A COMPARATIVE STUDY ON THE EFFECTS OF UNILATERAL INTRANIGRAL INJECTIONS OF SUBSTANCE P AND SUBSTANCE K ON CIRCLING BEHAVIOR

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A Comparative Study on the Effects of Unilateral Intranigral Injections of Substance P and Substance K on Circling Behavior

by

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ABSTRACT

The effects of unilateral intranigral injections of substance P and the newly discovered tachykinin, substance K, were compared using a rotometer to measure circling behavior in rats. Various solutions were injected directly into the right substantia nigra pars reticulata (SNR), via chronically implanted guide cannulae. Substance P, substance K, apomorphine and saline were administered sequentially in each rat. This repeated measures design allowed for comparisons to be made with respect to the effects of each solution on circling. Separate groups were formed so that three different concentrations of substance P and substance K could be tested (Groups A, B, C received 8, 12, 16 ug / .5 ul, in that order). Groups A, B, and C were pretreated with d-amphetamine, while Group D was not. All groups also received intranigral injections of apomorphine (15 ug / .5 ul) and saline. At a concentration of 12 ug, substance K significantly increased the amount of contralateral circling when compared with the effects of control administrations. These are the first data to indicate that substance K can elicit contraversive circling when administered unilaterally into the SNR. Concentrations of eight and 16 ug of substance K did not significantly increase contralateral circling. None of the three doses of SP elicited significantly more circling than did saline. Apomorphine was ineffective at increasing circling in three groups, while it significantly increased contraversive circling in one group. In examining the results of the experiment, the effects of each solution are discussed. Explanations that may serve to clarify inconsistencies are proposed, and an effort is also made to reconcile these results with those reported in the relevant literature.

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INTRODUCTION

Tachykinins

During the last decade there has been growing interest in neuropeptides and the role they may play in the neural control of behavior. A recent estimate suggests that over 40 brain and/or gut peptides may play roles in a number of important behaviors (Krieger, 1983). Little is known about many of these neuropeptides although a number of them have been implicated in the control of specific behaviors (eg. angiotensin and drinking (Fitzsimons, 1980)).

Recently interest has been generated by one particular family of neuropeptides, the tachykinins. Members of this family share a common amino-acid sequence at their carboxyl-terminus (C-terminus) (Phe-X-Gly-Leu-Met-NH2, where X is a hydrophobic or aromatic residue). Substance P (SP), the most studied of the tachykinins, was discovered by von Euler and Gaddum (1931) in alcoholic extracts of equine brain and intestine. Its structural characterization followed later, in 1971 (Chang, Leeman, and Niall, 1971). Other members of the tachykinin family include eledoisin, physaelemin, kassinin, substance K (SK, neuromedin L, neurokinin A) and neuromedin K (NK, neurokinin B). Table 1 shows from which type of tissue each of the latter neuropeptides was first isolated.

SP and SK can, in certain cases, be part of the same polypeptide precursor molecule. Nawa, Hirose, Takashima, Inayama, and Nakanishi (1983) were able to clone cDNA sequences that were complementary to mRNA sequences encoding for SP. Following the analysis of the nucleotide sequences of the

TABLE 1: Some members of the tachykinin family and the corresponding types of tissue from which they were first isolated

Substance P	Horse brain and intestine
Eledoisin	Mediterranean octopod salivary gland
Physaelemin	Amphibian skin
Kassinin	Amphibian skin
Substance K	Pig spinal cord
Neuromedin K	Pig spinal cord

cDNA it was discovered that bovine brain SP precursors are encoded by at least two different mRNAs. It is known that the precursor Alpha-preprotachykinin (A-PPT: 119 amino acids) is a precursor to SP, while a second precursor, Beta-preprotachykinin (B-PPT: 130 amino acids) contains copies of both SP and SK. Both preprotachykinins have 111 amino acids in common; and within these 111 resides the SP peptide. The remaining 19 amino acids present in B-PPT alone contain those 10 which characterize SK. Thus, certain neurons that contain B-PPT are candidates for releasers of SP and SK.

Tachykinin Receptors and Immunoreactive Brain Sites

A number of reports have described the binding properties of tachykinins and the one or more receptors that mediate their effects. Various pathways containing tachykinins have also been identified. Results from these studies will be described since they bear on the experiment to be presented later.

Both peripheral and brain tissue preparations have been studied in attempts to identify tachykinin receptors and probable subtypes. The guinea pig ileum, urinary bladder, and rat cerebral cortex are examples of such preparations. Based on experimental work using a number of bioassays and other pharmacological systems two tachykinin receptors were originally proposed: the SP-P and SP-E receptors. The SP-P receptor ('P' designating physaelemin), it was shown, bound physaelemin, SP, kassinin, and eledoisin without preference for any one of these tachykinins. The SP-E receptor ('E' designating eledoisin), on the other hand, was found to bind eledoisin and kassinin preferentially (Iversen, Hanley, Sandberg, Lee, Pinnock, and Watson, 1982).

Recent work on the receptor binding characteristics of radiolabelled tachykinins has, however, suggested binding patterns indicative of a third tachykinin receptor (Buck, Burcher, Shults, Lovenberg, and O'Donohue, 1983; Mantyh, Maggio, and Hunt, 1984). Buck et al.(1983) used the process of competitive inhibition as a tool in binding studies and reported binding patterns shown in Table 2; it therefore appears that SP-P, SP-E, and SP-K receptors exist (the endogenous ligands being SP, NK, and SK, respectively). Quirion (1985) has noted that a more logical classification scheme would have the SP-E term replaced with a more general term. The reasoning is that the SP-E receptor binds a number of tachykinins, and shows the highest affinity for NK; suggesting that it is more likely to be a NK receptor. Hence, the three receptors would be SP, SK, and NK receptors. It should be noted that the possibility that only one receptor type exists (with multiple binding sites) has been raised (Growcott, 1983).

In the rat brain autoradiography has been used to locate specific tachykinin binding sites; whereas cell bodies, fibers and terminals containing these peptides have been located using immunocytochemistry. SP binding sites are more widespread than SK binding sites (Shults, Buck, Burcher, Chase, and O'Donohue, 1985). Areas showing a high density of SP binding sites include the striatum, dentate gyrus, certain regions of the amygdala and septal area, locus coeruleus, superior colliculus and dorsal parabrachial nucleus (Quirion, Shults, Moody, Pert, Chase, and O'Donohue, 1983; Mantyh et al., 1984; Shults et al., 1985). Areas with a high density of SK binding sites include the substantia nigra (pars compacta), interpeduncular nucleus, suprachiasmatic nucleus, supraoptic nucleus, and certain areas of the cortex (Shults et al., 1985; Mantyh et al., 1984). The

TABLE 2: The relative binding potencies of a number of tachykinins with respect to three proposed tachykinin receptors (based on work by Buck et al., 1983; and Quirion, 1985)

RECEPTOR TYPE	RANK ORDER (BINDING POTENCY)
Substance P	SP > Phys > SK > Ele > Kass > NK
Substance K	SK > Kass > Ele > NK > SP > Phys
Neuromedin K	NK > Kass > Ele > Phys > SK > SP

external layer of the olfactory bulb, and the dorsal horn of the spinal cord both show a large number of SP and SK binding sites.

Although sites that bind SP are more widespread than those that bind SK, the distributions of both peptides overlap to a large extent. Shults, Yajima, Gullner, Chase, and O'Donohue (1985) have shown that concentrations of SP and SK were maintained at a fixed ratio throughout the majority of 20 brain areas dissected (SP:SK, 2:1).

Some Behavioral Effects of SP and SK

Work on mammals, more specifically rats, has tended to focus on three tachykinins---SP, SK and NK. SP has been implicated in a number of behaviors. For example, depending on the dose and the particular pain threshold of an animal an intrathecal injection of SP can bring about either an analgesic or hypoalgesic response (Frederickson, Burkes, Harrell, and Edwards, 1978). In addition, SP has also been found to facilitate lordosis when injected into the midbrain central gray (Dornan, Malsbury, and Penney, submitted for publication); elicit circling when injected into the substantia nigra (Olpe and Koella, 1977; James and Starr, 1979); and increase locomotor activity when administered into the ventral tegmental area (VTA) (Stinus and Kelly, 1978). Administration of SK into the VTA has also been reported to increase locomotor activity, and a comparison with SP has shown SK to be 10 times more potent in this respect (Kalivas, Deutch, Maggio, Mantyh, and Roth, 1985). This latter form of comparison, in conjunction with reports that suggest that many types of tachykinin receptors exist, made it of interest to compare the effects of SP and SK on circling behavior. Prior to describing this experiment, information relevant to the anatomy of the

substantia nigra, and its function in circling behavior, will be presented.

The Substantia Nigra

The SN is a bilateral ventral midbrain structure that lies dorsal to the cerebral peduncles. It has commonly been included in descriptions of the basal ganglia, along with the caudate nucleus, putamen and globus pallidus of the forebrain.

In order to fully understand the role of the substantia nigra (SN) with respect to behavior, especially circling behavior, knowledge pertaining to its structural and chemical anatomy is essential. The various afferent and efferent connections that involve the SN and electrophysiological data also give clues with regard to its function.

Cytoarchitecture

Cytoarchitecturally the SN has traditionally been separated into three regions: pars compacta (SNC), pars reticulata (SNR) and pars lateralis (SNL) (Gillian, 1943). The SNC lies dorsally and is made up of a thin layer three to five cells thick. This layer contains mainly dopamine (DA) cell bodies, and is sometimes referred to as A9 (according to the nomenclature of Dahlstrom and Fuxe (1964)). The SNR, on the other hand, makes up the ventral portion of the SN and contains a lower density of cells in comparison to the SNC. Although the SNR is the largest region of the SN, it contains relatively few DA cell bodies, the bulk of it being neuropil. Lastly, the SNL is phylogenetically the oldest region of the SN and contains a relatively small number of neurons (Dray, 1980).

