Anatomical, Pharmacological And Phenomenological Characteristics Of Morphine-Induced Circling Elicited From The Ventral Mesencephalon

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ABSTRACT

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Crystalline morphine applied unilaterally to the ventral mesencephalon induced circling directed contralateral to the side of injection. Circling was seen after morphine application to either the ventral tegmental area or the zona compacta of the substantia nigra, however latency was shorter and rates higher following ventral tegmental stimulation. Only a few sites supported circling when morphine was applied to the zona reticulata of the substantia nigra, or to other regions outside the dopamine cell layer. When morphine-induced circlers were placed in an open field they consistently followed the perimeter of the enclosure in a contralateral direction; the radius of the circle was determined by the size of the enclosure. Morphine induced little postural asymmetry and induced forward locomotion in all four limbs. The opiate receptor antagonists naltrexone and naloxone blocked morphine-induced circling whether given before or during the sessions. Pimozide pretreatment also blocked morphine-induced circling. Thus circling following unilateral morphine application was dependent on both an opiate receptor mechanism

and dopaminergic activation. In contrast to morphine-induced circling, unilateral application of muscimol at the same sites resulted in more circumscribed contralateral circling that was resistant to dopamine receptor blockade. Muscimol-induced circling was qualitatively distinguishable from morphine-induced circling. Rather than travelling over the entire test area, muscimol-treated animals maintained a relatively fixed position within either the small test box or the large open field, circling with a diameter of travel on the order of 12 cm. In this case, the hind limb contralateral to the side of injection was engaged in backward stepping movements while the hind limb ipsilateral to the side of injection served as a pivot. A strong postural asymmetry accompanied muscimol-induced circling.

These studies demonstrate that two midbrain mechanisms can mediate circling. Morphine-induced circling results from dopaminergic activation and results in behaviour that is compatible with forward locomotion. In contrast, muscimol-induced circling activates a mechanism that is independent of, or efferent to, the dopamine pathways and appears incompatible with simple forward locomotion. Muscimol-induced circling, unlike that induced by morphine, appears relatively independent of environmental objects and events.

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INTRODUCTION

Frame of Reference

Rewarding microinjections of morphine into the ventral tegmental area both reinforce preceeding responses and elicit unconditioned responses of their own (Bozarth, 1983). The unconditioned response to rewarding stimuli represents the motivating effects of such stimuli; some theorists would argue that forward search activity necessarily comprises this unconditioned response (Glickman and Schiff, 1967; Bindra, 1969).

Schneirla (1959) first argued that all motivated behaviours involve either approach or withdrawal responses. Interestingly, sites in the brain where electrical brain stimulation is positively reinforcing elicits forward approach movements when the animals are stimulated under other test conditions (Valenstein, 1964). Such movements are accompanied by search behaviours and these behaviours are clearly environmentally elicited. For example, when a goal object is placed in the environment and the animal is electrically stimulated in brain areas subserving positively reinforcing functions, the animal approaches the object and engages in appropriate goal-directed activities (i.e. Caggiula and Hoebel, 1966; Vaughn and Fisher, 1962; Wise, 1974). Bindra (1969) has argued that such rewarding

electrical stimulation renders more effective the environmental stimuli eliciting exploratory responses.

Central drug application can also elicit forward search activity (Pert and Sivit, 1977; Broekkamp, Phillips and Cools, 1979). Such activity is observed following the bilateral application of opioids to the mesolimbic dopamine cells (Joyce and Iversen, 1979; Broekkamp et al., 1979), an area implicated in several reward functions (Wise, 1980). Recently, it has been noted that when rewarding micro-injections of morphine into the mesolimbic area are unilateral, the animal engages in asymmetrical movements (Bozarth, 1983). Such movements are best described as 'circling'; it is of interest to study such circling responses for they may provide potential clues as to the nature and mechanism of motivational processes.

Phenomenological Characteristics of Circling

The term circling is a general term used to describe directionally-biased movements. Such movements can take qualitatively different forms; the size and number of circles being described by an animal reflect such differences. For example, it has been reported that a strong postural asymmetry often accompanies circling such that the size of the circle being described by the animal is little more than the length of its' body (Pycock, 1980). Animals circling

with a strong postural asymmetry generally circle many times in a given time period (Scheel-Kruger, Arnt and Magelund, 1977). Circling has also been demonstrated that is accompanied by only a mild postural asymmetry (Roffman, Bernard, Dawson, Sobiski and Shelens, 1978). In this case the animal describes very large diameter circles and generally describes substantially fewer such circles within the same time period. While circling accompanied by a strong postural asymmetry and circling accompanied by only a mild postural asymmetry have both been repeatedly reported in the literature, it is as yet unclear what neural mechanisms mediate these qualitatively different responses.

Recently, directionally-biased movements have been classified into two types according to the limb movements made by the animals during the movement. These two types have been labelled 'circling' and 'pivoting' (Teitelbaum, Szechtman, Sirkin and Golani, 1982). Circling is directionally-biased forward locomotion in that all four limbs move in a forward direction. In pivoting, on the other hand, the hind limb ipsilateral to the direction of movement engages in backward stepping movements, while the opposite hind limb serves as a pivot (Teitelbaum, personal communication). The animal thus remains in a fixed position. While the distinction between circling and pivoting is an important one, the body

of this introduction will use circling to refer to all directionally-biased movements. The majority of those investigators who study circling quantify the behaviour while the qualification of the behaviour has been relatively neglected. It is thus unclear whether most experimentally-induced asymmetrical movements that have been reported in the literature are of the circling or the pivoting type.

Pharmacological Basis of Circling

The original model of circling was based on an inter-hemispheric imbalance in brain dopamine activity (Anden, Dahlstrom, Fuxe and Larsson, 1966). Such an imbalance can be created by either unilaterally denervating the dopaminergic system or by unilaterally activating the dopamine system.

The most popular model used to create a unilateral denervation of the dopamine system is the six-hydroxydopamine (6-OHDA) lesioning technique. Six-OHDA is a neurochemical toxin that selectively destroys catecholamine-containing neurons (Ungerstedt, 1968). Injections of 6-OHDA unilaterally into the dopaminergic system leads to reductions in dopamine concentrations in the ipsilateral hemisphere while other neurotransmitter systems remain relatively intact (Pycock, Tarsey and Marsden, 1975; Costall, Marsden, Naylor and Pycock, 1976).

In animals with a unilateral 6-OHDA lesion of the

ascending dopamine pathways, drugs that increase dopaminergic activity induce circling towards the side of the brain with less dopamine activation (i.e. Anden, 1970; Christie and Crow, 1970; 1973; Ungerstedt, 1971a; Von Voigtlander and Moore, 1973; Pycock et al., 1975). There are two classes of dopamine-facilitating drugs that have been tested in the unilateral 6-OHDA preparation; drugs increasing dopamine activity by a presynaptic mechanism and drugs that act as agonists directly on the postsynaptic receptors.

Amphetamine has been the most extensively studied drug in the unilateral 6-OHDA circling model that increases dopamine by a presynaptic mechanism; amphetamine both releases dopamine from dopaminergic nerve terminals and inhibits dopaminergic reuptake thus increasing its' availability within the synaptic gap (Moore, 1978). When amphetamine is injected peripherally in an animal with a unilateral 6-OHDA lesion of the dopamine system the animal exhibits large diameter circles (Pycock, 1980); only little postural asymmetry accompanies the circling. The direction of circling is ipsilateral to the side of the lesion (Anden, 1970; Christie et al., 1971; 1973; Unger- stedt, 1971a; Von Voigtlander et al., 1973; Pycock et al., 1975; Costall et al., 1976). The animal turns ipsilateral to the side of the lesion because the nerve terminals on this side of the brain

have been denervated; the contralateral dopamine system is thus more strongly activated. Amphetamine-induced ipsilateral circling is blocked by alpha-methyl-para-tyrosine, a dopamine synthesis inhibitor (Anden, 1970; Christie et al., 1970; Ungerstedt, 1971a; Von Voigtlander et al., 1973) which illustrates that a presynaptic mechanism is involved in this response. Dopamine receptor blockers also attenuate amphetamine-induced ipsilateral circling (Ungerstedt, 1971a; Pycock et al., 1975) demonstrating its' dependence upon dopaminergic activation.

There are a number of other pharmacological agents that increase dopaminergic neurotransmission by a presynaptic mechanism that have been tested in the unilateral 6-OHDA circling model. Such drugs include methylamphetamine (Christie et al., 1970), ephedrine (Christie et al.,1970; Boulu, Rapin, Lebas and Jacquet, 1972) and methylphenidate (Von Voigtlander et al., 1973); each has been shown to produce circling ipsilateral to the side of the lesion.

Apomorphine is considered the classical postsynaptic dopamine receptor agonist and this agent has been extensively investigated in the unilateral 6-OHDA circling model (i.e. Ungerstedt, 1971b; Mendez, Gotzias, Finn and Dahl, 1975; Anlezark, Pycock and Meldrum, 1976; Costall et al., 1976). In contrast to drugs that act presynaptically to

increase dopamine neurotransmission, apomorphine results in contralateral circling. The circles are of a small diameter and accompanied by a tight postural asymmetry (Pycock, 1980). Apomorphine-induced contralateral circling is blocked by dopaminergic antagonists (Ungerstedt, 1971b; Pycock et al., 1975; Nakamura, Engel and Goldstein, 1978), demonstrating that this behaviour is dependent upon dopaminergic activation.

