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Dissociation of the Anatomical, Pharmacological and Phenomenological Characteristics of Circling Elicited By Morphine and Muscimol Applied to the Ventral Mesencephalon

Larry John Holmes

A Thesis

in

The Department

of

Psychology

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ABSTRACT

Dissociation of the Anatomical, Pharmacological and Phenomenological Characteristics of Circling Elicited by Morphine and Muscimol Applied to the Ventral Mesencephalon

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Concordia University, 1986

A series of experiments was undertaken in order to establish whether circling elicited by unilateral muscimol injections into the ventral mesencephalon had similar anatomical, pharmacological and phenomenological characteristics to circling elicited by unilateral morphine injections to the same regions as reported in earlier studies. Muscimol (25, 50 and 100 ng) was applied unilaterally to sites in the ventral mesencephalon in rats. It caused contraversive or ipsiversive circling, depending on the site of injection. Muscimol induced strong contraversive circling when applied to the substantia nigra pars reticulata (SNR) and weak ipsiversive circling when applied to some parts of the SNC; only the ipsiversive circling was blocked by pimozide (0.25, 0.50 and 1.0 mg/kg). There was very little contraversive circling noted when injections sites were located outside of the central SNR; when circling was recorded from extra-nigral sites, it was only with the highest doses of muscimol. The phenomenological characteristics of muscimol-induced contraversive circling were different from those of morphine-induced contraversive circling in several respects. First, in contrast to morphine-induced

circling, muscimol-induced circling was accompanied by a strong postural asymmetry (even when the doses were adjusted to produce similar rates of circling). Second, muscimol-induced circlers reared less often than morphine-induced circlers but engaged in more stereotyped biting responses. Finally, whereas the direction of morphine-induced circling depended upon environmental contingencies, the direction of muscimol-induced circling did not. These studies suggest that three types of circling can be elicited from stimulation of the ventral mesencephalon. Morphine-induced contraversive circling is thought to activate dopaminergic cells, muscimol-induced ipsiversive circling is thought to inhibit dopaminergic cells and muscimol-induced contraversive circling is thought to result from activation of GABAergic systems which seem to serve as output pathways mediating dopamine-induced circling.

This thesis is dedicated to my parents Jack and Marilyn

and to my sisters, Sharon, Gail, Jane, Joy, Nancy and Kathryn

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INTRODUCTION

Frame of Reference

In 1873 Ferrier demonstrated that unilateral electrical stimulation of the caudate nucleus in dogs caused a contralateral postural asymmetry bringing the head of the dog in close proximity to its tail (Pycock, 1980); when these animals moved forward, they moved with a directional bias. Since Ferrier's time numerous reports have appeared which suggest that unilateral stimulation of many different central sites cause directionally-biased movements in a variety of species (Forman and Ward, 1957; White and Himwich, 1957; Barnett and Goldstein, 1975, Slater and Lee, 1980). These directionally-biased movements have become known as 'circling behaviour' and they have proved to be a useful behavioural assay for quantifying movements elicited by basal ganglia stimulation.

While the circling model has provided investigators with much information with respect to basal ganglia mechanisms of movement, the phenomenon, in and of itself, remains somewhat of a mystery. There have been few reports investigating the psychological concommitants associated with circling (but see Szechtman, 1983; Holmes and Wise, 1985a). It is likely that asymmetrical movements, like symmetrical ones, can occur for a variety of reasons. For example, recent work has demonstrated that whereas some directionally-biased movements are determined

by sensory stimuli (Szechtman, 1983; Holmes and Wise, 1985a), other directionally-biased movements seem sensory-independent (Holmes and Wise, 1985a). It makes sense that movements might take qualitatively different forms since the central nervous system mechanisms of movement are hierarchically organized; stimulation of different levels might produce different types of movements.

Recent work in this laboratory has demonstrated that animals will learn a lever-pressing response for unilateral morphine injections into the ventral tegmental area (VTA), the origin of mesolimbic/mesocortical dopamine cells. Interestingly, these animals engage in directionally-biased movements between bouts of lever-pressing (Bozarth, 1983). Close behavioural observations of these asymmetrical movements have demonstrated that they consist of forward locomoting responses that are environmentally-determined (Holmes, Bozarth and Wise, 1983; Holmes and Wise, 1985a).

In an attempt to analyze the pharmacological characteristics of morphine-induced circling elicited from the ventral mesencephalon, circling rates have been compared between rats injected with morphine following neuroleptic pretreatment and rats injected with muscimol following neuroleptic pretreatment. In addition to pharmacological differences between these two behaviours, it was found, serendipitously, that these agents also produced strikingly different circling behaviours (Holmes and Wise, 1985a). Whereas morphine-induced circling involved

a forward locomoting response that was environmentally-dependent, muscimol-induced circling appeared 'forced' and stimulus-independent. It could not be concluded however, that these qualitative differences were not simply due to differential circling rates since muscimol-induced circling was ten to twenty time faster than morphine-induced circling. These initial observations did however, lead to a testable hypothesis that such differences might exist, even when the rate of circling was equated.

The present experiments were designed to test the hypothesis that qualitatively different types of circling behaviour could be elicited by stimulation of two different chemical systems (dopaminergic and GABAergic) within the same brain region when rate of circling was equated. In addition, the anatomical boundaries mediating muscimol-induced circling and the pharmacological profile of this behaviour was explored so that comparisons could be made with similar observations on morphine-induced circling that were reported earlier (Holmes and Wise, 1985a). It was felt that such comparisons were warranted because knowing whether or not circling elicited by muscimol and morphine were anatomically, pharmacologically and phenomenologically differentiable would be important to the understanding of circling in general as well as to the understanding of basal ganglia mechanisms of movement.

Phenomenology of Circling

Circling has been used as a general term to describe

directionally-biased movements in animals. It has been used synonymously with several other terms; these include rotating, pivoting, turning, swivelling, circus movements and circumambulation. While these terms do not necessarily reflect the same, single function, in the present thesis circling will be used to describe all such movements reported in the literature.

The direction of circling is generally described in relation to the side of the brain that was manipulated. Thus, if an animal moves away from the side that was manipulated, investigators speak of contraversive circling. Movements toward the side of manipulation are labelled ipsiversive circling. These terms are used interchangeably with contralateral and ipsilateral. If circling is caused by peripheral injections of drugs in an intact animal, investigators speak of right and left side circlers or clockwise and counterclockwise circlers.

It has been suggested that at least two components underly the circling response: a postural component and a locomotor component (Kelly and Moore, 1977; Pycock and Marsden, 1978). This idea suggests that animals have a curvature in the longitudinal axis of their body (postural) which gets translated into circling when the animals move forward (locomotion). There are some studies which have reported that circling is accompanied by a 'strong' postural asymmetry (i.e. Arnt and Scheel-Kruger, 1979a), while others have reported that circling is accompanied by only a

'mild' postural asymmetry (i.e. Roffman, Bernard, Dawson, Sobiski and Shelens, 1978). Circling can also occur in the absence of any postural asymmetry (Holmes et al., 1983; Holmes and Wise, 1985a; 1985b) except when the animal encounters a corner. It is as yet unclear what neural mechanisms mediate these differences.

Circling can be described in terms of the size and number of circles being made by an animal. For example, some animals make very small circles that are little more than the length of their body ('tight' circles); in general, these animals circle very fast (i.e. Arnt and Scheel-Kruger, 1979a). Other animals can make large diamter circles ('wide circles') (i.e. Roffman et al., 1978); usually animals making large circles make fewer of them in the same time period (i.e. Holmes and Wise, 1985b).

Circling can also be differentiated in terms of the types of limb movements that the animals make. Their are several potential ways that an animals could use its limbs while moving with a directional bias. Two of these types have been reported in the circling literature. In one case, all four limbs move in a forward direction (although there is a bias in the direction of forward movement) (Holmes et al., 1983; Holmes and Wise, 1985a; 1985b). In the other case, the hind limb ipsilateral to the direction of movement steps backwards while the other hind limb serves as a pivot; the forelimbs of the animal generally move foreward (Teitelbaum, Szechtman, Sirkin and Golani, 1982). These animals thus

remain in a relatively fixed location in the test environment.

A review of the literature suggests that the size and number of circles made by an animal may be related to both the postural asymmetry accompanying the movements as well as to the limb movements made by the circling animals. For example, in some studies that report fast circling, it is noted that animals move in 'tight' circles and are strongly posturally asymmetric (i.e. Arnt and Scheel-Kruger, 1979b; Kilpatrick and Starr, 1981); sometimes a pivoting action of the limbs is noted (Teitelbaum, et al., 1982). In other studies where circling is slow, animals make large-diameter circles and have little postural asymmetry. These animals generally move all four limbs forward (Holmes et al., 1983; Holmes and Wise, 1985a; 1985b). While it has been unclear whether these factors (i.e. postural asymmetry, particular limb movements, size and speed of circling) necessarily coexist, at least two laboratories have demonstrated that postural asymmetries can occur independent of the direection of circling and that circling can occur independent of postural asymmetries. Thus, when some intact animals are injected with apomorphine, the side of postural bias and the direction of circling are not correlated (Szechtman and Pisa, 1984). Conversely, unilateral morphine or neurotensin injections into the VTA cause circling with essentially no postural asymmetries (Holmes et al., 1983; Holmes and Wise, 1985a; 1985b).

At least some types of circling are sensory-dependent movements. For example, when animals are given moderate dose systemic injections of apomorphine, they engage in forward progression that includes snout-to-ground exploration (Teitelbaum et al., 1982). If the sensory input to the head is unilaterally eliminated by bandaging one-half of the animal's head, the forward progression following apomorphine injections becomes directionally-biased (Szechtman, 1983). Furthermore, in some intact animals that are systemically injected with dopaminergic agonists, the direction of circling is dependent upon particular environmental cues (Pisa and Szechtman, 1984). Unilateral application of morphine (Holmes et al., 1983; Holmes and Wise, 1985a) or neurotensin (Holmes et al., 1985b) to the VTA in rats also causes environmentally-directed circling since the size of the circles depends upon the size of the test enclosure. The direction of morphine-induced circling elicited from the VTA also depends upon environmental contingencies (Wise and Holmes, in press). It has not been established whether all types of circling are sensory-dependent.

Pharmacological Basis of Circling

The earliest mechanistic interprettation of circling was that it resulted from an interhemispheric imbalance in brain dopamine function. According to this hypothesis, if there is a net difference in the functional level of brain dopamine activity on

the two sides of the brain the animal will tend to circle (Ungerstedt, 1971a; 1971b). While some animals have been shown to have natural asymmetries in brain dopamine activity (Glick, Zimmerberg and Greenstein, 1976), most investigators experimentally manipulate these systems to create an interhemispheric dopaminergic imbalance. Thus, investigators either unilaterally destroy the ascending dopamine neurons or unilaterally activate them.

The most popular method used to create a unilateral denervation of the ascending dopamine system is the six-hydroxydopamine (6-OHDA) lesioning technique. Six-OHDA selectively destroys catecholamine-containing neurons (Ungerstedt, 1968). Unilateral injections of 6-OHDA into the dopaminergic cells or terminal regions destroys these cells in the ipsilateral hemisphere while other neurotransmitter systems remain relatively intact (Pycock, Tarsey and Marsden, 1975; Costall, Marsden, Naylor and Pycock, 1976).

Immediately following a unilateral 6-OHDA lesion, animals show a marked postural asymmetry; in this case, the head and tail deviate ipsilaterally (towards the side of the lesion) while the contralateral limbs are in an extended position (Ungerstedt, 1971a). For the first twenty-four hours following the lesion, the postural asymmetry is tonic, that is, head movements in the opposite direction are never seen. Two days following the lesion, the postural asymmetry is not apparent while the animals are at rest; stimuli which disturb the animal however (i.e. tail

pinch) effectively reinstate the asymmetrical postrue which becmoes circling as the animal moves forward (Ungerstedt, 1971a). Interestingly, these same 6-OHDA lesions, in addition to causing postural and movement asymmetries, produce sensory neglect towards stimuli presented on the side of the body contralateral to the lesion (i.e. Ljungberg and Ungerstedt, 1976; Marshall, 1979); no sensory neglect on the side of the body ipsilateral to the lesion is apparent.

In animals with a unilateral 6-OHDA-induced lesion of the dopaminergic pathways, drugs which increase dopaminergic activity cause asymmetrical movements (i.e. Anden, 1970; Christie and Crow, 1971; 1973; Ungerstedt, 1971a; 1971b; Von Voigtlander and Moore, 1973; Pycock et al., 1975). This is true whether the lesion is placed at the level of the dopamine cell bodies, the fibers of passage or the terminal region (Crow, 1971). The number and direction of circles is a function of the dose of the drug and the time since the lesion (Ungerstedt, 1971b). Generally, however, when amphetamine (a presynaptic dopaminergic agonist) is injected to rats with a unilateral 6-OHDA lesion, animals circle towards the side of the lesion (ipsilateral) (i.e. Anden, 1970; Christie and Crow, 1971; 1973; Ungerstedt, 1971a, Von Voigtlander and Moore, 1973; Pycock et al., 1975; Costall et al., 1976). Amphetamine causes animals to turn towards the side of the lesion because the nerve terminals on this side of

the brain have been denervated; thus only the contralateral dopamine system is activated. Apomorphine (a direct acting dopaminergic agonist), on the other hand, causes either ipsiversive or contraversive circling, depending on the location of the lesion and the time since the lesion. Thus, when the dopamine terminal region is destroyed, apomorphine causes ipsiversive circling presumably because there would be more dopaminergic receptors in the intact hemisphere (Anden, 1970). Apomorphine causes contraversive circling however, when the 6-OHDA lesion is placed at the level of the dopamine cell bodies or fibers (Ungerstedt, 1971b; Mendez, Cotzias, Finn and Dahl, 1975; Anlezark, Pycock and Meldrum, 1976; Costall et al., 1976) apparently because of unilateral dopamine receptor supersensitivity (Ungerstedt, 1971b); this renders the lesioned side of the brain more effective upon apomorphine administration. This contraversive circling following apomorphine injections occurs on the second postoperative day and later; it is presumed that this reflects the process of neuronal degeneration following the 6-OHDA lesions (Ungerstedt, 1971b). Both amphetamine-induced ipsiversive circling and apomorphine-induced contraversive circling are dopamine receptor specific effects since the behaviour is blocked by treatments which disrupt dopaminergic functioning (Ungerstedt, 1971a; 1971b; Pycock, Donaldson and Marsden, 1975; Christie and Crow, 1973; Von Voigtlander and Moore, 1973; Nakamura, Engel and Goldstein, 1978).

In addition to amphetamine and apomorphine, other presynaptic and postsynaptic agents have been tested in the unilateral 6-OHDA model of circling. The presynaptic agents include methylamphetamine (Christie and Crow, 1971), ephedrine (Christie and Crow, 1971; Boulu, Rapin, Lebas and Jacquet, 1972) and methylphenidate (Von Voigtlander and Moore, 1973). Other postsynaptic agonists include N-propyl-norapomorphine (Costall, Naylor and Neumeyer, 1975; Mendez, Cotzias, Fihn and Dahl, 1975; Neumeyer, Dafeldecker, Costall and Naylor, 1977), diacetylapomorphine (Baldessarini, Walton and Borgman, 1976), diisobutyryl apomorphine (Tye, Horsman, Wright, Large and Fuller, 1977) and piribadel (Costall and Naylor, 1974a; Thornburg and Moore, 1974). Each of these agents induces circling in the direction predicted by the dopaminergic imbalance model, that is, towards the side of the lesion following injection of indirect acting agonists and away from the side of the lesion following injection of direct acting agonists.

Unilateral stimulation of brain regions that include dopaminergic cells also causes circling. Electrical stimulation results in circling when the tip of the electrode is aimed at the dopamine cell body region (Arbuthnott, Crow, Fuxe and Ungerstedt, 1970; Arbuthnott and Crow, 1971; Arbuthnott and Ungerstedt, 1975; Roffman et al., 1978, Vaccarino and Franklin, 1982a; 1982b; Gratton and Wise, 1984), the medial forebrain bundle (Arbuthnott

and Ungerstedt, 1975) and the striatum (i.e. Zimmerberg and Glick, 1974); in general, this circling is tight and accompanied by a clear head deviation (Pycock, 1980). While the direction of circling elicited from electrical stimulation of the medial forebrain bundle and striatum is contralateral to the side of stimulation, the direction of circling elicited from electrical stimulation of the cell body region is either contralateral or ipsilateral, depending on both the exact location of the electrode (Vaccarino and Franklin, 1982a; 1982b; Gratton and Wise, 1985) and the stimulation parameters used to elicit the circling (Gratton and Wise, 1985). While electrically-induced circling may not necessarily result from direct activation of dopaminergic neurons (i.e. Gallistel, Shizgal and Yeomans, 1981), at least some studies have demonstrated indirect activation since contraversive circling induced by electrical stimulation in the dopamine cell body region is accompanied by release of dopamine in the striatum (Arbuthnott and Crow, 1971; Von Voigtlander and Moore, 1971). Furthermore, at least some behavioural evidence has demonstrated that electrically-induced circling is dependent upon the integrity of these neurons since neuroleptics block the behaviour (Roffman et al., 1978; Vaccarino and Franklin, 1982a).

Unilateral application of chemical agents that are known to increase dopaminergic neurotransmission also cause circling when applied to either the dopaminergic cells or terminal regions.