Although there have been numerous studies of the morphological characteristics of SN neurons, a controversy exists regarding the exact dimensions of neurons found in the three regions of the SN. For example, Gulley and Wood (1971) identified three types of neurons in the SN: small (11-26 micrometers (um)), medium (19-46 um) and large (45-74 um). Small and medium-sized neurons were found to be present in all three regions of the SN; whereas large neurons were found mainly in the SNR and SNL. Hanaway, McConnel, and Netsky (1970) could only identify two types of neurons in the SN: a small-sized type (8-11 um) present in the SNR and SNL, and a medium-sized type (12-17 um) present in all three regions of the SN. Juraska, Wilson, and Groves (1977) identified three types of neurons: small-sized neurons (11-20 um) present in all three areas of the SN; medium-sized neurons (19-46 um) present in the SNC and SNR; and finally, large-sized neurons (45-74 um) present in the SNR and SNL.

Recently, Poirier, Giguere, and Marchand (1983) have suggested two possible factors that might explain many of the discrepancies in the results reported above. To begin with, the fact that too few neurons were sampled from each of the SN regions may have led to inaccurate generalizations with regards to their size and distribution. Secondly, tissue preparation methods varied considerably between studies so that variability in the results could be expected.

According to Poirier et al. (1983), the size of a neuron cannot be used as a reliable predictor of its location in the SN. In their own study of the SN Poirier and his colleagues identified four types of neurons based on a number of cytological characteristics (size, shape and amount of Nissl substance). The neurons were categorized as being either compacta-type

neurons, reticulata-type neurons, intermediary-type neurons or globular-type neurons. The name of each type of neuron implies its location within the SN, except in the case of the globular-type neuron. Table 3 summarizes the characteristics of each type of neuron and their number in the rat SN.

Efferent Projections of the SN in the Rat

Beckstead, Domesick, and Nauta (1979) used the method of autoradiography to map out the efferent connections of the SN in the rat. Small amounts of tritiated amino acids (leucine and proline) were injected into both the SNC and SNR.

Both the SNC and SNR were found to be the origins of ascending and descending fiber projections. With regards to the SNC the ascending projections were observed to terminate in the striatum, globus pallidus, and ventromedial and mediodorsal thalamic nuclei. The nigrostriatal projection was organized so that fibers originating from medial regions of the SN terminated in medial parts of the striatum while fibers originating from more lateral regions of the SN terminated in more lateral parts of the striatum. In terms of the rostro-caudal organization of the projections the authors concluded that the location of a cell body in the SNC was not a good predictor of its terminal field in the striatum. Essentially, all parts of the SN project to various areas of the striatum along the rostro-caudal plane. Descending projections of the SNC were also found to terminate in a wide variety of areas. These included the median raphe nucleus, dorsal raphe nucleus, pedunculopontine nucleus, central grey matter, and locus coeruleus.

With respect to the SNR both its ascending and descending projections were found to be somewhat different from those of the SNC, although some

TABLE 3: Cytological characteristics corresponding to each of the four types of neurons identified in the substantia nigra by Poirier et al.(1983), and their number in the rat

Neuron Type and number: COMPACTA (9,925: 44% of total)
Shape Of Soma: triangular, fusiform, pyramidal, polygonal, ovoid or 'plump'
Other Cytological Characteristics: i) relatively large pale nucleus that is often not centrally located, ii) large amounts of Nissl substance distributed in patches and iii) mean largest diameter of 17.2 um

Neuron Type and number: RETICULATA (3,122: 14% of total)
Shape Of Soma: multipolar, triangular or ovoid
Other Cytological Characteristics: i) large amounts of Nissl substance, but not as much as the compacta-type neuron, ii) a more centrally located nucleus and iii) a mean largest diameter of 20.0 um

Neuron Type and number: INTERMEDIATE (3,227: 14% of total)
Shape Of Soma: elongated, triangular or fusiform
Other cytological Characteristics: i) thinner processes than either of the above neuron types, ii) the darkest nucleus of all types and iii) a mean largest diameter of 16.0 um

Neuron Type and number: GLOBULAR (6,258: 28% of total)
Shape Of Soma: round
Other Cytological Characteristics: i) a high nuclear/cytoplasmic ratio, ii) a clear nucleus and iii) a mean largest diameter of 12.1 um

overlap was evident. For example, although the SNR projects to the striatum, the size of the projection is relatively smaller. Other ascending projections from the SNR include those going to the paralamellar zone, ventromedial, mediodorsal and parafascicular nuclei of the thalamus, and globus pallidus. The descending projections of the SNR terminate mostly in the superior colliculus ,pedunculopontine nucleus, periaqueductal grey, and reticular formation.

To more accurately identify the location of nigral neurons projecting to the superor colliculus, thalamus and striatum (three major recipients of nigral output) Faull and Mehler (1978) injected horseradish peroxidase (HRP) into the three latter regions. Since the enzyme is taken up by terminals and is subject to retrograde axonal transport, the cell distribution of the three specific efferent systems could be mapped. The authors reported that i) distinct cell aggregations characterized each of the three projections and ii) the mean size of the cell diameters was different for each aggregation. Nigrotectal cells were of medium size (16 um) and were found mainly in the ventral part of the SNR. Nigrothalamic cells had the largest mean cell diameters (19 um) and were located in the lateral and central SNR. Lastly, nigrostriatal cells had the smallest mean cell diameter (14 um) and were almost exclusively located in the SNC. It is uncertain whether these cells correspond in any way to those described earlier, based on the work of Poirier et al. (1983). In addition to all of the above it has been reported by other authors that the SN also projects to the cerebellum (Chan-Palay, 1977 in Dray, 1979), amygdala (Fallon, Riley, and Moore, 1978) and cortex (Avendano, Reinoso-Suarez, and Llamas, 1976).

Afferent Projections to the SN in the Rat

Following the microiontophoretic administration of HRP into the SN Bunney and Aghajanian (1976) were able to identify sources of afferents to the SN. The authors categorized the density of the sources of afferent projections as either high or low density areas. High density areas suggest larger SN afferent pathways, while lower density areas suggest a smaller number of projecting fibers are involved. High density areas included the caudate nucleus, globus pallidus, nucleus accumbens, central nucleus of the amygdaloid complex and dorsal raphe nucleus. Low density areas included the cortex (layer 5), habenula and hypothalamus.

Other authors have reported that the cerebellum (Snider, Maiti, and Snider, 1976), locus coeruleus (Sakai, Touret, Salvert, Leger, and Jouvet, 1977) and subthalamus (Kanazawa, Marshall, and Kelly,1976) also project to the SN. Table 4 summarizes some of the nigral afferent projections and the transmitters believed to be of relevance in each of these.

Putative Neurotransmitters in the SN and Effects Following the Microiontophoretic Administration of Some of These

A wide variety of potential neurotransmitters have been identified in the SN. These include SP (Powell, Leeman, Tregear, Niall, and Potts, 1973); SK (Shults et al., 1975); GABA (Perry, Hansen, and Kloster (1973); DA (Anden, Fuxe, Hamberger, and Hokfelt (1966); acetylcholine (Cheney, Lefevre, and Racagni, 1975); serotonin and glycine (Dray and Straughan, 1976); enkephalin (Inagaki and Parent, 1984); and dynorphin (Khachaturian; Watson, Lewis, Coy, Goldstein, and Akil (1982).

In a review, Dray and Straughan (1976) summarized the effects of some

TABLE 4: Some sources of nigral afferents and neurotransmitters believed to be of importance

Origin of Nigral Afferents	Neurotransmitter Used	Reference
Striatum	GABA	Brownstein et al.(1977)
	Substance P	Cuello et al.(1982)
	Met-Enkephalin	Khachaturian et al.(1982)
	Acetylcholine	Olivier et al.(1970)
Globus Pallidus	GABA	Hattori et al.(1973)
	Substance P	Cuello et al.(1982)
Nucleus accumbens	GABA	Fung et al.(1979)
Raphe	Serotonin	Fibiger and Miller (1977)
Amygdala	Unknown	
Locus Coeruleus	NA	Collinbridge et al.(1979)
Hypothalamus	Unknown	

of these substances following iontophoretic administration into the SN. GABA, noradrenaline, and glycine, when administered into the SNC, all produced inhibition; whereas acetylcholine excited cells. In the SNR acetylcholine also increased the baseline firing rates of neurons, while GABA and glycine inhibited neurons. Serotonin excited and inhibited cell firing in the SNR.

Data concerning the effects of iontophoretically applied SP on single unit activity in the SN are more difficult to interpret. It has been reported that iontophoretically applied SP excited more than 85% of cells tested (Walker, Kemp, Yajima, Kitagawa, and Woodruff, 1976). However, Pinnock and Dray (1982) could only demonstrate excitation in 30 to 40% of the cells they tested. More recent reports have either implied that SP has no effect on cells situated in the SN (Innis et al.,1984) or, at best, very inconsistent and weakly excitatory effects (Lanthorn, O'Donohue, Shults, Chase, and Walters, 1984).

Jones and Olpe (1985) have hypothesized that inhibitory responses following iontophoresis of SP in various areas of the CNS, including the SN, may be due to a SP-dependent release of an inhibitory substance. It has also been suggested that SP's excitatory effects might be mediated by some other excitatory transmitter (Davies and Dray, 1976). Cellular responses following SP iontophoresis in the SN are characterized by a slow onset and prolonged duration (Lanthorn et al., 1984). These observations, in addition to those regarding the scarcity of SP receptors in the SN (Quirion et al.,1983), have provided support for the view that SP potentiates the release of other substances that in turn affect receptors in the SN (Jones and Olpe, 1985). Others have suggested that cellular responses to SP are slower than

those seen with other substances possibly because SP is a larger molecule, and therefore exhibits a slower rate of diffusion (Guyenet, Mroz, Aghajanian, and Leeman, 1979).

The effects of iontophoretically applied SK on SN neurons also seem variable, although very little research has been done with regard to SK. Innis et al.(1985) reported that SK produced excitation in approximately 50% of DAergic and non-DAergic SN cells sampled; whereas inhibitory responses were recorded in only 3% of cells. Both SNR and SNC neurons responded in similar ways to SK. This latter observation does not agree with results which suggest that a cell's anatomical location within the SN correlates with its response to SK (Lanthorn et al.,1984). Cells in and around the SNC were reported to be excited by SK; although these cells were not believed to be DAergic. Within the SNR Lanthorn et al.(1984) reported inhibitory responses. Therefore, although it can be suggested that SK has both excitatory and inhibitory effects on SN neurons, conclusions regarding both the neuron types affected and their location in the SN can only be tentative.

