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Effects of Dopamine and Dopaminergic Agents on Neuronal Activity of the Ventral Pallidum/Substantia Innominata

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EFFECTS OF DOPAMINE AND DOPAMINERGIC AGENTS ON NEURONAL ACTIVITY OF THE VENTRAL PALLIDUM/SUBSTANTIA INNOMINATA

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I am dedicating the dissertation to the memory of my father, Joseph, who passed away in December of 1990, because he taught me that the secret to success is to always do the hardest job first.

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LIST OF ABBREVIATIONS

A	ampere
AC	anterior commissure
AChE	acetyl choline esterase
ACy	adenylate cyclase
ANOVA	analysis of variance
AMN	amygdaloid nuclei
AMP	adenosine monophosphate
APO	apomorphine
ATP	adenosine triphosphate
blA	basolateral nucleus of the amygdala
^{o}C	degrees Celcius
Ch4	primate cholinergic cell group, includes the cholinergic neurons of the ventral pallidum/substantia innominata
ChAT	choline acetyltransferase
cis-FLU	*cis*-flupentixol = (4-[3-[2-(trifluoromethyl)-9H-thioxanthen-9-ylidene] propyl]-1-piperazine ethanol dihydrochloride)
D_1	dopamine receptor subtype 1
D_2	dopamine receptor subtype 2
DA	dopamine = 3,4-dihydroxyphenethylamine

DARPP-32	dopamine regulated phosphoprotein with an apparent molecular weight of 32,000
df	degrees of freedom
E	expected value
E_{max}	largest observed effect, standardized as percent of baseline firing rate
ECu_{50}	stimulation current producing 50% of total effect
ED_{50}	dose required to induce 50% of the maximum response
EFF	effective
fmol	femtomoles
g	gram
GABA	gamma-aminobutyric acid
GP	dorsal globus pallidus
h	hour
H	hydrogen ion
HCl	hydrochloride solution
^3H	tritium ion
[^3H]SCH	tritium-labeled SCH23390
[^3H]SUL	tritium-labeled sulpiride
HRP	horse radish peroxidase
Hz	Hertz; frequency unit = one spike per second
IC_{50}	concentration of inhibitor producing 50% inhibition
i.p.	intraperitoneal
i.v.	intravenous
K^+	potassium ion
K_d	equilibrium dissociation constant

K_i	equilibrium dissociation constant for a competitive inhibitor
kg	kilogram
KHz	kilohertz
L	lateral
L-DOPA	levo-3,4-dihydroxyphenylalanine
mRNA	messenger ribonucleic acid
M	molar = moles per liter
MΩ	megohm
µg	microgram
µl	microliter
µm	micron = micrometer
µM	micromolar
µV	microvolt
mA	milliampere
mg	milligram
min	minute
ml	milliliter
mm	millimeter
ms	millisecond
n	number
N	normal
NA	nucleus accumbens
Na^+	sodium ion
NaCl	sodium chloride
NaOH	sodium hydroxide

NC	no change
nA	nanoampere
nM	nanomolar
O	oxygen ion
6-OH-DA	6-hydroxydopamine
p	probability value
P	posterior
PHA-L	*Phaseolus vulgaris* leucoagglutinin
QUIN or QUN	quinpirole = LY171555 = trans-(-)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5propyl-1H (or 2H)-pyrazolo-(3,4-g) quinoline monochloride
r	correlation coefficient
s	second
SCH	SCH23390 = (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hemimaleate
SEM	standard error of the mean
SI	substantia innominata
SKF	SKF38393; 2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine-7,8-diol hydrochloride
SNc	substantia nigra, pars compacta
SNr	substantia nigra, pars reticulata
STR	dorsal striatum
SUL	sulpiride = 5-(aminosulfonyl)-N-((1-ethyl-2-pyrrolidinyl) methyl)-2-methoxybenzamide
TH	tyrosine hydroxylase
TTA	tartaric acid solution

v	volt
V	ventral
VP	ventral pallidum or ventral pallidal
VTA	ventral tegmental area
WGA-HRP	wheat germ agglutinin conjugated to horseradish peroxidase
χ^2	chi-square

CHAPTER I

INTRODUCTION

Major neurological disorders that afflict humans include Alzheimer's disease, Parkinson's disease, Huntington's chorea, mood disorders and schizophrenia. Although each of these diseases has distinctive symptoms which allow for ultimate diagnosis, there is also a certain degree of similarity among the clinical manifestations. All of these disorders are characterized by cognitive impairments, abnormal movements, psychoses, and depression that are expressed with varying prevalence and severity in individual patients (for review see, Heimer *et al.*, 1991). In fact, two of these diseases, Alzheimer's disease and Parkinson's disease, are often diagnosed within the same patient (Boller, 1985; Jellinger, 1987; Quinn *et al.*, 1986). This has led to the theory that these two disorders may be part of a disease continuum, where each "pure" disease state forms the boundaries, and combinations of symptoms represent the remainder of the spectrum (Appel, 1981; Korczyn *et al.*, 1986).