Thus dopamine applied to the dopaminergic terminal fields in either crystalline form (Ungerstedt, Butcher, Butcher, Anden and Fuxe, 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 contraversive circling that is blocked by dopamine receptor antagonists (Ungerstedt et al., 1969). Similarly, unilateral application of amphetamine or methylphenidate (Costall and Naylor, 1974b; McKenzie, Gorden and Viik, 1972) also elicits contraversive circling when injected into dopaminergic terminal regions. Contraversive circling has also been observed following the unilateral application of either morphine (Iwamoto and Way, 1977; Pert, 1978; Holmes et al., 1983; Holmes and Wise, 1985a) or neurotensin (Holmes and Wise, 1985b) into the dopaminergic cell region, two chemical agents that are thought to increase dopaminergic activity in other behavioural (i.e. Joyce and Iversen, 1979; Vezina and Stewart, 1984; Kalivas, Burgess, Nemeroff and Prange, 1983), biochemical (Kalivas et al, 1983) and electrophysiological (Matthews and German, 1983) studies. Morphine and neurotensin-induced circling are each blocked by pimozide pretreatment (Holmes et al., 1983; Holmes and Wise, 1985a; 1985b).

There are several studies reporting that normal, intact animals will sometimes circle, although at lower rates, when either indirect or direct acting dopaminergic drugs are

administered peripherally (i.e.Jerussi and Glick, 1976; Glick et al., 1976; Glick, Jerussi, Cox and Fleisher, 1977; Glick, Hinds and Shapiro, 1983; Szechtman, 1983). Amphetamine, apomorphine, cocaine and l-dopa all induce circling in some intact rats; furthermore, there appear to be sex-dependent differences in the direction of circling (Glick, Hinds and Shapiro, 1983). As in lesioned rats the direction is consistent for some intact rats. When tested on several different occassions, some rats consistently circle to the right and some rats consistently circle to the left. Haloperidol antagonizes the circling response in these animals (Jerussi and Glick, 1976; Glick et al., 1977). The phenomenon of circling in normal animals has led to the hypothesis that there are sometimes normal intrinsic asymmetries in brain dopamine concentrations. Subsequent work has demonstrated such asymmetries (Jerussi and Glick, 1976).

While the traditional view of circling suggested that a dopaminergic imbalance in the two hemispheres produced asymmetrical movements in animals (i.e. Ungerstedt, 1971a; 1971b), a number of subsequent studies demonstrated that the unilateral manipulation of other neurotransmitter systems could also produce these movements. One of the major inhibitory transmitters implicated in these phenomena is gamma-amino-butyric-acid (GABA); brain areas containing high levels of dopamine also contain high levels of GABA (Okada, Nitsch-Hassler, Kim, Bak and Hassler, 1971).

Since the ventral mesencephalon is rich in inhibitory GABAergic neurons in close proximity to the ascending dopaminergic neurons, , it was originally thought this neurotransmitter served as a modulator of dopaminergic neuronal activity. GABA, within dopamine-rich areas, was thought to be contained primarily in terminals of feedback pathways (Precht and Yoshida, 1971; Kim, Bak, Hassler and Okada, 1971). This 'feedback' hypothesis was supported by early pharmacological (Crossman, Walker and Woodruff, 1973) and electrophysiological studies (Bunney and Aghajanian, 1976); thus, iontophoretically applied GABA into ventral mesencephalic regions was shown to cause decreases in dopaminergic cell firing (Crossman et al., 1973; Aghajanian and Bunney, 1975).

In its simplest form, the GABAergic feedback hypothesis predicted that unilateral application of GABAergic agonists would produce dopamine-dependent ipsiversive circling whereas similar application of GABAergic antagonists would produce dopamine-dependent contraversive circling. GABAergic agonists were predicted to cause ipsiversive circling because injection of these agents into the dopaminergic cell region should inhibit dopaminergic activity ipsilaterally thus rendering the dopamine activity in the contralateral hemisphere more effective. GABAergic antagonists, on the other hand, were presumed to cause the opposite phenomenon.

Initial work investigating the direction of circling following

unilateral GABAergic manipulations supported the inhibitory feedback hypothesis. Thus, unilateral application of picrotoxin, a GABAergic antagonist, was shown to evoke contraversive circling that was dependent upon the integrity of dopaminergic neurons since prior 6-OHDA lesions eliminated the response (Tarsy, Pycock, Meldrum and Marsden, 1975). Conversely, elevation of GABA concentrations in one hemisphere following microinjections of ethanolamine-O-sulphate (EOS) (a GABA transaminase inhibitor) caused ipsiversive circling (Dray, Fowler, Oakley and Simmonds, 1975a; 1977; Dray, Oakley and Simmonds, 1975b; Horton and Pycock, 1977). These observations complemented the electrophysiological (Bunney and Aghajanian, 1976) studies which demonstrated a GABAergic inhibitory influence on dopamine activity.

While the GABAergic feedback hypothesis received strong electrophysiological (Aghajanian and Bunney, 1974) and behavioural (i.e. Dray, Oakley and Simmonds, 1975; Dray, Fowler, Oakley, Simmonds and Tanner, 1977; Horton and Pycock, 1977) support, a number of studies began to appear which seemed to seriously challenge the simple form of this hypothesis. For example, several studies appeared which demonstrated that unilateral injections of the potent GABA agonist muscimol induced strong, dose-dependent, head-to-tail contraversive circling (Oberlander, Dumont and Boissier, 1977; Scheel-Kruger, Arnt and Magelund, 1977; Martin, Papp and Bacino, 1978). Conversely, the unilateral application of picrotoxin or

bicuculline (GABAergic antagonists) was shown to produce ipsiversive circling (Olpe, Schellenberg and Koella, 1977; Scheel-Kruger et al., 1977; James and Starr, 1978; Olianas, De Montis, Mulas and Tagliamonte, 1978). The circling activity elicited by both the GABA agonists and antagonists seemed independent of dopaminergic functioning since the behaviour still occurred following neuroleptic treatment (Olpe et al., 1977; Olianas et al., 1978; Reavill, Leigh and Marsden, 1979), blockade of presynaptic catecholaminergic transmission by prior treatment with alpha-methyl-para-tyrosine or reserpine (Scheel-Kruger et al., 1977; Arnt and Scheel-Kruger, 1979a) or following ablations of the telencephalon (Papadopoulos and Huston, 1980).

A reconciliation of the conflicting reports concerning both the direction of circling elicited by GABAergic agents and its dependence on dopaminergic functioning became evident when detailed analysis of the specific GABA injection sites within the ventral mesencephalon were undertaken. Thus, injection of GABAergic agents into either the dorsal parts of the ventral mesencephalon (where the dopaminergic cells are located) or the ventral parts (where non-dopaminergic neurons are located) produce opposite directions of circling (James and Starr, 1978; Arnt and Scheel-Kruger, 1979b; Reavill, Jenner, Marsden and Leigh, 1979; Kozlowski and Marshall, 1980; Kilpatrick and Starr, 1981; Havemann, Turski, Schwarz and Kuschinsky,

1983). In general, only injections of these agents into the dopaminergic cell region are blocked by treatments which inactivate dopaminergic neuronal function (James and Starr, 1978; Reavill et al., 1979).

The observation that GABAergic agents can produce both dopamine-dependent and dopamine-independent circling when applied to the ventral mesencephalon has led to the idea that this neurochemical system serves dual functions within this region. This idea fits well with data implicating both GABAergic local circuit neurons and projection neurons within this midbrain region. The local circuit GABA neurons are presumed to inhibit dopaminergic cell activity; the projecting GABA neurons, on the other hand seem to relay some dopamine-induced messages beyond the level of the striatum (Dray, 1979). In support of this latter suggestion, a number of studies have appeared demonstrating that denervation of these presumed GABAergic output pathways inhibits the expression of striatal dopamine-induced behaviours such as circling, stereotyped gnawing and catelepsy (see Di Chiara, Porceddu, Imperato and Morelli, (1981), for review).

In addition to dopamine and GABA, several other neurotransmitter systems appear capable of producing circling when unilaterally manipulated. Included in the list are norepinephrine (Pycock, Donaldson and Marsden, 1975), acetylcholine (Kelly and Miller, 1975a; Muller and Seeman, 1974; Glick, Jerussi, Walters and Green, 1974), serotonin (Green and

Grahame-Smith, 1975), glycine (Mendez, Cotias, Finn and Dahl, 1975) and substance P (James and Starr, 1977; Arnt and Scheel-Kruger, 1979). It is generally thought that these chemical systems modulate dopaminergic or GABAergic-induced circling (Pycock, 1980).

Anatomical Basis of Circling

I. Dopamine-Induced Circling

It has been strongly argued that at least some types of directionally-biased movements in animals result from an imbalance in dopaminergic activity. It is the nigrostriatal dopamine system, rather than the other ascending dopamine pathways that has received most attention in this behaviour.

The ascending dopamine systems involve different pathways; these pathways are identified in terms of their main projection targets. The nigrostriatal dopamine system arises primarily in the pars compacta of the substantia nigra (SNC) and projects primarily to the striatum. The mesolimbic and mesocortical dopamine systems both arise primarily in the 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). There is a small percentage of nigrostriatal dopamine cells that projects to

the VTA; similarly, there is a small percentage of mesolimbic dopamine cells that projects to the striatum (Fallon and Moore, 1978).

The emphasis placed on the nigrostriatal dopamine system in circling behaviour stemmed from the early studies that demonstrated that animals would 'turn' when injected systemically with indirect and direct acting dopaminergic agonists when the nigrostriatal dopamine system was unilaterally denervated (Anden, 1966). It was an imbalance in striatal dopamine activity that was presumed to cause asymmetrical movements and animals were thought to circle towards the side of the brain with weaker dopaminergic functioning. A flurry of reports followed Anden's work on dopamine and circling behaviour and in all of the early studies the unilateral lesion was placed in the nigrostriatal dopamine system (i.e. Ungerstedt, 1971a; Lotti, 1971; Costall et al., 1974c; 1976; 1979; Waddington, 1977).

A number of biochemical studies support the striatal imbalance theory of dopamine-induced circling. First, there is a positive correlation between the rate of circling when dopaminergic agonists are administered to animals with a unilateral 6-OHDA lesion and the levels of striatal dopamine on the side of the brain ipsilateral to the lesion (Thornburg and Moore, 1975; Costall et al., 1976). Second, intact animals that circle in response to systemic dopaminergic agonists have a

ten to fifteen percent difference in interhemispheric striatal dopamine (Glick et al., 1974); animals circle towards the side of the brain with lower striatal dopamine levels. Third, when embryonic dopamine cells are implanted in the dorsal striatum of adult rats that have a unilateral nigrostriatal lesion, the circling in response to peripherally administered amphetamine is abolished and normal locomotion results (Bjorklund, 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 dopamine systems either electrically or chemically. Thus, when dopamine or one its agonists is applied unilaterally to dopaminergic terminal regions it is generally applied to the striatum (Ungerstedt et al., 1969; Costall et al., 1974a; 1974b; Setler et al., 1978). Similarly, early work on morphine-induced circling stressed nigrostriatally-mediated activation (Iwamoto et al., 1977; Pert, 1978). Electrically-induced circling has also been primarily studied with the electrode in the vicinity of the nigrostriatal system (Arbuthnott et al., 1970; 1971; 1975; Roffman et al., 1978; Vaccarino et al., 1982a; 1982b). It is only recently that attention has been given to the role of mesolimbic systems in the circling phenomenon (Holmes et al., 1983; Holmes and Wise, 1985a; 1985b; Gratton and Wise, 1985).

While the mesolimbic dopamine system has been generally neglected in the circling model, recent work suggests that this system may play an important role, at least in some types of circling. It is surprising that this system has been neglected, in fact, because circling is presumed to result from an interaction of locomotion and postural asymmetry (Kelly and Moore, 1977; Pycock and Marsden, 1978); it is the mesolimbic dopamine system that has been most implicated in locomotion and not the striatum. For example, dopamine applied bilaterally to the nucleus accumbens septi, a major terminal region of the mesolimbic dopamine system, causes 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); similar injections into the striatum cause sterectypies, behaviours incompatible with simple forward locomotion (Costall et al., 1977). Bilateral injections of low doses of haloperidol into the nucleus accumbens antagonize the effects on locomotor activity elicited by peripheral amphetamine (Pijnenburg, Honig and Van Rossum, 1975) as do bilateral 6-OHDA lesions to this nucleus (Kelley, Seviour and Iversen, 1975). These same 6-OHDA lesions enhance apomorphine-induced locomotion (Kelley et al., 1975), an observation consistent with the notion of dopamine receptor supersensitivity; lesions of the striatum, however, do not.

Several investigators have also demonstrated that the

bilateral application of opioids into either the nucleus accumbens septi (Pert and Sivit, 1977) or into the mesolimbic dopamine cell region (Joyce and Iversen, 1979; Broekkamp et al., 1979; Kelley, Stinus and Iversen, 1980; Vezina et al., 1984) also results in increased locomotion. Interestingly, microinjections of opioids into the mesolimbic cells, which primarily project to the nucleus accumbens septi and frontal cortex (Fallon and Moore, 1978) are accompanied by exploratory activities (Kelley et al., 1980; Broekkampp et al., 1979) and it is these dopamine cells that are implicated in opiate reward (Bozarth, 1983). Similar bilateral application of opioids into the nigrostriatal dopamine cells results in stereotypy (Iwamoto and Way, 1977; Pert, 1978).

While it is clear that the bilateral manipulation of the mesolimbic dopamine system results in increased locomotion, it has only recently been appreciated that unilateral manipulation of this system can cause circling. Unilateral electrical stimulation in the region of the mesolimbic dopamine cells has been reported to cause contraversive circling (Roffman et al., 1978), either contraversive or ipsiversive circling (depending on the location of the electrode and the stimulation parameters) (Gratton and Wise, 1985) or no circling (Arbuthnott and Ungerstedt, 1978). Interestingly, the contraversive circling elicted by unilateral electrical stimulation of the mesolimbic region is accompanied by only a mild postural asymmetry (Roffman et al., 1978). Unilateral

application of either morphine (Holmes et al., 1983; Holmes and Wise, 1985a) or neurotensin (Holmes and Wise, 1985b) into the VTA causes contraversive circling that is accompanied by very little postural asymmetry and involves environmental exploratory responses.

While unilateral manipulation of the VTA can cause circling, it has been reported that unilateral application of dopamine to the nucleus accumbens septi cannot (Elkhawad and Woodruff, 1975). Similarly, if the nucleus accumbens septi is unilaterally destroyed with 6-OHDA and animals are treated systemically with amphetamine, they reportedly do not circle (Kelly, 1975). While these data are inconsistent with the idea that unilateral manipulations of the mesolimbic dopamine system can cause circling, it would be interesting to retest animals with these preparations more closely since, at the time, circling was thought to necessarily consist of a postural asymmetry. Bilateral 6-OHDA lesions to the nucleus accumbens have been shown to abolish amphetamine-induced circling in rats that also have a unilateral 6-OHDA nigrostriatal lesion (Kelly and Moore, 1977; Pycock and Marsden, 1978); similar lesions enhance apomorphine-induced circling, presumably due to the development of receptor supersensitivity. These data are consistent with the idea that the mesolimbic dopamine system plays some role in circling.

II. Gaba-Induced Circling

Similar to the organization of the ascending dopamine systems, the descending output pathways of the striatum are also organized into separate systems; these pathways are named with respect to the location of their cell bodies and terminal fields. There are two major GABAergic pathways that arise in the striatum and terminate in more posterior nuclei. The striatopallidal GABAergic pathway sends projections to both the internal and external segment of the globus pallidus. The other major striatally-derived GABAergic pathway, the striatonigral GABAergic system, terminates in the pars reticulata of the substantia nigra (SNR) (Dray, 1979).

In addition to the projecting GABAergic neurons that arise in the striatum and terminate in more caudal parts of the brain, there is also extensive evidence for the existence of GABAergic interneurons within the substantia nigra and VTA (Dray, 1979; Bartholini, Scatton, Worms, Zivkovic and Lloyd, 1981). Thus, the substantia nigra contains, in addition to the numerous GABAergic terminals that form part of the striatonigral GABAergic system, a large number of local circuit GABA neurons. The VTA, on the other hand, has been argued to contain only local circuit GABA neurons (Scatton et al., 1981). There is biochemical evidence to support the existance of differential GABAergic innervations of the substantia nigra and VTA. Thus, while cerebral hemitransection results in a marked reduction of glutamic acid decarboxylase (GAD) (the enzyme responsible for the conversion of l-glutamic acid to GABA) in the substantia nigra, there is no effect on GAD activity in the VTA

following these transections (Bartholini, Scatton, Worms, Zivkovi and Lloyd, 1981).

Since GABA was initially seen as a primary modulator of nigrostriatal dopamine activity (i.e. Aghajanian and Bunney, 1974), it is not surprising that most investigators studying GABA-induced circling restricted their injections to the substantia nigra. In all of the early work, GABA, or agents increasing or decreasing the functional level of GABAergic systems, were unilaterally injected into the region of the SNC (Tarsy et al., 1975; Dray et al., 1975; Horton and Pycock, 1977). Consistent with the electrophysiological work (Bunney and Aghajanian, 1976), behavioural effects following unilateral manipulation of GABA mechanisms within the SNC suggested a dopamine-inhibiting effect of the drug. Thus, the unilateral elevation of GABA concentrations following injections of EOS caused ipsiversive circling (Dray et al., 1975; Horton and Pycock, 1977), whereas similar injections of picrotoxin caused contraversive (Tarsy et al., 1975) that was abolished by a 6-OHDA lesion of the nigrostriatal dopamine pathway. Later, however, many studies appeared which suggested that GABA agonists injected unilaterally into the SN could induce contraversive circling (Oberlander et al., 1977; Scheel-Kruger, Arnt and Magelund, 1977; Martin, Papp and Bacino, 1978) that was unaffected by systemic administration of the neuroleptic haloperidol (Olpe et al., 1977; Olianas et al., 1978b; Reavill et al., 1979) or by blockade of presynaptic catecholaminergic transmission by prior treatment

with alpha-methyl-para-tyrosine or reserpine (Scheel-Kruger et al., 1977; Arnt and Scheel-Kruger, 1979a). Similarly, GABAergic antagonists were shown capable of producing ipsiversive circling when injected unilaterally into the SN (Olpe et al., 1977; Scheel-Kruger et al., 1977; James and Starr, 1978; Olianas et al., 1978b) that was also unaffected by prior infusion of 6-OHDA into the nigrostriatal pathway (Wolfarth et al., 1979). These observations were opposite to the ones predicted by the GABAergic inhibitory feedback hypothesis (Bunney and Aghajanian, 1976).