It has been reported that DA neurons in the SNC are depressed when DA is iontophoretically applied (Ruffieux and Schultz, 1980); whereas neurons in the SNR are both inhibited and excited by DA (Dray and Straughan, 1976).

DA has been regarded as having a crucial role in the SN. It has been shown to release [3H]-GABA from nigral slices (Reubi, Iversen, and Jessell, 1977), as well as modulate GABA's activity in the SN (Waszcack and Walters, 1983). Perhaps most importantly it has been shown that DA is released by dendrites in the SN. This release from non-terminal structures has been hypothesized to have a number of possible functions, the most intriguing of which are auto-inhibition and lateral inhibition of SNC cells that project to

the striatum (Cheramy, Leviel, and Glowinski, 1981). Geffen, Jessell, Cuello, and Iversen (1976) have also suggested that dendritic release of DA in the SN may have a role to play in the control of regenerative sprouting and the establishment of new synapses.

The Neural Basis of Circling

Turning produced by the stimulation or lesioning of various brain sites has been reported over the course of more than 50 years. Ingram, Ranson, and Hannett (1932) reported the ipsiversive turning of head and trunk in the cat following the electrical stimulation of the tegmentum dorsal and lateral to the red nucleus. Ipsiversive turning has also been reported following either the removal of the head of the caudate nucleus or the globus pallidus (Mettler and Mettler, 1942). A large number of other structures have been implicated, at one time or other, in turning or circling behavior; these include the substantia nigra (Anden, Dalhstrom, Fuxe, and Larsson, 1966); ventral tegmental area (Holmes and Wise, 1985); nucleus accumbens (Kelly and Moore, 1976); thalamus (Garcia-Munoz, Patino, Wright, and Arbuthnott, 1983); periaqueductal grey (Reavill, Muscatt, Leigh, Jenner, and Marsden, 1984); superior colliculus, reticular formation (DiChiara, Morelli, Imperato, and Porceddu, 1982); red nucleus (Mussen, 1934); and zona incerta (Hess, 1956).

Based on the research tools used, studies concerned with circling or turning behavior can be divided into two categories. The first category includes those studies based on methods which destroy tissue (extirpation, electrolytic lesions, or chemical lesions); while the second category

includes those studies which involve the direct stimulation of brain tissue (electrical or chemical stimulation). A brief look at the results of some studies will follow.

It should be noted at this point that a distinction is usually made between turning and circling (rotating) behaviors. Turning involves postural deviation or bias, whereas circling typically involves full 360-degree rotations.

Studies Based On The Destruction Of Tissue

The first study to implicate a specific pathway in turning behavior was that of Anden et al.(1966). Using electrolytic lesions at the level of the mammilary bodies the investigators were able to produce unilateral damage to DA pathways travelling in the crus cerebri. The systemic administration of different drugs following the latter experimental manipulation was found to affect the direction of turning. For example, injections of l-DOPA, a DA precursor, in combination with a monoamine oxidase inhibitor produced a turning of the head and tail towards the lesioned side (ipsiversive turning). The authors concluded that the asymmetries observed following the experimental manipulations were presumably due to an effect on the function of DA in the striatum, and that lesions of the nigrostriatal pathway were responsible for those asymmetries.

Since the nigrostriatal pathway was presumed to play a role in circling behavior, subsequent studies by other investigators attempted to confirm and add to the data provided by Anden et al.(1966). The dopaminergic nature of the nigrostriatal pathway (Dalhstrom and Fuxe, 1964) allowed for the use of more specific lesioning techniques, namely the use of 6-

hydroxydopamine (6-OHDA). 6-OHDA has been shown to destroy catecholaminergic neurons when injected into the brain. Therefore, its administration into the pars compacta of the substantia nigra, where DA cell bodies are located, enabled the specific destruction of DA cells.

Through the use of 6-OHDA lesions Ungerstedt and Arbuthnott (1970) showed that unilateral damage to DA-containing cell bodies of the substantia nigra (pars compacta) caused animals to circle towards their lesioned side when disturbed by handling or when injected with d-amphetamine. The authors hypothesized that such ipsiversive circling might be due to a DA imbalance between the left and right striata. This explanation suggested that the intact, non-lesioned side contained more DA than the lesioned side so that the influence of the innervated striatum "overpowered" that of the denervated side, thus resulting in a circling bias.

However, although this traditional view has maintained that an animal will circle towards the 6-OHDA-lesioned side, it was reported by Anden et al. (1966) and Ungerstedt (1971) that the direction of circling was also a function of the amount of time that had elapsed between the day of the lesion and the test session. For example, Anden and his colleagues reported that l-DOPA sometimes elicited turning contraversive to the lesion site (away from the lesioned side). The concept of supersensitivity, well-documented in studies of the peripheral nervous system was used to explain such circling behavior. It was reasoned that the lack of DA action on striatal receptors on the lesioned side caused striatal tissue to become more sensitive to DA than would normally be the case were the striatum innervated. It was subsequently shown that the number of DA receptors under such circumstances increases (Creese, Burt, and Snyder, 1977). It would follow then that any

administration of DA or DA precursors would have a stronger effect on the denervated striatum.

Ungerstedt (1971) found that DA terminals lost their contents 24 to 48 hours following the administration of 6-OHDA. A period of weeks followed during which the sensitivity of striatal tissue to DA and DA agonists increased. Ungerstedt reported contraversive circling following the administration of apomorphine and l-DOPA. Additional evidence that contralateral circling was due to supersensitive DA receptors in the ipsilateral striatum came from the fact that when the ipsilateral striatum was electrolytically coagulated following the 6-OHDA lesion low doses of apomorphine had no effect on circling. The destruction of potentially supersensitive striatal receptor sites eliminated the possibility that they might be stimulated by, in this case, a post-synaptic DA agonist.

Once the role of the SN in circling behavior was firmly established research efforts were directed towards a further elaboration of the neural basis of circling behavior. Efforts were made to identify other important pathways and areas in the brain that are involved in circling behavior.

Marshall and Ungerstedt (1977) blocked the rotation which followed unilateral 6-OHDA lesions of the nigrostriatal system by using selectively placed knife-cuts. They reported that cutting striatal efferents to the substantia nigra which travel in the internal capsule, or destroying a major part of the striatum, blocked circling.

The role of the striatonigral projection discussed above is not completely understood. A number of investigators have suggested that the striatonigral projection serves as a feedback loop, and in this way provides DA cells in the SNC with information that affects DA turnover largely in the

ipsilateral striatum. A second group of investigators has shown that a striatonigral projection which is GABAergic carries striatal output to the SNR. In this case the SNR could act as a relay station that sends its own efferents to other areas (i.e. thalamus, superior colliculus and reticular formation), and, in this way, may itself affect circling behavior. The fact that little change in DA metabolism results from the unilateral transection of the GABAergic pathway, while at the same time ipsiversive circling is elicited, supports this view (Garcia-Munoz et al., 1977).

While the nigrostriatal pathway is involved in turning, the full circling response involves other structures as well; these include the mesolimbic DA system, nucleus accumbens, ventromedial thalamus, superior colliculus, and reticular formation.

It has been suggested that circling can be elicited when two conditions are satisfied: postural asymmetry (turning) and a state of increased locomotor activity. The postural bias determines the direction of circling while the level of locomotor stimulation will determine the magnitude of circling. The bilateral destruction of the nucleus accumbens (a forebrain structure that receives DA projections from the VTA and that sends efferents to the SN) in unilaterally lesioned 6-OHDA animals reduced or completely abolished any circling previously elicited by DA agonists. Unilateral lesions of the nucleus accumbens did not affect circling responses. Kelly and Moore (1976) regarded this as support for the argument that activity in the nigrostriatal DA system determines the direction of circling whereas activity in the mesolimbic DA system determines the magnitude of circling seen. This line of reasoning is embodied in what is sometimes referred to as the two-component system. One component, the SN,

exerts an effect on the striatal DA balance and produces turning, whereas the other component exerts an effect on the level of locomotor activity (Pycock and Marsden, 1978).

Another important line of research has focused on a second functionally distinct system in the SN that was alluded to earlier. DiChiara and his colleagues have proposed that a nigral efferent system that originates in the SNR functions in a manner opposite to the nigrostriatal DAergic system. It has been demonstrated that the superior colliculus, thalamus, periacqueductal grey, and reticular formation all receive input from cells that originate in the SNR (Beckstead et al.,1979).

DiChiara et al.(1977) first reported that the administration of kainic acid (a neurotoxic analog of glutamate) into the SN resulted in contraversive circling (6-OHDA lesions produce ipsiversive turning). When rats were given bilateral 6-OHDA lesions of the SNC following a unilateral kainic acid lesion of the SNR, the contraversive circling which first appeared following the kainic acid lesion persisted. This suggested that a non-DA system was involved since 6-OHDA had no effect on the circling initially elicited by the kainic acid lesion.

The 6-OHDA lesions lowered nigral tyrosine-hydroxylase activity (the rate-limiting step in the biosynthesis of DA) by more than 85 percent. Glutamic-acid-decarboxylase activity (reflecting GABA synthesis), however, was not affected. These results suggested that GABA may play an important role in this particular SN system, which is functionally opposed to the role of the DAergic nigrostriatal system. It has been generally accepted that excitation of the nigrostriatal pathway produces contraversive circling. A similar form of contraversive circling can be elicited by the destruction of a

presumed nigral GABAergic efferent pathway (DiChiara et al.,1977). This GABAergic pathway is believed to strongly inhibit activity in other areas receiving the projection (eg., ventromedial nucleus of the thalamus (VMT)). One hypothesis tested by Garcia-Munoz et al.(1983) was that if the VMT had an excitatory influence on other areas (cortex ?), and was normally inhibited by the GABAergic projection in question, then destruction of the ipsilateral VMT should drastically reduce the amount of contralateral circling seen when animals were administered apomorphine systemically following unilateral 6-OHDA lesions of the nigrostriatal pathway. The hypothesis was confirmed.

