fibers of the more medial, lateral, and caudal cells aggregate in this region, the stab-wound caused by the most on-target injection cannulae may have caused especially significant damage. Recently, damage to the DA system has been observed in animals implanted with unilateral cannulae in this brain region (D. Devine and R.A. Wise, personal communication, 1991). Damage to this region, then, could conceivably have counteracted the actions of cytisine (such damage may perhaps even account for the slight inhibition of locomotion that was observed in some animals).

It is also possible that injections into the most dense areas of the cell region overstimulated the DA system, causing it to go into a condition of depolarization inactivation that is known to accompany over-activation of DA neurons (Grace & Bunney, 1986), or perhaps even causing nicotinic receptors in the region to become desensitized (Grenhoff & Svensson, 1989). This could explain the cases of decreased locomotion seen in a few cases of very central injection sites. , With slightly off-target injections, the concentration of cytisine at the critical receptors would be less and the DA system would be maximally activated. While it is known that the DA system can be driven into depolarization inactivation with a variety of treatments, it is not known that nicotine stimulation is capable of producing this phenomenon.

Another possibility, albeit a remote one, is that nicotine acts on receptors that are not localized on the DA cell bodies but rather on their axons. It has been suggested that there are nicotinic receptors on the non-myelinated axons (Armett & Ritchie, 1961); since DA neurons are unmyelinated the possibility exists that injections of cytisine into the caudal diencephalon activated mesolimbic DA neurons by acting at receptors localized just anterior to, rather than directly on, the DA cell bodies. This possibility does not fit easily with the finding of strong locomotor effects in response to some of the more posterior injections sites in the present study.

Thus far, the possibility that the mesolimbic DA system mediated the locomotor-activating effects of cytisine was considered. Because no attempt was made in the present study to determine the exact nature of the substrate, the possibility that cytisine also increased locomotion through its direct actions on other transmitter systems cannot be ruled out. Specifically, the direct actions of cytisine on either opioid or GABA transmission may be important. There are reports that nicotine influences the function of opioid (Holt and Horn, 1989; Pierzchala, Houdi, & Van Loon, 1987) and GABA (Freund, Jungschaffer, Collins, & Wehner, 1988) systems, and since changes in opiate or GABA transmission in the ventral tegmentum are believed to bring about changes in locomotion (Joyce & Iversen, 1979; Kalivas et al., 1986; Mogenson , 1984), there is a possibility that these non-DA systems were directly activated by injections of cytisine.

In summary, the present investigation was primarily designed to explore the relationship between the anatomical location of the injection sites and the locomotion caused by injections into these sites. The findings served primarily to identify a region of the ventral tegmentum and caudal diencephalon in which nicotinic actions increased locomotion. No direct evidence was obtained as to the neurochemical identity of the one or more substrates that mediate the locomotion caused by ventral tegmental injections of cytisine. But to the extent that the present data could be used to evaluate the dominant, current hypothesis—the DA hypothesis—the results were inconclusive; whereas some of the present findings were consistent with the idea of mesolimbic DA involvement, other findings were not.

EXPERIMENT 4:

CYTISINE MICROINJECTIONS INTO THE VENTRAL TEGMENTUM: EFFECTS ON CONDITIONED PLACE-PREFERENCE

It has been hypothesized that the neural substrate that mediates locomotion also subserves some reinforcement-related function (Glickman & Schiff, 1967; Schneirla, 1957; Wise & Bozarth, 1987). The purpose of the present experiment was to determine whether ventral tegmental injections of cytisine, in addition to increasing locomotion, also have reinforcing effects; the conditioned place-preference procedure was used to measure reinforcement.

METHOD

Subjects

Sixteen male Long-Evans rats that weighed between 400 and 450 g at the time of surgery were used as subjects. The animal supplier and the housing conditions were the same as in Experiment 1.

Surgical Procedure

The general surgical procedure was the same as in Experiment 1. The guide cannulae were implanted bilaterally, with the tips of the injector cannulae located either in the ventral tegmentum or in sites dorsal to it. The tips of the guide cannulae were lowered stereotaxically to a location 1.5 mm short of the final injection sites. The stereotaxic coordinates for the injection sites located in the ventral tegmentum were as follows: 3.0 mm posterior to bregma,

2.2 mm lateral to bregma, and 8.8 mm below the skull surface. The coordinates for the dorsal placements were similar to the latter group's, with the exception that the guide cannulae were only lowered 7.3 mm. Animals were allowed at least one week to recover from the surgical procedure.

Apparatus

Each of the eight boxes used in the experiment was constructed of wood and consisted of three interconnected compartments; two main compartments (26 x 24 x 40 cm) were joined by a side compartment (12 x 35 x 4 cm). The compartments differed from one another in terms of floor texture and wall color. Whereas the floor of one of the main compartments consisted of a wire mesh layed over Plexiglas, the floor of the other main compartment consisted of steel rods layed out in parallel order (one rod ⁻ :⁻ 1.5 cm); the side compartment had a Plexiglas floor. The walls of a main compartment were either all painted with 2 cm-wide horizontal black stripes that alternated with 2 cm-wide light brown stipes, or they were all painted light brown; the side compartment of each place-preference apparatus also had its walls painted light brown. To minimize any visual distraction, a sheet of wax paper was placed atop each compartment, thus creating a diffuse, low level of illumination.

The point where the wire mesh floor met the steel rod floor served as the fulcrum of a cantilever-like floor system. When an animal entered a compartment, its weight displaced the floor system which, in turn, caused one of the two switches to be

depressed (a switch was positioned at either end of the floor system). The depression of any of the two switches closed an electrical circuit, and the amount of time an animal spent in each of the two main compartments was recorded by a computer located in the testing room. If the animal exited any one of the two main compartments, the floor-system automatically repositioned itself; under such conditions neither switch was depressed and the passage of time was recorded and allotted to the side compartment.

Procedure

The experiment consisted of three phases: the pre-conditioning, conditioning, and post-conditioning phases. During the pre-conditioning phase, baseline measures were taken to determine whether animals exhibited a particular preference for any of the three compartments. Each animal was placed in the apparatus for 15 min and the amount of time spent in each of the three compartments was recorded.

During the conditioning phase, the animals received drug as well as saline injections (the injection procedure was identical to the one decribed in the pilot experiments). Cytisine injections were paired with one of the main compartments on four separate occasions, and on four other occasions saline injections were paired with the other main compartment. On the first day, each animal was injected with cytisine (10 nmol per 0.5 μL per side) and immediately placed in one of the main compartments for the duration of the 20 min session. On alternate days, animals received

saline injections (0.5 µL per side) and were placed in the other main compartment for 20 min.

Since animals were injected serially (at an approximate rate of one animal every four minutes) white noise was used to mask any sound produced by the injection pump, or while other animals were being placed in the apparatus. White noise was also present during the pre-conditioning and post-conditioning phases of the experiment.