In an attempt to clarify the confusion with respect to the direction and pharmacology (i.e. dopamine-dependence) of circling elicited by both GABAergic agonists and antagonists, a number of reports appeared which provided close histological reconstructions of the precise injection sites within this region. These studies pointed to the duality of GABAergic function within the substantia nigra. Thus, GABA agonists or antagonists applied to the SNC caused ipsiversive and contraversive circling respectively (Reavill, Jenner, Leigh and Marsden, 1978; Havemann, Turski, Schwarz and Kuschinsky, 1983) that was disrupted by dopaminergic inactivation (Reavill et al., 1979). Similar injections of GABAergic agonists into the SNR were shown to elicit contraversive circling whereas GABAergic antagonists were shown to elicit ipsiversive circling (Reavill et al., 1978; Havemann et al., 1983); neither direction of circling was blocked by neuroleptic pretreatment (Reavill et

al., 1978).

Whereas unilateral application of GABAergic agents to the SNR cause circling, the bilateral application of such drugs cause stereotypy (Koob, Del Fiacco and Iversen, 1978; Arnt and Scheel-Kruger, 1980; Taha, Dean and Redgrave, 1982; Jackson and Kelly, 1984; Baumeister and Frye, 1984). The behavioural syndrome elicited by bilateral application of GABAergic agents into this nucleus resembles the behavioural effects following high systemic doses of dopamine receptor agonists (i.e. Ellinwood and Kilbey, 1974) or intrastriatal injections of these agents (Costall et al., 1977). Animals given injections of muscimol or EOS into the SNR exhibit stereotypic sniffing and biting responses (Taha et al., 1982) that are unaffected by injections of neuroleptics (Taha et al., 1982; Arnt and Scheel-Kruger, 1980) or by prior injections of reserpine plus alpha-methyl-para-tyrosine (Taha et al., 1982).

While the GABAergic mechanisms located in the SN has been strongly implicated in circling, there has been very few reports examining the effects of GABAergic agents applied unilaterally to the VTA; in the two studies that did make injections there, some circling was reported (Kilpatrick et al, 1981; Holmes and Wise, 1985a). Such circling might be expected to result following unilateral manipulation of GABAergic mechanisms in the VTA, since, similar to bilateral activation of dopamine mechanisms in that region, bilateral VTA application of GABAergic agents also increases locomotion.

Interestingly, however, GABA (Tanner, 1979) and its antagonist picrotoxin (Mogenson, Wu and Manchanda, 1979) have each been shown to elicit increases in locomotion. The increased locomotor activity following bilateral injection of picrotoxin is blocked by bilateral injections of spiroperidol, a dopaminergic antagonist, into the nucleus accumbens septi (Mogenson et al., 1979).

Present Investigations

Although there is a substantial body of literature on circling, there are at least two assumptions that have been made regarding this behaviour that have not, or only recently, been empirically tested. One of these assumptions is that mechanisms located in the substantia nigra are the prime mediators of circling. Another assumption is that circling generally consists of a postural component coupled with a locomotor component. Recently, both of these assumptions have been challenged, at least with respect to morphine-induced circling elicited from the ventral mesencephalon; unilateral injections of morphine cause circling most strongly when injections are aimed at the VTA and not the SNC. Secondly, the behaviour is accompanied by very little postural asymmetry (Holmes and Wise, 1985a).

While comparing the pharmacological characteristics of morphine-induced circling to those of muscimol-induced circling

it was noted that these two agents produced strikingly different types of circling behaviour. Whereas morphine-induced circling resembled a forward locomoting response that was environmentally-elicited, muscimol-induced circling appeared forced and stimulus-independent. This led to the hypothesis that different types of circling might result from stimulation of ventral mesencephalic structures and that these differences might be apparent anatomically, pharmacologically and phenomenologically. Indeed, the literature suggested that anatomical and pharmacological differences did exist between the circling elicited by these two agents, although it was never tested directly. The present set of experiments were designed to establish the anatomical, pharmacological and phenomenological characteristics of muscimol-induced circling elicited from ventral mesencephalic regions so that comparisons could be made with similar observations on morphine-induced circling that were reported earlier (Holmes and Wise, 1985a) and partially replicated here.

GENERAL METHODS

Subjects

One hundred ten 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 immediately after surgery that extended 0.5 mm beyond the guide cannulae. The dummy cannulae were kept in place until behavioural testing began. The animals were allowed to recover from surgery for at least one week before intracranial drug injections were given.

Apparatus

The apparatus for measuring circling 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 box. The head pedestal of the animal was attached to a cable which was mounted onto a shaft hanging from a ball bearing that was secured

into the top of the wooden test box. 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 box. 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.

To measure rearing activity, animals were placed in a circular plexiglas apparatus that measured 30 cm high and 24 cm in diameter. Near the top of the cylinder (10 cm) there was an indented ledge that was 6 cm deep and 6 cm in diameter. The ledge was large enough that stimulus objects (such as a food pellet) could be placed there. This served as a potential motivating factor for the animal to encourage rearing activity directed towards the ledge. The total number of rears elicited in 30 minutes was recorded.

In order to determine whether environmental factors influenced the direction of circling, animals were placed in three different environments. Environment A consisted of the circular plastic buckets described above that were used for quantifying circling. Environment B consisted of a 120 cm square wooden surface with a 30 cm high wall around the perimeter and a 76 cm square open hole in the center. Environment C consisted of a 180 cm square wooden surface with the outer perimeter open to the floor (one meter below)

and a 30 cm high 120 cm square inner perimeter. The animals were placed in each of these environments for a 30 minute period on each of two days and the number and direction of circles per minute (net) were recorded.

Behavioural Testing

Each animal was tested over at least an eight week period. On each test day the animals were placed in the buckets for two hours and the total number and direction of circles were recorded. Each animal was tested once with saline or muscimol (25, 50 and 100 ng) in a counterbalanced order. At least seven days separated the injections. After all data were collected for the anatomical localization study, some animals were selected, based on the presumed site of injection and their circling rates, for further pharmacological and phenomenological testing.

Intracranial Injections

Each animal received unilateral injections of either muscimol (25, 50 or 100 ng), saline or morphine (5 or 10 ug) into some part of the ventral mesencephalon. For injection, the dummy cannula was removed and replaced by the injection needle which extended 0.50 mm beyond the tip of the guide cannula. Injections were made using a 1 ul Hamilton microsyringe connected via poly-ethylene (PE-10) tubing. The total volume injected was 0.5 ul. Injections were made over one minute. The injection needle was kept in place for a further minute following the injection procedure. After drug injection

the dummy cannula was fitted back into the guide cannula. The rats were manually restrained during the time that the injection needle was pushed through the guide cannula but were allowed to move about on a platform (45 cm x 24 cm) during the injection period.

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 MUSCIMOL-INDUCED CIRCLING

Methods

Guide cannulae were implanted at different levels of the ventral mesencephalon. The coordinates ranged between 2.0 and 5.0 mm posterior to bregma, 0.2 and 3.0 mm lateral to the midsagittal suture and 6.0 and 9.0 mm ventral to dura. These coordinates were chosen because they were also the sites that had been tested in an earlier study (Holmes et al., 1985a) for morphine-induced circling.

The total number of circles following central muscimol injections was determined for the two hour test period at each of the three doses. The subjects were classified into five groups

according to the number and direction of circles elicited in the two hour session: no circling (less than 25 in either direction in two hours), ipsilateral circling, low contralateral circling (26-100 in two hours), medium contralateral circling (101-250 in two hours) and high contralateral circling (>250 in two hours).

Cannula placements were verified histologically for each subject and compared with the Pellegrino, Pellegrino and Cushman Atlas of the Rat Brain (1979). The region mapped was divided into four sections. The first section represents cannula placements that were located anterior to the dopamine cell containing region (-2.8 mm posterior to bregma). The second and third sections represent anterior and posterior zones within the dopamine cell containing region (-3.2 and -3.6 mm posterior to bregma) (Holmes, Bozarth and Wise, 1983). The fourth section represents cannula placements that were posterior to the dopamine cell containing region (-4.0 mm posterior to bregma).

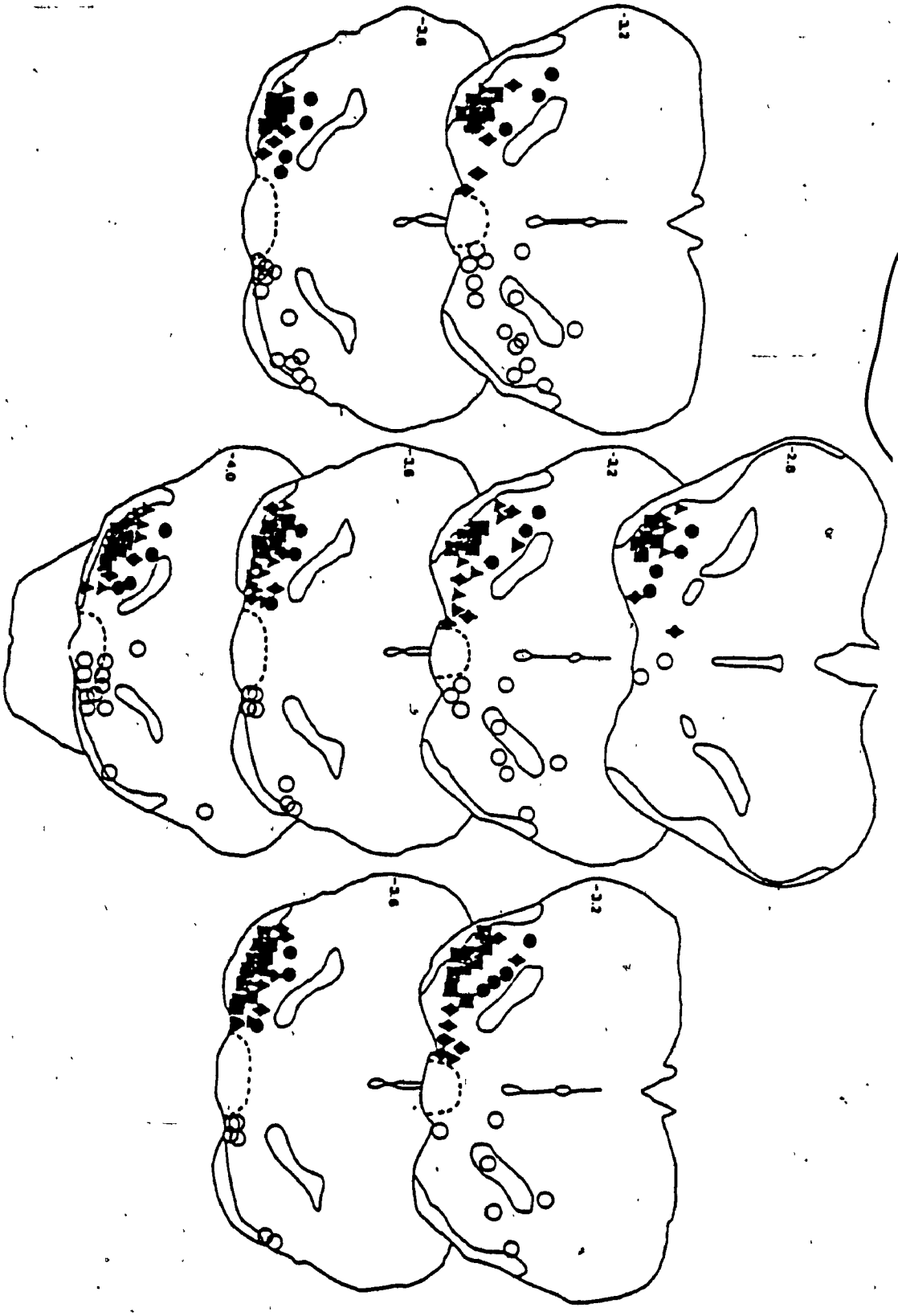
Results

Circling elicited by muscimol injections into either the rostral or caudal SNR was directed contralateral to the side of injection; ipsilateral circling resulted from injection into some parts of the SNC. There was very little circling noted when cannulae were located medial to the the SNR. As the dose of muscimol was increased, muscimol caused circling from more

sites (Figure 1).

At the lowest dose of muscimol (25 ng) only animals with rostral and caudal SNR placements showed strong contraversive circling. Forty-one animals had cannulae located in this region; 21 of these demonstrated strong contraversive circling, eight demonstrated moderate contraversive circling, six demonstrated weak contraversive circling and six did not circle. Some animals circled when muscimol was applied to sites medial to the SNR, but always at a lower rate relative to the SNR sites. As the dose of the drug was increased, the circling rates also increased. Thus, muscimol elicited strong contraversive circling in 28 animals with SNR placements following injections of the 50 ng dose and elicited strong contraversive circling in 35 animals following injections of the 100 ng dose. This same pattern was also seen at sites that were located medial to the SNR. Thus, for example, some animals with cannulae in the VTA did not circle following injections of the lowest dose of muscimol, circled at a low rate with the 50 ng dose and circled at an even higher rate with the 100 ng dose. Sites that demonstrated high circling rates with the lowest dose of muscimol, demonstrated even higher circling rates as the dose increased. The circling rates following injection of the highest dose was often five times the circling rate following injection of the lowest dose. Thus, an animal

Figure 1. Histological reconstructions of muscimol application sites, indicating the vigor of the circling associated with muscimol injections in each site. The rates are the total number of circles elicited in two hours. Cannula tips from animals that circled are represented on the left, cannula tips from non-circlers are represented on the right. Only the complete mapping with the 50 ng dose is represented; for comparison purposes however, the two middle sections are shown for the 25 and 100 ng doses. Solid dots (●) represent cannula tips from animals that circled ipsiversively, solid diamonds (♦) represent cannula tips from animals that showed weak contraversive circling, solid triangles (▲) represent moderate contraversive circling and solid squares (■) represent strong contraversive circling.


25 ng
50 ng
100 ng

that circled as many as 300 times with the lowest dose circled as many as 1,500 times with the highest dose.

In contrast to the clear dose-dependent effects of muscimol-induced contraversive circling from the SNR, the rate of ipsiversive circling following muscimol injections into the SNC was not monotonically related to the dose of the drug. In point of fact, whereas the lowest dose of muscimol effectively produced ipsiversive circling at some sites in the SNC, higher doses of muscimol into two of these sites caused contraversive circling. Similarly, at least one animal circled contraversively with the lowest dose of muscimol and circled ipsiversively with the highest dose of muscimol. Eighteen animals had cannulae located in the SNC. The lowest dose of muscimol caused ipsiversive circling in seven, low contraversive circling in six and no circling in five of the animals. The 50 nanogram dose caused ipsiversive circling in twelve, low contraversive circling in two, medium contraversive circling in one and no circling in two animals. The 100 ng dose elicited ipsiversive circling in ten, low contraversive circling in six, moderate contraversive circling in one and no circling in one.

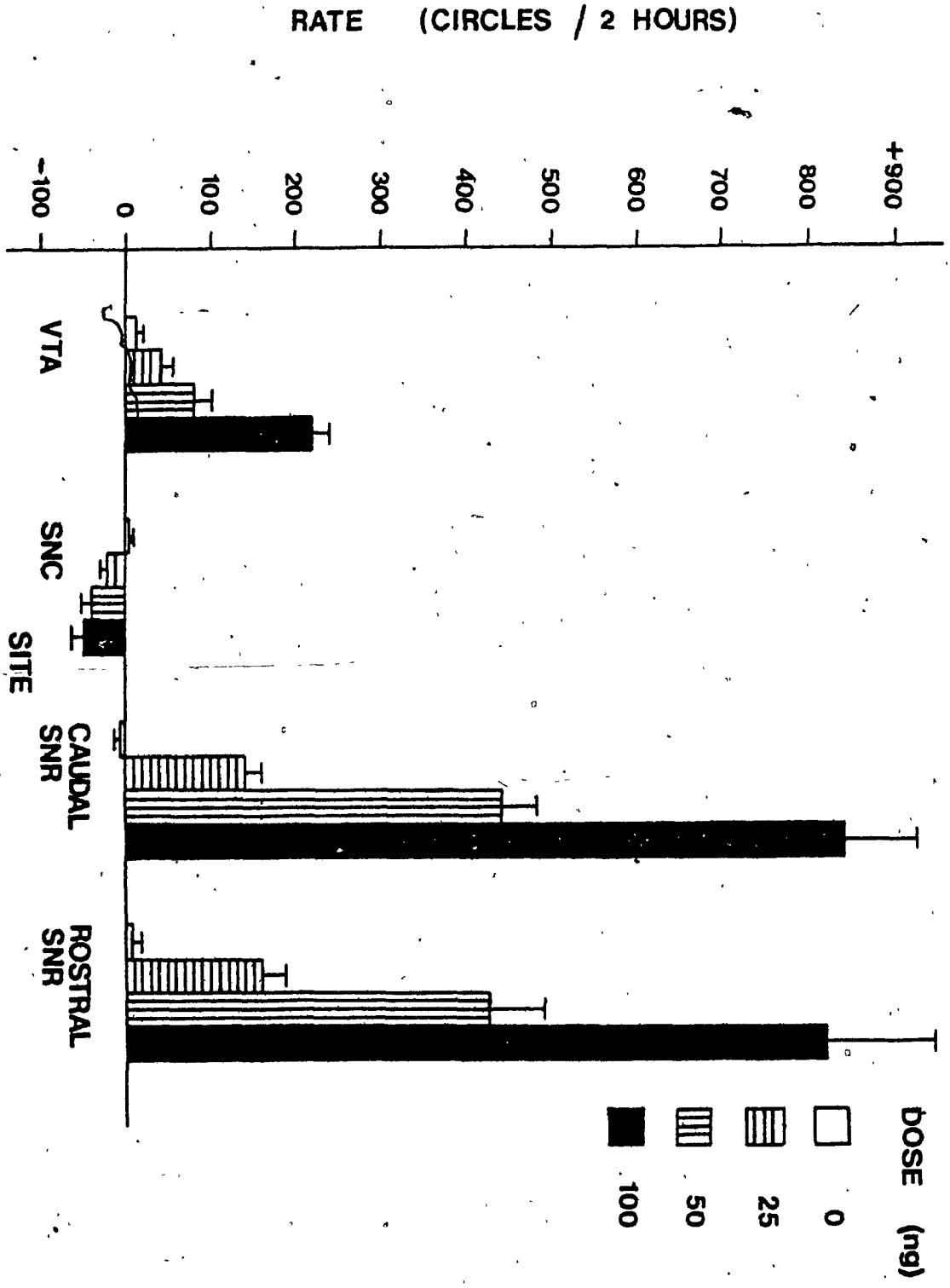
The data from subjects whose cannulae were verified to be within either the rostral SNR, caudal SNR, SNC or VTA were collapsed for each dose of muscimol and for the saline condition. The total number and direction of circles elicited in the two hour test

sessions were determined for each of these groups.