The pathology of both Parkinson's disease and Alzheimer's disease involve the loss of neurons that are located within discrete brain regions and contain specific neurotransmitters. In Parkinson's disease, abnormalities of the dopaminergic (*i.e.* dopamine (DA) containing) system that originates within the midbrain are observed with the concomitant loss 60 to 85% of the substantia nigra neurons (for review see Boller, 1985; Jellinger, 1986). Clinical signs of Parkinson's disease include: resting tremor, rigidity and the impaired ability to initiate and execute voluntary movement (Cote and Crutcher, 1985). In Alzheimer's disease, which accounts for about 70% of all cases of

dementia or progressive decline of mental function (Cote, 1985), cell loss is the most dramatic among the cholinergic (acetylcholine-containing) neurons (Cote, 1985; Lehericy *et al.*, 1991).

The majority of cholinergic neurons affected by Alzheimer's disease are located at the base of the forebrain in the nucleus basalis of Meynert (Whitehouse *et al.*, 1981). Parkinson's patients can also demonstrate cognitive impairments and dementia associated with loss of forebrain cholinergic neurons (Boller, 1980; Candy *et al.*, 1983; Whitehouse, 1986; Whitehouse *et al.*, 1983); and Alzheimer's patients can also present with rigidity and impaired movement related to loss of dopaminergic neurons (Boller, 1985; Jellinger, 1987; Mayeux and Stern, 1986; Uchihara *et al.*, 1992). This overlap in clinical manifestations suggests that there may be some strategically located anatomical systems mediating the cognitive and motoric behaviors that have become deficient in these disease states.

As a basis for understanding the clinical findings of the aforementioned neurological diseases, many studies have emphasized the importance of an integrative knowledge of the normal anatomical connections of the brain regions affected by these diseases, as well as the functional significance of these innervations. One potential locus for dysfunction that is similar among these diseases is the basal nucleus of Meynert or substantia innominata, and also may involve the midbrain dopaminergic system (for review see, Heimer *et al.*, 1991). Parkinson's disease and Alzheimer's disease, as well as animal models of these disease states, also demonstrate alterations in the density of specific DA receptor subtypes (Cortes *et al.*, 1988; Gagnon *et al.*, 1990; Graham *et al.*, 1990; Seeman *et al.*, 1987). This dissertation is based upon the hypothesis that the output (measured as changes in neuronal firing rate) of the rat brain region comparable to the human nucleus basalis of Meynert, the ventral pallidum and the

adjacent substantia innominata (VP/SI), is altered by DA and dopaminergic agents acting through specific receptor subtypes.

Recent molecular biological studies have revealed that at least four DA receptor subtypes exist in the rat brain (Civelli *et al.*, 1991), and possibly five receptors in the human brain (Grandy *et al.*, 1991). Previously, DA was considered to act through two receptor subtypes, the D_1 and the D_2 subtype (Kebabian and Calne, 1979). Both receptor subtypes have been identified within the VP/SI, and either the D_1 or the D_2 receptor can mediate the actions of DA applied within the VP/SI. The effect of separate activation of these receptors by selective agonists on VP/SI firing rate has not been previously determined. Furthermore, DA may also act as neuromodulator, altering the effects of other afferent systems on neuronal activity of VP/SI, similar to its action in other dopaminoceptive brain regions (Graybiel, 1990; Le Moal and Simon, 1991; Yim and Mogenson, 1982, 1983). The distribution and functional relevance of the other DA receptor subtypes await future studies with receptor subtype specific dopaminergic agents.

The hypotheses of this dissertation are: 1) that the D_1 and the D_2 DA receptor subtypes mediate DA-induced effects on VP/SI neuronal activity, and 2) that DA is a neuromodulatory transmitter within the VP/SI altering neuronal activity evoked in this brain region by electrical stimulation of afferents from the amygdaloid nuclei (AMN). To investigate these hypotheses, the following specific aims were proposed:

Specific Aim 1: To characterize the DA receptor subtypes that mediate the responses of single VP/SI neurons to systemic administration of DA agonists.

In vivo electrophysiological experiments were performed on anesthetized rats. Spontaneously active VP/SI neurons were characterized by their action potential

properties (configuration, amplitude and duration) and activity (firing rate and pattern). Agonists that selectively activate D_1 or D_2 DA receptor subtypes were injected intravenously in increasing doses. VP/SI neuronal activity was measured by alterations in firing rate in response to increasing concentrations of DA agonists. If any significant rate changes occurred, the antagonist specific for the activated receptor subtype was administered to determine if the rate alterations were mediated by that specific receptor subtype. To determine whether activation of one receptor subtype was sufficient to mediate the actions of a nonselective DA agonist (*i.e.*, one that mimics the actions of endogenous DA within the brain), the combined D_1 and D_2 DA agonist apomorphine was administered, and any effects induced were tested for receptor subtype specificity by administration of selective D_1 or D_2 antagonists.