Apomorphine-induced contralateral circling is presumed to result as a consequence of unilateral dopamine receptor supersensitivity (Ungerstedt, 1971b). When the natural action of a neurotransmitter is inhibited chronically, the postsynaptic neuron compensates. New receptors proliferate and the postsynaptic neuron shows an enhanced response to the transmitter substance if it is suddenly reintroduced into the system (Creese and Snyder, 1978). Apomorphine induces contralateral circling, therefore, because the lesioned side of the brain (with the supersensitized receptors) shows an enhanced response to this agent.

A host of other postsynaptic dopamine receptor agonists have also been tested in animals with a unilateral 6-OHDA lesion of the dopaminergic system. Such agents include N-propyl-noraporphine (Costall, Naylor and Neumeyer, 1975; Mendez et al., 1975; Neumeyer, Dafeldecker,

Costall and Naylor, 1977), diacetyl-apomorphine (Baldessarini, Walton and Borgman, 1976), diisobutyryl apomorphine (Tye, Horsman, Wright, Large and Puller, 1977) and piribedil (Costall and Naylor, 1974a; Thornburg and Moore, 1974). Each of these agents induces contralateral circling in the unilateral 6-OHDA preparation.

A number of other methods have been employed to inactivate the dopaminergic system unilaterally. Circling activity in rats following treatment with dopaminergic-facilitating drugs has been demonstrated when the dopaminergic system is denervated unilaterally by either aspiration (Anden et al., 1966; Lotti, 1971), electrolytic lesions (Naylor and Olley, 1972; Costall, Fortune, Naylor and Nohria, (1979), injections of hypertonic KCl solutions (Costall, Naylor and Pettit, 1974c), copper sulfate injections (Costall et al., 1976), or ascorbic acid injections (Waddington and Crow, 1979). In all unilateral dopamine lesion models, drugs that increase dopaminergic activity induce circling towards the side of the brain with less dopamine activation.

Unilateral activation of the dopaminergic system also results in circling and such circling is observed following either electrical or chemical stimulation. Electrical stimulation results in circling when the electrode is aimed at the dopamine cell body region and such circling was originally reported to be contralateral to the side of stimulation

(Arbuthnott, Crow, Fuxe and Ungerstedt, 1970; Arbuthnott and Crow, 1971; Arbuthnott and Ungerstedt, 1975; Roffman et al., 1978). Electrically-induced contralateral circling was shown to be blocked by either clozapine or haloperidol (Roffman et al., 1978), both of which are dopamine receptor antagonists. Recently, it has been reported that unilateral electrical stimulation in the area of the dopamine cell bodies elicits either contralateral or ipsilateral circling, suggesting that the dopamine system may be heterogeneous in function (Vaccarino and Franklin, 1982a). Both the contralateral and ipsilateral circling were dose-dependently blocked by pimozide (Vaccarino and Franklin, 1982b), a dopamine receptor antagonist, which supports the hypothesis that it is a dopaminergic imbalance that underlies this activity.

Unilateral chemical application of pharmacological agents increasing dopaminergic neurotransmission results in circling when such agents are applied to the dopamine terminal fields. Dopamine applied unilaterally in either crystalline form (Ungerstedt et al., 1969), or as a liquid (Ungerstedt et al., 1969; Costall and Naylor, 1974b; Costall, Naylor and Pinder, 1974d; Setler, Malesky, McDevitt and Turner, 1978) results in contralateral circling and such activity is blocked by dopamine receptor antagonists (Ungerstedt et al., 1969). Amphetamine and methylphenidate

(Costall et al., 1974b; McKenzie, Gordon and Viik, 1972) also elicits contralateral circling when injected unilaterally into the terminal fields of the dopaminergic system.

Unilateral chemical stimulation at the level of the dopamine cell body also results in circling. Such circling results from the unilateral application of morphine, as well as a host of other opioid compounds (Iwamoto and Way, 1977; Pert, 1978) and the direction of circling is contralateral to the side of application. The demonstration that opioid-induced circling is contralateral to the side of injection suggests that these agents facilitate dopaminergic neurotransmission, at least at the level of the dopamine cell body. Opioid-induced contralateral circling is blocked by haloperidol, as well as by a unilateral 6-OHDA lesion of the dopamine system on the side that the opioid is administered (Iwamoto et al., 1977); these observations support the hypothesis that opioid-induced circling depends upon dopaminergic activation.

There are several studies reporting that normal, intact animals will circle, although at lower rates, when administered drugs increasing dopamine activity (Jerussi and Glick, 1976; Glick, Zimmerberg and Greenstein, 1976; Glick, Jerussi, Cox and Fleisher, 1977). Amphetamine, apomorphine and l-dopa all induce circling in normal rats; as in

lesioned rats the direction is consistent for normal rats. When tested on several different occasions, some rats consistently rotate to the left and some rats consistently rotate to the right. Haloperidol antagonizes the circling response to these agents (Jerussi et al., 1976; Glick et al., 1977). The phenomenon of circling in normal animals suggested that intact animals may have an intrinsic asymmetry in brain dopamine concentrations; subsequent studies demonstrated such an asymmetry (Glick, Jerussi, Walters and Green, 1974).

In addition to dopamine, several other of the central neurotransmitter substances appear capable of producing circling. Included in the list are norepinephrine (Pycock, Donaldson and Marsden, 1975), acetylcholine (Kelly and Miller, 1974; Muller and Seeman, 1974; Glick et al., 1974), serotonin (Green and Grahame-Smith, 1975), gamma-amino-butyric-acid (GABA) (Tarsey, Pycock, Meldrum and Marsden, 1975; Dray, Oakley and Simmonds, 1975), glycine (Mendez, Cotzias, Finn and Dahl, 1975) and substance P (James and Starr, 1977; Arnt and Scheel-Kruger, 1979). It is generally assumed however, that these chemical messengers act by either modulating dopamine-induced circling (Glick, Jerussi and Fleisher, 1975), or by stimulating output pathways mediating dopamine-induced circling (Arnt et al., 1979).

Anatomical Basis of Dopamine-Induced Circling

An interhemispheric imbalance in brain dopamine activity has been strongly argued to underlie circling; it is the nigrostriatal dopamine system, rather than the other ascending dopamine pathways, that has received most attention in this behaviour.

The organization of the ascending dopamine projections to the forebrain are generally viewed to consist of three pathways that are best described in terms of their sites of projection. The nigrostriatal dopamine system arises primarily in the pars compacta of the substantia nigra (SNC) and terminates primarily in the striatal nuclei. The mesolimbic and mesocortical dopamine systems both arise primarily in the ventral tegmental area (VTA) and project widely to both isocortical and allocortical telencephalic regions. Brain areas innervated by the mesolimbic dopamine system include the nucleus accumbens septi, central amygdala, lateral septum and olfactory tubercle. The dopamine terminals of the mesocortical dopamine system have been identified in the medial sulcal and prefrontal cortices and in the cingulate cortex (Lindvall and Bjorklund, 1978). The dopamine cells of the SNC have been labelled the A-9 dopamine cells whereas those of the VTA have been designated A-10 (Ungerstedt, 1971c).

The interest in the nigrostriatal dopamine system in

the circling model stemmed from the original work of Anden (1966) who demonstrated that animals would circle when injected systemically with agents that increase dopaminergic activity when the nigrostriatal dopamine system was unilaterally denervated. It was an imbalance in striatal dopamine activity that was presumed to underlie circling and it was thought that the animal circled towards the side of the brain with less striatal dopamine activation. A number of investigators subsequently lesioned the dopamine system unilaterally and in all of the early work the lesion was placed in the nigrostriatal dopamine system (i.e. Ungerstedt, 1971a; Lotti, 1971; Naylor et al., 1972; Costall et al., 1974a; 1976; 1979; Waddington et al., 1979).

There are a number of observations that support the striatal imbalance theory of circling. First, there is a positive correlation between the rate of circling when dopaminergic agonists are administered in animals with a unilateral nigrostriatal lesion and the reduction in striatal dopamine concentration on the side ipsilateral to the lesion (Thornburg et al., 1975; Costall et al., 1976). Second, in intact animals that circle in response to dopaminergic agents there has been found a ten to fifteen percent difference in interhemispheric striatal dopamine concentration (Glick et al., 1974); the animal circles towards the side of the brain with

less striatal dopamine. Third, when embryonic dopamine cells are implanted in the dorsal neostriatum of adult rats that have a unilateral nigrostriatal lesion, the circling in response to amphetamine is abolished and normal locomotion results (Bjorklund, Dunnett, Stenevi, Lewis and Iversen, 1980). Subsequent removal of the implanted dopamine cells reinstates amphetamine-induced circling.

It is also the nigrostriatal dopamine system that has been most extensively investigated in studies that unilaterally activate the dopamine system either electrically or chemically. For example, it is clear that unilateral electrical stimulation of the A-9 dopamine cells results in circling (Arbuthnott et al., 1970; 1971; 1975; Roffman et al., 1978), and it is within these dopamine cells that directional differences have been observed (Vaccarino et al., 1982a, 1982b); more specifically, it is stimulation of the medial SNC dopamine cell area that results in contralateral circling, whereas stimulation of the far lateral dopamine cell area elicits ipsilateral circling.

Unilateral chemical activation of the dopaminergic system results in contralateral circling when applied to the nigrostriatal dopamine system as well. Dopamine, amphetamine and methylphenidate elicits such activity when applied to the striatum (Ungerstedt et al., 1969; Costall et al., 1974a; 1974b; Setler et al., 1978), and the opioids elicit contralateral

circling when applied to the SNC cell bodies (Iwamoto et al., 1977; Pert, 1978). Furthermore, coadministration of naloxone and morphine into the SNC blocks intranigral morphine-induced circling (Iwamoto et al., 1977).