Muscimol-induced circling rates were highest (as shown in the mapping of effective sites) when the drug was injected into either the rostral or caudal SNR (Figure 2). The Scheffe post hoc test for multiple comparisons (Roscoe, 1979) showed no differences in the circling rates for these two sites at any of the doses tested ($p>.05$). Animals with cannulae in one of these areas circled about 175 times with the lowest dose of muscimol, 400 times with the middle dose of muscimol and 900 times with the highest dose of muscimol. Animals with cannulae in the VTA circled about 40 times with the lowest dose, 80 times with the middle dose and 210 times with the highest dose. SNC animals circled approximately 25 times with the lowest dose, 40 times with the middle dose and 50 times with the highest dose. A two-way analysis of variance revealed a main effect of site ($F(3,79)=28.77$, $p<.001$), a main effect of dose ($F(3,9)=91.33$, $p<.001$) and a dose by site interaction ($F(3,237)=20.43$, $p<.001$). Scheffe post hoc tests for multiple comparisons (Roscoe, 1979) revealed that the rate of circling differed significantly between all drug doses (0,25,50,100) for all sites except the SNC. There was no difference found between the circling rates at the 50 ng and 100 ng dose of muscimol for animals with SNC placements.

Some circling was also elicited when muscimol was applied to sites external to the SNC, SNR or VTA. Of particular interest was

Figure 2. Mean muscimol-induced circling rates (± SEM) as a function of placement and dose. Data were taken from animals having cannula tips either in the VTA, SNC, rostral SNR or caudal SNR. Data from animals with cannula tips outside of these regions were excluded. Positive scores represent net contraversive scores; negative scores represent net ipsiversive scores.


RATE (CIRCLES / 2 HOURS)
-100
0
100
200
300
400
500
600
700
800
+900
VTA
SNC
CAUDAL SNR
ROSTRAL SNR
SITE
100
50
25
0
DOSE (ng)

the strong ipsiversive circling noted in two animals whose cannulae were later determined to be within the deep layers of the superior colliculus (not shown on histological reconstruction). The circling activity shown by these two animals included a strange head-bobbing motion.

Discussion

Muscimol elicited circling when applied at different levels of the ventral mesencephalon. Contraversive circling resulted from injections of muscimol into either the rostral or caudal SNR and ipsiversive circling was elicited from some sites in the SNC. Muscimol produced very little circling when applied to sites external to either the SNR or SNC; when it did, it was only at the higher doses.

It seems clear that muscimol most effectively causes contraversive circling when applied to the SNR at all rostro-caudal levels. This is evident if one compares the anatomical mapping of this activity across all three doses of the drug. For example, although the VTA appears somewhat effective at the 50 and 100 ng doses, at the lower dose this site is relatively ineffective.

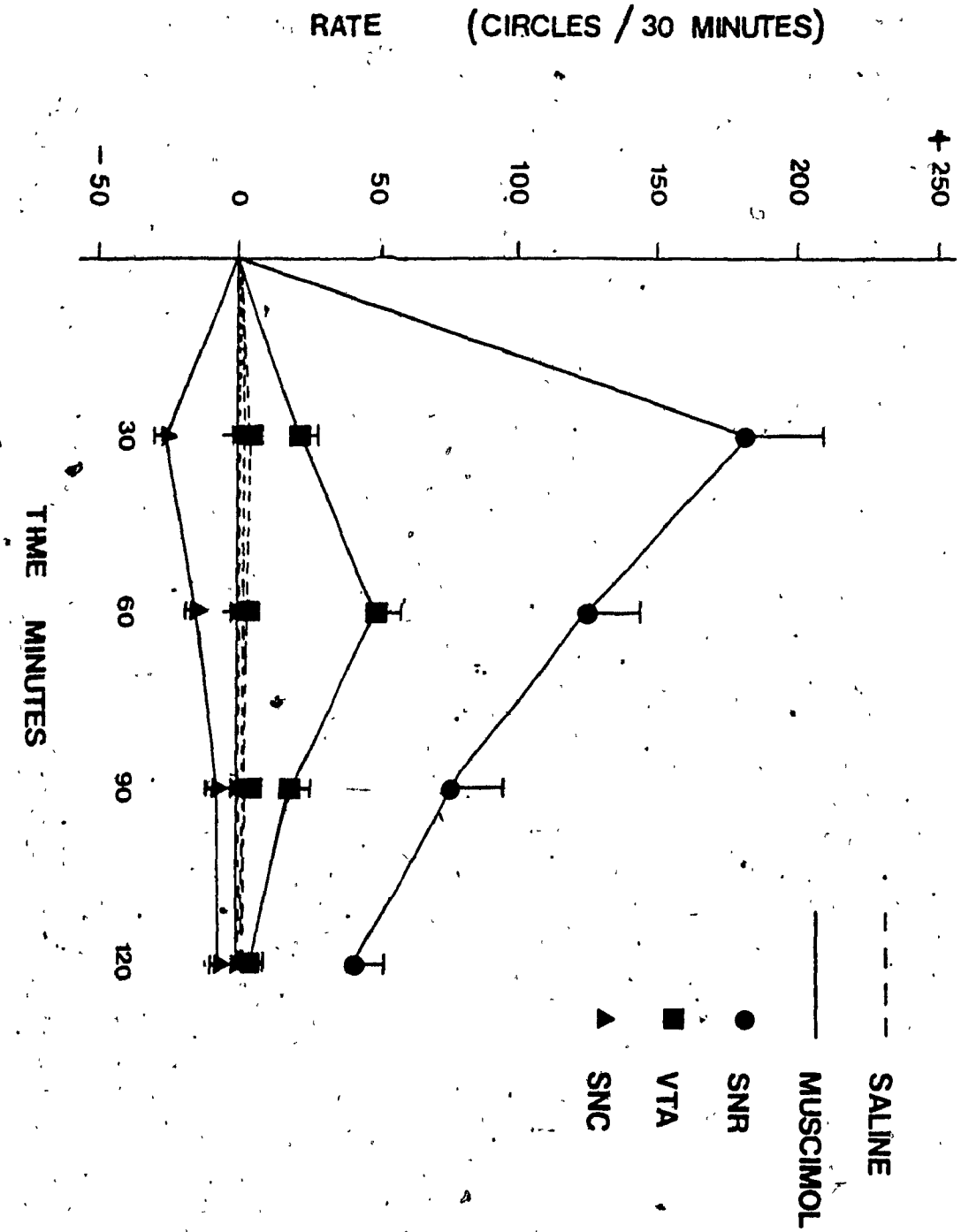
The fact that the circling is best with injections of muscimol into SNR could be due to one of two factors. First, circling may result from stimulation at all levels of the substantia nigra and VTA, but the number of GABAergic receptors might determine

which site is most effective. The central region of the SNR has the greatest number of GABAergic fibers in comparison to both the VTA and SNC (Ribak, Vaughan and Roberts, 1980); it might be expected then, that the density of postsynaptic receptors is highest in this region. Alternatively, circling might be determined by the nature of the chemical system post-synaptic to the GABAergic receptors in this region.

It is likely that muscimol causes the highest rates of contraversive circling when applied to the central region of the SNR because of the nature of the chemical system efferent to the GABAergic receptors and not simply because there may be more GABAergic receptors in that region. If it was simply the number of receptors that determined the rate of circling, one would not expect to find differences in latency to peak circling rates following stimulation of the VTA or SNR. In point of fact however, muscimol-induced circling elicited from the VTA has a longer latency to peak rate (Figure 3). This is probably due to spread of drug to SNR mechanisms.

It has been suggested that contraversive circling induced by muscimol injections into the SNR results from activation of descending GABAergic fibers that arise in the substantia nigra and terminate in three main target regions: the deep layers of the superior colliculus, the mesencephalic reticular formation and the thalamus (i.e. Reavill, Leigh, Jenner and Marsden, 1981).

Figure 3. Mean circling rates (±SEM) as a function of placement and time. The number of circles elicited over two hours in thirty minute blocks was compared between animals with cannula placements in either the VTA, SNR or SNC. The solid lines represent circling rates following muscimol injections (50 ng). The dotted lines represent the saline conditions.


RATE (CIRCLES / 30 MINUTES)
− 50
0
50
100
150
200
+ 250
TIME MINUTES
30
60
90
120
SALINE
MUSCIMOL
SNR
VTA
SNC

At least some of these pathways are known to contain GABA as their neurotransmitter (i.e. Scheel-Kruger, Magelund and Olianas, 1981) and are thought to play some role in circling behaviour. Thus, either lesions of these pathways or injections of GABAergic drugs into these terminal regions are known to influence circling that is mediated by the striatum or SNR (see De Chiara, Porceddu, Imperato and Morelli, 1981 for review). The GABAergic neurons of the VTA are thought to inhibit mesolimbic dopaminergic neurons (Wolf, Olpe, Avrith and Haas, 1977; Mogenson, Wu and Manchanda, 1979). If circling in the VTA was due to GABAergic inhibition of dopaminergic neurons, the direction of circling should have been ipsilateral to the side of drug injection since animals are known to circle away from the side of the brain with greater dopamine activation (i.e. Glick et al., 1974). Since the observed circling following injections of muscimol into the VTA was contralateral to the side of drug injection (except for one site), it suggests that this behaviour was not due to inhibition of dopaminergic neurons.

There are several reports that circling following the unilateral injection of GABAergic antagonists depends upon the anterior-posterior placement of the cannula within the SNR (James and Starr, 1978; Reavill et al., 1978; Scheel-Kruger et al., 1979). If anterior-posterior differences exist, it should be apparent with injections of GABAergic agonists. The investigator of the

present study intentionally varied the anterior-posterior co-ordinates to study this possibility (as well as to compare the effective sites with morphine-induced circling). No support for the claim of anterior-posterior differences was evident. Rather than an anterior-posterior difference within the nigra influencing direction of circling, it would appear that the SN zone (i.e. SNC-SNR) in which the cannula is located is the critical factor. The discrepancy between the results of the present experiment and those of others may be due to two factors. First, it is conceivable that there was no close analysis of the zone-specific effects reported in the earlier studies. For example, all of the experiments claiming anterior-posterior differences in direction of circling neglected to publish histological reconstructions of their cannula tips; it is conceivable that their anterior placements were located in the SNR and that their posterior placements were located in the SNC. Second, in one report (Scheel-Kruger et al., 1977) high doses of GABAergic antagonists produced odd types of behaviour (i.e. a contralateral postural asymmetry followed by ipsilateral turning and wild running seizures); these observations make the data difficult to interpret. The lack of an anterior-posterior differentiation in direction of circling found in the present study is consistent with work of Kozlowski and Marshall (1980); these authors did publish a confirmation of their histologies. As

in the present study, the direction of circling depended on the zone of the SN in which the drug was injected and no anterior-posterior differences were apparent.

In contrast to the dose-dependent contraversive circling following muscimol injections into the SNR, the ipsiversive circling showed no such orderliness. In fact, as mentioned earlier, there were at least three sites in the SNC that elicited either contraversive or ipsiversive circling depending on the dose of the drug. In two cases, the low dose produced ipsiversive circling whereas the higher doses produced contraversive circling. In the other case, the opposite phenomenon occurred with the highest dose eliciting ipsiversive circling and the lower dose eliciting contraversive circling. These results may be due to the heterogeneity of GABAergic mechanisms within this region. For example, it is well known that there are both intrinsic GABAergic neurons and extrinsic GABAergic neurons within the substantia nigra (Dray, 1979). The intrinsic neurons are thought to inhibit dopaminergic activity whereas the extrinsic neurons are thought to function independently of dopaminergic systems. It has been demonstrated that within particular regions of the substantia nigra (i.e. the border between compacta and reticulata) there are intrinsic and extrinsic GABAergic neurons in close proximity (Dray, 1979). When drug was applied to cannulae within this region, it may have

stimulated more intrinsic neurons on one occasion (causing ipsiversive circling) and more extrinsic neurons on another occasion (causing contraversive circling) simply by chance distribution of the drug. An even finer histological analysis of muscimol-induced ipsiversive and contraversive circling from areas rich in both intrinsic and extrinsic GABAergic neurons (such as the border between the SNC and SNR) might help to clarify the picture.

In addition to the controversy surrounding the anterior-posterior differences or similarities in muscimol-induced circling, there is also considerable debate regarding the direction of circling elicited from dorsal-ventral regions in the SN. At least two different stories have emerged from the literature. First, early reports suggested that muscimol induced contraversive circling at all levels of the SN (Oberlander et al., 1977; Scheel-Kruger et al., 1977; Martin et al., 1978) (although close histological analyses of injection sites were never reported). Later however, several reports appeared suggesting that unilateral GABAergic stimulation produced different directions of circling, depending on which SN zone was injected with drug (James and Starr, 1978; Reavill et al., 1979; Koslowski and Marshall, 1980; Havemann et al., 1983). As stated earlier, the present study supports observations that there are zone-specific differences in muscimol-induced

circling and is in agreement with the data that implicate the SNC in ipsilateral circling following muscimol injections and implicate the SNR in contralateral circling following these same injections (James and Starr, 1978; Reavill et al., 1979). These data also fit well with recent observations of muscimol-induced effects from the SN that use a new index of motility - tonic activity in the EMG (Havemann et al., 1983). These investigators have found that muscimol injections into the SNC produced tonic activity in the gastrocnemius-soleus muscle whereas similar injections into the SNR produced no tonic activity in these muscles; only the SNR injections produced circling (in the contralateral direction). Tonic activity in the gastrocnemius-soleus muscle is an index of immobility; thus stimulation of SNC renders animals somewhat immobile while stimulation of the SNR causes movement. This might possibly explain why only weak circling is generally observed with SNC stimulation while strong circling is generally observed with SNR stimulation. Interestingly, when morphine is applied to the SNR tonic activity is observed in the gastrocnemius-soleus muscle whereas no tonic activity in these muscles are observed with similar injections into the dopaminergic cell body region (Turski, Havemann and Kuschinsky, 1983).

Contraversive circling elicited by unilateral muscimol injections into ventral mesencephalic regions has a different

anatomical profile than morphine-induced contraversive circling elicited from the same brain areas (Holmes et al., 1985a). First, whereas muscimol is most effective when injected into the SNR at all anterior-posterior levels, morphine is relatively ineffective at these sites. Twenty-three animals with cannulae aimed at the SNR were tested for morphine-induced circling in an earlier study (Holmes and Wise, 1985a); only eight of these circled (always in the contralateral direction) and the rate of circling was low. Furthermore, histological analysis demonstrated that all eight animals had cannulae located in the medial SNR, encroaching upon the VTA. This part of the SNR contains some dopamine cells as demonstrated with histochemical flourescence (Fallon and Moore, 1978).

Whereas muscimol injections into the VTA are relatively ineffective for producing contraversive circling (except at the highest doses of the drug), stimulation of this region with morphine produces strong contraversive circling. In fact, whereas injections of the low dose of muscimol are ineffective in the medial VTA, injections of morphine into the medial VTA produces contraversive circling rates that are higher than circling rates following similar injections into more lateral parts of the SN (Holmes and Wise, 1985a). Furthermore, the latency to peak circling rate is shorter when morphine injections are placed in the medial VTA than when placed more laterally. The opposite

phenomenon occurred with unilateral muscimol injections; the peak circling rates were noted in the first 30 minutes following injections into the SNR, but they were not noted until 60 minutes following intracranial injections when cannulae were located in the VTA.

That morphine-induced contraversive circling has a different anatomical localization than muscimol-induced contraversive circling makes sense when one considers the nature of the chemical system believed to be activated either indirectly or directly by opiate injections into ventral mesencephalic regions. Morphine-induced circling is thought to rely on the integrity of dopaminergic neurons since treatments which abolish dopamine functioning block the behaviour (Iwamoto and Way, 1977; Pert, 1978; Holmes et al., 1983; Holmes and Wise, 1985a). Furthermore, iontophoretic application of opiates to this region increases dopaminergic cell firing (Matthews and German, 1983). The VTA contains a large number of dopamine cells that are clustered around the interpeduncular nucleus. In contrast, the dopamine cells of the SNC lie in a thin layer and are less numerous than are the VTA cells; only few dopaminergic cells reside in the SNR (Fallon and Moore, 1978). Since morphine-induced circling is fastest when drug is injected into the VTA, since it is blocked by dopaminergic antagonists and since it resembles a unilateral forward locomotion, it is likely that the behaviour is mediated by

ascending dopaminergic systems. This is in contrast to muscimol-induced contraversive circling which is thought to result from stimulation of GABAergic systems efferent to the ascending dopamine systems.