During the post-conditioning phase each animal was given access to all compartments and, as in the pre-conditioning phase, the amount of time spent in each of the three compartments during a 15-min session was recorded.

Statistical Analysis

The data for each group were analyzed separately with the repeated measures t-test. The calculations were based on scores obtained during the pre-conditioning and post-conditioning phase; for each of the two groups, the amount of time the animals spent in the side paired with saline was subtracted from the amount of time the animals spent on the side paired with cytisine; in this way, the difference scores for the pre-conditioning phase were compared to the difference scores for the post-conditioning phase.

Only animals with bilateral injector tips located in either the ventral tegmentum or in sites dorsal to the ventral tegmentum were included in the statistical analyses. The locations of the injector cannulae tips are shown in Figure 10. Of the sixteen animals implanted, four were not included in the statistical analyses: one

animal's guide cannula did not remain patent for the duration of the experiment, and three other animals had either one or both injectors penetrate the bottom of the brain. Of the twelve remaining animals, seven had the tips of the injectors positioned in the ventral tegmentum and five had the injectors positioned in a more dorsal region.

RESULTS

Prior to the conditioning phase, animals with injectors in the ventral tegmentum and animals with injectors dorsal to the ventral tegmentum showed no significant preference for either of the two main compartments (Figure 11A). Following the conditioning phase, however, animals injected into the ventral tegmentum spent significantly more time in the compartment previously paired with cytisine injections than they did in the compartment paired with saline injections, $t(6) = 2.55$, $P < .05$. Animals receiving injections dorsal to the ventral tegmentum spent as much time in the cytisine-paired compartment as they did in the compartment paired with saline, $t(4) = 0.37$, $P > .05$ (Figure 11B).

DISCUSSION

The rats showed conditioned preferences for the compartments paired with ventral tegmental injections of cytisine relative to the compartments paired with ventral tegmental injections of saline. When rats were given cytisine injections into sites dorsal to the ventral tegmentum, no such preference was established; this rules

Figure 10. (see page 96).   Anatomical localization of injector

cannulae tips in animals that were given injections into

either the ventral tegmentum (●) or more dorsal

sites (▼).   The number on the side of each section

designates the location behind bregma of the

coronal section (in millimeters). (Adapted from

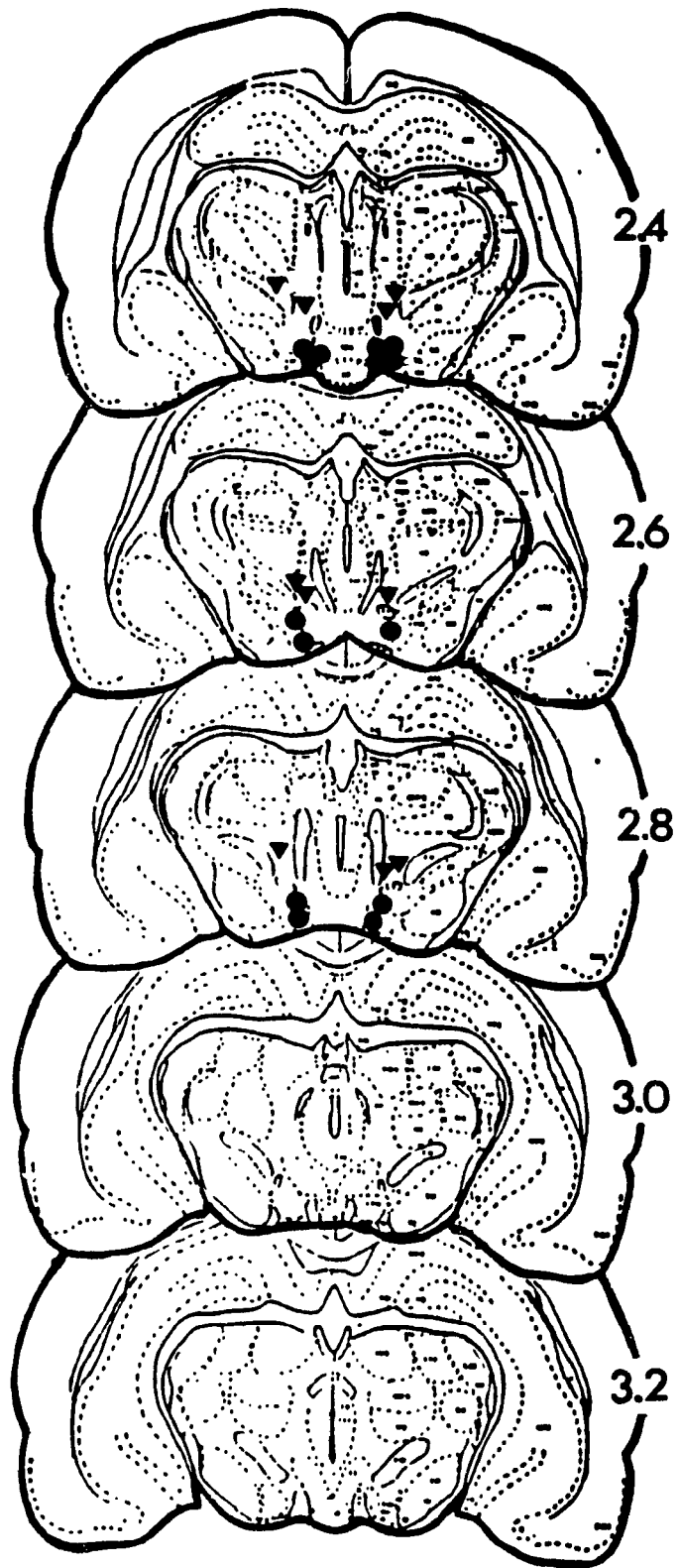Pellegrino et al., 1979.)