Additional tests of this SNR model have been carried out, again the idea being that if one removes the inhibitory link provided by the SNR efferent system (disinhibition), contraversive circling results; whereas the destruction of GABA-recipient areas will reduce their excitatory output, resulting in ipsiversive circling. DiChiara et al. (1982) used kainic acid to specifically lesion areas of the superior colliculus and reticular formation in normal rats. These groups of animals all expressed a significant tendency to circle ipsiversively following the systemic administration of low doses of apomorphine. These same lesions reduced the amount of contraversive circling when animals were administered apomorphine following unilateral 6-OHDA lesions of the rostral SN (where nigral efferents to the striatum converge prior to projecting to the striatum).

On the basis of all the experimental results discussed above it can be concluded that at least two distinct systems exist in the SN, and that both of these are involved in circling (Starr, Summerhayes, and Kilpatrick, 1983). However, the majority of the studies discussed so far have used various forms of SN lesions to affect circling behavior. The following subsection will

include studies dealing with direct stimulation of SN neurons.

Studies Based On The Direct Electrical And Chemical Stimulation Of The Substantia Nigra

Arbuthnott and Crow (1971) reported that electrical stimulation of the medial substantia nigra produced contraversive circling. Vaccarino and Franklin (1982) measured circling responses following the unilateral electrical stimulation of the SNC and reported results that suggested the existence of a functional difference between the lateral and medial SNC. Contraversive circling resulted from stimulating the medial SNC, whereas ipsiversive circling resulted from stimulating the lateral SNC. Gratton and Wise (1985), using a moveable electrode to stimulate various regions of the SN along the dorso-ventral plane could not reproduce Vaccarino's and Franklin's results. One possible explanation for this may be that the lateral stimulation sites reported by Gratton and Wise (1985) were not as lateral as those reported by Vaccarino and Franklin (1982) (Franklin, personal communication).

Although the contention that both ipsiversive and contraversive circling can be elicited, depending on which SNC region is stimulated, has not been fully confirmed, the elicitation of contraversive circling following the electrical stimulation of the SN in general has been widely accepted.

A large number of other studies have involved the use of direct chemical stimulation. Whereas electrical stimulation excites all types of neurons, chemical stimulation technique gives the investigator the option of stimulating a distinct type of cell within a larger neuronal population.

As described earlier, a large number of potential neurotransmitters

and/or neuromodulators are found in the SN. The list includes SP, SK, GABA, DA, norepinephrine, enkephalin, dynorphin, acetylcholine, glutamate, and serotonin. Table 5 summarizes the effects some of these have been reported to have on circling behavior when administered intranigrally.

The data shown in Table 5 can be explained if one makes use of findings discussed earlier (p. 21), which suggest that two functionally opposed systems are present in the SN. One system includes those DA cells that are present in the SNC and that make up the nigrostriatal projection; whereas the other system includes those GABA cells that are present in the SNR and that project to the ventromedial thalamus, superior colliculus, and reticular formation. In the case of the former system, intranigral administrations of drugs that unilaterally excite this DA system presumably increase impulse flow along the nigrostriatal pathway. As a consequence of this increased neuronal output, DA levels increase in the ipsilateral striatum and contraversive circling results (James and Starr, 1979). This is one hypothesized mode of action for substances such as SP, DA, amphetamine, morphine, dynorphin, and met-enkephalin. Using similar reasoning, it can be seen that unilateral intranigral administrations of drugs that inhibit impulse flow along the nigrostriatal pathway elicit ipsiversive circling (i.e. apomorphine).

With respect to the GABAergic system drugs that increase the output of GABA cells in the SNR (thereby increasing the inhibitory output to the ventromedial thalamus, superior colliculus, and reticular formation) elicit ipsiversive circling (e.g. picrotoxin); while those drugs that reduce the output of GABA cells originating in the SNR elicit contraversive circling (e.g. GABA and muscimol).

TABLE 5: Effects of various substances on circling following their intranigral administration

Substance	Site of Injection	Direction of Circling	Reference
Substance P	SNR	Contraversive	Olpe and Koella, 1977
	SNC	Ipsiversive	James and Starr, 1977
Dopamine	SNR	Contraversive	Mendez et al., 1976
Apomorphine	SNC (lateral)	No Effect	Vaccarino and Franklin, 1984
	SNC (medial)	Ipsiversive	Vaccarino and Franklin, 1984
Amphetamine	SNR	Contraversive	Jackson and Kelly, 1983
Morphine	SN	Contraversive	Jacquet, 1983
Dynorphin	SNR	Contraversive	Herrera-Marschitz et al.,1984
Met-Enkephalin	SN	Contraversive	Jacquet, 1983
GABA	SNR	Contraversive	Kamata et al.,1985
Muscimol	SNR	Contraversive	Oberlander et al.,1977
Picrotoxin	SNR	Ipsiversive	Olianas et al.,1978
Glycine	SNR	Contraversive	Mendez et al.,1976

Although SNR injections of SP have been hypothesized to increase impulse flow along the nigrostriatal pathway, SP's precise mode of action is not known. As mentioned earlier various tachykinin receptors are hypothesized, and in addition, the SNR has been shown to exhibit immunoreactivity to both SP and SK antisera (Shults et al.,1985). A consideration of the above, in conjunction with the fact that SK has already been shown to be 10 times more potent than SP in eliciting certain behavioral responses (Kalivas, Deutch, Maggio, Mantyh, and Roth, 1985) has led a number of investigators to express reserve concerning the interpretation of previous reports on the effects of SP administrations. The reasoning behind such reservations rests on the possibility that effects caused by SP injections might be due to the activation of receptors whose affinity might still be highest for other substances, i.e. SK or NK. (Innis, Andrade, and Aghajanian, 1985). Research suggesting that different tachykinin receptors exist and that each may be differentially sensitive to a number of tachykinins can help explain certain puzzling results. For example, when one compares published descriptions of the distribution of receptor binding sites with descriptions of the distributions of cellular immunoreactivity to SP and SK, striking differences are noted, especially with respect to the substantia nigra. Although the SN receives a dense SP innervation from the striatum, few SP receptors have been located in the SN (Quirion et al., 1983); whereas SK binding sites have been visualized in this area (Mantyh et al., 1984). The possibility therefore exists that some pathways containing SP may also contain SK; and that behavior reported to be due to SP might also involve SK (eg. circling behavior). Innis et al.(1985) have speculated, following the discovery of SK receptor sites in the SN, that SP effects in this region may

have been due to SP action on:

i) sparse but existing SP receptors, or,

ii)abundant, but SK-preferring receptors that show some affinity for SP.

An experimental model that may be used to test the hypothesis that SK can elicit behavioral responses similar to those elicited by SP is the circling or rotation model. It has been shown that microinjections of SP in the SNR elicit circling contraversive to the site of injection (Olpe and Koella, 1977; James and Starr, 1979). It was therefore of interest in the present study to microinject SK intranigrally and compare its effects to those obtained with SP injections. Such an experimental manipulation is described below.

METHODS

Animals and Housing

One hundred and twelve male rats of the Sprague-Dawley strain weighing between 250 and 350g were purchased from Charles River Inc. (Quebec). Upon their arrival at the laboratory animals were randomly divided into groups of three or four, and housed in a transparent plexiglas cages (44 cm x 23 cm x 23 cm). Food and water were available at all times. In addition, the light cycle in the animal/testing room was reversed (lights on at 21:00 hrs and off at 09:00 hrs), and the temperature was maintained at 20 degrees Celsius. Animals were given a minimum of five days to adapt to their new living quarters. This period enabled them to adjust to the reversed light/dark cycle and to recover from any stresses associated with their

transport.

Screening Test

Following the adaptation period some groups of rats were tested for a natural circling bias, expressed as a tendency to circle preferentially in a particular direction. On the day prior to the screening test each rat was handled for a period of five minutes at the end of which it was placed in a cylinder (30 min.) that was part of the apparatus used to quantify circling behavior (described below). The latter was carried out during the dark part of the light/dark cycle, as were all other test-related procedures.

On the screening test day each animal was given a subcutaneous injection of d-amphetamine (1 mg/kg) 20 minutes prior to being tested for a circling bias. D-amphetamine has its peak effect 20 minutes following its subcutaneous administration (N. White, personal communication). Following this 20 minute interval the animal was placed in the circling apparatus and the magnitude and direction of circling over a 20 minute period was recorded. Earlier work suggested that between 20 and 25% of animals within any group will show a marked tendency to circle repeatedly in one direction when administered a low dose of d-amphetamine. In order to screen out this latter group of animals, and yet retain a large enough sample to allow for subsequent testing, a criterion of 15 turns was adopted. That is, if an animal's cumulative number of turns was biased in one direction by more than 15 turns this animal was not included in the experiment. Certain subgroups of animals were not screened for circling biases since it was presumed at the time that a subcutaneous administration of a low dose of d-amphetamine would not by itself elicit circling.

Surgery

Prior to surgery each animal was weighed and anaesthetized using sodium pentobarbital (Somnotol, 60 mg/kg). A supplemental administration of the anaesthetic was given when required. Following the induction of anaesthesia hair on and around the planned site of incision was clipped using an electric hair clipper. The animal was then placed in a stereotaxic instrument (KOPF). A midline incision approximately 1.5 cm long was made and underlying membranes were cut and pushed aside to expose the coronal suture and bregma. Two holes, large enough to accomodate stainless steel screws (Plastic Products), were then drilled through the skull. Steel screws were used as anchoring sites for acrylic cement.

Following the placement of screws a 25 gauge guide cannula (Plastic Products) was lowered through a third drill hole. The guide cannula was placed using coordinates initially obtained from Paxinos and Watson (1982), and modified following a number of test operations. The coordinates for the substantia nigra pars reticulata were as follows: a.p. -5.4; m.l. 2.0; d.v. 9.3. All coordinates were measured in relation to bregma except the dorso-ventral coordinate. It was measured with respect to the surface of the skull overlying the substantia nigra. In addition, the nose bar was set at -3.5. The guide cannula was cut so that its tip would lie approximately one millimeter above the center of the substantia nigra.