PHARMACOLOGICAL CHARACTERISTICS OF MUSCIMOL-INDUCED CIRCLING ELICITED FROM THE SUBSTANIA NIGRA: COMPARISONS WITH VTA MORPHINE-INDUCED CIRCLING

There is now considerable agreement that at least some types of circling elicited by unilateral injection of agents influencing GABAergic mechanisms in the ventral mesencephalon can occur relatively independent of dopaminergic functioning (Olpe et al., 1977; Olianas et al., 1978; Reavill et al., 1979; Scheel-Kruger et al., 1977; Arnt and Scheel-Kruger, 1979); generally, injections of GABAergic compounds into the SNR are not completely blocked by treatments which disrupt dopaminergic functions whereas similar injections into the SNC are blocked by such treatments. While SNR circling is never completely blocked by treatments which disrupt dopaminergic functioning however, there are some studies which report slight attenuating effects (Arnt and Scheel-Kruger, 1979; James and Starr, 1979; Kilpatrick et al., 1980, Martin et al., 1978), no effects (Oberlander et al., 1977; Reavill et al., 1979; Waddington, 1979) or even facilitating effects (Olianas et al., 1978) following such treatments.

In contrast to the inconsistent effects of dopaminergic

inactivation on GABA-induced circling, morphine-induced circling elicited from either the SNC or VTA has been shown to depend entirely on dopaminergic functioning since treatments which result in dopaminergic inactivation block circling caused by unilateral morphine injections (Iwamoto and Way, 1977; Pert, 1978; Holmes et al., 1983; Holmes and Wise, 1985a). Recently, in fact, it was shown that doses of pimozide that effectively blocked VTA morphine-induced circling had no effects on muscimol-induced circling from the same brain region (Holmes and Wise, 1985a). These differential effects of pimozide on morphine and muscimol-induced circling may have resulted from the fact that muscimol-induced circling was much faster than morphine-induced circling (i.e. it may prove more difficult to block very fast circling).

The present study was designed to compare the effects of pimozide pretreatment on contraversive circling elicited by unilateral morphine injections into the VTA with contraversive circling by unilateral injections of muscimol into the SNR under conditions where the rate of circling between the two agents was equated. In addition, the effects of pimozide pretreatment on muscimol-induced ipsiversive circling was also noted.

Methods

Eight animals that circled contraversively following central

muscimol and six animals that circled ipsiversively following central muscimol were used in the present study. Eight animals were also implanted with cannulae in the VTA.

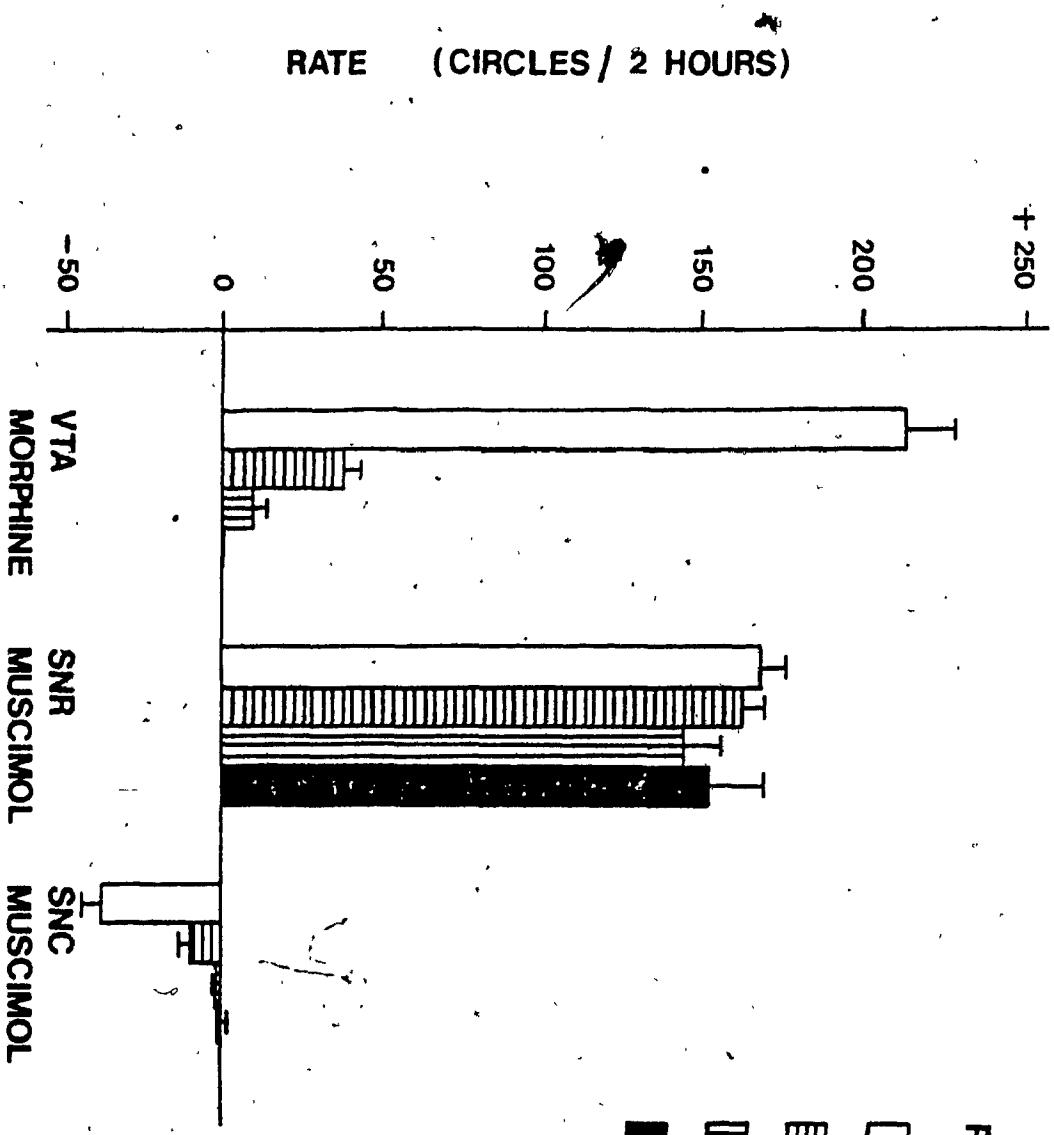
The animals with cannulae in the SNR or SNC were tested with 25 ng of muscimol. At this dose it was found in the mapping study that SNR animals circled approximately 175 times in two hours and SNC animals circled approximately 25 times in two hours. VTA animals were injected with 5 ug of morphine. These animals circle approximately 210 times in two hours (Holmes and Wise, 1985a).

Animals were tested over at least a four week period. They were injected with either pimozide (0.25, 0.50 or 1.0 mg/kg, IP) or saline, in a counterbalanced order, four hours prior to central morphine or muscimol (see Atalay and Wise (1982) for time course effects of pimozide). The animals were then placed in the plastic buckets used to measure circling; the total number and direction of circles elicited in the two hour test period were determined.

Results

Pimozide effectively blocked contraversive circling elicited by unilateral morphine injections into the VTA and circling elicited by unilateral muscimol injections into the SNC but had no effects on contraversive circling elicited by unilateral muscimol injections into the SNR (Figure 4). A two-way analysis of variance revealed significant

Figure 4. Mean circling rates (±SEM) following pimozide pretreatment. Pimozide was given four hours prior to central morphine or muscimol. Positive scores represent net circles in the direction contralateral to the side of drug injection; negative scores represent net circles in the direction ipsilateral to the side of drug injection.


RATE (CIRCLES / 2 HOURS)
-50
0
50
100
150
200
+ 250
VTA MORPHINE
SNR MUSCIMOL
SNC MUSCIMOL
PIMOZIDE DOSE (mg/kg)
0
0.25
0.50
1.0

main effects of site ($F(2,19)=41.61$, $p<.001$) and dose ($F(3,57)=30.30$, $p<.001$) and a site by dose interaction ($F(6,57)=37.95$, $p<.001$). Scheffe post hoc tests for multiple comparisons (Roscoe, 1975) revealed that both morphine-induced contraversive circling and muscimol-induced ipsiversive circling differed significantly from normal conditions following all doses of pimozide pretreatment ($p<.05$). Muscimol-induced contraversive circling, on the other hand, was no different from normal conditions at any dose of pimozide pretreatment ($p>.05$).

Discussion

Muscimol-induced ipsiversive circling seems to be due to dopaminergic receptor activation since all doses of pimozide blocked the behaviour. Muscimol-induced contraversive circling on the other hand appears relatively independent of dopaminergic functioning since no dose of pimozide influenced the behaviour. The failure to block muscimol-induced contraversive circling was not simply due to fast rates of circling since these same doses of pimozide either attenuated (0.25 mg/kg) or blocked (0.50 and 1.0 mg/kg) morphine-induced contraversive circling from the VTA even though animals circled slightly faster following morphine injections under normal conditions.

The fact that morphine induced contraversive circling was blocked by all doses of pimozide supports earlier electrophysiological (Matthews and German, 1982) and behavioural (i.e. Bozarth, 1983;

Holmes et al., 1983; Vezina and Stewart, 1984; Holmes and Wise, 1985a) work which suggests that morphine activates dopaminergic neurons. The fact that muscimol-induced ipsiversive circling elicited from some parts of the SNC is blocked by neuroleptics is consistent with the idea that some GABAergic mechanisms serve to regulate midbrain dopaminergic activity in a negative feedback fashion (Aghajanian and Bunney, 1974). Finally, the fact that muscimol-induced contraversive circling elicited from the SNR can occur independent of dopaminergic receptor blockade suggests that this activity results from activation of chemical systems efferent to the midbrain neurons (i.e. Starr and Kilpatrick, 1981). These differential effects of pimozide on muscimol-induced circling elicited from different zones within the SN are consistent with other reports (i.e. Reavill et al., 1978).

PHENOMENOLOGICAL CHARACTERISTICS OF CIRCLING ELICITED BY MUSCIMOL INJECTION INTO THE SNR AND MORPHINE INJECTIONS INTO THE VTA

Circling is a general term used to describe directionally biased movements. Such movements can be quantitatively and qualitatively different. One way to differentiate one type of circling activity from another is by the size and number of the circles being described by an animal. For example, it has been reported that a strong postural asymmetry often accompanied circling such that the size of the circles being made by the animals is little

more than the length of the animal's body (Pycock, 1980). Generally, animals circling with a strong postural asymmetry circle very fast (i.e. 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 makes very large diameter circles and moves slowly. Finally, circling has been described that is accompanied by essentially no postural asymmetry (Holmes et al., 1983; Holmes and Wise, 1985a; 1985b). In this case, when animals are removed from the circling apparatus (round plastic buckets) and placed in a large open space they tend to move forward in straight lines until they encounter a wall or other barrier. Once the barrier is met, the animals begin to traverse the environment in a directionally-biased fashion. Thus, the size of the circles made by these animals is dependent upon the size of the enclosure in which the animal is placed.

A second factor that can distinguish one type of circling from another is the type of limb movements that animals make. There are at least two types of limb movements have been reported in the literature. First, circling has been described in which an animal uses the hind limb contralateral to the direction of movement to support the body weight while the opposite hind limb steps backwards (Teitelbaum et al., 1982). Animals that engage in this type of movement remain in a relatively fixed

location within the test area and are generally strongly asymmetric posturally (Arbuthnott and Ungerstedt, 1975); in addition, these animals generally circle many times in a given test session. The other type of circling that has been reported involves a forward locomoting response in which all four limbs move in a forward direction (i.e. Holmes et al., 1983; Holmes and Wise, 1985a; 1985b). These animals describe large diameter circles and generally circle much slower than 'pivoting' animals.

A third factor that can potentially differentiate one type of circling from another is the degree to which the asymmetrical movements rely upon environmental information. For example, systemic injections of low dose apomorphine (which generally cause forward progression) causes circling when the sensory input to an animal's head is unilaterally eliminated (Szechtman, 1983); in this case, animals circle towards the side of the strong sensory stimulation. Similarly, the direction of spontaneous apomorphine-induced circling is dependent upon environmental information since environmental manipulations can change the direction of circling (Pisa and Szechtman, 1984). These are in contrast to the types of movements elicited by unilateral muscimol injections which appear forced and stimulus-independent (Holmes and Wise, 1985a).

Contralateral circling induced by VTA morphine has been shown to differ in all three ways from that induced by SNR muscimol. Whereas

morphine-induced circling is an environmentally-dependent behaviour that is generally slow, involves forward limb movements and little postural asymmetry, muscimol-induced circling seems relatively independent of environmental stimuli, is fast and involves a pivoting action of the limbs (Holmes and Wise, 1985a). Rate of circling however, may have been a potential confound in that study since the rate of muscimol-induced circling was fifteen to twenty times faster than the rate of morphine-induced circling. If muscimol-induced circling rates and morphine-induced circling rates were equated, there might be no qualitative differences in the circling.

The present experiments were designed to establish whether morphine-induced contraversive circling resulting from injections into the VTA is phenomenologically different from muscimol-induced contraversive circling resulting from injections into the SNR. Observations were made with respect to the degree of postural asymmetry accompanying the circling, the size of the circles, the type of limb movements made by the animals and the direction of circling as a function of environmental factors. In addition, observations were made on biting behaviour and rearing activity.

Experiment 1 - Open Field Observations of Morphine and Muscimol-Induced Contraversive Circling

Methods

Animals were injected with either muscimol (25 ng) in the SNR or morphine (5 ug) in the VTA. These doses were chosen

because the results of the present anatomical study demonstrated that the lowest dose of muscimol produced rates of circling comparable to those produced by morphine injections (Holmes and Wise, 1985a). Thirty minutes after the injection the animals were observed for fifteen minutes 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. In addition, a metal spatula (approximately 15 cm long) was brought towards the snout of the animal twice during the open field testing.

Results

Unilateral morphine injections into the VTA resulted in forward locomotion with turning being predominantly to the side contralateral to the morphine application. There was little postural asymmetry noted in the longitudinal axis of the animal's body and all four limbs moved forward. The size of the circles being made by the animals was almost as large as the size of the open field since there was a strong tendency to walk along the perimeter of the environment. When the metal spatula was brought towards the snout of the animals, they stopped circling, sniffed in the direction of the spatula and then continued to locomote. There were no biting responses directed towards the spatula.

Unilateral muscimol injections into the SNR did not produce

forward locomotion. In this case, the hind limb contralateral to the side of injection stepped backwards while the opposite hind limb served as a pivot; the animals thus tended to remain in a relatively fixed location. There was a tight postural asymmetry accompanying muscimol-induced circling. The animals circled in a diameter little more than the length of their bodies. When the metal spatula was brought towards their snout, they stopped circling (although still remained posturally asymmetric) and tended to engage in stereotyped biting responses for as long as the spatula was kept in place. When the spatula was not present in fact, the animals often chewed their forepaws.

Experiment II - Rearing Activity Following Morphine Injections into the VTA and Muscimol Injections into the SNR

Methods

Eight animals with cannulae in the VTA and eight animals with cannulae in the SNR were used in the present experiment. VTA animals were injected with saline or morphine (5 ug and 10 ug) in a counterbalanced order. SNR animals were injected with saline or muscimol (25 and 50 ng) in a counterbalanced order. Each animal was placed in the apparatus for measuring rearing (General Methods) 30 minutes following intracranial injections. A 60 gram food pellet was placed on a ledge near the top of the apparatus to encourage rearing. The total number of rears

elicited in 30 minutes was recorded. All of the animals had been habituated to the rearing apparatus for 60 minutes prior to any intracranial drug injections.

Results

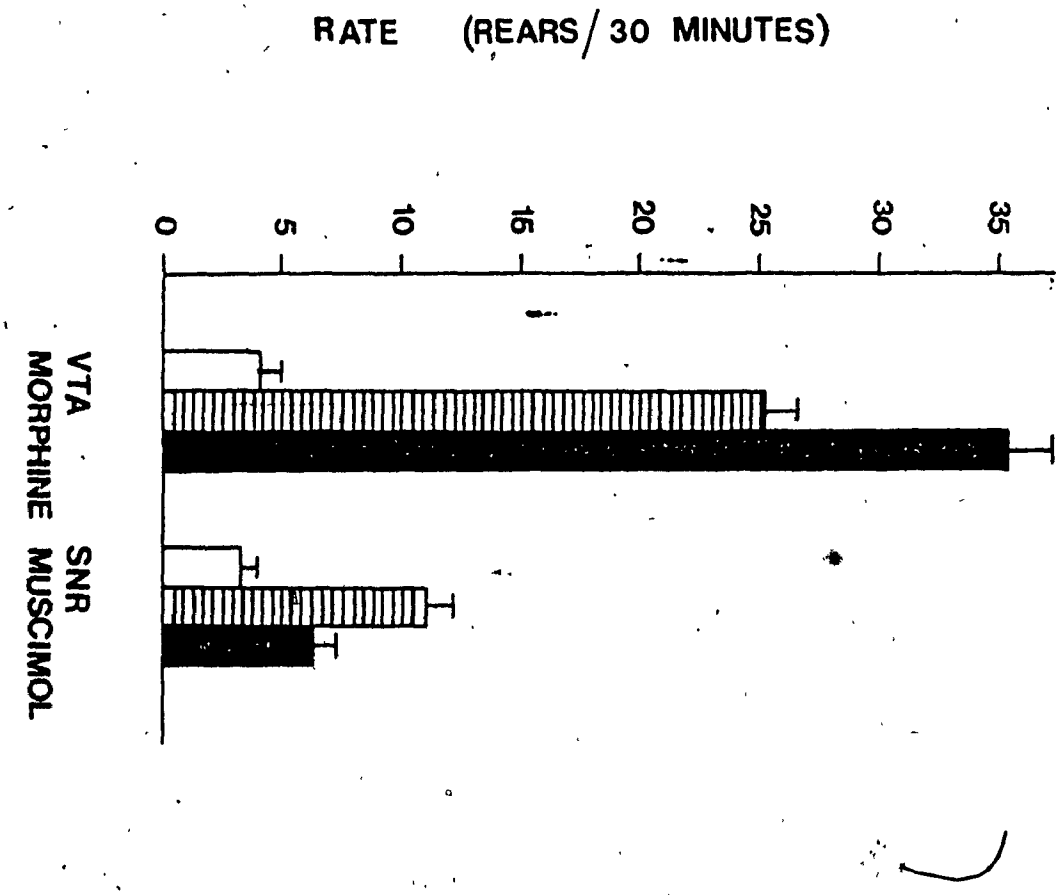
Animals injected with morphine engaged in directed rearing responses; the highest dose of morphine produced the most rearing. Animals injected with muscimol engaged in fewer directed rearing responses compared to morphine injected animals; the lower dose of muscimol produced more rearing than the higher dose (Figure 5). A two-way analysis of variance revealed a main effect of site ($F(1,14)=32.42$, $p<.01$), a main effect of dose ($F(2,28)=43.11$, $p<.001$) and a dose by site interaction ($F(2,28)=27.46$, $p<.01$). Scheffe post hoc tests for multiple comparisons (Roscoe, 1979) revealed significant differences between morphine-induced rearing activity at all doses ($p<.05$), between muscimol-induced rearing activity at all doses ($p<.05$) and between morphine-induced and muscimol-induced rearing activity at both the low and high doses of the drug ($p<.05$).