It is more recently that the mesolimbic dopamine system with projections to the nucleus accumbens septi has been implicated in circling and yet it is this dopaminergic system that has been strongly argued to mediate locomotor activity. For example, dopamine applied bilaterally to the nucleus accumbens septi results in increased locomotor activity as demonstrated by a number of investigators (Costall and Naylor, 1975; Costall, Naylor, Cannon and Lee, 1977; Pijnenburg, Honig, Van der Heyden and Van Rossum, 1976). Bilateral injections of low doses of haloperidol into the nucleus accumbens septi antagonize the locomotor effects of amphetamine (Pijnenburg, Honig and Van Rossum, 1975), as do bilateral 6-OHDA lesions to this nucleus (Kelley, Seviour and Iversen, 1975). Furthermore, bilateral 6-OHDA lesions to the nucleus accumbens septi enhance apomorphine-induced locomotion (Kelley et al., 1975), an observation consistent with the notion of dopamine receptor supersensitivity.

Several investigators have demonstrated that the bilateral application of opioids into either the nucleus septi (Pert et al., 1977) or into the A-10 dopamine cell

region (Joyce and Iversen, 1979; Broekkamp et al., 1979; Kelley, Stinus and Iversen, 1980) also results in increased locomotion. Interestingly, microinjections of opioids into the A-10 dopamine cell region are accompanied by exploratory activities (Kelley et al., 1980, Broekkmap et al., 1979) and it is these dopamine cells that have been implicated in morphine reward (Bozarth, 1983). Similar bilateral application of opioids into the A-9 dopamine cell region results in stereotypy however, (Iwamoto et al., 1977, Pert, 1978), a behaviour that is incompatible with forward locomotion.

While it is clear that bilateral manipulation of the mesolimbic dopamine system results in increased locomotion, it has not been clearly established whether the unilateral manipulation of this system will result in circling. There are only two reports of unilateral electrical stimulation in this region, and one study reports no circling (Arbuthnott et al., 1978), while the other study reports circling with only a mild postural asymmetry (Roffman et al., 1978). Animals do not circle when the nucleus accumbens is unilaterally denervated with 6-OHDA and subsequently treated with amphetamine (Kelly, 1975), nor do they circle when dopaminergic agonists are injected unilaterally into this region (Elkhawad and Woodruff, 1975).

Although the unilateral manipulation of the mesolimbic dopamine system does not generally evoke circling, it has been demonstrated that this system is important in the mediation of circling in animals with a unilateral nigrostriatal lesion. Bilateral 6-OHDA lesions to the nucleus accumbens septi abolished amphetamine-induced circling in animals with a unilateral 6-OHDA lesion of the nigrostriatal dopamine system (Kelly and Moore, 1977). These same lesions enhanced apomorphine-induced circling however, presumably as a consequence of dopamine receptor supersensitivity. Furthermore, bilateral electrolytic lesions of the nucleus accumbens septi abolish both amphetamine and apomorphine-induced circling (Pycock and Moore, 1978). Finally, the bilateral application of haloperidol into the nucleus accumbens septi abolished both amphetamine and apomorphine-induced circling (Kelly et al., 1977). The status to date seems to be that both the nigrostriatal and mesolimbic dopamine systems are essential for the mediation of dopamine-induced circling. Kelly et al. (1977) have described a model of this system where the imbalance in striatal dopamine activation feeds into an amplifier system located in the nucleus accumbens septi which regulates the rate of dopamine-induced circling.

Present Investigations:

Morphine-induced circling has been demonstrated foll-

owing stimulation of either the nigrostriatal (Iwamoto et al, 1977; Pert, 1978) or mesolimbic (Bozarth, 1983) dopamine cells. In order to more clearly establish the anatomical boundaries of this behaviour, unilateral crystalline morphine was applied throughout the ventral mesencephalon and circling activity was recorded. To establish the pharmacological specificity of morphine-induced circling, the animals were challenged with either opiate receptor antagonists or a dopaminergic antagonist. Finally, to establish phenomenological basis of morphine-induced circling, careful behavioural observations were undertaken.

GENERAL METHODS

Subjects

One hundred and twenty-five male Long Evans Hooded rats were housed individually with free access to food and water. They were maintained on a twelve hour light, twelve hour dark cycle. The mean preoperative weight of the animals was 300 grams.

Surgical Procedures

The rats were anesthetized with sodium pentobarbital (60 mg/kg) and positioned in a Kopf stereotaxic apparatus. The incisor bar was placed 5.0 mm above the interaural line. Twenty-two gauge guide cannulae were implanted, one per rat, throughout the ventral mesencephalon. The guide cannulae were fitted with dummy cannulae that extended 0.5 mm beyond the guide cannulae immediately after surgery. The dummy cannulae were kept in place until behavioural testing began.

Apparatus

The apparatus consisted of circular plastic buckets (40 cm high) with a flat bottom base (28 cm in diameter). The buckets were placed in a wooden test chamber. The head pedestal of the animal was attached to a cable which was mounted onto a shaft hanging from a ballbearing that was secured into the top of the wooden test chamber. If the animal moved in a consistent direction, the cable and shaft turned freely, winding

twines of thread from a spool on a spindle at the side of the wooden test chamber. The thread was taped to the shaft at periodic intervals. By counting the number of thread winds around the shaft after specified time periods, reliable measures of the net number and direction of circles could be recorded (Fig 1).

Behavioural Testing

The experiment was conducted over seven test days. On each test day the animals were placed in the buckets for two hours and the number and direction of circles were recorded. On the first test day, an empty injector cannula that was 0.5 mm longer than the guide cannula was placed inside the guide cannula and the direction and number of spontaneous circles were recorded. For the next four days of the experiment (days two to five), the injector cannula was tapped thirty times into crystalline morphine sulphate and then placed in the guide cannula. The total amount of morphine in the cannulae was approximately eighteen micrograms, as determine by weighing the injector cannulae with a Cahn 25 Automatic Electrobalance when the cannulae were empty and loaded with drug. The number and direction of circles were recorded at 20 minutes intervals from the time of drug application. On test day six, those animals that were used for subsequent studies were injected with either pimozide or naltrexone prior to morphine application or were treated with naloxone

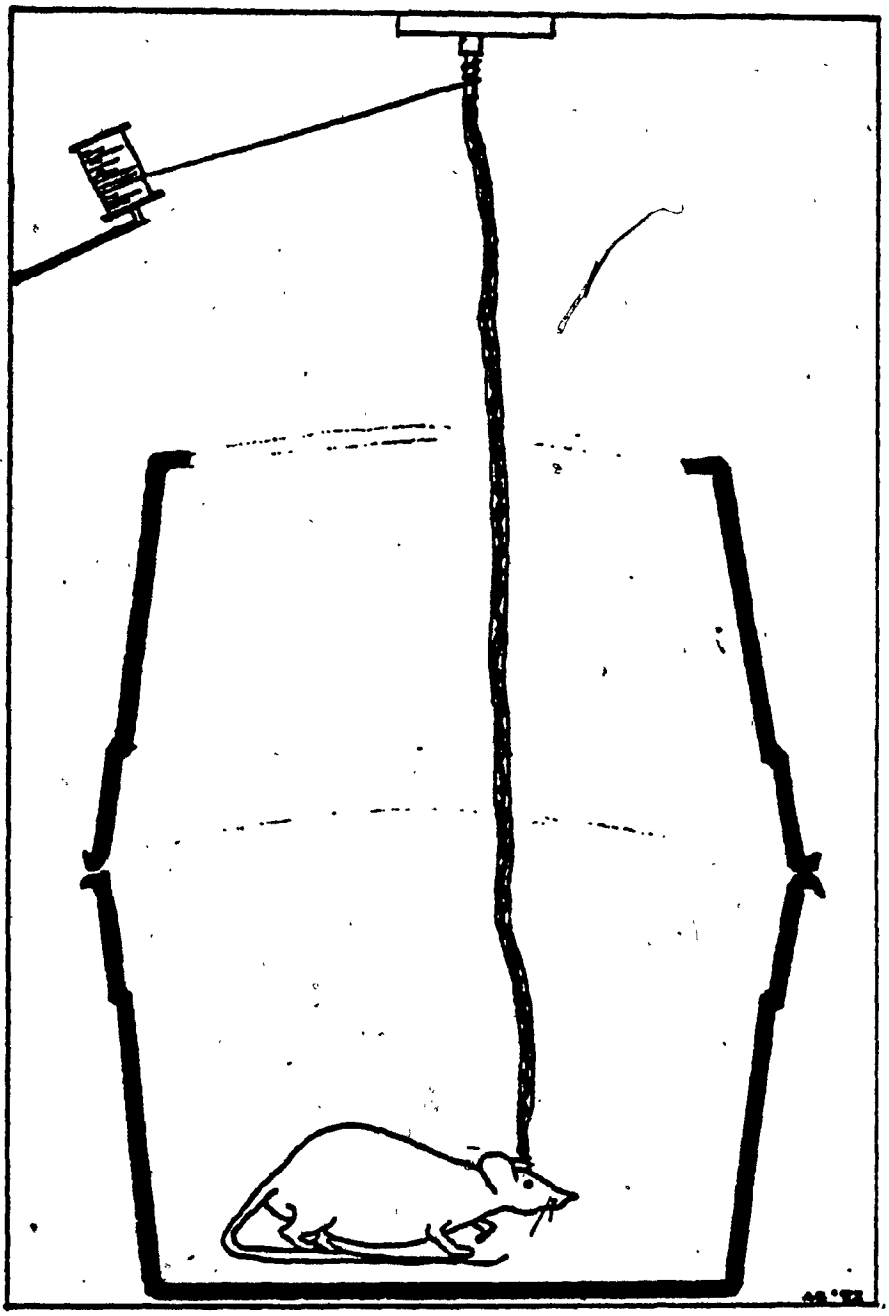

Fig. 1. Diagrammatic representation of apparatus used to quantify circling.

during the two hour test session. The number and direction of circles were recorded. On test day seven, the injector cannula was tapped three times into crystalline muscimol prior to similar testing.