2.4

2.6

2.8

3.0

3.2

Figure 11. (see page 98). The amount of time animals spent in either the cytisine-paired compartment or the saline paired compartment prior to the conditioning trials (A) and after conditioning (B). During conditioning trials one group was given injections into the ventral tegmental and the other group was given injections into more dorsal sites.
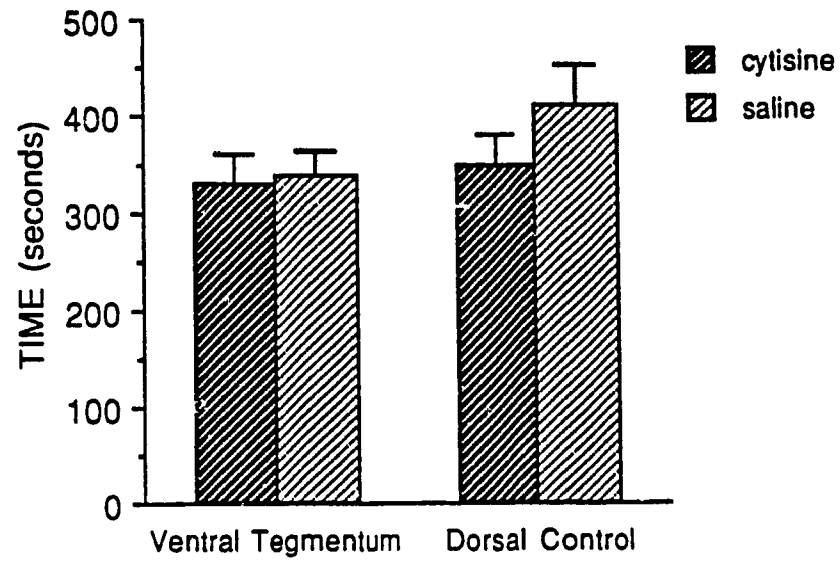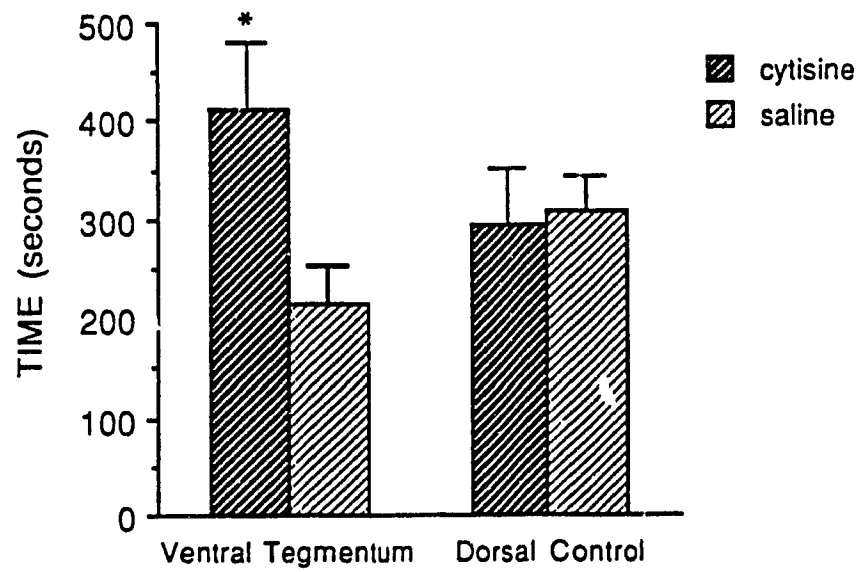
out the possibility that ventral tegmental injections of cytisine established conditioned place-preferences as a result of some action at more dorsal sites. Since drug efflux up the side of the injector or guide cannula shaft is the most likely path of drug diffusion from the injection site (Johnson & Epstein, 1975; Routtenberg, 1972), it seems likely that actions in or near the ventral tegmentum were responsible for the conditioned preferences.

Although animals spent more time in the cytisine-paired compartment than in the saline-paired compartment, the amount of time they spent in the cytisine-paired compartment did not increase dramatically as a result of conditioning. However, while the change in preference for the cytisine-paired compartment was not great, it was comparable to the changes seen with other drugs in this procedure; when the same apparatus and testing procedure are used, systemic injections of amphetamine (D. Hoffman, personal communication, 1990) and ventral tegmental injections of morphine (personal observation) establish preferences with magnitudes similar to those established by ventral tegmental injections of cytisine.

The finding that animals spent more time in the compartment paired with cytisine than in the compartment paired with saline, taken together with the finding that ventral tegmental injections of cytisine increase locomotion, adds to the evidence in support of the suggestion by Schneirla (1959) and others (Glickman & Schiff, 1967; Wise & Bozarth, 1987) that locomotion and reinforcement are

mediated by a common neural substrate. Although the present findings do not directly address the question of substrate, it is tempting to suggest that the mesolimbic DA system could be that common substrate, especially since the mesolimbic DA system is localized in this region (Lindvall & Bjorklund, 1974; Fallon & Moore, 1978) and is implicated in both locomotion (Fink & Smith, 1980; Kalivas et al., 1983; Kelley et al., 1980) and reinforcement (Lyness et al., 1979; Robbins et al., 1983; Yokel & wise, 1975).

Like ventral tegmental injections of cytisine, ventral tegmental injections of morphine increase locomotion (Pert & Sivit, 1977; Vezina & Stewart, 1984) and establish conditioned place-preferences (Phillips & LePiane, 1980; Bozarth, 1987). There is electrophysiological (Gysling & Wang, 1983; Matthews & German, 1984) and biochemical (Kalivas et al., 1983; Leone et al., 1991) evidence that opiates alter mesolimbic DA function. Perhaps more importantly, there is behavioral evidence that implicates the mesolimbic DA system in the reinforcing (Bozarth, 1987; Bozarth & Wise, 1981) and locomotor-activating (Holmes & Wise, 1985; Joyce & Iversen, 1979; Joyce et al., 1981; Kalivas et al., 1983; Kelley et al., 1980; Stinus et al., 1980) effects of ventral tegmental injections of morphine. Specifically, ventral tegmental injections of morphine increase locomotion (Joyce & Iversen, 1979; Vezina & Stewart, 1984) and establish conditioned place-preferences (Bozarth, 1987; Phillips & LePiane, 1980; Vezina & Stewart, 1987). With regard to the effects of morphine on the establishment of conditioned place-preferences, the location of the injection sites in

the ventral tegmentum correlate with the magnitude of the place-preferences; whereas injections within the mesolimbic DA cell-group establish place-preferences (Bozarth & Wise, 1987; Phillips & LePiane, 1980) injections anterior or posterior (Bozarth, 1987) or dorsal (Phillips & LePiane, 1980) to the DA cell group fail to establish place-preferences. A similar relationship between the location of injection sites relative to the DA cell-body region and the behavioral actions associated with injections into these sites has been reported in experiments involving unilateral ventral tegmental injections of morphine; when morphine is administered unilaterally, rats engage in circling behavior, a behavior that has been characterized as a biased form of locomotion (Holmes & Wise, 1985). Injections into the DA cell-body region are more effective at inducing circling behavior than are injections outside the cell-body region (Holmes & Wise, 1985).

Since nicotine, like morphine, influences mesolimbic DA function (Andersson et al., 1981; Clarke et al., 1988a; Grenhoff & Svensson, 1988; Imperato et al., 1986; Mereu et al., 1987), the possibility exists that the mesolimbic DA system also mediates the reinforcing and locomotor-activating effects of ventral tegmental injections of cytisine. As mentioned previously, there are nicotinic receptors in the ventral tegmentum (Clarke et al., 1984; Clarke & Pert, 1985) and the firing of mesolimbic DA cells increases following the systemic administration of nicotine (Grenhoff et al., 1986; Yoon & Westfall, 1989). An increase in DA levels in the NAS following ventral tegmental injections of cytisine has been reported

(Pert & Chiueh, 1986). Also, 6-OHDA-induced lesions of the NAS disrupt 1), the self-administration of nicotine (Singer, Wallace, & Hall, 1982) and 2), the locomotor effects associated with ventral tegmental area injections of cytisine (Pert & Clarke, 1987) and systemic injections of nicotine (Clarke et al., 1988a).