Specific Aim 2: **To determine the DA receptor subtypes involved in the VP/SI responses evoked by endogenously-released DA during stimulation of midbrain dopaminergic regions. To characterize the VP/SI responses evoked by electrical activation of the AMN. To determine if endogenously-released, and exogenously-applied DA within the VP/SI modulate AMN-evoked responses of VP/SI neurons.**

In vivo electrophysiological experiments were used to describe the effects of orthodromic (*i.e.*, trans-synaptic) stimulation of the AMN and two midbrain dopaminergic regions, the ventral tegmental area and the substantia nigra pars compacta (VTA/SNc), on the activity of VP/SI neurons. To verify whether VTA/SNc stimulation results in the release of DA in the VP/SI the following criteria were used: 1) exogenously-applied DA via microiontophoresis should mimic the effects of electrical stimulation and 2) exogenously-applied D_1 and/or D_2 DA antagonists within the VP/SI should attenuate the effects of VTA/SNc stimulation on VP/SI neuronal firing rate. The effects of microiontophoretic application of DA agonists selective for the D_1 or D_2

receptor subtypes were also assessed to determine the contribution of these subtypes to alterations of spontaneous activity of VP/SI neurons. In addition, possible modulatory effects of DA within the VP/SI on VP/SI responses evoked by AMN stimulation were examined to determine: 1) if electrical stimulation of the VTA/SNc (which presumably releases endogenous DA), prior to AMN stimulation alters the effects of AMN stimulation alone; and 2) if exogenous application of DA mimics the modulatory effects of endogenously-released DA on VP/SI neuronal activity evoked by AMN stimulation.

CHAPTER II

REVIEW OF RELATED LITERATURE

In Chapter I, the degeneration of the nucleus basalis of Meynert (or its rat analogue, the ventral pallidal/substantia innominata region; VP/SI) and the midbrain dopaminergic regions was described in relation to Alzheimer's disease and Parkinson's disease. The importance of understanding the normal anatomy and physiology of these systems was indicated as the foundation for this dissertation. Thus, to illustrate the relationship of dopamine (DA) to changes in neuronal activity of the VP/SI, this chapter is divided into three major subdivisions.

Initially, the **Anatomy of the Ventral Pallidum/Substantia Innominata** is described to identify the location of the VP/SI within the rat brain; the nomenclature used for this region is also described. An outline of the afferents to VP/SI is included, emphasizing the innervation from the amygdala and the midbrain dopaminergic regions, to provide the anatomical background for the electrophysiological studies in the following chapters. The anatomy of VP/SI efferents is summarized to suggest the potential consequences of altering the activity of the VP/SI neurons. In the second subdivision, **Dopamine Receptor Pharmacology**, the characteristics of the DA receptor subtypes (*i.e.*, the D_1 and D_2 DA receptors) are described in relationship to the effects of specific agonists and antagonists, and their location within the basal forebrain is also detailed. The final subdivision, **Neurochemical, Behavioral and Electrophysiological Studies Involving Dopamine**, summarizes the known

interactions between D_1 and D_2 receptor stimulation, and describes the potential relevance of these receptors for DA agonist-mediated effects on VP/SI output.

Anatomy of the Ventral Pallidum/Substantia Innominata

Introduction

The term "ventral pallidum" (VP) was first used by Heimer and Wilson (1975) to describe the rostroventral extension of the globus pallidus beneath the anterior commissure. The VP displays similar cell morphology to the dorsal globus pallidus (GP), and in fact is indistinguishable from the GP in Nissl-stained material (Nauta and Domesick, 1984). However, the VP can be differentiated from the GP by other histochemical methods, since the VP exhibits a dense plexus of substance P-positive striatopallidal fibers which readily demarcate it from the GP (Haber and Nauta, 1983; Heimer *et al.*, 1985). The identification of the VP has redefined the region ventral to the anterior commissure since the VP was previously considered the subcommissural part of the substantia innominata (SI; Switzer III *et al.*, 1982).

The SI is restricted to the caudal sublenticular gray (Alheid and Heimer, 1988; Heimer *et al.*, 1985), "a homogenous region underneath the caudal part of the globus pallidus" (Switzer III *et al.*, 1982). It is located medial to the caudal aspects of VP (Paxinos and Watson, 1986). Although anatomically distinct, the SI and VP regions exhibit similar afferent innervation (Haring and Wang, 1986), terminal configuration (Grove, 1988a) and electrophysiological characteristics (Napier *et al.*, 1991b). The cholinergic neurons contained within both regions receive midbrain dopaminergic and amygdaloid afferents (see review below). Furthermore, the cholinergic neurons of the VP (Zaborszky and Leranth, 1985) and the SI (Carlsen, 1985) both project to the basolateral nucleus of the amygdala. Recent anatomical evidence suggests that portions of the SI may form a continuum with the bed nucleus of the stria terminalis and the centromedial amygdaloid nuclei, which together are termed "the extended amygdala" (Alheid and Heimer, 1988). However, the former term of SI is maintained throughout

the following chapters since functional differences (*i.e.*, action potential duration, firing rate or pattern) were not observed between SI and VP neuronal populations.