Histological Procedures

After the completion of all behavioural testing the rats were anesthetized with sodium pentobarbital and intracardially perfused with saline followed by a 10% formalin solution. The brains were removed and stored in a 10% formalin solution for at least four days. The brains were then frozen, sectioned and stained with thionin for histological verification of cannula placements.

ANATOMICAL LOCALIZATION OF MORPHINE-INDUCED CIRCLING

Methods

Guide cannulae were implanted at different levels of the ventral mesencephalon. The coordinates ranged between 2.0 to 5.0 mm posterior to bregma, 0.2 to 3.0 mm lateral to the midsagittal suture and 6.0 to 9.0 mm ventral to dura.

The total number of circles following central morphine were determined for the two hour test period and averaged over the four morphine test days. The subjects were classified into four groups according to the total number of circles elicited in the two hour test session.

Cannula placements were verified histologically for

each subject and compared with the Pellegrino and Cushman Atlas of the Rat Brain (1979). The region mapped was divided into four planes. The first section represents cannula placements that were anterior to the dopamine cell containing region. The second and third sections represent anterior and posterior planes within the dopamine cell containing region. The fourth section represents cannula placements that were posterior to the dopamine cell containing region.

Results

Morphine-induced circling was always directed contralateral to the side of drug application. The best circling was seen when the drug was applied to the VTA (Figure 2). Thirty-three animals had cannulae located in this region and twenty-three of these subjects circled 200 times or more during the two hour sessions. Another five animals circled between 126 and 200 times and four circled between 26 and 125 times. Only one animal with a VTA placement failed to circle more than 25 times in response to morphine; no animal circled more than 25 net times in the empty cannula condition.

A number of subjects also demonstrated contralateral circling when cannulae were located in the SNC, however the rates were lower than they were from VTA sites. Of the thirty-two subjects that had cannulae located in the SNC, only two circled more than 200 times in the two hour session,

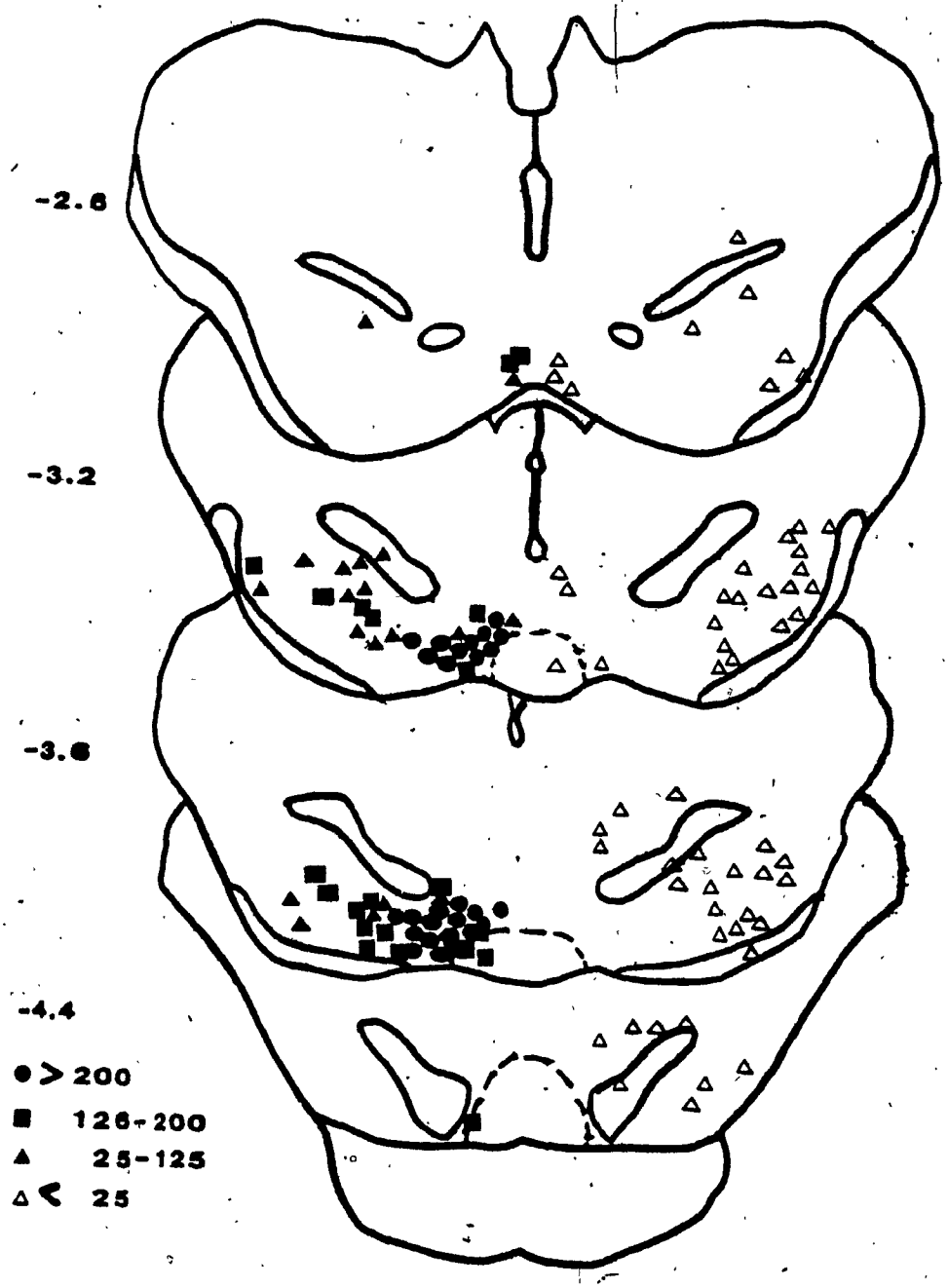

Fig. 2. Histological reconstruction of cannula placements throughout the ventral mesencephalon.

seven circled between 126 and 200 times, seven circled between 26 and 125 times and sixteen failed to demonstrate circling. Interestingly, of the nine subjects that circled more than 126 times, seven had cannulae located in the medial SNC, encroaching upon the VTA.

Morphine-induced contralateral circling was also observed when cannulae were located outside the VTA or SNC. Twenty-three subjects had cannulae located in the pars reticulata of the substantia nigra (SNR); two of these subjects circled between 126 and 200 times in the two hour session, six circled between 25 and 125 times and fifteen subjects did not circle.

Thirty-seven subjects had cannulae located in areas other than the VTA, SNC or SNR. Five of these subjects circled between 126 and 200 times, six circled between 25 and 125 times and twenty six demonstrated no circling. Five of the subjects that circled more than 25 times had cannula located within 0.5 mm of the midline, anterior to the interpenduncular nucleus. Another two were far lateral placements, dorsal to the cerebral peduncle. One placement was located in the zona incerta, one in the medial lemniscus, and one in the reticular formation.

A finer analysis of morphine-induced circling elicited from the dopamine cell containing region was undertaken. Subjects whose cannulae were verified to be within the dopamine cell area were divided into two groups. Those subjects whose

cannulae were located in the A-10 dopamine cell region were designated 'VTA' animals and those subjects whose cannulae were located in the A-9 dopamine cell region were designated 'SNC' animals. The mean number of circles was determined for these two groups every twenty minutes.

Morphine-induced circling rates were higher for the VTA group than for the SNC group. The latency to circle was also much shorter for the VTA group than for the SNC group (see Figure 3). Both groups of animals increased their circling rates over the two hour session such that the VTA animals were circling approximately fifty five times in the last twenty minute period of the two hour session, whereas SNC animals were circling approximately twenty times in the last twenty minute period. A two-way analysis of variance was performed on these data and there was found a main effect for placement ($F(1,64)=80.30$, $p<.00001$), a main effect for treatment ($F(1,64)=80.30$, $p<.00001$), a main effect for trials ($F(5,320)=37.16$, $p<.00001$), a treatment by placement interaction ($F(1,64)=25.22$, $p<.00001$), a trials by treatment interaction ($F(5,320)=11.63$, $p<.00001$) and a trials by treatment by placement interaction ($F(5,320)=20.7$, $p<.00001$). Scheffe post hoc tests revealed that VTA morphine circling rates differed significantly from VTA baseline rates at 40, 60, 80, 100 and 120 minutes of the two hour session ($p<.05$). SNC morphine circling