Once the cannula was lowered into place a weak stream of air was applied to the exposed skull to obtain a dry surface. This facilitated the adhesion of the dental cement [Sybron (Kerr)] when it was finally applied around the base of the cannula. Following the hardening of the acrylic cement

the cannula was released from the holder and a 28 gauge obturator was screwed on and remained in place until test day 1.

Animals were given a period of at least five days to recover from surgery. During this recovery period they were housed individually in plexiglas cages identical to those described earlier and handled at least once, for a period of five minutes. Following the recovery period, and one day prior to the first test session, each animal was placed in the circling cylinder for a period of 45 minutes.

Apparatus

The apparatus used to quantify circling was similar in construction to one developed by Vaccarino and Franklin (1982), and is shown schematically in Figure 1. The apparatus consisted of three major components: i) a plastic cylinder 30 cm in diameter and 38 cm high, ii) a harness attached to a plastic-covered wire that served to provide a connection between the animal and the circling counter, and iii) a circling counter consisting of a rotating tube, a silk thread, and a calibrated scale.

The harness consisted of a velcro strap attached to a piece of plastic-covered extension wire. The extension wire extended vertically and was attached to a metal clip, the latter being fastened to a cut 1 ml syringe shell which fit through a hole in a supporting wooden plank. Every turn completed by the animal caused a single turn of the syringe shell. Silk thread was connected to the syringe and wrapped around the latter in accordance with the number of turns completed by the animal. Rotations were represented in a cumulative fashion on a calibrated wall; this is also depicted schematically in Figure 1. The direction of turning could be

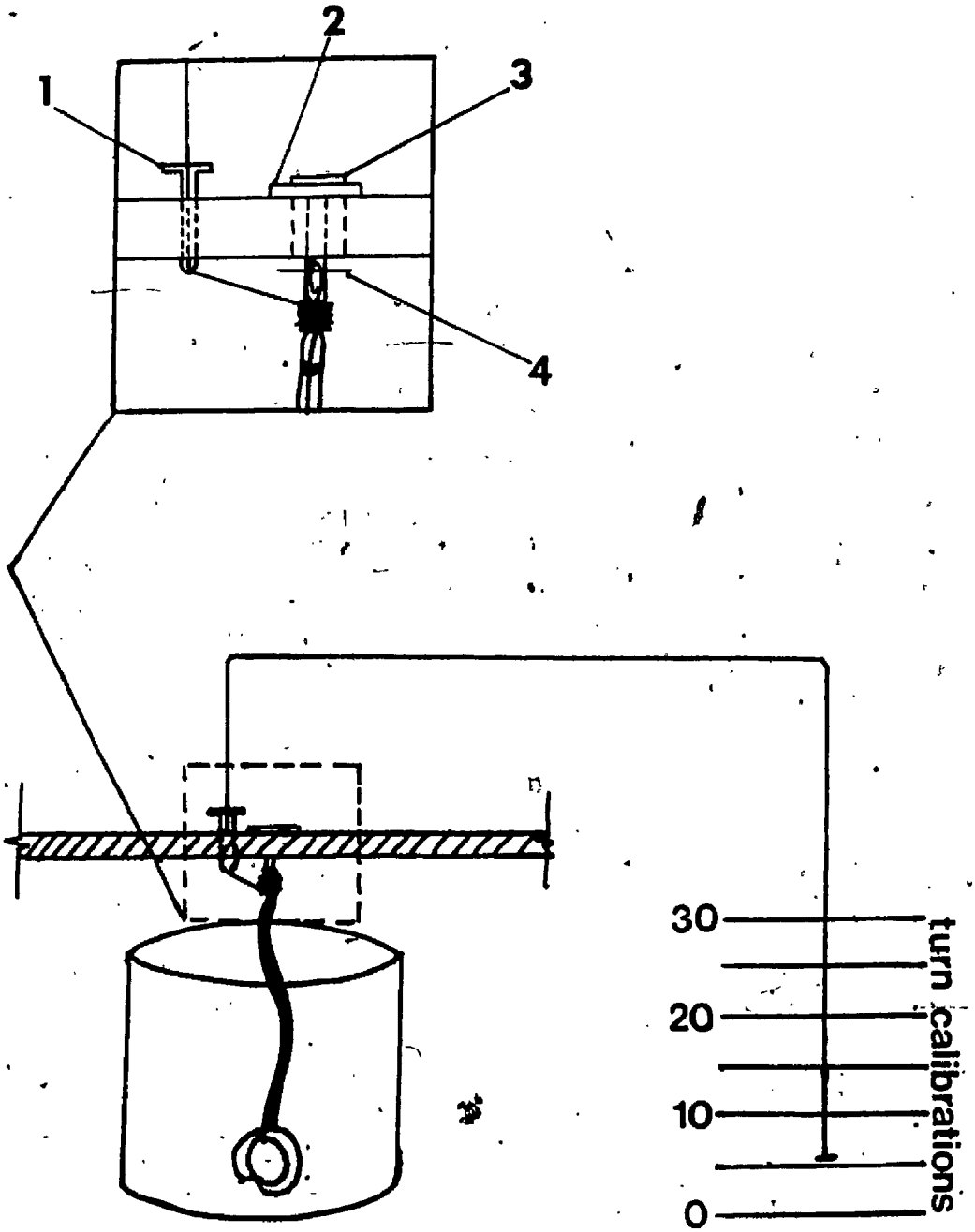

FIGURE 1 A schematic diagram of the apparatus used to measure the magnitude and direction of circling following the intranigral administration of four solutions. The basic apparatus is comprised of a plexiglas cylinder, turning mechanism, and a calibrated wall. The enlarged section shows (1) a 1 cc syringe shell serving as channel through plank; (2) a 5 cc syringe shell supporting (3), a 1 cc syringe shell allowed to rotate; and (4) a metal pin supporting a paper clip which in turn supports a plastic-covered metal wire.

ascertained by noting the direction in which the silk thread wrapped around the syringe.

Mixing Solutions

Five different solutions were used in the experiments. These included SP, SK, acidified saline, apomorphine and d-amphetamine solutions. Substance P was purchased from Sigma Chemical Company (Lot 124F-59201). Its molecular weight was 1348 ('bare bones': sum of amino acid residue weights). Its peptide content was 85% (sum of amino acid residue weights / weight of amino-acid residue weights + solvent used in lyophilization). Its purity was 99%. Substance K was purchased from Bachem Inc.(Lot 485A), and its peptide content was 85%. Apomorphine hydrochloride was also purchased from Sigma (Lot 14F-0555) and had a molecular weight of 303.8. D-amphetamine sulfate (Lot 51DXS) was kindly donated by Smith Kline and French Canada Ltd..

A number of important facts were kept in mind while making solutions containing SP or SK (Stewart, 1983):

i) both tachykinins behave like basic polypeptides,

ii) they are very hydrophobic and as a result do not dissolve well in water, especially when high concentrations are desired. Acetic acid helps to increase their solubility,

iii) low pH preserves their biological activity, and

iv) sterile techniques prevent bacterial degradation and preserve the biological activity of these peptides.

Peptide solutions were usually prepared shortly before they were administered.. In the few instances where this was not the case, aliquots containing 20-30 microliters (ul) of the solution were prepared in advance

and were stored in a liquid nitrogen freezer (-60 degrees Celsius). Also, solutions were kept in a refrigerator when not being used (i.e. during between-test intervals within one particular session).

As noted above, the use of acetic acid improves the solubility of SP and SK. Acidified physiological saline (0.9 % saline) was made by mixing 0.6 ml of glacial acetic acid into one litre of distilled water and adding 9 g of NaCl. A final upward adjustment of pH to 6.22 was arrived at by the dropwise addition of sodium hydroxide (in 0.9 % saline). Finally, the acidified saline was sterilized using a Sybron (Nalge) filter (pore size = 0.22 um).

The apomorphine hydrochloride was found to be most soluble in water. When attempts were made to mix small amounts in 0.9 % acidified saline, or 0.9 % physiological saline very poor solubility resulted. Since apomorphine is highly susceptible to auto-oxidation, solutions were used quickly (within 10 or 15 minutes). D-amphetamine was mixed in 0.9 % physiological saline in a concentration of 1 mg/ml.

Research Design and Testing Procedure

The research design used is shown in Table 6. Animals in Groups A, B, and C received d-amphetamine (sc); whereas animals in Group D did not. Saline (Sal) and apomorphine (Apo) were administered intranigrally to all groups. In addition Groups A, B, C, and D received 8, 12, 16, and 12 micrograms (ug) of both SP and SK respectively. SP was always administered first. All groups were further divided into two subgroups in an effort to counterbalance the order of intranigral administrations. The order of administration was Sal-SK-SP-Apo for one subgroup, and SP-SK-Sal-Apo for the other. Due to the fact that damage to neural tissue increases with the number

TABLE 6: Experimental research design depicting drug conditions associated with Groups A, B, C, and D. Groups A, B, and C received d-amphetamine (sc) prior to all intranigral injections (**); whereas Group D did not (#)

GROUP A**	SP	(8 ug)
(n=17)	SK	(8 ug)
	APO	(15 ug)
	Sal	
GROUP B**	SP	(12 ug)
(n=17)	SK	(12 ug)
	APO	(15 ug)
	Sal	
GROUP C**	SP	(16 ug)
(n=14)	SK	(16 ug)
	APO	(15 ug)
	Sal	
GROUP D#	SP	(12 ug)
(n=8)	SK	(12 ug)
	APO	(15 ug)
	Sal	

of injections given, and since our interest lay mainly in comparing SP with SK, Apo was given last. As mentioned earlier a number of animals were not prescreened for circling biases [subgroup of A (n=8); B (n=8); C (n=8); D (n=8)].