Neuronal Population of the VP/SI

The VP/SI is a heterogeneous cell population similar in composition to the GP, which is primarily comprised of diffusely arranged, large triangular cells with long radiating dendrites covered with extensive synaptic terminals. Scattered among this cell population are fewer small cells (Heimer *et al.*, 1985; Heimer and Wilson, 1975). Immunocytochemical studies of the neurotransmitter content of VP/SI neurons indicates that the large cell types are either cholinergic or GABAergic (Carlsen *et al.*, 1985; Kimura *et al.*, 1981; Zaborszky and Leranth, 1985; Zaborszky *et al.*, 1985). The cholinergic neurons within the VP/SI constitute part of the rat brain region comparable to the primate nucleus basalis of Meynert (Ch4 cholinergic cell group), and project widely throughout the cortex and amygdala (for review see, Mesulam *et al.*, 1983a; Zaborszky *et al.*, 1991).

Afferents of the VP/SI

Amygdaloid Afferents. The VP/SI regions in the monkey and the rat are innervated by amygdalofugal fibers originating from many amygdaloid nuclei (AMN; Fig. 1; Aggleton *et al.*, 1987; Fuller *et al.*, 1987; Haring and Wang, 1986; Kelley *et al.*, 1982; Krettek and Price, 1978; Price, 1986; Russchen *et al.*, 1985). The majority of this AMN projection in the monkey arises from the parvicellular basal nucleus (or posterior basolateral nucleus; Aggleton *et al.*, 1987; Price, 1986), magnocellular accessory basal nucleus (or basal medial) and the central nucleus (Price, 1986). Using retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) and anterograde axonal transport of *Phaseolus vulgaris*