The fact that ventral tegmental injections of cytisine increase locomotion and establish conditioned place-preferences fits with the idea that the mesolimbic DA system mediates the locomotor-activating and reinforcing effects associated of cytisine. There is evidence, then, that the locomotor-activating and reinforcing effects associated with ventral tegmental injections of cytisine could result from, as is the case with ventral tegmental injections of opiates, the activation of the mesolimbic DA system.

The idea that a DA mechanism mediates both the reinforcing and locomotor-activating effects of cytisine can be criticized, however, on the basis of the findings reported in Experiment 2. In that study, the boundaries of the mesolimbic DA cell group were found not to coincide precisely with the boundaries of the area into which injections of cytisine increased locomotion. In addition, injections into some areas of the DA cell-body region failed to increase locomotion. It would be of interest to determine whether a mapping of the same region with regard to the effects of cytisine injections on the establishment of conditioned place-preference would result in a similar distribution of effective sites. A similar distribution of sites would add further support to the idea that a common substrate mediates the locomotor-activating and

reinforcing effects associated with ventral tegmental injections of cytisine. The idea that the substrate includes a DA component would, however, still be subject to the same criticisms outlined in Experiment 2.

It remains that because the results of Experiment 2 did not rule out the DA hypothesis, additional tests of the idea that the mesolimbic DA system mediates the locomotor-activating and reinforcing effects associated with ventral tegmental injections of cytisine are needed. It would be of interest, for example, to determine whether the administration of DA antagonists systemically or intracranially—into the NAS, for example—blocks the effects of ventral tegmental injections of cytisine on locomotion and place-preference. Similarly, a study of the effects of 6-OHDA-induced lesions of the mesolimbic DA system would help determine whether DA transmission is necessary for the establishment of conditioned place-preferences the same way it appears to be important for the locomotion induced by ventral tegmental injections of cytisine (Pert & Clarke, 1987).

EXPERIMENT 5:
CYTISINE MICROINJECTIONS INTO DOPAMINE TERMINAL FIELDS:
EFFECTS ON LOCOMOTION

Although nicotine may increase locomotion by acting primarily on the cell bodies of DA neurons, it may also increase locomotion by acting directly on the terminals of DA neurons. This latter action is thought to induce the release of DA (Giorguieff-Chesselet et al., 1979; Misfud et al., 1988; see Westfall et al., 1987 for a review) and, in turn, to increase locomotion. Experiment 5 was designed to determine the extent to which actions at each of several mesocorticolimbic DA terminal regions might contribute to the locomotor-activating effects associated with systemic injections of nicotine. Toward this end, various doses of cytisine were injected into each of the following DA terminal regions: NAS, caudate putamen, olfactory tubercle, and medial prefrontal cortex.

METHOD

Subjects

Sixty male Long-Evans rats, weighing between 350 and 450 g at the time of surgery, were used for this experiment. The animal supplier and the housing conditions were the same as in Experiment 1.

Surgical implantation of guide cannulae

Each animal was implanted bilaterally with guide cannulae, using the general surgical procedure described in the methods section of the pilot experiments. With the exception of the animals

in the medial prefrontal cortex group, the guide cannulae were implanted at a 10-degree angle in order to avoid puncturing the ventricles. The tips of the guide cannulae were lowered stereotaxically to a location 1.5 mm short of the final injection sites. The stereotaxic coordinates for the injection sites were as follows: NAS (3.2 mm anterior to bregma (AP), 2.2 mm lateral to the midsagittal suture (ML) and 7.8 mm ventral to the skull surface (DV)); caudate putamen, (AP = 3.6, ML = 2.2, DV = 5.8); olfactory tubercle, (AP = 3.6, ML = 3.5, DV = 9.2); medial prefrontal cortex, (AP = 4.5, ML = 0.7, DV = 3.5). Animals were allowed at least one week to recover from the surgical procedure.

Testing Procedure

The injection method was the same as in Experiment 1. Each animal was injected with four doses of cytisine (0.1, 1,10 and 100 nmol) and the vehicle solution (0.9 % saline); the order of dosing was counterbalanced. Two days prior to the beginning of the experiment, animals were habituated to the apparatus for 60 min. On every test day, following a 30-min habituation period, each animal was administered one of the five treatments, and locomotor activity was recorded during a 60-min session.

Confirmation of cannulae placements

Following the experiment, and while under deep chloral hydrate anaesthesia, animals were perfused transcardially with 50 mL of saline followed by 50 mL of 10% formalin. A thionin solution was then injected intracranially in a volume of 0.5 μL in order to help locate the injection site. Cannula placements were determined by

examining a series of 40 μ frozen coronal sections obtained from each animal.

<u>Statistical Analysis</u>

The data for each site were analyzed separately using one-way analyses of variance with repeated measures; post-hoc comparisons were made using Tukey's Honestly Significant Difference (HSD) test. Only animals with both injector tips located in the same structure were included in the data analyses. Of the 60 animals implanted with guide cannulae 12 animals were excluded on the basis of this criterion: NAS (n = 20; Figure 12) and caudate putamen (n = 9; Figure 12), olfactory tubercle (n = 8; Figure 15), medial prefrontal cortex (n = 10, Figure 17).

<u>RESULTS</u>

Cytisine injections into the NAS increased locomotion, $F_{(4, 76)} = 5.97$, $P < .01$. This effect was seen across a large range of doses; each of the three highest doses of cytisine (1, 10 and 100 nmol) produced statistically reliable increases in locomotion (Tukey's HSD test, $P < 0.05$). The administration of cytisine into sites dorsal to the NAS, into the caudate putamen, did not increase locomotion, $F_{(4, 32)} = 0.13$, $P > .05$. Also ineffective were injections into the olfactory tubercle, $F_{(4, 28)} = 0.86$, $P > .05$ and medial prefrontal cortex, $F_{(4, 44)} = 0.76$, $P > .05$.

Figure 12. (see page 108). Anatomical localization of injector cannulae tips in animals that received injections into either the nucleus accumbens (●) or caudate putamen (▼). The number on the side of each section designates the location behind bregma of the coronal section (in millimeters). (Adapted from Pellegrino et al., 1979.)

4.0

3.8

3.6

3.4

3.2

3.0

Figure 13. (see page 110). Locomotor effects associated with injections of cytisine into the nucleus accumbens (NAS) as a function of dose (nmol/0.5 µL/side) and time after injection: (A) the number of photocell interruptions associated with each treatment (plotted at 10-min intervals), and (B) the total number of interruptions for the 60-min sessions.