The testing procedure included the following:

i) a subcutaneous injection of d-amphetamine (1 mg/kg) (Groups A,B,C) followed by a 20 minute waiting period,

ii) an intranigral injection of one of four substances: a vehicle solution, SP, SK or apomorphine, and

iii) a 15 minute post-injection period during which circling was recorded.

All injections were made using a 2 ul glass syringe (Dynatech). Tubing (0.5 mm = i.d., 0.9 mm = o.d.) was attached to the injector cannula at one end, and, at the other end, to the glass microsyringe. Prior to connecting the glass microsyringre the tubing was cleaned with a few injections of 95% alcohol; followed by injection of air; and finally, the tubing was filled with sterile distilled water. The distilled water was passed through the tubing until the investigator was confident, following visual inspection, that air bubbles were not present along the length of the tubing. The tip of the 2 ul syringe was then inserted into the end of the tubing just as the syringe containing distilled water was being removed. Again, particular care was taken to ensure that no air bubbles were present along the length of the tubing. The plunger of the 2 ul syringe was then slowly pulled back so as to allow 0.5 ul of air into the injector cannula. Following this, 1.5 ul of the solution to be administered intranigrally was drawn up into the injector cannula. Prior to and following each intranigral

injection a check was carried out to make sure the cannula was not blocked. All injections were made manually. A volume of 0.5 ul was injected over a period of 60 seconds, and the injector cannula was left in place for an additional 60 seconds in order to avoid an improper diffusion of the administered substance. Animals were restrained as little as possible during this 2 minute period. Following the removal of the injector cannula the obturator was replaced and the animal was placed in the apparatus so that circling during the following 15 minutes could be measured. Approximately 48 hours elapsed between intranigral injections.

Perfusion and Histology

Animals were first deeply anaesthetized using sodium pentobarbital (Somnotol 100 mg/kg). Immediately prior to perfusion each animal was injected with a cresyl violet dye (.5 ul). This allowed for a more accurate assessment of the injector cannula (i.c.) tip location. Each animal was then perfused intracardially with 0.9 % saline (for approximately 30 seconds) and 10 % phosphate-buffered formalin (for approximately 10 minutes). Following decapitation brains were removed and kept in a 10 % formalin solution for at least one day.

Using a cryostat, sections 46 um thick were collected on glass slides. Every third or fourth section of the brain area of interest was taken and, on average, ten sections per brain were collected. Sections were left to dry for at least one day, following which they were stained using a metachromatic Nissl stain procedure. Cannula tip locations were identified using a microprojector.

HISTOLOGY AND RESULTS

Histology

A total of 94 animals were implanted with cannulae. Three died shortly after surgery, while another 4 animals had cannulae that dislodged during testing. Of the remaining 88 animals, 56 were found to have the injector cannula (i.c.) tip in the SNR. Two main criteria were used in deciding whether or not an animal was included in subsequent data analyses: i) the location of the i.c. tip (based on stain diffusion) and ii) the extent of nigral damage. Twenty-eight animals were rejected on the basis of the location of the i.c. tips: 13 were found to have the i.c. tip located outside and dorsal to the SNR; 11 had the i.c. tip ventral to the SNR; and 4 had the i.c. tip in the region caudal to the SNR. With respect to the second criterion, 3 animals were rejected due to massive destruction of the SN, due to the fact that a small number of guide cannulae were cut 1 mm too long.

The i.c. placements of the 56 animals considered in subsequent data analyses were categorized on the basis of their medio-lateral and rostro-caudal location in the SNR. With respect to the medio-lateral plane the i.c. tip was considered to be either in the medial, central or lateral SNR as depicted in Figure 2. In addition, i.c. tips were judged to lie mostly in one of three planes along the rostro-caudal axis, as shown in Figure 3. Table 7 shows the distribution of placements, and includes all animals whose data was used in the statistical analyses. All groups showed similar placement distributions, with the majority of the sites occupying the central and medial portions of the SN at a level 5.8 mm caudal to bregma (based on Paxinos and Watson, 1982).

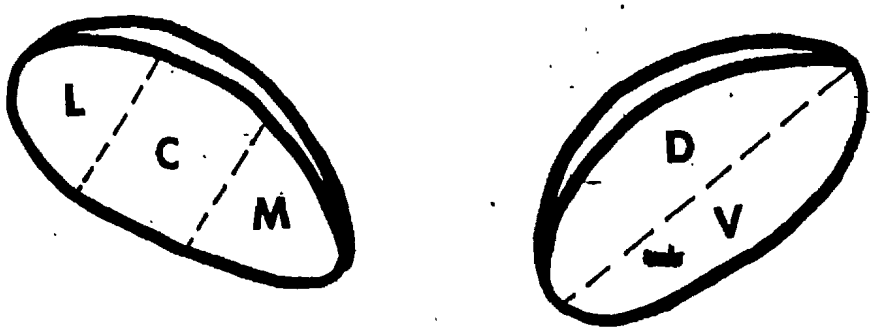

FIGURE 2 Coronal section of the SNR depicting the arbitrary classification system used to describe the location of injection sites along the medio-lateral plane.

FIGURE 3 Three coronal sections upon which the classification of injection sites along the rostro-caudal plane were based (the top section is the most rostral). Abbreviations- cp, cerebral peduncle; in, interpeduncular nucleus; ml, medial lemniscus; mn, mammillary nucleus; pag, periaqueductal grey; p, pons; rn, red nucleus; snc, SN pars compacta; snr, SN pars reticulata; vta, ventral tegmental area (based on Paxinos and Watson, 1982).

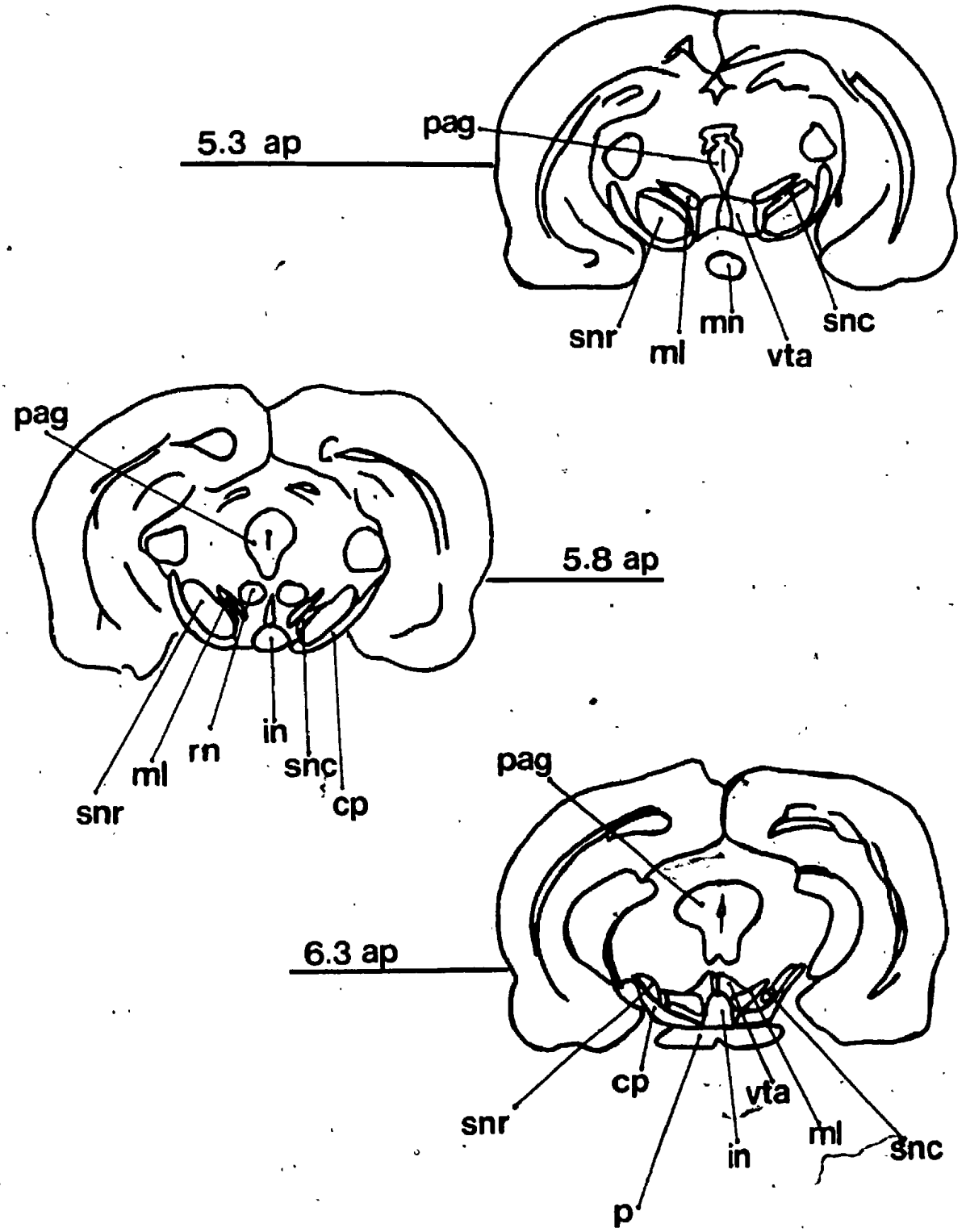
5.3 ap
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TABLE 7 Distribution of SNR injection sites in animals tested

		MEDIAL	CENTRAL	LATERAL
5.3 ap	DORSAL	1	1	0
	VENTRAL	1	2	0
5.8 ap	DORSAL	1	4	0
	VENTRAL	15	25	1
6.3 ap	DORSAL	0	0	0
	VENTRAL	1	4	0

Results

Statistical analysis: Figure 4 summarizes the mean circling totals for Groups A,B,C, and D during a 15 minute period following intranigral injections of either saline, SP, SK or Apo. The Friedman two-way ANOVA was used to test for within-group differences: four computations were carried out, one for each of the 4 groups. A significant difference between solution effects was found with respect to Group B ($p<.02$); however, analysis of the data from Groups A,C, and D showed that within each of these groups animals responded to all intranigrally administered solutions in a relatively similar fashion.