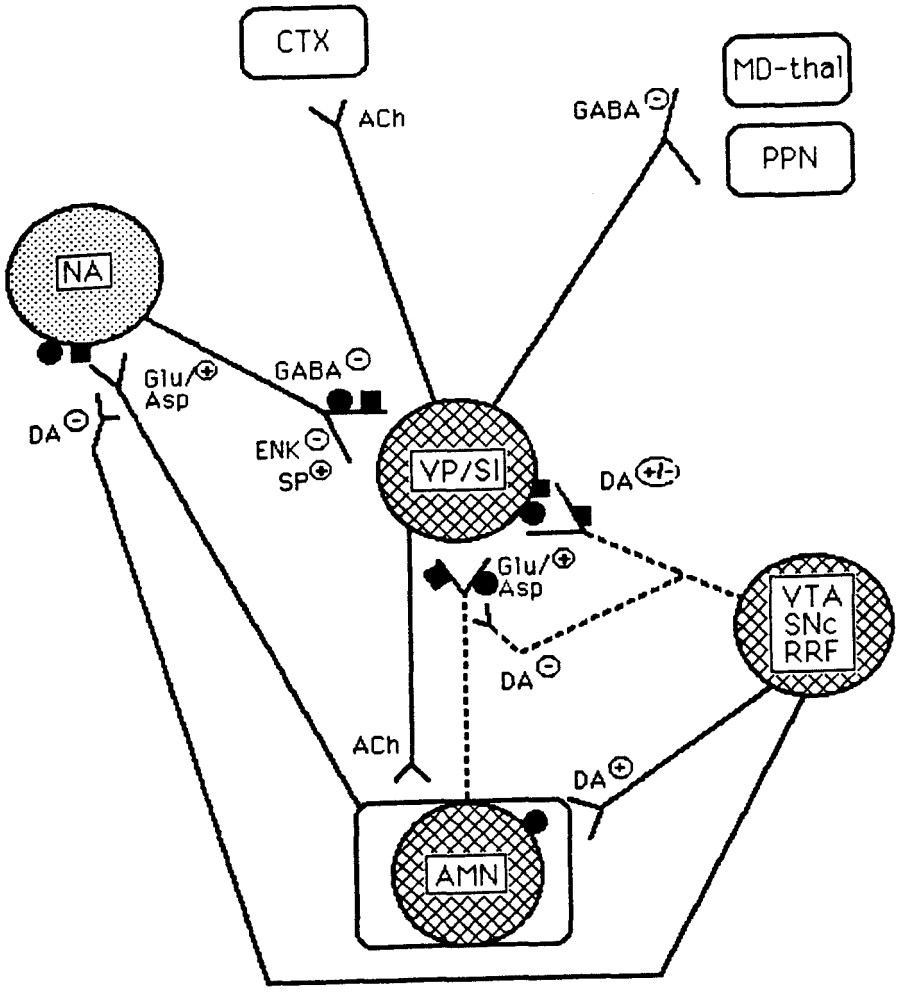

Fig. 1. SCHEMATIC ILLUSTRATION OF THE RELATIONSHIP BETWEEN VP/SI AFFERENT AND EFFERENT PATHWAYS. The VP/SI receives excitatory (plus symbol) input from the AMN, both inhibitory (minus symbol) and excitatory inputs from the NA and from the midbrain dopaminergic regions. VP/SI neurons innervate the cortex (CTX), the AMN, the mediodorsal nucleus of the thalamus (MD - thal) and the pedunculopontine nucleus (PPN). Biochemical and molecular studies suggest that D_1 receptors (filled circles) and D_2 receptors (filled squares) are located on pre- and postsynaptic membranes of the VP/SI (and the NA). Figure is not drawn to scale, and, for clarity, all inputs are not shown. ACh - acetylcholine; Asp - Aspartate; DA - dopamine; ENK - enkephalin; GABA - gamma-aminobutyric acid; Glu - glutamate; RRF - retrorubral field; SNc - substantia nigra, pars compacta; SP - substance P; VTA - ventral tegmental area.

leucoagglutinin (PHA-L), Grove (1988b) observed similar AMN projections to the VP/SI in the rat. HRP injections into the rat basolateral nucleus of the amygdala (blA) anterogradely label VP/SI neurons, some of which are immunoreactive for the enzyme that synthesizes acetylcholine, choline acetyltransferase (ChAT; Zaborszky *et al.*, 1984,1991). In addition, some of the AMN afferents to the VP/SI are thought to be glutamatergic and/or aspartergic, since [^{3}H]D-aspartate and WGA-HRP injections into the VP/SI retrogradely label neurons within the AMN (Fig. 1; Fuller *et al.*, 1987). Thus, some of the AMN afferents are excitatory and innervate both cholinergic and noncholinergic neurons of the VP/SI.

Midbrain Dopaminergic Afferents. Recent anatomical evidence indicates that the VP/SI is innervated by midbrain dopaminergic neurons. Catecholaminergic innervation to the VP/SI in the rat and the monkey originates primarily from the substantia nigra pars compacta (SNc; Fig. 1; Fallon and Moore, 1978; Haring and Wang, 1986; Jones and Cuello, 1989; Martinez-Murillo *et al.*, 1988b; Russchen *et al.*, 1985; Semba *et al.*, 1988; Zaborszky, 1989; Zaborszky *et al.*, 1991), ventral tegmental area (VTA; Fig. 1; Grove, 1988b; Haring and Wang, 1986; Jones and Cuello, 1989; Russchen *et al.*, 1985; Semba *et al.*, 1988; Zaborszky, 1989; Zaborszky *et al.*, 1991) and the retrorubral field (Fig. 1; Deutch *et al.*, 1988; Jimenez-Castellanos and Graybiel, 1987; Jones and Cuello, 1989; Zaborszky, 1989; Zaborszky *et al.*, 1991). The presence of immunoreactivity against the rate-limiting enzyme for catecholamine synthesis, tyrosine hydroxylase (TH) in VTA and SNc (Semba *et al.*, 1988), as well as retrorubral projections to the VP/SI (Deutch *et al.*, 1988) indicates that these afferents are catecholaminergic, and are likely dopaminergic based on the origin of the projections. Finally, Voorn *et al.* (1986), used antibodies against DA to demonstrate that "a dense plexus of thin, varicose dopaminergic fibers" is present in the VP/SI,

suggesting that this region is a target of these DA antibody-labeled fibers. Thus, dopaminergic afferents originating from the VTA, the SNc, and the retrorubral field innervate neurons within the VP/SI.

The dopaminergic region of the midbrain also innervates in particular the cholinergic subpopulation of VP/SI neurons. Retrograde and anterograde tracing studies using WGA-HRP and PHA-L, respectively, demonstrate that VTA afferents occasionally approach individual neurons within the cholinergic-rich regions of the SI (Grove, 1988b). TH-immunoreactive cell fibers from the VTA, SNc and the retrorubral field are located near ChAT-immunoreactive VP/SI somata (Jones and Cuello, 1989; Martinez-Murillo *et al.*, 1988b; for review see, Zaborszky, 1989; Zaborszky *et al.*, 1991). Further studies using electron microscopy indicate that TH-containing terminals contact ChAT-positive neurons in the rat VP/SI (Zaborszky *et al.*, 1992). These observations suggest that ascending dopaminergic fibers of SNc, VTA and retrorubral field synapse on cholinergic neurons in VP/SI. In addition, lesions of the ascending catecholaminergic bundles produced by the toxin, 6-hydroxydopamine (6-OH-DA) result in reductions of ChAT levels in the VP/SI (Zaborszky *et al.*, 1992). Thus, the midbrain dopaminergic innervation of cholinergic neurons within the VP/SI may modulate the activity of these cholinergic neurons.