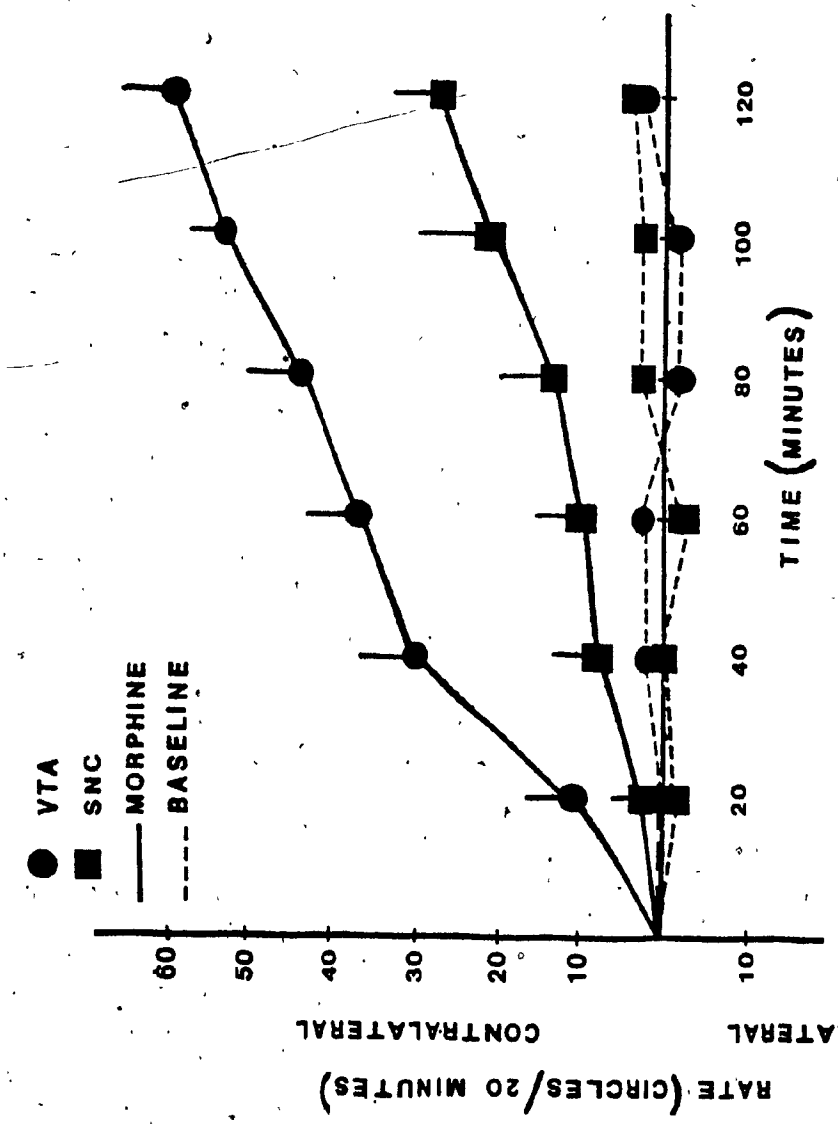

Fig. 3. Comparisons between morphine-induced circling elicited from the VTA and the SNC

rates differed significantly from SNC baseline circling rates at 80, 100 and 120 minutes of the two hour session ($p<.05$). VTA morphine circling rates differed significantly from SNC morphine circling rates at 40, 60, 80, 100 and 120 minutes following the injection ($p<.05$).

Discussion

Morphine applied unilaterally throughout the ventral mesencephalon elicited contralateral circling. Such contralateral circling was most evident when cannulae were placed in the dopamine cell containing region, and it was the VTA placements that supported the best circling.

Three possibilities exist with regard to the mechanism of action of morphine-induced circling. First, the critical mechanism may be located in the VTA in which case circling elicited from the SNC results from significant diffusion of drug to the VTA. Second, the critical mechanism may be located in the SNC in which case circling elicited from the VTA results from significant diffusion of drug to the SNC. Alternatively, the mechanisms responsible for morphine-induced contralateral circling may reside in both the VTA and SNC; the degree of circling may thus be related to the number of cells that are stimulated.

The present study suggests that the VTA may be more importantly involved in the action of morphine-induced

circling than is the SNC. First, thirty-two of the thirty-three VTA sites supported morphine-induced circling whereas only sixteen of the thirty-two SNC sites supported circling. Second, morphine resulted in much higher circling rates when applied to the VTA than it did when applied to the SNC. Third, the latency to circle was shorter following VTA application than following SNC application.

It is unlikely that morphine-induced circling elicited from the VTA results from significant drug diffusion to the SNC. Injection sites that were as close as 0.2 mm from the midsaggital suture supported high circling rates with a relatively short onset compared to most morphine-induced circlers. If there were major diffusion of drug following VTA stimulation, then the drug should diffuse across the midline as well as into the SNC and this should reduce the contralateral bias of the animals with cannulae near the midline; this was not the case however. Furthermore, Broekkamp (1976) has estimated that the rate of diffusion of liquid morphine is on the order of about 1 mm per hour; crystalline application of drug should result in even slower spread of drug than following liquid injections. Some of the VTA sites associated with high circling rates were at least 1 mm away from the SNC; these subjects often demonstrated significant circling twenty minutes following morphine application however.

Morphine-induced circling elicited from the SNC had a latency of at least sixty minutes which suggests that spread of drug to the VTA may have accounted for SNC circling. There were a number of subjects whose cannulae were clearly within 1 mm of the VTA that demonstrated no circling following morphine application however, even at the end of the two hour test session. If spread of drug to the VTA could account for SNC circling then these subjects should have circled in response to morphine. Furthermore, there were at least two SNC subjects whose cannulae were located as far as 2.0 mm from the VTA and these subjects did circle after sixty minutes. Further work might more closely assess the role of the VTA in SNC circling by lesioning the A-10 dopamine cells and determining the response to unilateral morphine applied to the SNC.

The most parsimonious explanation is that both the VTA and SNC mediate morphine-induced circling and that it is the number of cells that are activated that determines both the rate of circling and the latency to circle. The VTA contains a large number of dopamine cells that are clustered around the interpenduncular nucleus. In contrast, the dopamine cells of the SNC lie in a thin layer and are less numerous than are the VTA cells (Fallon and Moore, 1978). The crystalline morphine would probably recruit a greater number of cells in a shorter time period within the VTA simply because the cells are more

numerous there, and this may reflect both the shorter latencies to circle as well as the higher circling rates.

Iwamoto et al. (1977) and Pert (1978) have both demonstrated that morphine applied unilaterlly to the SNC results in contralateral circling. In the Iwamoto study, as many as twelve circles per minute were reported as early as ten minutes following drug injection; the rats in this study however were anesthetized when stereotaxically injected with a liquid morphine solution. The author of the present study has noted that when the animals in the present study were implanted with cannula when anesthetized, they often circled as the anesthetic was wearing off, even in the absence of further drug treatment. Thus, the fact that Iwamoto's rats were anesthetized may serve as a potential confound; the confound is the possible interaction that may have occurred between the two drug treatments. Pert's rats circled at rates comparable with the present study, although Pert also used liquid injections. The spread of drug from liquid injections would not be similar to the spread following crystalline injections; hydraulic pressure might be expected to lead to greater and more rapid diffusion. Thus, the drug in both the Iwamoto et al. study (1977) and the Pert study (1978) may have spread to stimulate cells in the adjacent VTA.

It is unclear as to the mechanism of action of morphine-induced circling elicited from areas external to the A-9 or A-10

dopamine cells, however, Fallon et al., (1978) have demonstrated some fluorescence of dopamine cells in the SNR. Similarly, dopamine cells have been reported in the medial zona incerta and the medial lemniscus. Both of these sites supported some circling in the present experiment, although generally at low rates. Further work on the anatomical localization of morphine-induced circling from areas external to the A-9 or A-10 dopamine cells might be complemented with histofluorescence to determine if the dopamine-fluorescing cells correspond to sites eliciting morphine-induced circling.

PHARMACOLOGICAL CHARACTERISTICS OF MORPHINE-INDUCED CIRCLING

Experiment 1 - Naltrexone Challenge

It has been demonstrated that opiates have effects on the central nervous system that are independent of opiate receptor mechanisms. Such non-specific effects of opiates include influences on membrane stabilization and electrolytic balance as well as disturbances in pH (Bozarth, 1983). If morphine-induced circling is due to an action at the opiate receptor, and not due to some non-specific effect of the drug, then pretreating an animal with an opiate receptor antagonist prior to intracranial morphine should block morphine-induced circling. The present experiments were designed to assess whether circling following central morphine was specific to an action at the opiate receptor.

Methods

Sixty-five animals with cannulae aimed for either the VTA or SNC were found, in the anatomical localization study, to respond to unilateral morphine by circling contralateral to the side of injection. Ten of these subjects were chosen at random and served as subjects in the present experiment. The day following the last morphine treatment day, the animals were pretreated with naltrexone (3 mg/kg i.p.) one hour prior to central morphine application. The animals were then tested for two hours and the number and direction of circles were recorded every twenty minutes. These data were compared to the mean number of morphine-induced circles as averaged over the four morphine test days.

Results

Pretreatment with naltrexone blocked morphine-induced circling (see figure 4). A two-way analysis of variance demonstrated a main effect of treatment ($F(1,9)=42.12, p<.00001$), a main effect for trials ($F(5,45)=27.57, p<.00001$), and a treatment by trials interaction ($F(5,45)=13.2, p<.00001$). Post hoc tests revealed significant differences between morphine circling in naltrexone treated rats and untreated rats 40, 60, 80, 100 and 120 minutes after the injection.