A

B

Figure 14. (see page 112). Locomotor effects associated with bilateral injections of cytisine into the caudate-putamen (CPU) as a function of dose (nmol/0.5 µL/side) and time after injection: (A) the number of photocell interruptions associated with each treatment (plotted at 10-min intervals), and (B) the total number of interruptions for the 60-min sessions.

A
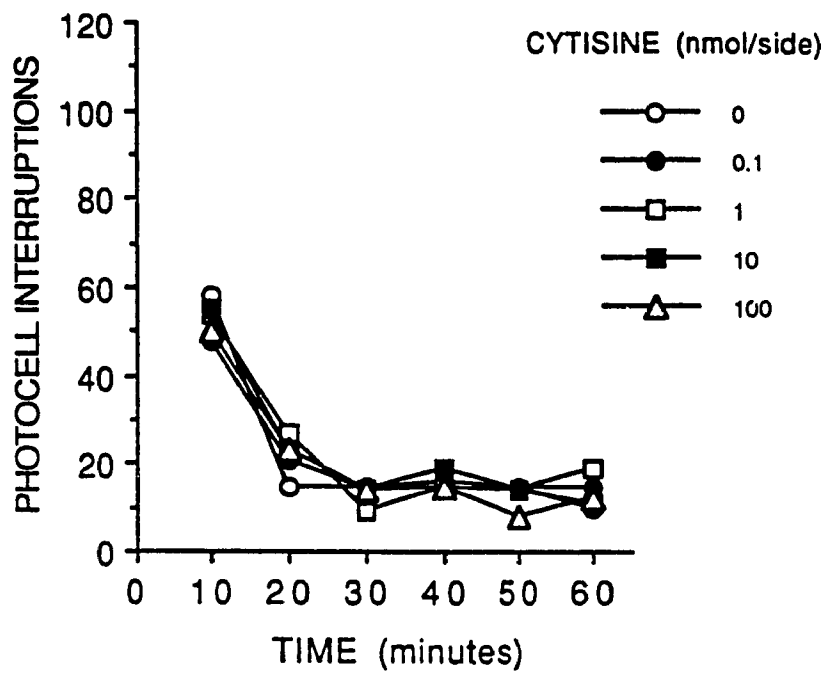
B
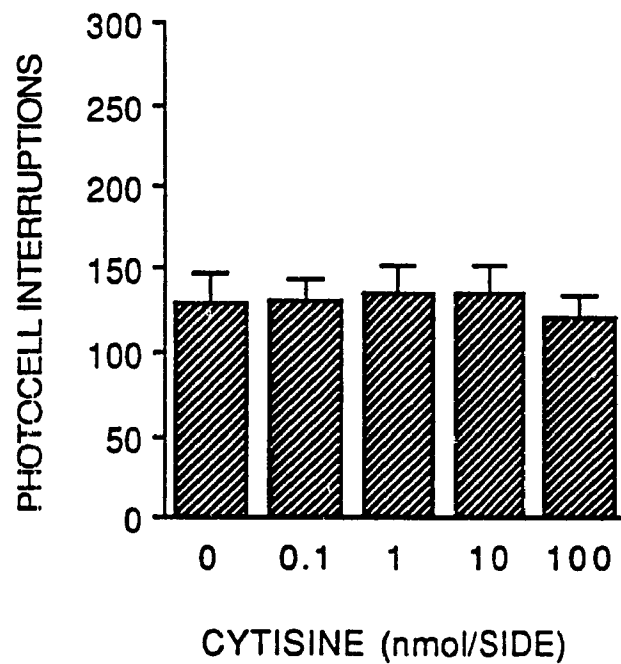
Figure 15.   (see page 114).   Anatomical localization of injector