Other Afferents. The nucleus accumbens (NA) and the remainder of the ventral striatum provide the most extensive innervation of the VP/SI in the rat and the monkey (Fig. 1; Grove, 1988b; Haber *et al.*, 1990; Heimer and Wilson, 1975; Mogenson *et al.*, 1983; Nauta *et al.*, 1978; Walaas and Ouimet, 1989; for review also see, Heimer and Alheid, 1991; Heimer *et al.*, 1991; Parent, 1990; Zaborszky *et al.*, 1991). The NA innervation of the VP/SI is topographically distributed such that "core" and "shell" regions of the NA project to dorsolateral and ventromedial VP/SI,

respectively (Zahm and Heimer, 1990). In addition, NA nerve terminals to VP/SI cholinergic and non-cholinergic neurons presumably contain the inhibitory neurotransmitters GABA and enkephalin, as well as the excitatory neurotransmitter substance P (Fig. 1; Bolam *et al.*, 1986; Martinez-Murillo *et al.*, 1988a; Wood and McQuade, 1986; Zaborszky *et al.*, 1986).

The cortical innervation of the VP/SI originates from the medial prefrontal, insular, perirhinal and entorhinal cortices in the rat (Grove, 1988b). In the monkey, projections from the orbitofrontal, insular and temporal cortices project to cholinergic and non-cholinergic neurons of the VP/SI (Mesulam and Mufson, 1984; Russchen *et al.*, 1985).

The non-telencephalic inputs to the rat and monkey VP/SI include the pedunculopontine nucleus (Russchen *et al.*, 1985), the dorsal raphe (Grove, 1988b; Russchen *et al.*, 1985), the locus ceruleus (Russchen *et al.*, 1985), much of the hypothalamic and midline thalamic nuclei (Groenewegen and Berendse, 1990; Grove, 1988b; Russchen *et al.*, 1985), the subthalamic nucleus (Groenewegen and Berendse, 1990), the parabrachial nucleus (Bernard *et al.*, 1991; Grove, 1988b; Russchen *et al.*, 1985), and the nucleus of the solitary tract (Grove, 1988b; Russchen *et al.*, 1985). Similarly, VP/SI cholinergic neurons are innervated by the dorsal raphe nucleus (Grove, 1988b), and hypothalamus (Grove, 1988b; Mesulam and Mufson, 1984), as well as noradrenergic neurons from the locus ceruleus (Chang, 1989; Zaborszky *et al.*, 1992). Thus, these afferents convey multifarious information that may regulate the final output of the VP/SI to its efferent targets.

Efferents from the VP/SI

Efferents to the Amygdala. Basal forebrain efferents, including those from the VP/SI, to the AMN (Fig. 1; Aggleton *et al.*, 1980, 1987; Carlsen *et al.*, 1985; De Olmos *et al.*, 1985; Emson *et al.*, 1979; Grove, 1988a; Haber *et al.*, 1985; Koliatsos *et al.*, 1988; Mesulam *et al.*, 1983a; Nagai *et al.*, 1982; Nauta and Domesick, 1984; Troiano and Siegel, 1978; Woolf and Butcher, 1982; Zaborszky *et al.*, 1986; Zaborszky and Leranth, 1985) arise from both cholinergic and noncholinergic neurons (Carlsen *et al.*, 1985; Woolf *et al.*, 1986; Zaborszky and Leranth, 1985). Combined retrograde transport and ChAT histochemical studies demonstrate that cholinergic projections to the basolateral amygdala (blA) originate predominantly from the dorsal subcommissural part of the VP (Zaborszky and Leranth, 1985) and the sublenticular SI (Carlsen *et al.*, 1985). Non-cholinergic neurons from the same region of the VP constitute approximately 25% of VP/SI efferents projecting to blA (Carlsen *et al.*, 1985). Thus, a substantial portion of the cholinergic efferents to the AMN is from the VP/SI (Aggleton *et al.*, 1987; Kitt *et al.*, 1987; Mesulam *et al.*, 1983a, 1983b; Russchen *et al.*, 1985), and the main target of these efferents in the AMN is the blA (Aggleton *et al.*, 1987; Carlsen *et al.*, 1985; Haber *et al.*, 1985; Kordower *et al.*, 1989; Otterson, 1980; Woolf and Butcher, 1982; Woolf *et al.*, 1984; Zaborszky *et al.*, 1986; for review see, Carlsen, 1989). Additionally, the blA contains more radioactive label for both AChE and ChAT than any other amygdaloid nucleus tested (Emson *et al.*, 1979). Since few cholinergic cell bodies have been found in the AMN (Carlsen, 1989; Carlsen and Heimer, 1986; Kimura *et al.*, 1981), and knife cuts of the ventral pathway that connects the VP/SI with the AMN produce large depletions of ChAT activity in the AMN (Emson *et al.*, 1979), it is considered to be cholinoceptive (Amaral and Bassett, 1989; Kimura *et al.*, 1981; Nagai *et al.*, 1982). There appears to be reciprocal innervation between blA neurons and VP/SI neurons.