Since the Friedman two-way ANOVA suggested that animals within Group B showed circling rates that varied depending on which solution was administered intranigrally, further tests were carried out to compare the various solution effects within Group B. For such tests involving related samples, the Wilcoxon matched-pairs signed-ranks test was used. Statistical differences were found between the circling rates of animals injected with SK and Sal ($p<.005$), Apo and Sal ($p<.005$), SK and SP ($p<.005$), and finally with Apo and SP ($p<.005$). Differences between Sal and SP, and between SK and Apo were not significant.

Table 8 shows the number of animals that turned contraversively, ipsiversively, or that did not turn at all following the intranigral administration of the tested solutions.

An examination of the mean circling totals shown in Figure 2, in addition to the distribution of turning tendencies, suggested that animals receiving a d-amphetamine pretreatment (Groups A,B,C) on average, circled more than Group D which did not receive a pretreatment. Since these groups represented unrelated samples the Kruskal-Wallis One-Way ANOVA was used to

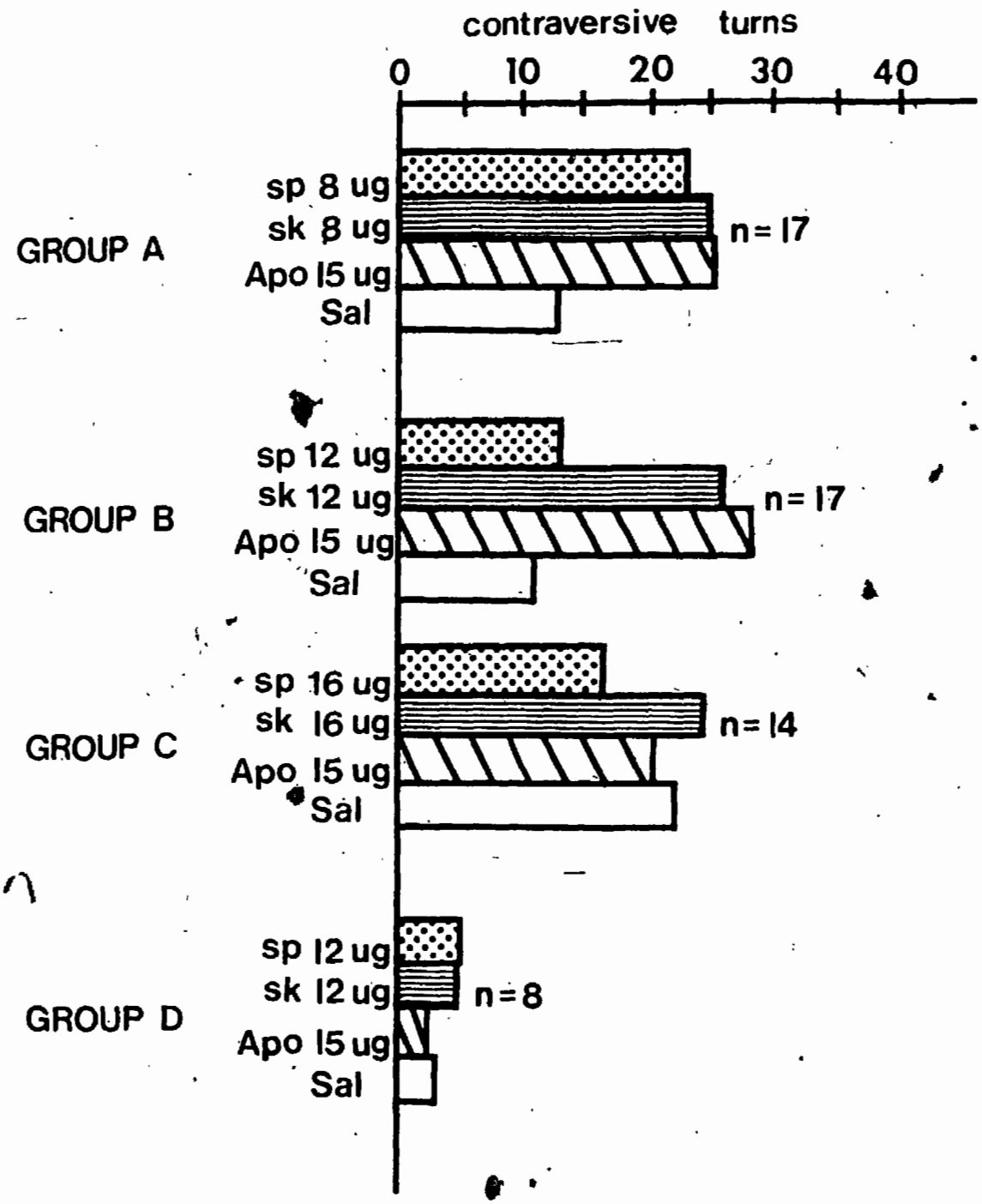

FIGURE 4 Mean circling totals for Groups A, B, C, and D following a 15 minute testing period. A statistical difference was found in Group B (Friedman two-way ANOVA, $p<.02$).

TABLE 8 Direction of circling following the intranigral administration of SP, SK, Apo, and Sal. Circling tendencies of animals not pretreated with d-amphetamine are also shown (**)

SUBSTANCE	IPSIVERSIVE	0	CONTRAVERSIVE
SP 8 ug (n=17)	1	1	15
SP 12 ug (n=17)	3	2	12
SP 12 ug ** (n=8)	1	3	4
SP 16 ug (n=14)	4	1	9
SK 8 ug (n=17)	2	1	14
SK 12 ug (n=17)	3	1	13
SK 12 ug ** (n=8)	1	2	5
SK 16 ug (n=14)	1	2	11
APO 15 ug (n=48)	3	5	40
APO 15 ug ** (n=8)	2	1	5
SAL (n=48)	7	8	33
SAL ** (n=8)	1	2	5

determine whether animals' turning rates were affected by d-amphetamine. The difference was not significant. However, in this respect, the small number of animals in Group D may have prevented any statistically detectable difference.

DISCUSSION

Within-group comparisons showed that in Group B contraversive circling increased significantly following the administration of a 12 ug dose of SK. These are the first data to indicate that SK may act within the SNR to produce contraversive circling. Within Group B Apo also elicited contraversive circling. Animals tested with 8, 12, and 16 ug of SP; or 8 and 16 ug of SK did not show significant differences in the magnitude or direction of circling with respect to saline injections. Apo's effects, like those of SK, were inconsistent across groups.

Increasing the doses of SP and SK (8, 12, 16 ug) did not bring about proportional increases in circling. Dose-related increases in circling have, nevertheless, been reported using different concentrations of SP injected intranigrally (2.5, 5, and 10 ug / 2 ul: Olpe and Koella, 1977; 1, 2, 4, 10 ug / .1 ul: James and Starr, 1979).

In examining SP's apparent lack of effect on circling behavior, a number of factors will be taken into consideration. These will include questions regarding the biological activity of the peptide solutions and their enzymatic degradation, injection sites, the injection procedure, and lastly the d-amphetamine pretreatment.

Intranigral peptide injections produced visible flushing of the ears

and paws in the vast majority of animals tested. This peripheral action has been documented in other studies for both SP and SK (Pernow, 1983; Holzer-Petsche, Schimeck, Amann, and Lembeck, 1985) and suggests that the peptide solutions were biologically potent. Procedures assuring sterility were also adopted to minimize the possibility of bacterial degradation. In light of these observations and precautions, an argument based on the assumption that the peptide solutions used were not biologically active cannot be forcefully made. In addition, although peptidases have been reported to rapidly degrade intranigrally injected peptides, data reported by Olpe and Koella (1977) and James and Starr (1977; 1979) suggest that this type of inactivation was not likely to have occurred within the time period used for measuring circling rates.

The anatomical location of injection sites was also unlikely to have been a contributing factor in SP's apparent lack of effect on circling. Although injections of SP in the SNR have been reported to elicit contraversive circling regardless of their location with respect to the rostro-caudal and medio-lateral planes (James and Starr, 1979), the injection sites reported here (the majority of which were distributed throughout the caudal half of the SNR) did not elicit circling.

Certain data suggested, however, that the procedure used may have influenced the results of the experiment. Table 8 (p. 43) shows that animals injected intranigrally with saline displayed a distinct bias in their circling: 7 animals turned ipsiversively, 8 did not turn, and 31 turned contraversively. These data are markedly different from those reported by Olpe and Koella (1977) and James and Starr (1979), which suggested that, as a group, animals showed very little bias. A number of factors may help to

explain the circling response to saline seen here: i) the saline injection, ii) natural animal biases, iii) nigral damage or iv) any combination of the above.

The effect produced by control injections is unlikely to have been due solely to saline's action on the neural circuitry involved in circling, mainly because saline has been routinely used as a control substance and has been shown to have no effect on circling (James and Starr, 1977). One likely cause of the circling that followed control injections involves animal biases. Postural deviation and circling can be elicited in intact animals when they are administered DA agonists subcutaneously, eg. Apo or d-amphetamine (Jerussi and Glick, 1976; Pisa and Szechtman, 1985). It has been suggested that this may be due to a natural hemispheric asymmetry of DA receptors or terminals. There is a possibility, therefore, that pretreatment with d-amphetamine may have accentuated such biases. If this hypothesis is correct, then it should follow that d-amphetamine's effect on circling should not have differed significantly from the effect of d-amphetamine when the latter was coupled with an intranigral saline injection. Using the Neuman-Keuls test (post hoc) data from each subgroup of animals that had been prescreened with d-amphetamine were used (A, n=9; B, n=9; C, n=6) so that comparisons could be made between the circling rates of animals receiving d-amphetamine alone and the same animals' circling rates following d-amphetamine and saline. Subgroups of Groups A and B showed differences that were not significant, suggesting that saline had no effect on circling. However, in the case of subgroup C, although animals turned ipsiversively following d-amphetamine, later tests using d-amphetamine and saline resulted in contraversive circling. This difference was significant ($p<.05$) and

supports one hypothesis that was formulated during the analysis of the data; that is, some animals were more likely to circle contraversively due to unilateral nigral damage. Although a number of factors may have intervened between the prescreening and the intranigral saline tests (eg. surgery, habituation), it was thought that damage to the SNR due to repeated injections was the factor most likely to have produced a contraversive circling bias in this subgroup. It is possible that since the i.c. tips were generally located in the central portion of the SNR, where nigrothalamic projections are known to originate (Faull and Mehler, 1978), damage in this region may have adversely affected nigral efferents. Kainic acid lesions in this area have been shown to produce contraversive circling (DiChiara et al.,1977).