cannulae tips of animals that were given injections of

cytisine into the the olfactory tubercle. The number on

the side of each section designates the location behind

bregma of the coronal section (in millimeters). (Adapted
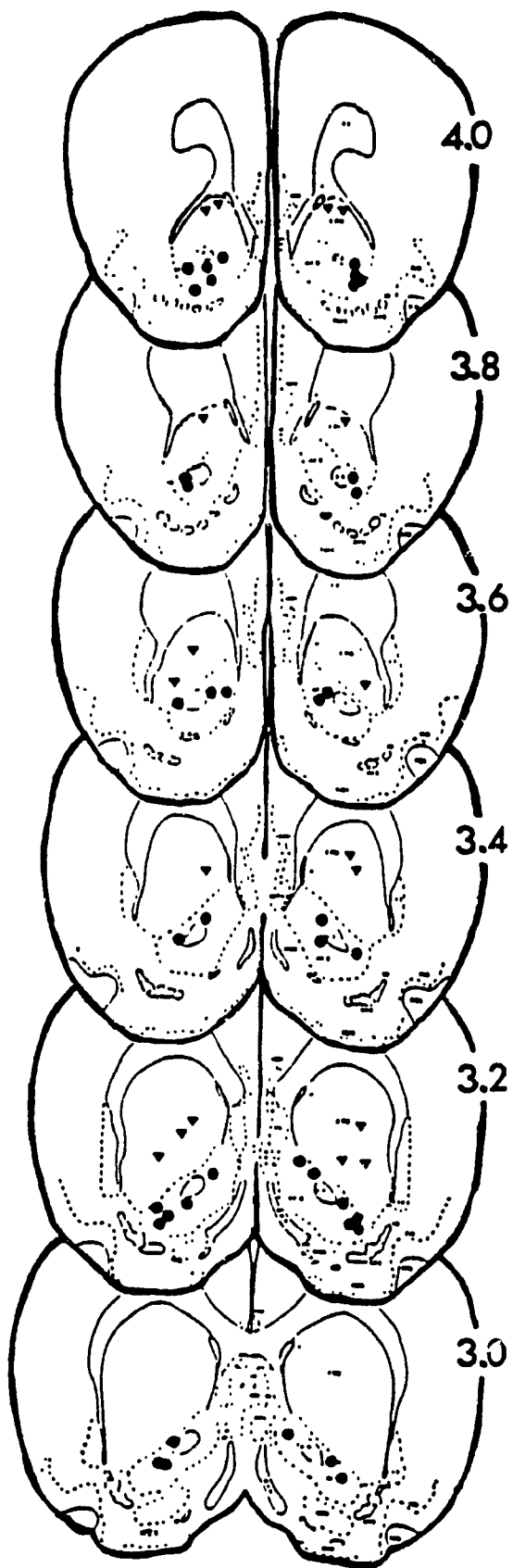
from Pellegrino et al., 1979.)
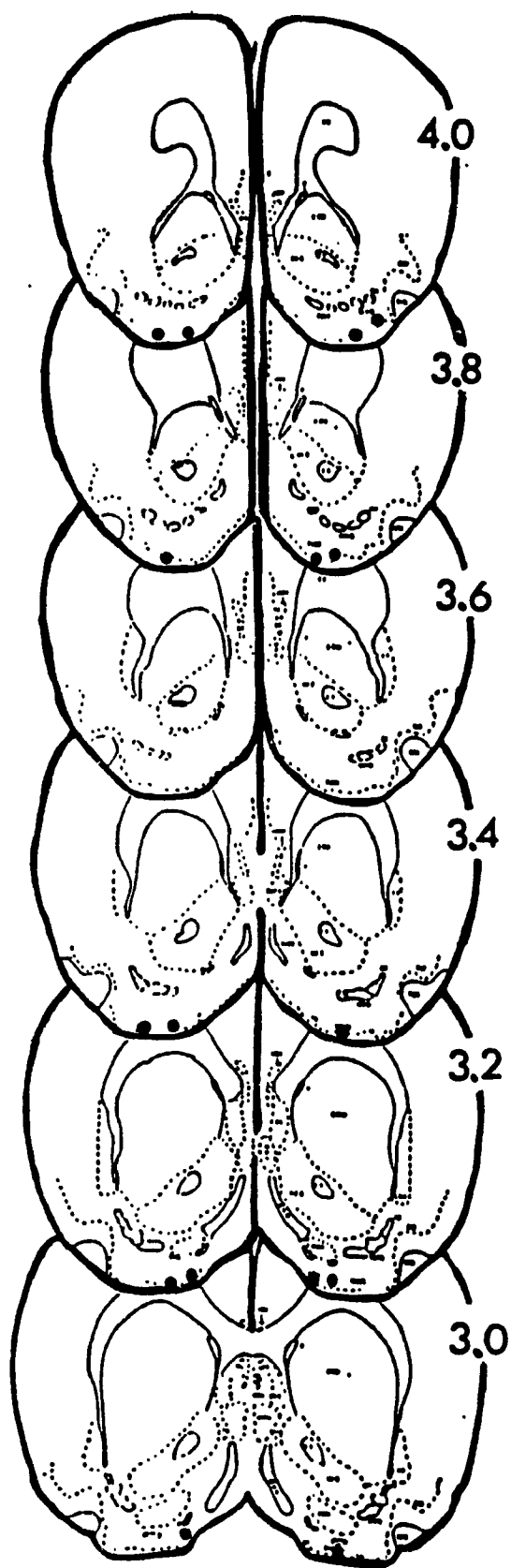
Figure 16. (see page 116). Locomotor effects associated with injections of cytisine into the olfactory tubercle as a function of dose (nmol/0.5 µL/side) and time after injection: (A) the number of photocell interruptions associated with each treatment (plotted at 10-min intervals), and (B) the total number of interruptions for the 60-min sessions.
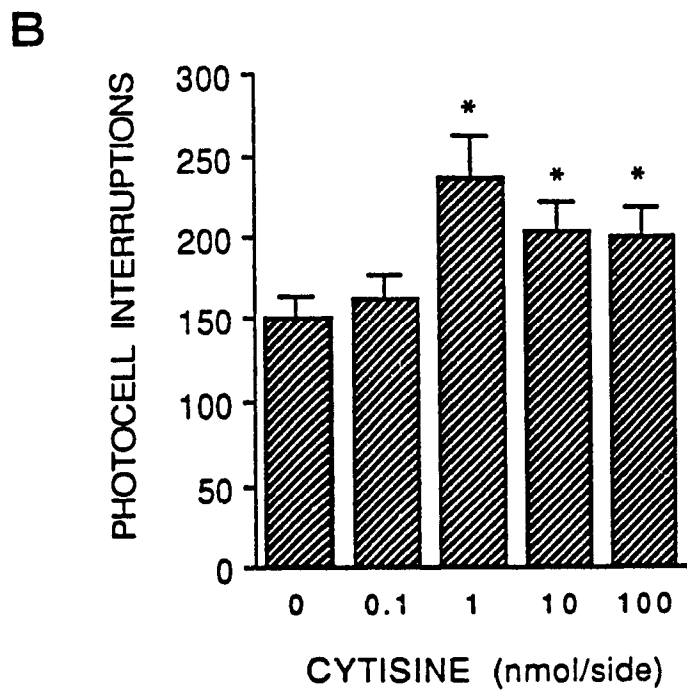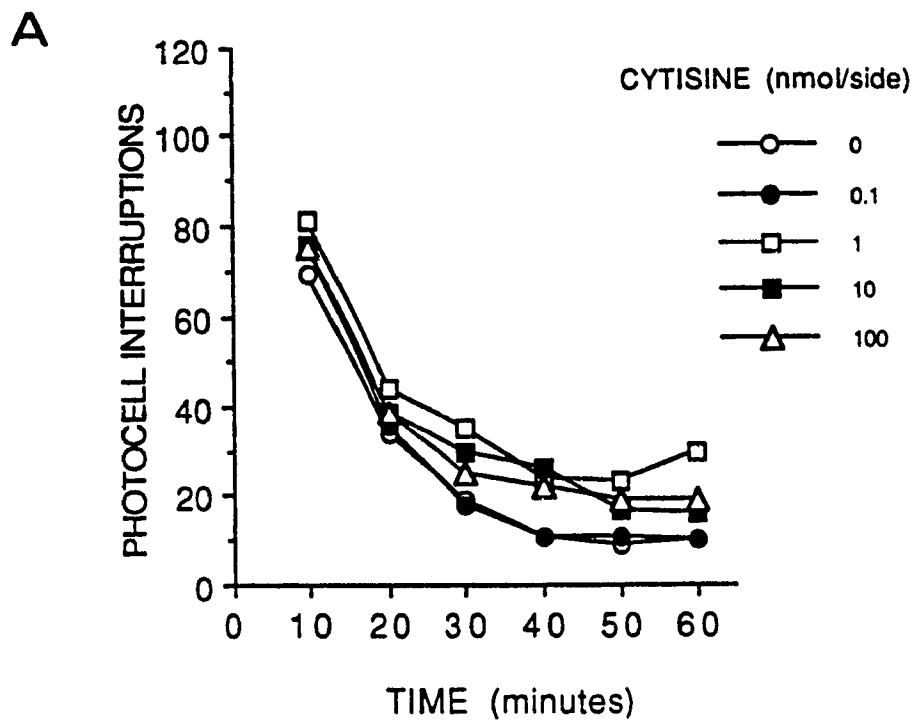
A
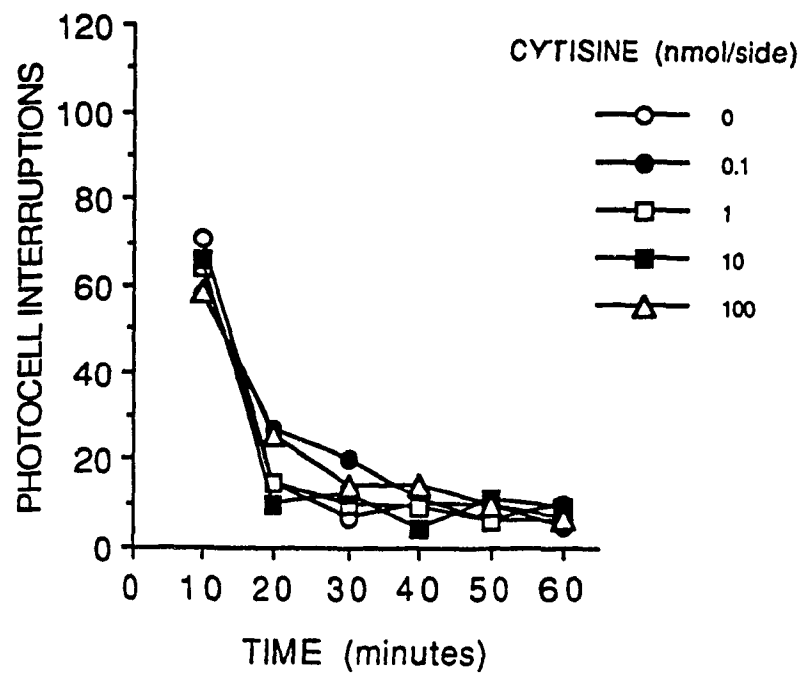
B
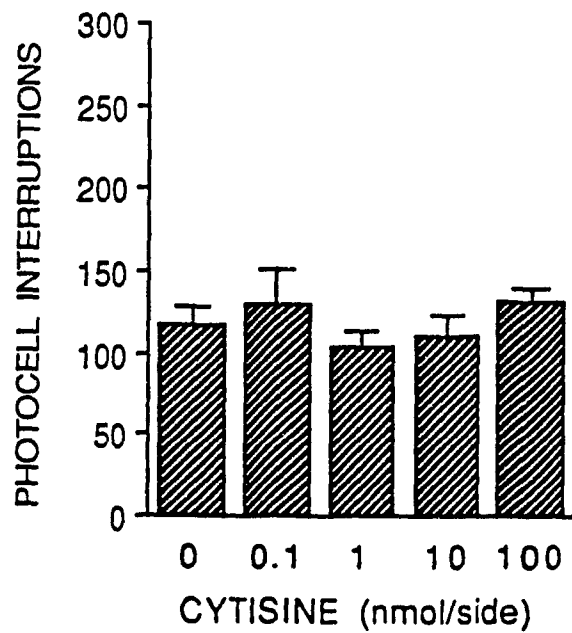
Figure 17.   (see page 118).   Anatomical localization of injector