Other Telencephalic Efferents. The VP/SI projections to the hippocampus (Aggleton *et al.*, 1987; Amaral and Cowan, 1980; DeVito, 1980; Koliatsos *et al.*, 1988; Woolf *et al.*, 1984) are in part cholinergic (Woolf *et al.*, 1984). Similarly, the cortical mantle receives innervation from neurons within the VP/SI (Fig. 1; Divac, 1975; Haber *et al.*, 1985), 80-90% of which are cholinergic (Ch4; Rye *et al.*, 1984). In fact, the VP/SI provides a major source of the cholinergic innervation of the rat, monkey, as well as human neocortices (Bigl *et al.*, 1982; Grove, 1988a; Koliatsos *et al.*, 1988; Lehmann *et al.*, 1980; Mesulam and Geula, 1988; Mesulam *et al.*, 1983a; Stewart *et al.*, 1985; Switzer III *et al.*, 1982; Woolf *et al.*, 1983, 1984).

Non-cortical, telencephalic projections from the VP/SI include a reciprocal innervation to the NA and the remainder of the ventral striatum. In contrast to the substantial afferent innervation of the VP/SI from the NA and the remainder of the ventral striatum, the VP/SI provide only minor efferent projections to these regions (Haber *et al.*, 1985; Spooren *et al.*, 1991b; Woolf *et al.*, 1984).

Non-telencephalic Efferents. The medial dorsal nucleus of the thalamus of the rat and monkey receives VP/SI efferents (Fig. 1; Grove, 1988a; Haber *et al.*, 1985; Hreib *et al.*, 1988; Mogenson *et al.*, 1987; Nauta and Domesick, 1984; Young *et al.*, 1984), as does the subthalamic nucleus (Canteras *et al.*, 1990; Groenewegen and Berendse, 1990; Haber *et al.*, 1985; Zahm, 1989) and the zona incerta (Mogenson *et al.*, 1985). There is also evidence for diencephalic projections from the VP/SI to the lateral habenular nucleus (Haber *et al.*, 1985; Nauta and Domesick, 1984), and to the reticular nucleus of the thalamus (Grove, 1988a; Heimer *et al.*, 1985; Jourdain *et al.*, 1989; Levey *et al.*, 1987), approximately 20% of these VP/SI neurons are ChAT-

positive (Jourdain *et al.*, 1989). Thus, most of the descending efferents of the VP/SI appear to arise primarily from noncholinergic cells (Grove, 1988a).

The SNc (Grove, 1988a; Haber *et al.*, 1985; Zahm, 1989) and the VTA (Grove, 1988a; Haber *et al.*, 1985; Nauta and Domesick, 1984; Zahm, 1989) receive VP/SI efferents which suggests reciprocal connections between the VP/SI and these regions. The pedunculopontine nucleus (Fig. 1; Semba *et al.*, 1989), which includes the mesencephalic locomotor region (Mogenson *et al.*, 1985; Mogenson and Wu, 1986), and the hypothalamus (Grove, 1988a; Haber *et al.*, 1985) also receive VP/SI efferents.

Through the cortical and the NA, the AMN, and midbrain dopaminergic afferent innervations of the VP/SI (Fig. 1), the VP/SI may receive information concerned with cognitive (for review see Richardson and DeLong, 1988; Salamone, 1986), motivational (for review see Richardson and DeLong, 1991), and motoric behaviors (for review see Heimer *et al.*, 1982; Kalivas *et al.*, 1991; Mogenson and Yang, 1991), respectively. In turn, the efferent connections of the VP/SI to the cortex, the AMN, the mediodorsal thalamic and pedunculopontine nuclei (Fig. 1), and the hypothalamus suggest that the VP/SI may be able to influence activity of brain regions concerned with cognition (Pirch *et al.*, 1986, 1991), motor behavior (for review see Mogenson and Yang, 1991), motivation (Richardson and DeLong, 1991), and possibly even visceral function (Grove, 1988; Haber *et al.*, 1985) . In summary, the VP/SI is located such that it can convey the output of "limbic antecedents not only into extrapyramidal circuits but also back into the circuitry of the limbic system" (Haber *et al.*, 1985).