Experiment II - Naloxone Challenge

The opiate receptor antagonist naloxone has been shown to have a fast rate of onset and a short duration of action (Ngai,

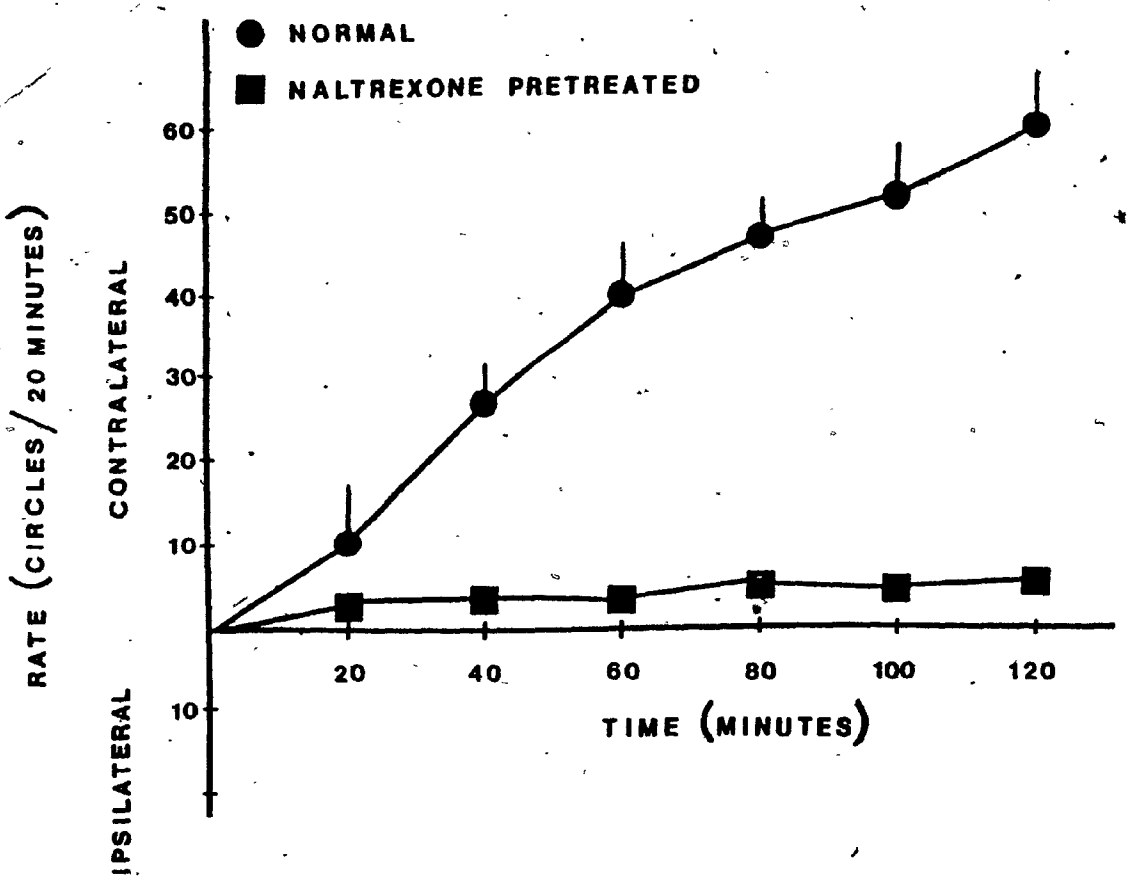

Fig. 4. The effects of naltrexone (3 mg/kg) pretreatment on morphine-induced circling.

Berkowitz, Yang, Hampstead and Spector, 1976). Treating an animal that is circling in response to central morphine with naloxone should reverse this activity if morphine-induced circling is specific to an action at the opiate receptor. The present experiment was designed to further assess whether morphine-induced circling was an opiate receptor specific effect.

Methods

Twelve other animals that demonstrated high circling rates following central morphine were used as subjects in the present experiment. On the last morphine test day the animals were challenged with naloxone (3mg/kg s.c.) two hours subsequent to central morphine. The animals were tested for a further twenty minutes and the number of circles was recorded.

Results

Naloxone completely disrupted morphine-induced circling (Figure 5). The animals were circling approximately sixty times in the last twenty minute period of the two hour test session. There was no circling activity registered at the end of the next twenty minute period.

Experiment III - Neuroleptic Challenge

Iwamoto et al. (1976) and Pert (1978) have both demonstrated that circling elicited by morphine injections in the SNC dopamine cell region is blocked by dopamine receptor antagonists. Furthermore, Bozarth (1983) has demonstrated that reward-

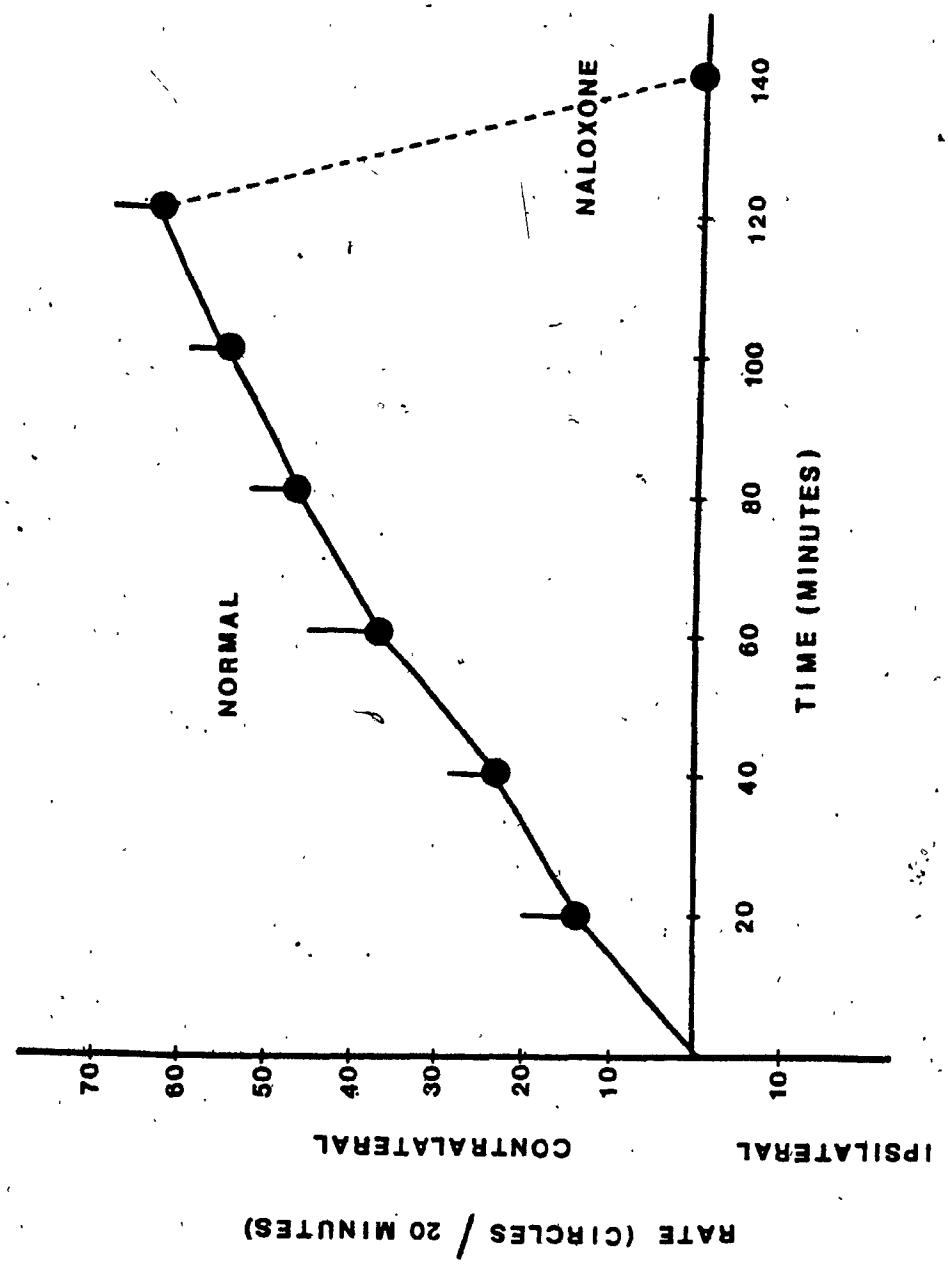

Fig. 5. The effects of naloxone (3mg/kg) challenge on morphine-induced circling.

ing microinjections of morphine are dependent upon a dopaminergic substrate. The present experiment was designed to assess whether morphine-induced circling elicited from the dopaminergic cell region could be blocked by a dopamine receptor antagonist.

Methods

Ten more of the animals that demonstrated strong circling in response to unilateral morphine were used as subjects in the present experiment. The day following the last morphine treatment day, these animals were pretreated with pimozide (0.5 mg/kg i.p.) four hours prior to central morphine; the circling was recorded every twenty minutes for a two hour test session.

Results

Pimozide blocked morphine-induced circling (see Figure 6). A two-way analysis of variance demonstrated a main effect for treatment ($F(1,9)=32.73$, $p<.01$), a main effect for trials ($F(5,45)=10.49$, $p<.01$) and a treatment by trials interaction ($F(5,45)=10.80$, $p<.01$). Post hoc tests revealed significant differences between morphine-induced circling in pimozide treated rats and non treated rats at 40, 60, 80, 100 and 120 minutes after morphine application.