Experiment III - Environmental Dependence of VTA Morphine-Induced Circling but not SNR Muscimol-Induced Circling

Methods

The same sixteen animals that were used in experiment II were used in the present experiment. The animals with VTA placements were injected with morphine (5 ug) and the animals with SNR placements

Figure 5. Mean number of rears (±SEM) following injection of saline, morphine (5 or 10 ug) into the VTA or muscimol (25 or 50 ng). The animals were habituated to the apparatus (in undrugged conditions) for a total of 60 minutes prior to testing with saline or drug.

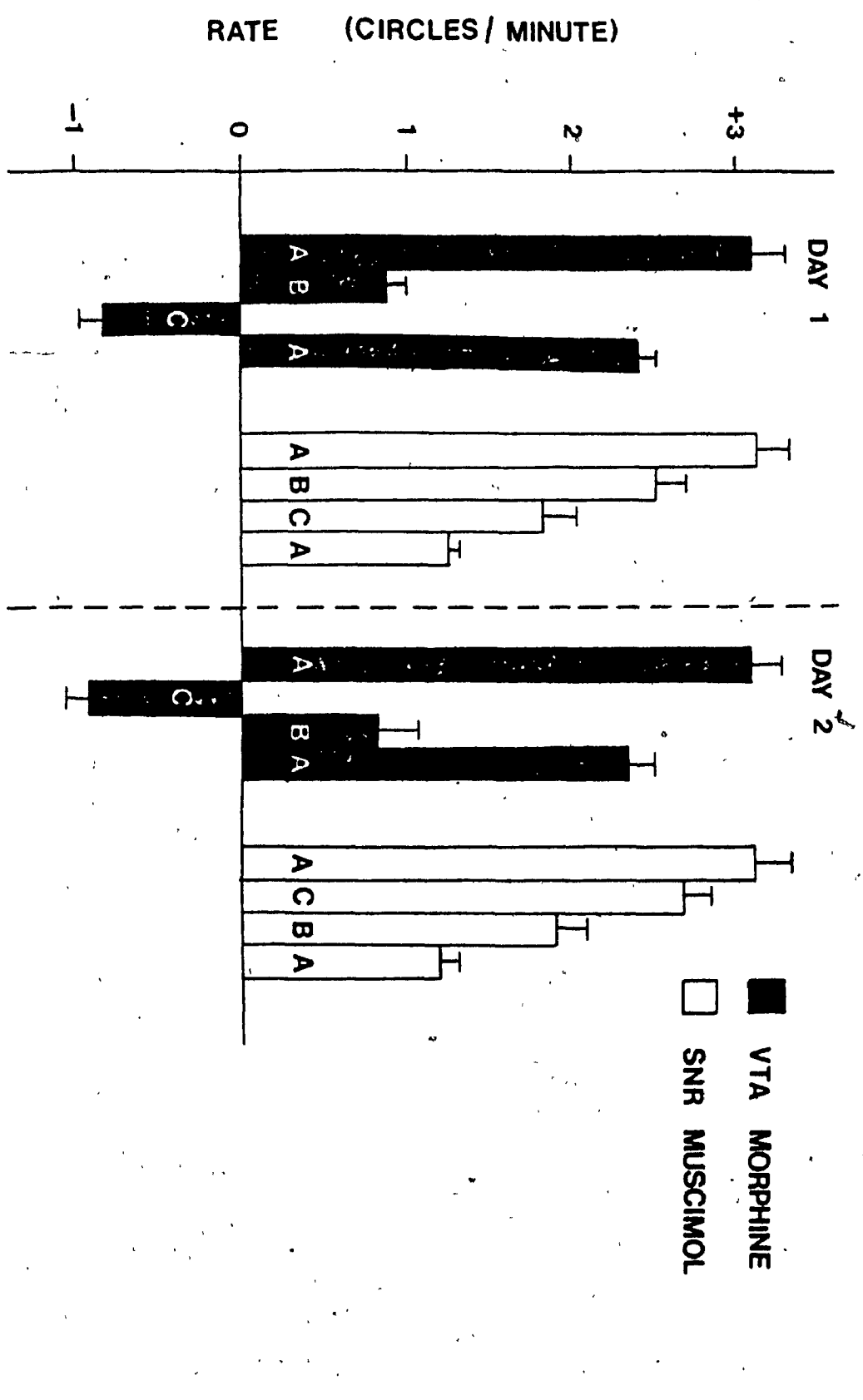

RATE (REARS/30 MINUTES)
0
5
10
15
20
25
30
35
VTA
MORPHINE
SNR
MUSCIMOL
SALINE
LOW DOSE
HIGH DOSE

were injected with muscimol (25 ng); these doses were used because they produce comparable rates of contralateral circling. The animals were tested in the apparatus used to measure direction of circling (General Methods) immediately following intracranial injections. The animals were tested on two separate days. On day one, the animals were placed in the round buckets for the first thirty minutes, on a 120 cm square wooden surface that had 30 cm high walls around the perimeter and a 76 cm square wooden hole in the center for the second thirty minutes, on a 180 cm square wooden surface with the outer perimeter open to the floor (one meter below) and a 30 cm high 76 cm square inner perimeter for the third thirty minutes and in the round plastic buckets for the last thirty minutes (A,B,C,A). On the second test day the animals were exposed to environments B and C in the opposite order (A,C,B,A). The total number and direction of circles per minute in each of the environments was determined.

Results

Animals given injections of morphine into the VTA circled contralateral to the side of injection when placed in environments A and B; they circled ipsilateral to the side of injection when placed in environment C. This was true regardless of the order of testing. Animals given injections of muscimol into the SNR circled contralateral to the side of injection in all three environments (Figure 6). This was also true regardless of the

Figure 6. Direction of circling as a function of environment and drug. Animals with cannulae aimed for the VTA were tested with morphine in three different environments; similar testing was performed on animals with cannulae in the SNR. The animals were tested on two different days. On the first day the order of exposure to the different environments was A,B,C,A; on the second day the order of exposure was A,C,B,A.

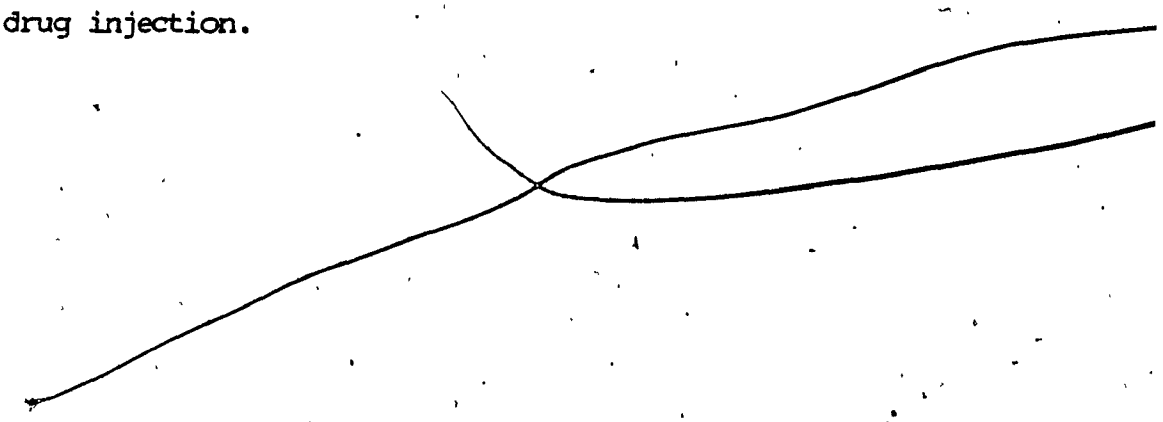

RATE (CIRCLES / MINUTE)
-1
0
1
2
+3
DAY 1
DAY 2
A B
C
A
A B C A
A
C
B A
A C B A
VTA MORPHINE
SNR MUSCIMOL

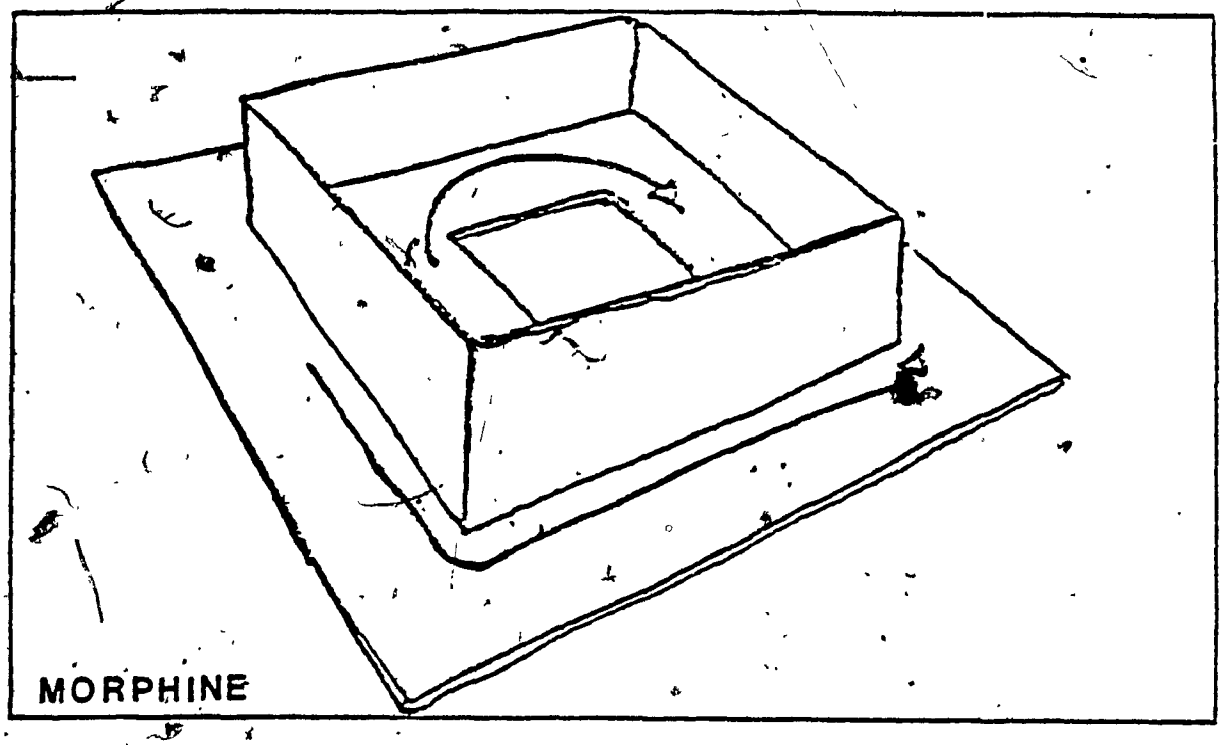
order of testing. Morphine-induced circlers tended to explore all parts of environments B and C, moving either around the square hole in the center of environment B or around the perimeter of the walls in environment C. Muscimol-induced circlers remained in a fixed location in both environments B and C (Figure 7). Most muscimol-injected animals fell through the square hole when tested in environment B; morphine-injected animals never fell through the hole. A three way analysis of variance revealed a main effect of site ($F(1,14)=581.46, p<.0001$), a main effect of environment ($F(3,42)=515.97$), $p<.0001$), and a site by environment interaction ($F(3,42)=679.04$, $p<.0001$).

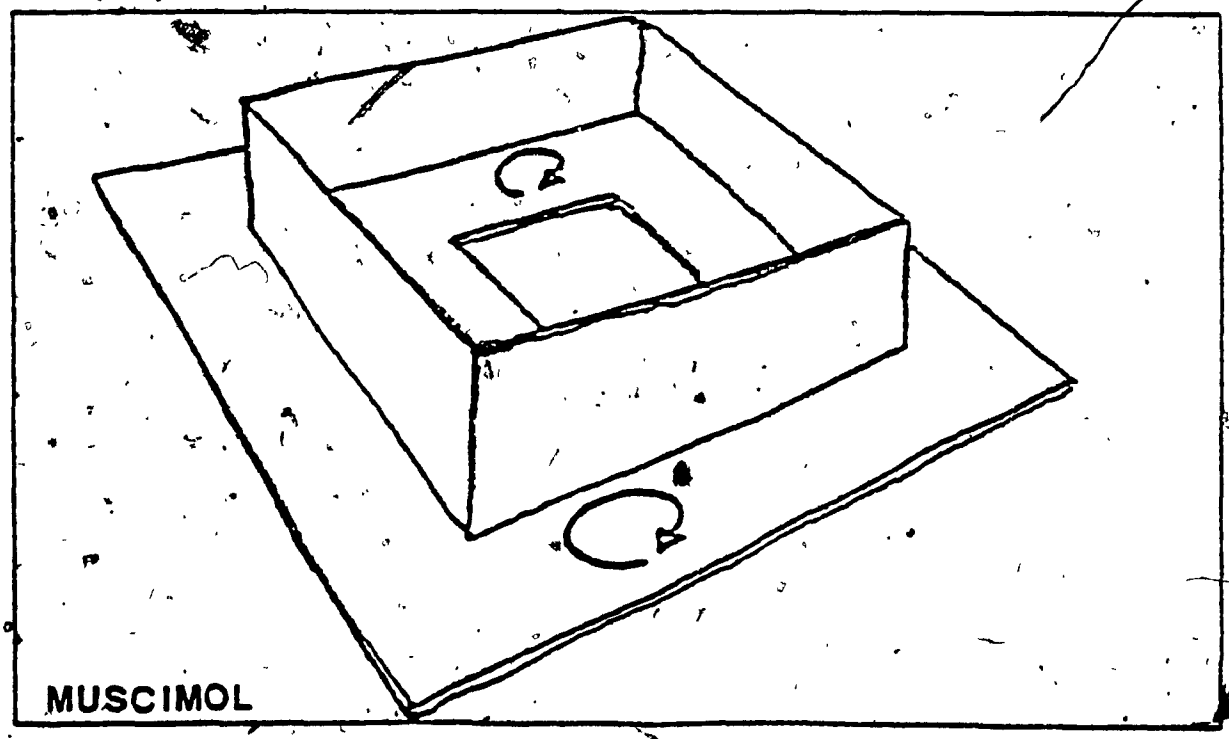
Discussion

Muscimol-induced circling appears to be relatively stimulus independent. At least three observations lead to this suggestion. First, the size of the circles remain constant regardless of the size of the test environment in which the animals are placed; environmental cues do not seem to encourage forward locomotion. Second, there is a strong 'forced' postural asymmetry in the longitudinal axis of the animals' body. This is true when the animals are at rest and not simply when they are moving. Third, the animals do not engage in directed rearing activity; in fact, as the dose of muscimol is increased, less rearing activity occurs. The circling seems to compete with the rearing response. Thus, even though rearing activity is often initiated, it less often gets expressed to completion

Figure 7. A diagrammatic representation of the type of circling activity elicited by either morphine in the VTA or muscimol in the SNR in two different environments. When morphine or muscimol-induced circlers are placed in enviroment B, they circle contralateral to the side of drug injection; in environment C however, animals given morphine circle ipsilateral to the side of drug injection and animals given muscimol circle contralateral to the side of drug injection.




MORPHINE


MUSCIMOL

(i.e. animals try to stand on their hind legs but fall down and begin to circle). Finally, the direction of muscimol-induced circling remains constant regardless of the environment; in fact, in all of the test environments, muscimol-induced circlers tend not to explore and remain in a relatively fixed location. It seems obvious when one watches them fall through a hole in the center of their environment (to a floor one meter below) that their forced, centrally generated movements caused their misfortune.

The suggestion that SNR muscimol injections cause 'forced-like' movements accompanied by strong postural asymmetries fits well with data demonstrating that unilateral stimulation or ablation of the striatum (the origin of the GABAergic fibers which synapse onto SNR neurons) causes asymmetries in the longitudinal axis of an animal's body. Part of these asymmetries are due to 90 degree head turns that are invariably elicited by either chemical (Ungerstedt, Butcher, Butcher, Anden and Fuxe, 1969) or electrical stimulation (Crossman, Lee and Slater, 1977). In addition to head turns, electrical stimulation in the area of the SNR are accompanied by driven contralateral activity in several groups of muscles including the trapezius, biventer cervicus, rectus capitis and scalenus dorsalis (Asdourian, Guela, Kelland, Shen, Zawisa and Lipinski, 1985). It is as yet unclear however, how these nigrally-derived muscular contractions gain access to the motor periphery since lesions of the main output pathways of the SNR (i.e. nigro-reticular,

nigro-thalamic, nigro-tectal) have no effects on muscular activity driven by SNR stimulation.