cannulae tips of animals that were given injections of

cytisine into the the medial prefrontal cortex.

The number on the side of each section designates the

location behind bregma of the coronal section (in

millimeters). (Adapted from Pellegrino et al., 1979.)

5.4

5.2

5.0

4.8

Figure 18.   (see page 120).   Locomotor effects associated with injections of cytisine into the medial prefrontal cortex as a function of dose (nmol/0.5 μL/side) and time after injection: (A) the number of photocell interruptions associated with each treatment (plotted at 10-min intervals), and (B) the total number of interruptions for the 60-min sessions.
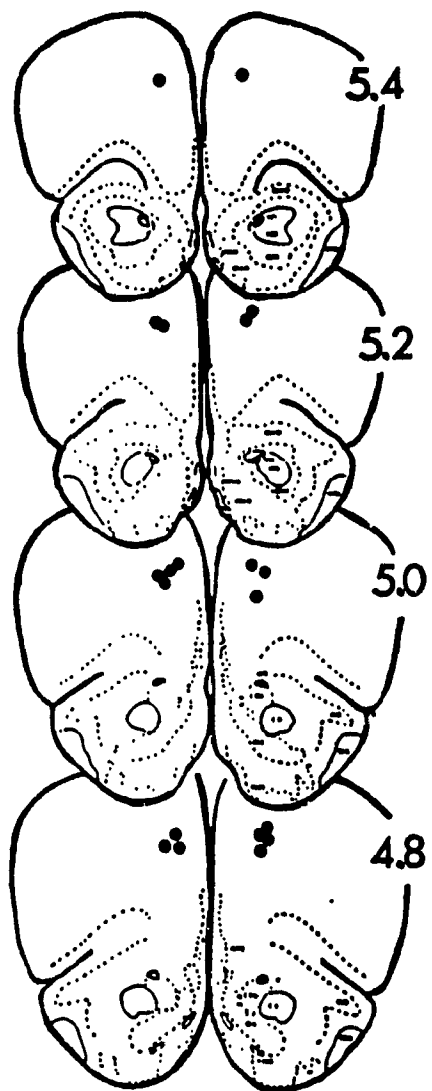
A
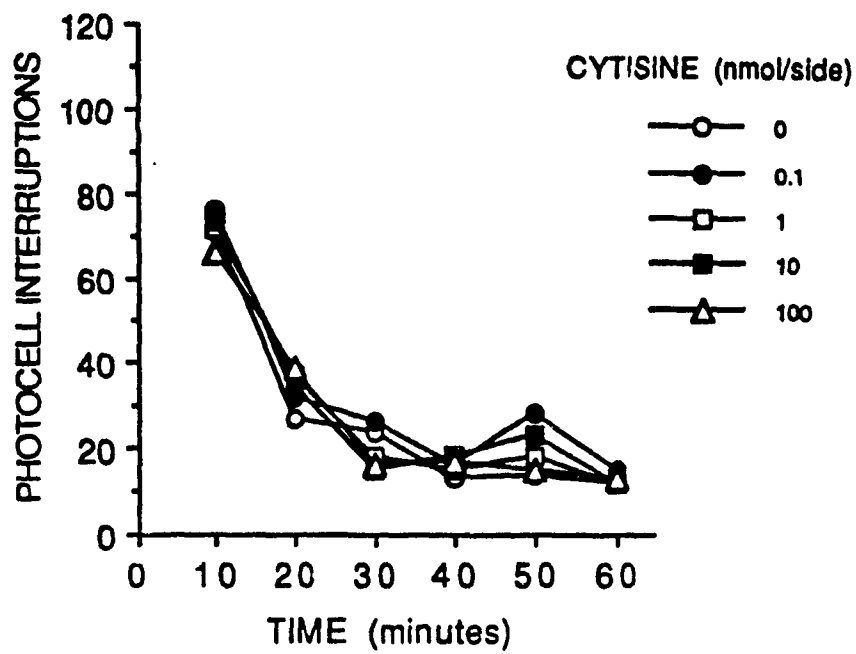
B
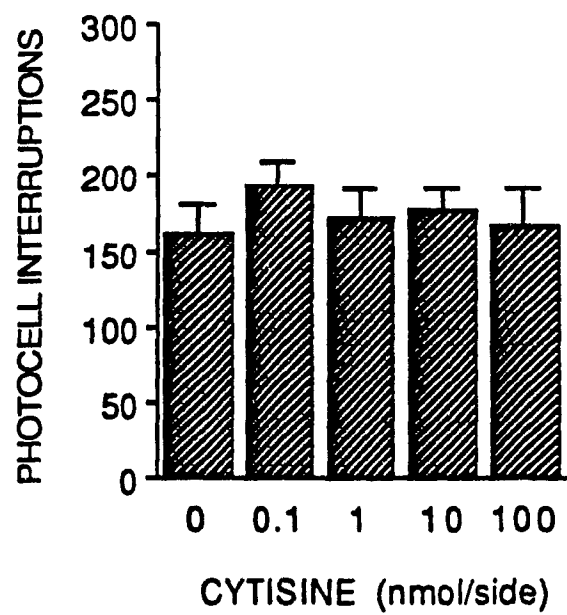
DISCUSSION