Discussion

Morphine-induced circling is an effect specific to the opiate receptor and is not due to some non-specific effect of the drug such as a disturbance in pH or an influence in membrane

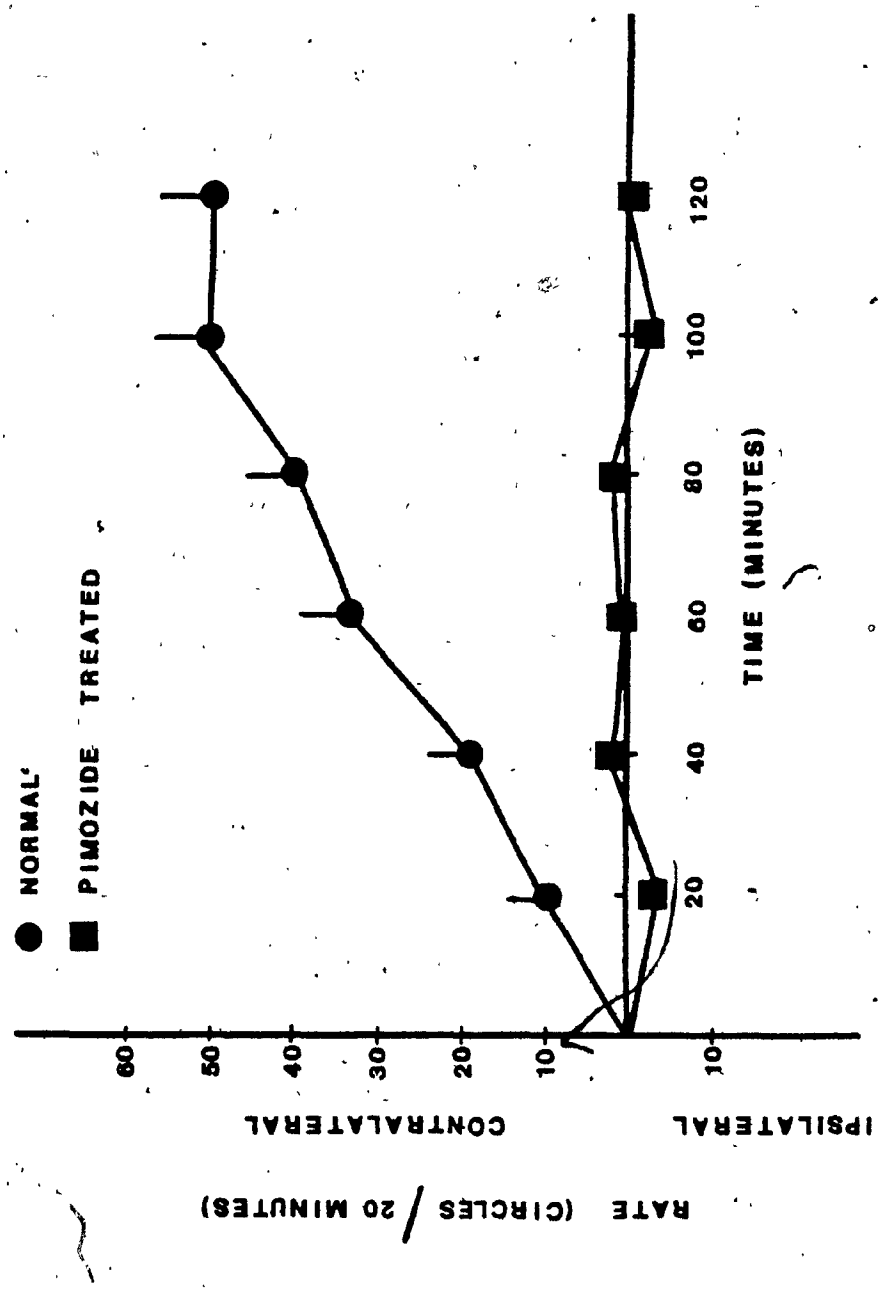

Fig. 6. The effects of pimozide (0.5 mg/kg) pretreatment on morphine-induced circling.

stabilization or osmolarity, since naltrexone and naloxone each blocked the effect. Morphine-induced circling is also dependent upon activation of a dopaminergic substrate since pimozide blocked the response. It should be noted that the animals treated with 0.5 mg/kg of pimozide appeared to move about freely and did not resemble akinetic animals; indeed, there was no obvious behavioural difference between pimozide treated animals and normal animals when both groups were placed in a bucket together.

PHENOMENOLOGICAL CHARACTERISTICS OF MORPHINE-INDUCED CIRCLING

A distinction between 'circling' and 'pivoting' (Teitelbaum et al., 1982) has been made based on the type of limb movements made by the animal. Circling is directionally-biased forward locomotion with all four limbs moving forward; pivoting is directionally-biased movement without the forward locomotion (Teitelbaum et al., 1982). The type of limb movements determine both the distance travelled by the animal and the degree of postural asymmetry in the longitudinal axis of its' body. In order to determine the type of movements elicited by unilateral morphine injections, the following experiments were designed.

Experiment one - Open Field Observations of Morphine-Induced Circling

Methods

Animals that were circling in response to morphine were removed from the buckets after the two hour test period and placed in an open field that measured 60 cm by 54 cm with 15 cm walls. The type of limb movements made by the animals, as well as the degree of postural asymmetry and the size of the circles were noted.

Results

Unilateral morphine applied to the dopamine cell region results in forward locomotion with turning being predominantly to the side contralateral to the morphine application. There was little postural asymmetry in the longitudinal axis of the animals' body and all four limbs moved forward. The size of the circles being described depended upon the size of the enclosure in which the animal was placed; the animals moved around the perimeter of either the test box or the open field, turning at the corners or with the curve of the perimeter.

The behaviour of the animals in the open field following unilateral morphine was consistent between subjects. When the animals were placed in the centre of the open field, they did not demonstrate clear movement asymmetries initially, but rather, appeared to locomote normally, exploring their environment. As soon as the animals encountered one of the walls of the enclosure however, they began to follow the perimeter of the enclosure in the direction contralateral to the side of morphine

application (Figure 7). The movements appeared environmentally elicited rather than forced. For example, if an object was placed in the animals' path, the animal would stop, explore the object, and then continue around it. As the animal encountered one of the corners of the enclosure it showed vertical and lateral scanning movements, behaviours that are consistent with exploration (Teitelbaum et al., 1982), before turning and continuing.

Experiment II - A Comparison of Morphine-Induced Circling with Muscimol-Induced Circling

There is now substantial evidence that a descending pathway containing GABA arises in the striatum and terminates in the pars reticulata of the substantia nigra (Ribak, 1981). The unilateral manipulation of this pathway and its' efferents results in circling that is resistant to dopamine receptor blockade (Oberlander, Dumont and Boissier, 1977; Scheel-Kruger, Arnt and Magelund, 1977). This striatonigral GABA pathway is thought to serve as a major output pathway for behaviour and motor patterns arising in the striatum (Di Chiara, Porceddu, Imperato and Morelli, 1981). Lesions of the striatonigral GABA pathway reduce or abolish the contralateral circling elicited by apomorphine in the unilateral 6-OHDA model (Marshall and Ungerstedt, 1977).

It has been reported that unilateral application of

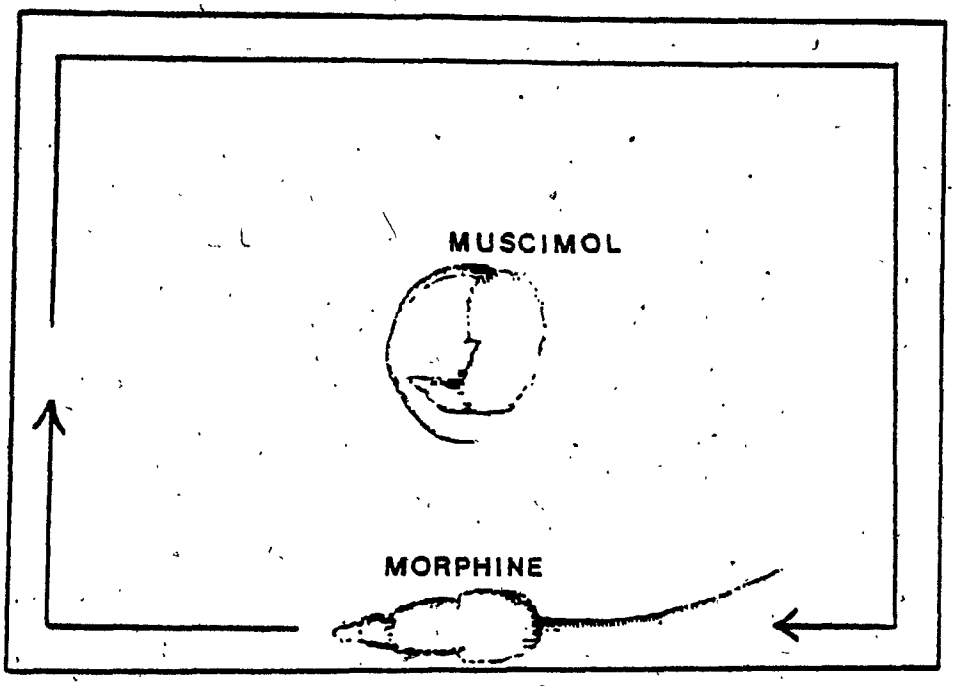

Fig. 7. A diagrammatic representation of circling in the open field elicited by either morphine or muscimol.

GABAergic agonists into the SNR results in very high circling rates (Scheel-Kruger et al., 1977); the circling is accompanied by a tight postural asymmetry.

The present experiment was designed to compare morphine-induced circling to GABA-induced circling in an attempt to determine the qualitative as well as quantitative differences in circling elicited by these drugs.