Although muscimol-induced circling seems relatively independent of environmental stimuli it may not be completely so since similar injections are known to cause some contralateral sensory enhancement. For example, animals given unilateral muscimol into the SNR demonstrate a contralateral enhancement of the perioral biting reflex (Huston, Nef, Papadopoulos and Welzl, 1980) such that they respond to tactile stimulation of the mouth area on the side of the body opposite to the drug injection more effectively than to similar stimulation on the side of the body ipsilateral to the drug injecton. These animals respond to tactile stimulation with a withdrawal of the lip followed by a vigorous biting of the probe. Similar bilateral injections of GABAergic agents are known to cause oral stereotypies including gnawing behaviours (Arnt and Scheel-Kruger, 1980; Huston, Nef, Papadopoulos and Welzl, 1980; Taha, Dean and Redgrave, 1982; Childs and Gale, 1983; Baumeister and Frye, 1984) The present experiments support the observation that such injections cause stereotyped biting behaviours since in all cases, animals in the open field made clear biting responses towards the spatula. Furthermore, when there was no stimulus in the environment on which the animal could gnaw, it often chewed its own paws. This phenomenon has also been reported previously in the literature (Baumeister and Frye, 1984). In these experiments animals gnaw at their bodies

even to the extent of causing lesions. While the observation that muscimol can enhance sensory responsiveness around the snout suggests that some sensory functions are subserved by SNR GABAergic systems, it does not necessarily mean that circling following such injections is also sensory dependent; it only suggests that there may be a sensory component to this activity.

Whereas SNR muscimol-induced contraversive circling appears to be relatively stimulus-independent, VTA morphine-induced contraversive circling appears to be environmentally-directed. At least three observations lead to this suggestion. First, in contrast to muscimol-induced circling, the size of the circles elicited by morphine injections depends upon the size of the test enclosure. Thus, when animals are placed in the small plastic buckets they make small circles; when placed in the large open field, they make large circles. Secondly, doses of morphine which cause strong contraversive circling tend to cause directed rearing responses as well. In this case, the rearing was directed towards the food stimulus located near the top of the apparatus. Finally, the direction of morphine-induced circling depends upon environmental stimuli since they circle contraversively when placed in some environments and ipsiversively when placed in others.

The fact that morphine-induced circling is dependent upon dopaminergic activation fits well with the suggestion that the

behaviour is dependent on environmental information. For example, it is well known that animals with a unilateral nigrostriatal dopamine lesion exhibit a unilateral sensory neglect in addition to the extensively studied movement asymmetries (Ljungberg and Ungerstedt, 1979; Marshall, 1980). As might be expected, the animal circles away from the visual field that is neglected, turning towards objects in the affective visual field. Thus, asymmetrical movements and asymmetrical sensations may be closely related, at least dopamine-induced ones. Recent evidence supports this suggestion. For example, an animal treated with moderate doses of apomorphine engages in forward locomotion (Teitelbaum et al., 1982) that includes snout-to-ground exploration. If the sensory input to the snout is unilaterally blocked by bandaging one half of the animals head, the direction of locomotion following apomorphine injections becomes biased towards the sensory intact side (Szechtman, 1983). Since morphine applied to the dopamine cell region increases dopaminergic activity as demonstrated electrophysiologically (Matthews and German, 1983) and behaviourally (i.e. Vezina and Stewart, 1984; Bozarth, 1983), unilateral injections should cause unilateral sensory enhancement. That this enhancement interacts with particular environmental stimuli to determine the direction the circling will take is evident since animals will circle a particular way in one environment

and the other way in another environment. While it is not clear why this is so, it has been observed previously that animals circling spontaneously following injections of dopaminergic agonists also tend to circle a particular way depending on environmental information; in these studies animals keep a particular side of the body exposed to edges (Pisa and Szechtman, 1984). These investigators suggest that dopaminergic agonists causing spontaneous directionally-biased movements do so by creating lateralized investigatory responses. With morphine injections into the VTA, animals tend to circle in the direction that keeps their 'enhanced' visual field away from walls. This too may be due to some sort of lateralized investigatory response; further work needs to address this issue.

GENERAL DISCUSSION

The basal ganglia comprise a complex set of neuronal systems that seems to play a primary role in motor behaviour. In an attempt to elucidate precise functions of the basal ganglia, many investigators have used the circling paradigm in which either unilateral activation or inactivation of different neural systems within basal ganglia structures causes animals to move with a directional bias.

While the evidence has shown that circling can result from manipulation at different levels of the basal ganglia, this does not mean that all levels of this complex system subserve the same motor functions. For example, stimulation of the ascending dopamine

systems (some of which form close connections with the basal ganglia) (Nauta, Smith, Faull and Domesick, 1978) causes behavioural activation accompanied by exploratory responses (i.e. Broekkamp et al., 1979; Kelley et al., 1980); when stimulation is unilateral, animals explore with a directional bias (Holmes and Wise, 1985a). Stimulation of the SNR (a major output pathway of the basal ganglia) seems to cause forced, stimulus independent (i.e. centrally generated) movements. While the dependent measure used to study these directionally-biased movements is the same (circling), it seems clear that they result from different underlying processes.

Investigators that make use of the circling paradigm should be cognizant of the fact that directionally-biased movements can can be both qualitatively and quantitatively different. It is the qualitative differences that may provide cues as to the potentially subtle functions subserved by different neurochemical systems. Awareness of these subtleties might lead to a clearer understanding of the underlying organization of the central nervous system mechanisms of movement.

REFERENCES

Aghajanian, G.K. and Bunney, B.S. (1974). Dopaminergic and non-dopaminergic neurons of the substantia nigra: Differential responses to putative transmitters. In, Proceedings of the 9th International Congress of the Collegium Internationale Neuropsychopharmacologicum, J. R. Boissier, H. Hippius and P. Pichot (Eds.), Exerpta Medica Foundation, Amsterdam.

Anden, N.E., Dahlstrom, A., Fuxe, K. and Larsson, K. (1966). Functional role of nigro-neostriatal dopamine neurons. Journal of Pharmacy and Pharmacology, 19, 627-629.

Anden, N.E. (1970). Effects of amphetamine and some other drugs on central catecholamine mechanisms. In E. Costa and S. Garattini (Eds.). Amphetamine and related compounds. New York: Raven Press, 1970.

Anlezark, C., Pycock, C. and Meldrum, B. (1976). Ergot alkaloids as dopamine agonists: comparisons in two rodent models. European Journal of Pharmacology, 37, 295-302.

Arbuthnott, G.W., Crow, T.J., Fuxe, K. and Ungerstedt, U. (1970). Behavioural effects of stimulation in the region of the substantia nigra. Journal of Physiology, London, 210, 61-62P.

Arbuthnott, G.W. and Crow, T.J. (1971). Relation of contraversive turning from unilateral release of dopamine from the nigrostriatal pathway in rats. Experimental Neurology, 30, 484-491.

Arbuthnott, G.W. and Ungerstedt, U. (1975). Turning behaviour

induced by electrical stimulation of the nigroneostriatal system of the rat. Experimental Neurology, 47, 162-172.

Arnt, J. and Scheel-Kruger, J. (1979a). GABAergic and glycerginergic mechanisms within the substantia nigra: Pharmacological specificity of dopamine independent contralateral turning behaviour and interactions with other neurotransmitters. Psychopharmacolgia, Berlin, 62, 267-277.

Arnt, J. and Scheel-Kruger, J. (1979b). Behavioural differences induced by muscimol selectively injected into pars compacta and pars reticulata of substantia nigra. Journal of Pharmacy and Pharmacology, 31, 306.

Arnt, J. and Scheel-Kruger, J. (1980). Intranigral GABA antagonists produce dopamine-independent biting in rats. European Journal of Pharmacology, 62, 51-61.

Asdourian, C., Geula, M.D., Kelland, M.D., Shen, R.Y., Zawisa, D.J. and Lipinski, W. (1985). Nigral influences on neck and shoulder muscles in the rat. Neuroscience Abstracts, 11, 364.

Atalay, J. and Wise, R.A. (1983). Time course of pimozide effects on brain stimulation reward. Pharmacology, Biochemistry and Behaviour, 18, 655-658.

Baldessarini, R.J., Walton, K.G. and Borgman, R.J. (1976). Prolonged apomorphine-like behavioural effects of apomorphine esters. Neuropharmacology, 15, 471-478.

Barnett, A. and Goldstein, J. (1975). Head-turning induced by electrical stimulation of the caudate nucleus and its antagonism by antiparkinson

drugs. Journal of Pharmacology and Experimental Therapeutics, 194, 296-302.

Bartholini, G., Scatton, B., Worms, P., Zivkovic, B. and Lloyd, K.G. Interactions between GABA, dopamine, acetylcholine and glutamate-containing neurons in the extrapyramidal and limbic systems. In G. Di Chiara and G.L. Gessa (Eds.), GABA and the Basal Ganglia: Advances in Biochemical Psychopharmacology, Vol. 30, New York, Raven Press, 1981, pp. 119-128.

Baumeister, A.A. and Frye, G.D. (1984). Self-injurious behaviour in rats produced by intranigral microinjection of GABA agonists. Pharmacology, Biochemistry and Behaviour, 21 (1), 89-95.

Bjorklund, A., Dunnett, S.B., Lewis, M.E. and Iversen, S.D. (1982). Reinnervation of the denervated striatum by substantia nigra transplants: functional consequences as revealed by pharmacological and sensorimotor testing. Brain Research, 199, 307-333.

Boulu, E., Rapin, J.R., Lebas, M. and Jacquet, C. (1972). Actione centrale de l'amphetamine de l'epinephrine et seur derive p-hyroxyle, apres lesion unilaterale du fasceau nigrostriatal chez le rat. Psychopharmacologia, 26, 54-61.

Bozarth, M.A. Opiate Reward Mechanisms Mapped by Intracranial Self-Administration. In Smith and Lane, (Eds.), The Neurobiology of Opiate Reward Pocesses. Elsevier Biomedical Press, 1983.

Broekkamp, C.L.E., Phillips, A.G. and Cools, A.R. (1979). Stimulant effects of enkephalin microinjection into the dopaminergic A-10

area. Nature, 278, 560-562.

Bunney, B.S. and Aghajanian, G.K. (1976). Dopaminergic influence in the basal ganglia: evidence for striatonigral feedback regulation. In The Basal Ganglia, M.D. Yahr (Ed.), Raven-Press: New York. pp. 249-267.

Childs, J.A. and Gale, K. (1983). Evidence that the nigrotegmental GABAergic projection mediates stereotypy induced by apomorphine and intranigral muscimol. Life Science, 33 (10), 1007-1010.

Christie, J.E. and Crow, T.J. (1971). Turning behaviour as an index of the action of amphetamines and ephedrines on central DA-containing neurons. British Journal of Pharmacology, 43, 658-667.

Christie, J.E. and Crow, T.J. (1973). Behavioural studies of the actions of cocaine, monoamine oxidase inhibitors and iminodibenzyl compounds on central dopamine neurons. British Journal of Pharmacology, 47, 39-47.

Costall, B. and Naylor, R. (1974a). Dopamine agonist and antagonist activities of piribidel (ET 495) and its metabolites. Naunyn-Schmiedebergs Archives of Pharmacology, 32, 87-92.

Costall, B. and Naylor, R. (1975). The behavioural effects of dopamine applied intracerebrally to areas of the mesolimbic system. European Journal of Pharmacology, 32, 87-92.

Costall, B., Naylor, R. and Neumeyer, J.L. (1975). Differences in the nature of the stereotyped behaviour induced by apomorphine derivatives in the rat and in their actions in

the extrapyramidal and mesolimbic brain areas. European Journal of Pharmacology, 31, 1-16.

Costall, B., Naylor, R., Cannon, J. and Lee, T. (1977). Differentiation of the dopamine mechanisms mediating stereotyped behaviour and hyperactivity in the nucleus accumbens and caudate putamen. Journal of Pharmacy and Pharmacology, 29, 337-342.

Costall, B., Marsden, C.D., Naylor, R.J. and Pycock, C.J. (1976). The relationship between striatal and mesolimbic dopamine dysfunction and the nature of circling responses following 6-OHDA and electrolytic lesions of the ascending dopamine systems of the rat brain. Brain Research, 118, 87-113.

Costall, B. and Naylor, R.J. (1974b). Specific asymmetric behaviour induced by the direct chemical stimulation of neostriatal dopaminergic mechanisms. Naunyn-Schmeidebergs Archives of Pharmacology, 285, 83-91.

Costall, B., Fortune, D.H., Naylor, R.J. and Nohria, V. (1979). A study of drug action on normal and denervated striatal mechanisms. European Journal of Pharacology, 56, 207-216.

Costall, B., Naylor, R.J. and Pettit, J.C. (1974c). Changes in dopamine agonist and antagonistic activity following temporary inactivation of the striatum. Naunyn-Schmiedebergs Archives of Pharmacology, 285, 103-126.

Costall, B., Naylor, R.J. and Pinder, R.M. (1974d). Design of agents for stimulation of neostriatal dopaminergic mechanisms. Journal of

Pharmacy and Pharmacology, 26, 753-762.

Coward, D. M. (1982). Nigral actions of GABA agonists are enhanced by chronic fluphenazine and differentiated by concomitant flurazepam. Psychopharmacology, 76, 294-298.

Crossman, A.R., Lee, L.A. and Slater, P. (1977). Effects of manipulating pallidal and nigral GABA on striatally-mediated head turning in the rat. British Journal of Pharmacology, 27, 943-946.

Crossman, A.R, Walker, R.J. and Woodruff, G.N. (1973). Picrotoxin antagonisms of gamma-aminobutyric acid inhibitory responses and synaptic inhibition in the rat substantia nigra. British Journal of Pharmacology, 49, 696-698.

Crow, T.J. (1971). The relationship between lesion site, dopamine neurons and turning behaviour in the rat. Experimental Neurology, 32, 247-255.

Di Chiara, G., Porceddu, M.L., Imperato, A. and Morelli, M. Role of GABA in the Striatal output system: Globus Pallidus, Nucleus Entopeduncularis, Substantia Nigra and Nucleus Subthalamicus. In G. Di Chiara and G.L. Gessa (Eds.), Gaba and the Basal Ganglia: Advances in Biochemical Psychopharmacology, Vol. 30, New York: Raven Press, 1981.

Dray, A. (1979). The striatum and substantia nigra: a commentary on their relationship. Neuroscience, 4, 1407-1439.

Dray, A., Fowler, L.J., Oakley, N.R., Simmonds, M.A. and Tanner, T. (1979). Regulation of nigro-striatal dopaminergic

neurotransmission in the rat. Neuropharmacology, 16, 511-518.

Dray, A., Oakley, N.R. and Simmonds, M.A. (1975). Rotational behaviour following inhibition of GABA metabolism unilaterally in the rat substantia nigra. Journal of Pharmacy and Pharmacology, 27, 627-629.

Elkhawad, A.O. and Woodruff, G.N. (1975). Studies on the behavioural pharmacology of a cyclic analogue of dopamine following its injection into the brain of conscious rats. British Journal of Pharmacology, 54, 107-114.

Ellinwood, E.H. and Kilbey, M.M. (1975). Amphetamine stereotypy: The influence of environmental factors and prepotent behavioural patterns on its topography and development. Biological Psychiatry, 10 (1), 3-16.

Fallon, J.H. and Moore, R.Y. (1978). Catecholamine innervation of the basal forebrain and neostriatum. Journal of Comparative Neurology, 180, 545-580.

Feltz, P. (1971). Gamma-aminobutyric acid and a cadato-nigral inhibition. Canadian Journal of Physiology and Pharmacology, 49, 1113-1115.

Forman, D. and Ward, W.D. (1957). Responses to electrical stimulation of caudate nucleus in cats in chronic experiments. Journal of Neurophysiology, 20, 230-244.

Gallistel, C.R., Shizgal, P. and Yeomans, J. (1981). A portrait of the substrate for self-stimulation. Psychological Review, 88 (3), 228-273.

Glick, S.D., Jerussi, T.P., Cox, R.D. and Fleisher, L.N. (1977). Pre and

post synatpic actions of apomorphine: differentiation by rotatory effect in normal rats. Archives International Pharmacodynamics and Therapeutics, 225, 303-307.

Glick, S.D., Zimmerberg, B. and Greenstein, S. (1976). Individual differences among mice in normal and amphetamine-enhanced locomotor activity: relationship to behavioural indices of striatal asymmetry. Brain Research, 105, 362-364.

Glick, S.D., Jerussi, T.P., Waters, D.H. and Green, J.P. (1974). Amphetamine induced changes in striatal dopamine and acetylcholine levels and relationship to rotation (circling behaviour) in rats. Biochemistry and Pharmacology, 23, 3223-3225.

Glick, S.D., Hinds, P.A. and Shapiro, R.M. (1983). Cocaine-induced rotation: Sex-dependent differences between left- and right-sided rats. Science, 221, 775-777.

Grace, A. A. and Bunney, B.S. (1979). Paradoxical GABA excitation of nigral dopaminergic cells: indirect mediation through reticulata inhibitory neurons. European Journal of Pharmacology, 59, 211-218.

Gratton, A. and Wise, R.A. (1985). Mapping of contraversive and ipsiversive circling responses to ventral tegmental and substantia nigra electrical stimulation. Physiology and Behavior, 31, 61-65.

Greene, A.R. and Grahame-Smith, D.G. (1975). 5-Hydroxytryptamine and other indoles in the central nervous system. In: Handbook of Psychopharmacology, Iversen, L.L., Iversen, S.D. and Snyder,

S.H. (Eds.), Plenum Press, New York, pp. 169-245.

Havemann, U., Turski, L., Schwarz, M. and Kuschinsky, K. (1983). Nigral GABAErgic mechanisms and EMG activity in rats: differences between pars reticulata and pars compacta. European Journal of Pharmacology, 92, 49-56.

Holmes, L.J., Bozarth, M.A. and Wise, R.A. (1983). Circling from intracranial morphine applied to the ventral tegmental area in rats. Brain Research Bulletin, 11, 295-298.

Holmes, L.J. and Wise, R.A. (1985a). Contralateral circling induced by teg- mental morphine: Anatomical localization, pharmacological specificity and phenomenology. Brain Research, 326, 19-26.