Injections of cytisine into the NAS—but not into the caudate putamen, olfactory tubercle or medial prefrontal cortex—increased locomotion. Since cytisine injections into the caudate putamen were ineffective, it appears that drug efflux up the cannula shaft—the most likely path of diffusion from the effective injection site (Johnson & Epstein, 1975; Routtenberg, 1972)—cannot account for the effectiveness of NAS injections.

Injections of the cholinergic agonist carbachol into the NAS also increase locomotion (Austin & Kalivas, 1988). Because the locomotor effects of carbachol are attenuated by mecamylamine, it appears that the activation of nicotinic receptors in the NAS is sufficient to increase locomotion (Austin & Kalivas, 1988). The observations of two other groups of investigators, however, are inconsistent with this view: injections of nicotine (Welzl, Battig, & Berz, 1990) and cytisine (Reavill & Stolerman, 1990) have been reported not to increase locomotion. It is uncertain whether differences in the apparatus and testing procedures might account for these observed differences. Welzl et al. (1990), for example, only tested one dose of nicotine, and the effects of this dose were determined during a much shorter testing session (4-min). And while Reavill and Stolerman (1990) administered doses within the range of doses that was found to be effective in the present study, the animals in that study had previously been exposed to nicotine (0.4 mg/Kg/day, 5 days out of the week, for 2 weeks), and were well habituated to the activity cages before ever receiving injections of

cytisine into the NAS. It is uncertain whether these or any other differences might account for the discrepancies between the present findings and those reported in these two studies.

The mechanism through which cytisine increased locomotion in the present study is not known. It has been suggested, however, that the activation of cholinergic receptors in the NAS can increase locomotion by altering the activity of a GABA pathway that originates in the NAS and that terminates in the substantia innominata (Austin & Kalivas, 1988). Treatments that increase the release of DA in the NAS are thought to inhibit the activity of this GABA pathway; the reduction in activity translates into a reduction in the release of GABA in the substantia innominata/ventral pallidal region, and this, in turn, is thought to increase locomotion (see Mogenson, 1984 for a review). Since injections of the GABA agonist muscimol into the substantia innominata antagonize the locomotion associated with injections of carbachol into the NAS, it appears that carbachol is producing its effects by activating a GABA pathway that originates in the NAS (Austin & Kalivas, 1988). Whether cholinergic stimulation activates this pathway directly (by activating nicotinic receptors on the NAS GABA efferents) or indirectly (by activating nicotinic receptors on DA terminals) is not known.

SUMMARY

The primary findings of this thesis are that injections of the nicotinic agonist cytisine into the ventral tegmentum, where the cell bodies of mesolimbic DA neurons are located, are sufficient to increase locomotion and to establish conditioned place-preferences. It was also found that cytisine increased locomotion when injected into the NAS, a major terminal region of mesolimbic DA neurons. These findings suggest that the increases in locomotion and the establishment of conditioned place-preferences reported following the systemic administration of nicotine may be mediated by actions on these DA neurons. Unlike systemic injections of nicotine which have been reported to have locomotor depressing and excitatory actions, and both aversive and reinforcing actions, the actions in the ventral tegmentum and NAS appear to have been solely excitatory and reinforcing.

It was also found that repeated injections of cytisine into the ventral tegmentum led to progressive increases in locomotion, raising the possibility that sensitization of the locomotor actions of nicotine involve changes in the mesolimbic DA system. Unlike the sensitization found with compounds such as amphetamine, cocaine and morphine, the basis of the sensitization found with cytisine is unknown. The possibility exists that at some level, chronic nicotine injections produce sensitization through its actions on the mesolimbic DA system, but perhaps not in exactly the same manner that amphetamine and cocaine do.

The data from Experiment 3 were the least consistent with the idea that DA cells are directly involved in the locomotor effects associated with injections of cytisine. The results of Experiment 3 did not provide unambiguous support of this hypothesis, however; for example, whereas injections anterior to the DA cell-body region increased locomotion, some injections in the DA cell-body region failed to increase locomotion. Although these findings might suggest that the DA hypothesis is incorrect, other factors could also account for them. It is possible that the spread, or diffusion, of drug to the cell-body region could account for the effectiveness of injections outside the cell-body region. It is also possible that the high concentration of cytisine at the site of injection might have induced depolarization block, thereby inhibiting neural transmission (Grace & Bunney, 1986). Alternatively, high concentrations might have produced tachyphylaxis, or acute tolerance; such a response could be brought on by the rapid desensitization that nicotinic receptors are known to undergo following excessive stimulation (Grenhoff & Svensson, 1989). Because of these possibilities, it is difficult to argue strongly against DA involvement in the effects observed following ventral tegmental injections of cytisine.

Although the majority of the experiments involved the measurement of the effects of cytisine on locomotion, the results of Experiment 4 involved the effects of cytisine on the establishment of conditioned place-preferences. Ventral tegmental injections of cytisine established conditioned place-preferences, thereby suggesting that an action at the level of the ventral tegmentum can

have reinforcing effects. It appears that it is less difficult to demonstrate place-preferences using intracranial injections than it has been using systemic nicotine injections. Because preferences can be established with only one intraventricular injection of nicotine (Iwamoto, 1990), the possibility is raised that by administering drugs centrally, one bypasses effects that otherwise make difficult the establishment of conditioned place-preferences.

To conclude, locomotion is increased by injections of cytisine into the ventral tegmentum and NAS; in the case of the ventral tegmentum, injections of cytisine into this region were sufficient to establish conditioned place-preferences. These regions may therefore mediate the locomotor-activating and reinforcing effects associated with systemic injections of nicotine.

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