Methods

Twenty animals that demonstrated high circling rates in response to unilateral morphine were used in the present experiment. Animals were tested the day following completion of testing with morphine. The animals were pretreated with pimozide (0.5 mg/kg i.p.) and four hours later the injector cannula was loaded with crystalline muscimol, a GABA agonist, and placed in the guide cannula. The animals were tested for twenty minutes. At the end of the twenty minute test period, animalswere placed in the open field. The type of movements made by the limbs of the animal, as well as the size of the circles and the degree of postural asymmetry were noted. The animal's reaction to environmental objects placed in its' path of travel were also noted.

Results

Crystalline muscimol induced strong contralateral circling that was resistant to dopamine receptor blockade (Figure 8). Such

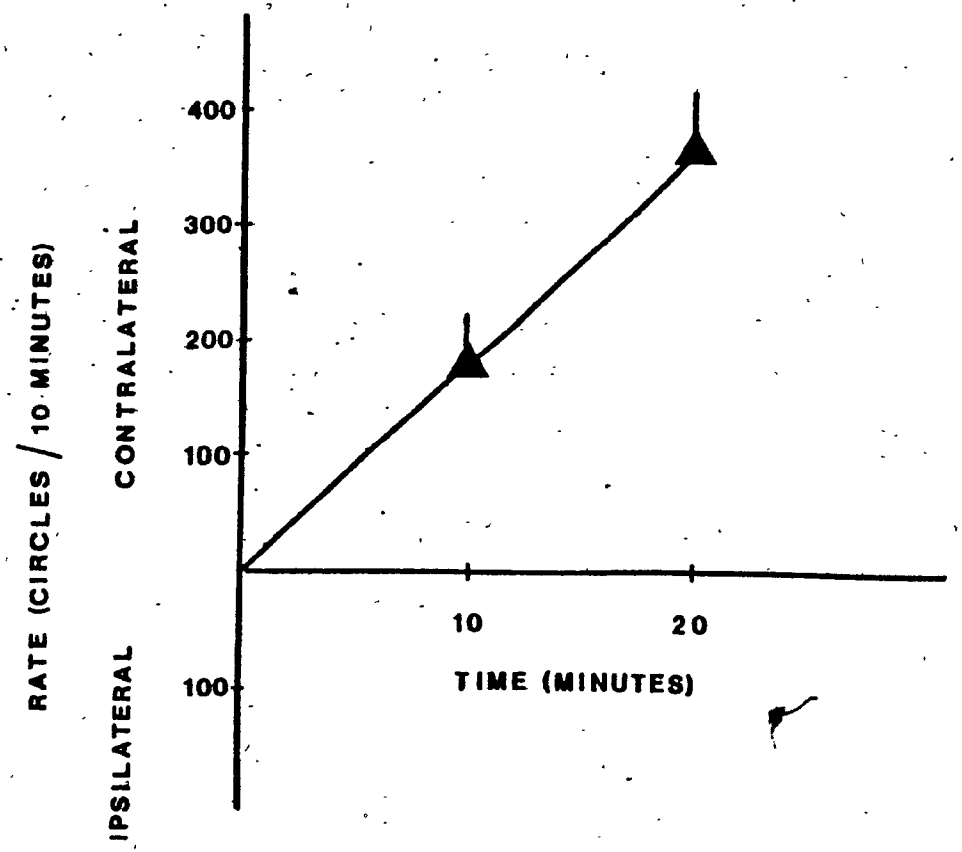

Fig. 8. Muscimol-induced circling following pimozide (0.5 mg/kg) pretreatment.

activity was observed following application of muscimol to placements in either the VTA, the SNC or the SNR (Figure 9). The type of movements made by muscimol-treated animals was qualitatively different from morphine-induced movements. First, the animals hind limbs were not moving forward; rather, the hind limb contralateral to the side of injection was engaged in backward stepping movements while the hind limb ipsilateral to the side of injection served as a pivot. The animal thus remained in a fixed position both in the rotometers and in the large open field. There was a very tight postural asymmetry accompanying muscimol-induced circling such that the size of the circles being described by the animals was little more than the length of its' body (see Figure 7). The animal appeared unresponsive to environmental stimuli; when a food pellet, a block of wood or even the experimenter's foot was placed in the animal's path of travel, the animal simply continued circling.

Discussion

Circling can be either stimulus-directed or stimulus-independent. For example, if an animal with a unilateral sensory neglect or a unilateral sensory enhancement were placed in an environment capable of eliciting exploratory responses, the animal might engage in directionally-biased exploration; the direction of the bias would be towards the visual field experiencing the stronger sensory reception.

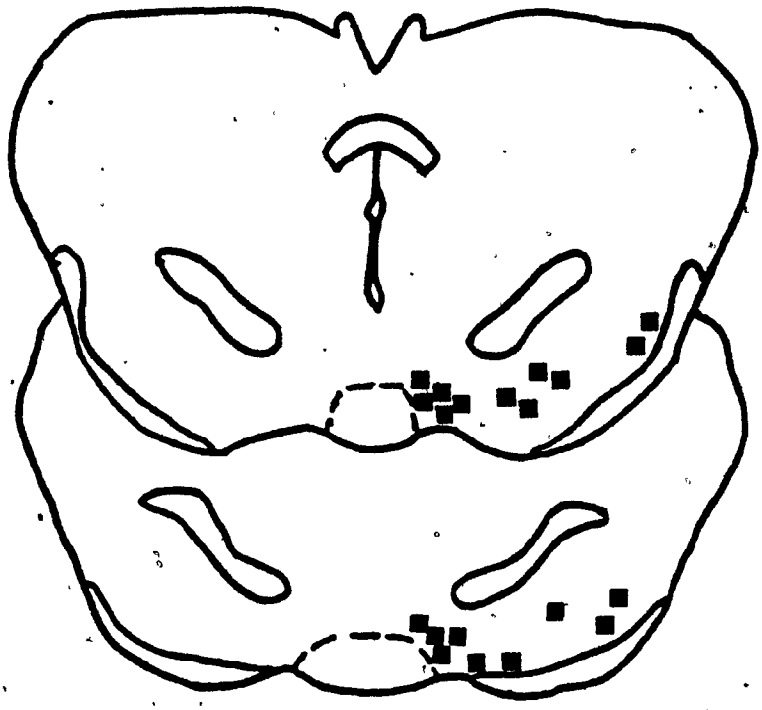

Fig. 9. Histological reconstruction of cannula placements tested for muscimol-induced circling

Such directionally-biased exploratory responses would probably result in directionally-biased movements; these movements would thus be stimulus-directed. The size and number of circles be made by the animal would likely depend on the environment in which the animal is placed. A large enclosure would encourage large diameter circles, presuming the animal explores all parts of its environment. Such an enclosure would also probably elicit fewer net circles than would a small enclosure; it takes more time to travel a large area than a small one. Finally, the number of stimulus objects might also influence the rate of circling. An environment with few stimulus objects might elicit many more circles than would a stimulus-rich environment; the distribution of time spent among the many environmental objects would reduce the animal's net movement time.

Stimulus-independent circling would be internally-generated. This type of circling might result from the unilateral activation of motor output programs; such circles would thus be 'forced' or 'driven' movements. The size of the circles would likely be constant for a given animal and not vary according to the size of the environment. The number of circles might depend on how fast the motor programs are being driven. Animals engaging in internally-generated circles would be relatively unresponsive to environmental

stimuli.

Morphine-induced circling seems to be stimulus-directed since the size of the circles made by the animals depend upon the size of the enclosure; furthermore, the animals demonstrate clear exploratory responses as reflected in both the animal's body movements (i.e. forward limb movements, lateral, vertical and horizontal scans) and its' responses to environmental stimului (i.e. sniffing, oral contact). The fact that this behaviour is dependent upon dopaminergic activation fits well with this suggestion. For example, it is well known that an animal with a unilateral nigrostriatal dopamine lesion exhibits a unilateral sensory neglect in addition to the extensively studied movement asymmetries (Ljungburg and Ungerstedt, 1979). As might be expected, the animal circles away from the visual field that is neglected, turning towards objects in the effective visual field. Thus asymmetrical movements and asymmetrical sensation may be closely related. Recent evidence would support this suggestion. For example, an animal treated with low doses of apomorphine engages in forward locomotion (Teitelbaum et al., 1982). Such locomotion includes snout-to-ground exploration. If the sensory input to the snout is unilaterally blocked by a local anesthetic, the animal's locomotion changes to circling and the direction is towards the side of the normal

sensory snout stimulation (Szechtman, as cited in Teitelbaum et al., 1982). Dopamine-induced lesions may thus operate to create sensory deficits. Morphine applied unilaterally to the dopamine cell region increases dopaminergic activity which should presumably create a sonsory enhancement on the side of the body contralateral to the side of injection. This fits with the direction of circling seen in the present study.

GABA-induced circling, on the other hand, appears to be a forced type of circling that is relatively independent of environmental objects and events. This suggestion also fits well with the literature. For example, it has been demonstrated that animals with a unilateral electrolytic lesion of the substantia nigra demonstrate both a contralateral sensory neglect (presumably reflecting nigro-striatal dopamine damage) and a strong spontaneous contra-lateral circling bias (reflecting striatonigral output activity) (Schallert, Upchurch, Lobaugh, Farrar, Spirduso, Gilliam, Vaughn and Wilcox, 1982). Thus, while dopamine dependent circling is environmentally elicited, GABA induced circling is not. The present set of experiments support this idea.

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