Holmes, L.J. and Wise, R.A. (1985b). Dopamine-dependent contralateral circling induced by neurotensin applied unilaterally to the ventral tegmental area in rats. Brain Research Bulletin, 15, 537-538.

Horton, R.W. and Pycock, C.J. (1977). Circling behaviour in rodents following an imbalance of basal ganglia GABA concentrations. Pharmacology, Biochemistry and Behaviour, 6, 493-497.

Huston, J.P., Nef, B., Papadopoulos, G. and Welzl, H. (1980). Activation and lateralization of sensorimotor field for perioral biting reflex by intranigral GABA agonist and by systemic apomorphine in the rat. Brain Research Bulletin,

5, 745-749.

Iwamoto, E.T. and Way, E.L. (1977). Circling behaviour and stereotypy induced by intranigral opiate microinjections. Journal of Pharmacology and Experimental Therapeutics, 203, 347-359.

Jackson, E.A. and Kelly, P.H. (1984). Effects of intranigral injections of dopamine agonists and antagonists, glycine, muscimol and N-methyl-D,L-aspartate on locomotor activity. Brain Research Bulletin, 13 (2), 309-317.

James, T.A. and Starr, M.S. (1977). Behavioural and biochemical effects of Substance P injected intoo the substantia nigra of the rat. Journal of Pharmacy and Pharmacology, 29, 181-182.

James, T.A. and Starr, M.A. (1978). The role of GABA in the substantia nigra. Nature (London), 275, 229-230.

Jerussi, T.P. and Glick, S.D. (1976). Drug-induced rotation in rats without lesions: behavioural and neurochemical indices of a normal asymmetry in nigro-striatal function. Psychopharmacology, 47, 249-260.

Joyce, E.M. and Iversen, S.D. (1979). The effect of morphine applied locally to the mesencephalic dopamine cell bodies on spontaneous locomotor activity in the rat. Neuroscience Letters, 14, 207-212.

Kalivas, P.W., Burgess, S.K., Nemeroff, C.B. and Prange, A.J. Jr.

(1983). Behavioural and neurochemical effects of neurotensin microinjections into the ventral tegmental area of the rat. Neuroscience, 8, 495-505.

Kelley, A.E., Stinus, L. and Iversen, S.D. (1980). Interactions between d-ala-met-enkephalin, A-10 dopaminergic neurons and spontaneous behaviour in the rat. Behavioural and Brain Research, 1, 1-34.

Kelly, P.H. (1975). Unilateral 6-hydroxydopamine lesions of nigrostriatal and mesolimbic dopamine-containing terminals and drug-induced rotation in rats. Brain Research, 100, 163-169.

Kelly, P.H. and Moore, K.E. (1977). Mesolimbic dopamine neurons: Effects of six-hydroxydopamine induced destruction and receptor blockade on drug-induced rotation in rats. Psychopharmacology, 55, 45-51.

Kelly, P.H. and Moore, K.E. (1976). Mesolimbic dopamine neurons in the rotational model of nigrostriatal function. Nature, 263, 695-696.

Kelly, P.H. and Miller, R.J. (1975a). The interaction of neuroleptic and muscarinic agents with central dopaminergic systems. British Journal of Pharmacology, 54, 115-121.

Kelly, P.H., Seviour, P.W. and Iversen, S.D. (1975b). Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Research, 94, 507-522.

Kilpatrick, I.C., Starr, M.S., Fletcher, A., James, T.A. and MacLeod, N.K. (1980). Evidence for a GABAergic nigrothalamic pathway in the rat. I. Behavioural and biochemical studies. Experimental Brain Research, 40, 45-54.

Kilpatrick, I.C. and Starr, M.S. (1981). Involvement of dopamine in circling responses depends upon intranigral site of injection. European Journal of Pharmacology, 69, 407-419.

Kim, J.S., Bak, I.J., Hassler, R. and Okada, Y. (1971). Role of gamma-aminobutyric acid (GABA) in the extrapyramidal motor system. II. Some evidence for existence of a type of GABA-rich striato-nigral neurons. Experimental Brain Research, 14, 95-102.

Koob, G.F., Del Fiacco, M. and Iversen, S.D. (1978). Spontaneous and amphetamine-induced behaviour after bilateral injection of ethanolamine-O-sulphate into the substantia nigra. Brain Research, 146 (2), 313-323.

Kozlowski, M.R. and Marshall, J.F. (1980). Rotation induced by intranigral injections of GABA agonists and antagonists: zone-specific effects. Pharmacology, Biochemistry and Behaviour, 13 (4), 561-567.

Lee, L.A. and Slater, P. (1981a). Role of globus pallidus and substantia nigra efferent pathways in striatally evoked head turning in the rat. Experimental Brain Research, 44, 170-176.

Lee, L.A., Slater, P. and Crossman, A.R. (1981b). The role of the

substantia nigra in a striatally-evoked motor response in the rat: effects of intranigral drugs and lesions. Neurosci[illegible]6, 359-366.

Lee, R.G. (1984). Physiology of the Basal Ganglia: An overview. Canadian Journal of Neurological Sciences, 11, 124-128.

Lindvall, O. and Bjorklund, A. (1978). Organization of catecholamine neurons in the rat central nervous system. In L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.), Handbook of Psychopharmacology, Volume 9, New York: Plenum Press.

Ljungberg, T. and Ungerstedt, U. (1976). Sensory inattention produced by six-hydroxydopamine-induced degeneration of ascending dopamine neurons in the brain. Experimental Neurology, 53, 585-600.

Lotti, V.J. (1971). Actions of various acting agents in mice with unilateral caudate brain lesions. Life Science, 10 (1), 781-789.

Marsden, C.D. (1982). The mysterior motor function of the basal ganglia: The Robert Wartenberg Lecture. Neurology, 32(5), 614-639.

Marshall, J.F. (1979). Somatosensory inattention after dopamine-depleting intracerebral 6-OHDA injections: spontaneous recovery and pharmacological control. Brain Research, 177, 311-324.

Marshall, J.F. and Ungerstedt, U. (1977). Striatal efferents play a role in maintaining rotational behaviour in the rat. Science, 199, 62-64.

Martin, G.E., Papp, N.I. and Bacino, C.B. (1978). Contralateral

turning evoked by intranigral microinjection of GABA and other agonists. Brain Research, 155, 297-312.

Matthews, R.T. and German, D.C. (1982). Electrophysiological evidence for morphine excitation of ventral tegmental area dopamine neurons. Neuroscience Abstracts, 8; 777.

McKenzie, G.M., Gordon, R.J. and Viik, K. (1972). Some biochemical and behavioural correlates of a possible animal model of human hyperkinetic syndromes. Brain Research, 47, 439-456.

Mendez, J.S., Cotzias, G.C., Finn, B.W. and Dahl, K. (1975). Rotatory behaviour induced in nigra-lesioned rats by N-propyl-noraporphine, apomorphine and L-dopa. Life Science, 16, 1737-1742.

Mogenson, G.J., Wu, M. and Manchanda, S.K. (1979). Locomotor activity initiated by microinfusions of picrotoxin into the ventral tegmental area. Brain Research, 161, 311-319.

Muller, P. and Seeman, P. (1974). Neuroleptics: relation between cataleptic and anti-turning actions, and role of the cholinergic system. Journal of Pharmacy and Pharmacology, 26, 981-984.

Nakamura, A., Engel, J. and Goldstein, M. (1978). Blockade of lergotril or apomorphine induced turning behaviour by haloperidol and clozapine. Communications in Psychopharmacology, 2, 185-190.

Nauta, W.J.H., Smith, P.H., Faull, R.L.M. and Domesick, V.B.

(1978). Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. Neuroscience, 4, 385-401.

Neumeyer, J.L., Dafeldecker, W.P., Costall, B. and Naylor, R.J. (1977). Aporphines 21. Dopaminergic activity of aporphine and benzylisoquinoline derivatives. Synthesis of 8 hydroxy-aporphines and 1-(hydroxybenzyl)-2-n-propyl-1,2,3,4-tetra-hydroisoquinolines. Journal of Medicinal Chemistry, 20, 190-196.

Oberlander, C., Dumont, C. and Boissier, J.R. (1977). Rotational behaviour after unilateral intranigral injection of muscimol in rats. European Journal of Pharmacology, 43, 389-390.

Okada, Y., Nitsch-Hassler, C. Kim, J.S., Bak, I.J., and Hassler, R. (1971). Role of gamma-aminobutyric acid (GABA) in extrapyramidal motor system. I. Regional distribution of GABA in rabbit, rat, guinea pig and baboon CNS. Experimental Brain Research, 13, 514-523.

Olianas, M.C., De Montis, G.M., Muulas, G. and Tagliamonte, A. (1978). The striatal dopaminergic function is mediated by the inhibition of a nigral, non-dopaminergic neuronal system via a strio-nigral GABA pathway. European Journal of Pharmacology, 45, 291-294.

Olpe, H.-R., Schellenberg, H. and Koella, W.P. (1977). Rotational behaviour induced in rats by intranigral application of GABA-related drugs and GABA antagonists. European Journal of Pharmacology, 45, 291-294.

Papadopoulos, G. and Huston, J.P. (1980). Removal of the telencephlon

spares turning induced by injection of GABA agonists and antagonists into the substantia nigra. Behavioural Brain Research, 1 (1), 25-38.

Pellegrino, L.J., Pellegrino, A.S. and Cushman, A.J. (1979). A Stereotaxic Atlas of the Rat Brain. New York: Plenum Press.

Pert, A. The effects of opiates on nigrostriatal dopamine function. In N.L. Van Ree and L. Terenius (Eds.), Developments in Neuroscience, Vol. 4, Elsevier, North Holland Biomedical Press, 1978.

Pert, A. and Sivit, C. (1977). Neuroanatomical focus for morphine and enkephalin hypermotility. Nature, 265, 645-647.

Pijnenburg, A.J.J. and Van Rossum, J.M. (1973). Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. Journal of Pharmacy and Pharmacology, 25, 1003-1005.

Pijnenburg, A.J.J., Honig, W.M.M. and Van Rossum, J.M. (1975). Inhibition of d-amphetamine induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. Psychopharacologia, 41, 87-95.

Pisa, M. and Szechtman, H. (1984). Lateralization of attention in apomorphine-treated rats. Neuroscience Abstracts, 10, 1068.

Precht, W. and Yoshida, M. (1971). Blockade of caudate-evoked inhibtion of neurons in the substantia nigra by picrotoxin. Brain Research, 32, 229-232.

Pycock, C.J. (1980). Turning behaviour in animals. Neuroscience, 5, 461-514.

Pycock, C.J., Donaldson, I.M. and Marsden, C.D. (1975). Circling behaviour produced by unilateral lesions in the rgion of the locus coeruleus in rats.. Brain Research, 97, 317-319.

Pycock, C.J. and Marsden, C.D. (1978). The rotating rodent? A two component system? European Journal of Pharmacology, 47, 167-175.

Pycock, C.J., Tarsey, D. and Marsden, C.D. (1975). Inhibition of circling behaviour with neuroleptic drugs in mice with unilateral 6-hydroxydopamine lesions of the striatum. Psycholpharmacology, 45, 211-219.

Reavill, C., Jenner, P., Leigh, N. and Marsden, C.D. (1979). Turning behaviour induced by injection of muscimol or picrotoxin into the substantia nigra demonstrates dual GABA components. Neuroscience Letters, 12, 323-328.

Reavill, C., Leigh, N., Jenner, P. and Marsden, C.D. Critical role of midbrain reticular formation in the expression of dopamine-mediated circling behaviour. In G. Di Chiara and G.L. Gessa, (Eds.), GABA and the Basal Ganglia, Advances in Biochemical Psycholpharmacology, Vol. 30, New York: Raven Press. 1980, 187-204.

Ribak, C.E. The GABAergic neurons of the extrapyramidal system as revealed by immunocytochemistry. In G. Di Chiara and G.L. Gessa

(Eds.), GABA and the Basal Ganglia, Advances in Biochemical Psychopharmacology, Vol. 30, New York: Raven Press, 1980, pp. 23-36.

Ribak, C.E., Vaughn, J. E. and Roberts, E. (1980). GABAergic nerve terminals decrease in the substantia nigra following hemitransections of the striatonigral and pallidonigral pathways. Brain Research, 192, 413-420.

Roffman, M., Bernard, P.S., Dawson, K.M., Wobiski, R.E. and Shelens, J.K. (1978). The effects of haloperidol and clozapine on circling induced by electrical stimulation of the ventral tegmentum. Neuropharmacology, 17, 943-946.

Roscoe, J.T. Fundamental Research Statistics For The Behavioral Sciences. Holt, Rinehart and Winston: New York. 1975.

Scheel-Kruger, J., Arnt, J. and Magelund, G. (1977). Behavioural stimulation induced by muscimol and other GABA agonists into the substantia nigra. Neuroscience Letters, 4, 351-356.

Scheel-Kruger, J., Magelund, G. and Olianas, M.C. Role of GABA in the striatal output system: Globus pallidus, nucleus entopeduncularis, substantia nigra and nucleus subthalamicus. In G. Di Chiara and G.L. Gessa (Eds.), GABA and the Basal Ganglia, Advances in Biochemical Psychopharmacology, Volume 30, New York: Raven Press, 1980, pp. 165-186.

Setler, P.E., Malesky, M., McDevitt, J. and Turner, K. (1978). Rotation produced by administration of dopamine and

related substances directly into the supersensitive caudate nucleus. Life Science, 23, 1277-1284.

Starr, M.S. and Kilpatrick, I.C. (1981). Nigral-derived muscimol circling - ipsiversive, contraversive or contraversial? Nature, 290, 610.

Szechtman, H. (1983). Peripheral sensory input directs apomorphine-induced circling in rats. Brain Research, 264, 332-335.

Szechtman, H. and Pisa, M. (1984). Apomorphine induces postural asymmetries independent of asymmetries in the direction of turning. Neuroscience Abstracts, 10, 1069.

Taha, E.B., Dean, P. and Redgrave, P. (1982). Oral behaviour induced by intranigral muscimol is unaffected by haloperidol but abolished by large lesions of the superior colliculus. Psychopharmacology, 77, 272-278.

Tarsy, D., Pycock, C., Meldrum, B. and Marsden, C.D. (1975). Rotational behaviour induced by intranigral picrotoxin. Brain Research, 89, 160- 165.

Teitelbaum, P., Szechtman, H., Sirkin, D.W. and Golani, I. Dimensions of movement, movement subsystems and local reflexes in the dopaminergic systems underlying exploratory locomotion. In M.Y. Spiegelstein and A. Levy (Eds.), Behavioural Models and the Analysis of Drug Action. Elsevier Scientific, Amsterdam, 1982, pp. 357-385.

Thornburg, J.E. and Moore, K.E. (1974). A comparison of effects of apomorphine and ET 495 on locomotor activity and circling behaviour.

Journal of Pharmacy and Experimental Therapeutics, 192, 42-49.

Turski, L., Havemann, U. and Kuschinsky, K. (1983). The role of the substantia nigra in motility of the rat: muscular rigidity, body asymmetry and catalepsy after injection of morphine into the nigra. Neuropharmacology, 22 (9), 1039-1048.

Tye, N.C., Horsman, L., Wright, F.C., Large, B.T. and Pullar, J.A. (1977). Two dopamine receptors: supported evidence with the rat rotational model. European Journal of Pharmacology, 82, 69-93.

Ungerstedt, U. (1968). 6-Hydroxydopamine induced degeneration of central monoamine neurons. European Journal of Pharmacology, 5, 107-110.

Ungerstedt, U. (1971a). Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. Acta Physiologica Scandinavia, Supplement 367, 49-68.

Ungerstedt, U. (1971b). Post-synaptic supersensitivity after six-hydroxy-dopamine induced degeneration of the nigrostriatal dopamine system in the rat brain. Acta Physiologica Scandinavia, Supplement 367, 69-93.

Ungerstedt, U., Butcher, L.L., Butcher, S.G., Anden, S.E. and Fuxe, K. (1969). Direct chemical stimulation of dopaminergic mechanisms in the neostriatum of the rat. Brain Research, 14, 461-471.

Vaccarino, F.J. and Franklin, K.J. (1982a). Self-stimulation and circling reveal functional differences between medial and lateral substantia nigra. Behavioural Brain Research, 17, 431-434.

Vaccarino, F.J. and Franklin, K.J. (1982b). Dopamine mediates ipsi and contraversive circling elicited from the substantia nigra. Pharmacology, Biochemistry and Behaviour, 17, 431-434.

Vezina, P. and Stewart, J., (1984). Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA. Pharmacology, Biochemistry and Behaviour, 20, 925-934.

Von Voigtlander, P.F. and Moore, K.E. (1971). Nigro-striatal pathway: Stimulation-evoked release of (β H)dopamine from caudate nucleus. Brain Research, 35, 580-583.

Von Voigtlander, P.F. and Moore, K.E. (1973). Turning behaviour of mice with unilateral 6-hydroxydopamine lesions in the striatum: effects of apomorphine, amphetamine and other psychomotor stimulants. Neuropharmacology, 12, 451-462.

Waddington, J.L. (1980). GABAergic mechanisms in the substantia nigra. Nature (London), 282, 696.

White, R.P. and Himwich, H.E. (1957). Circus movements and excitation of striatal and mesodiencephalic centers in rabbits. Journal of Neurophysiology, 21, 81-90.

Wise, R.A. Brain neuronal systems mediating reward processes.

In: The Neurobiology of Opiate Reward Processes, J.E. Smith and J.D. Lane (Eds). Amsterdam: Elsevier/North Holland Biomedical Press, 1983, pp. 405-437.

Wise, R.A. and Holmes, L.J. Circling from unilateral VTA morphine injections: Direction is controlled by environmental stimuli. Brain Research Bulletin, in press.

Wolfarth, S., Kolasiewicz, W. and Sontag, K.H. (1981). The effects of muscimol and picrotoxin injections into the cat substantia nigra. Archives of Pharmacology, 7 (1), 54-60.