Therefore, both animal biases and nigral damage may have affected circling responses, and hence could have made SP's and SK's effects more difficult to demonstrate. Animal biases tend to be relatively permanent since they are based on a biological asymmetry. However, the effect of the presumed nigral damage is unknown. All groups of animals sustained comparable damage to the SNR; the majority of animals had approximately 15 % of the SNR damaged, while a small number of animals showed either very little damage (5 %) or much larger damage (40 %) (the variability in damage may be related to the fact that injections were made by hand, rather than by a motorized pump). If one considers the potential effect of this damage the possibility is raised that SP's effect on circling could have been overshadowed by a circling tendency resulting from the damage that was enhanced by d-amphetamine. That is, in a number of instances where nigral damage was considerable, a circling response elicited by any particular solution may have been largely due to damage to nigral efferents, or some

other circuit important in circling. Consequently, if the latter hypothesis is correct the circling responses elicited in some animals may have been the result of unilateral damage combined with the effects of systemic d-amphetamine injections, as opposed to the effect of any intranigral injection.

SP's mode or mechanism of action is unknown. However, if one assumes for a moment that SP's effect in certain regions of the SNR is inhibitory, and that this effect is mediated by an inhibitory transmitter, (in a manner similar to that suggested by Jones and Olpe (1985) in the cingulate cortex), a number of conditions follow. For example, one way SP (or SK) could elicit contraversive circling would be by inhibiting GABA nigral efferents in the SNR that project to the thalamus, superior colliculus, and reticular formation. This type of inhibition has been hypothesized to have a disinhibitory effect on the three structures; and if this disinhibition is unilateral, contraversive circling results (Starr et al.,1983). Now, if this source of efferents were destroyed, as is thought to have been the case in a number of animals, SP (or SK) may have little additional effect on circling if it were administered in this region. This explanation applies mainly to animals that sustained nigral damage. Statistically, only a subgroup of Group C showed differences between pretreatment (d-amphetamine alone) and control (Sal and d-amphetamine) circling rates. Therefore, if considering whole groups, Groups A, B, and D did not seem to be affected by nigral damage significantly. In these animals a natural bias was most certainly the cause of circling following control injections.

Similar reasoning applies to SK. However, the fact that SK was able to produce significantly more circling than that seen following saline

injections suggests that it may be more potent than SP in this regard, at least at the 12 ug dose. Experiments comparing the effects of a larger number of doses along a wider spectrum (i.e. including smaller doses in the nanogram range) would help elucidate the question of relative potency with regard to SP and SK.

It should also be noted that other investigators have used identical strains of rats (Olpe and Koella, 1977) and similar volumes and rates of infusion (James and Starr, 1977; 1979). These two factors, in addition to those of biological activity, enzymatic degradation, and sites of injection were unlikely to have affected the results significantly. However, a basic assumption made was that all injections would be localized completely within the SNR. The possibility nevertheless exists, at least in the case of the more dorsal SNR injections, that SP and SK were diffusing dorsally along the cannula tract and into the SNC. This type of diffusion, which is difficult to predict, would complicate the interpretation of the results, especially since it is well known that neurons in the SNR and SNC often respond in opposite ways to the iontophoretic administration of particular substances (Dray and Straughan, 1976); and especially since there are more SK than SP receptors in the SNC (Mantyh et al., 1984).

An additional factor that merits consideration is the d-amphetamine treatment. The working assumption used during the design of the experiment was based on the two-component model of circling behavior (Pycock and Marsden, 1978). This model suggests that SN activity, in particular, is responsible for the direction of circling; whereas activity in other structures (striatum, nucleus accumbens, and VTA) is responsible for the

spontaneous motor activity that accompanies the circling response. Since pilot studies suggested that intranigral injections of SP alone had no apparent effect on circling, it was presumed that this might be due to the lack of motor stimulation (that is, although a bias may have resulted from the intranigral administraton of SP, its expression may have been difficult to measure in the absence of locomotor stimulation). It was therefore thought that the use of a low dose of d-amphetamine would serve to increase locomotor activity without having any effect on the direction of circling. Although contraversive circling following unilateral intranigral administraton of SP in the absence of d-amphetamine pretreatmerent has been reported (Olpe and Koella, 1977; James and Starr, 1977; 1979) the line of reasoning expressed above is widely accepted, and d-amphetamine has been used for the same purpose by others (Vaccarino and Franklin, 1984). The results of one other study (James and Starr, 1979) suggest, however, a more complex interaction between intranigrally administered substances and d-amphetamine. James and Starr (1979) reported that pretreatment with d-amphetamine (sc) coupled with unilateral intranigral administration of SP elicited a biphasic response. Following the SP injection, animals first circled contraversively, but a few minutes later began to circle ipsiversively. The authors did not attempt to explain this effect. In light of these surprising data (that fail to be explained by the two-component model of circling) the d-amphetamine pretreatment may also have played an important role in circling, a role that was not originally assumed.

With respect to Apo, unilateral intranigral administration of this substance has been reported to have no effect on the circling behavior of

intact animals (Glick and Crane, 1978; Kelley and Moore, 1978; Kozlowski, Sawyer, and Marshall, 1980). However, Apo injections into the medial SNC elicited ipsiversive circling when animals were given d-amphetamine prior to Apo (Vaccarino and Franklin, 1984). Kozlowski et al. (1980) reported that intranigrally administered Apo elicited contraversive circling in animals that were pretreated with a DA receptor blocker for one week prior to, and at the time of Apo injection. The contraversive circling that followed the latter manipulation suggested that Apo may have acted on non-DAergic cells believed to be GABAergic since the circling was blocked by picrotoxin (sc). Kozlowski et al. hypothesized that Apo could act on GABAergic nigral afferents (striatonigral) or GABAergic nigral efferents (nigrothalamic).

In the present study Apo significantly increased contraversive circling in only one of four groups tested. This inconsistent Apo effect is difficult to interpret for a number of reasons. To begin with, since our interest lay mainly in comparing the effects of SP to those of SK, peptides were injected early in the series of injections (keeping in mind that the probability of producing neural damage increased with the number of injections made). The fact that no comparison could be made between Apo's effect when injected first in the series, and its effect when injected last makes it difficult to draw a firm conclusion with regard to its effect on circling. Vaccarino and Franklin (1984) have suggested that d-amphetamine can condition animals to the effects produced by other drugs; that is, if one consistently elicits turning in one direction by administering a substance intranigrally after an animal has been administered d-amphetamine, d-amphetamine alone (i.e. in the absence of an intranigral injection) could later elicit a response similar to the one previously elicited by the

intranigrally administered substance. Therefore Apo's effect on circling could be explained by the latter conditioning effect, or, could have been due to some of the factors mentioned earlier (i.e. animal bias and nigral damage).

General Observations

Circling responses elicited by the intranigral solutions administered here lacked a number of features commonly observed following chemical stimulation of the SNR (Olpe and Koella, 1977), unilateral 6-OHDA lesions of the nigrostriatal pathway (Ungerstedt, 1971) or electrical stimulation of the SN (Arbuthnott and Crow, 1971). These latter manipulations have been reported to produce a form of circling characterized by rigidity and tight circling, such that the affected animal's hind paws remain relatively stationary, while its forelimbs provide the required force for movement. In our hands unilateral 6-OHDA lesions and electrical stimulation of the SN also elicit this form of circling; whereas intranigral administration of the various solutions described here did not have a similar effect. Animals in this experiment showed wide circling that was frequently interrupted by grooming and exploratory behavior. The compulsion to turn seen, for example, following electrical stimulation of the SN was clearly absent. It has been suggested that stimulation of the nigrostriatal pathway is more likely to produce circling that is of the ambulatory type (wide circling); whereas stimulation of pathways or structures closer to neuron pools located in the spinal cord will bring about tighter and more compulsive circling. This is presumably due to the fact that less fine tuning occurs the 'further' we move downstream from the basal ganglia (Franklin, personal communication).

In addition to these behavioral observations flushing of the ears and paws was noticeable following the intranigral administration of SP and SK. Although flushing was not quantified, SP showed a tendency to produce more intense vasodilation than SK. This observation agrees with recent findings (Holzer-Petsche et al.,1985). Flushing normally appeared 30 to 60 seconds following the start of the intranigral injection and on average lasted 60 seconds. Saline and Apo were never observed to produce visible signs of flushing.

General Conclusions

Although a large number of studies have focused on the neuroanatomy and electrophysiology of the SN, descriptions of neuronal mechanisms and interactions within the SN are, at the moment, largely hypothetical. Both SP and SK probably play a role in the processing of information within the SN. The importance and nature of this role still remain to be elucidated. For example, although intranigral administration of SP increases the amount of DA released in the striatum (James and Starr, 1977) and decreases the amount of dendritically released DA in the SNR (Cheramy et al.,1981), its mode of action within the SN is unknown. Does it act presynaptically or postsynaptically? Does SP serve a neuromodulatory function? If so, how does it interact with other neurotransmitters known to be present in the SN? These questions remain for the most part unanswered.

With respect to circling behavior, of key importance is the notion that this behavior is the product of complex neuronal interactions that take place within the SN as well as between the SN and various other structures (eg. striatum, thalamus, and superior colliculus). While studies based on

manipulations of intranigral systems benefit our understanding of circling behavior, the complex interplay involving a number of structures described above is of crucial importance.

The study presented here focused on the intranigral effects of SP and SK on circling behavior, and an interpretation of the results suggested that SK was more potent than SP in eliciting circling at a particular dose. The importance of this new finding will only become clear as additional research involving SK is carried out.

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