Dopamine Receptor Pharmacology

Introduction

Multiple subtypes of the DA receptor have been described (for review see, Civelli *et al.*, 1991; Clark and White, 1987). The generally accepted classification, that was used for this dissertation is into two subtypes designated D_1 and D_2 (Kebabian and Calne, 1979; Stoof and Kebabian, 1981). This classification is based upon differences in the biochemical (Kebabian and Calne, 1979), pharmacological (Billard *et al.*, 1984; Creese *et al.*, 1983; Stoof and Kebabian, 1984), anatomical (Creese *et al.*, 1983), and behavioral (Arnt, 1985; Barone *et al.*, 1986) profiles of these receptor subtypes. In addition, the D_1 and D_2 receptors are deemed distinct entities since they can be separated using biochemical techniques (Dumbrille-Ross *et al.*, 1985), and have different DNA and protein sequences (Civelli *et al.*, 1991). The original classification was based upon the differential manner by which these subtypes affect the activation of adenylyl cyclase (ACy). The D_1 receptor subtype stimulates (Hyttel, 1978; Kebabian and Calne, 1979) and the D_2 receptor subtype inhibits (Battaglia *et al.*, 1985; Onali *et al.*, 1984; Stoof and Kebabian, 1981) the production of cyclic AMP by ACy. The classification was refined to include later findings that some D_2 receptors were not linked to this enzyme (Memo *et al.*, 1986b; Stoof and Kebabian, 1982; Stoof and Verheijden, 1986), and that agonist-mediated effects at these D_2 receptors are independent of ACy inhibition (Memo *et al.*, 1986a). These functional studies, combined with the results of radioligand binding studies, provide a means for assessing the apparent selectivity of agonists and antagonists that interact with either D_1 or D_2 receptor subtypes.

Identification of D_1 and D_2 DA Agonists and Antagonists

The non-selective and D_1 or D_2 selective agonists and antagonists used in the experiments for this dissertation are described below. The D_1 DA receptor agonist and antagonist, used throughout these studies, were SKF38393 (SKF) and SCH23390 (SCH), respectively; the D_2 DA receptor agonist and antagonist were quinpirole (QUIN) and sulpiride (SUL), respectively. In addition, apomorphine (APO), a non-selective DA agonist, was tested in experiment 2 (Chapter IV); and a non-selective DA antagonist, *cis*-flupentixol (*cis*-FLU) was tested in experiment 3 (Chapter V).

D_1 or D_2 Receptor Selectivity of Dopaminergic Agents. The selectivity of SKF for the D_1 receptor is defined by its ability to stimulate ACy in homogenates of rat caudate, and by its inability to cause emesis, stereotypic behavior or to inhibit prolactin release, all of which are associated with activation of the D_2 receptor (Setler *et al.*, 1978). SKF is considered a partial agonist for the D_1 receptor since its efficacy for stimulating DA-sensitive ACy from rat striatum is about 45% of that observed for DA (Andersen and Jansen, 1990; Battaglia *et al.*, 1986). Similarly, SCH is considered selective for the D_1 receptor based on its failure to induce prolactinemia, its potent blockade of DA-stimulated ACy, and its weak displacement of [^{3}H]spiperone bound to D_2 receptor sites (Hyttel, 1983; Hyttel, 1984; Iorio *et al.*, 1983; and see below).

QUIN (Bach *et al.*, 1980) is a relatively selective and potent D_2 receptor agonist since it stimulates the D_2 receptor in the intermediate lobe of the rat pituitary gland (the pituitary gland serves as a model for D_2 receptors), inhibits ACy activity in homogenates (Tsuruta *et al.*, 1981); the latter effect is attenuated by SUL and other DA antagonists which compete for the D_2 receptor (Tsuruta *et al.*, 1981). Likewise, SUL (Spano *et al.*, 1979) is considered a selective D_2 antagonist since it stimulates prolactin

release, attenuates the effects of DA agonists on locomotor activity, which mimics the actions of classical neuroleptic agents, and potently inhibits the binding of non-selective and selective D_2 DA antagonists (O'Connor and Brown, 1982). In contrast to these selective compounds, the classical DA agonist, APO, is considered to be non-selective at these two DA receptors since its effects can be mediated through either receptor subtype, and *cis*-FLU is a non-selective DA antagonist which binds to both subtypes with similar affinity (Creese *et al.*, 1983; and see below).

Binding Characteristics of Selective D_1 and D_2 Agonists and Antagonists

Indirect Binding Assays for Dopaminergic Agents. The interaction of a dopaminergic agent with a receptor can be characterized by assessing its ability to inhibit the binding of a radioligand. The pharmacological specificity of the D_1 or the D_2 receptor site is described based on the dissociation constants for a variety of dopaminergic agents determined from inhibition of the binding of a radioligand. The equilibrium inhibition constant or K_i of the unlabeled competing dopaminergic agent is related to the concentration required to inhibit 50% of the binding of a radioligand to the same site (McGonigle and Molinoff, 1989) . In rat striatum (STR), the ratio of K_i values for the D_1 versus the D_2 binding sites (and the relative potency of the dopaminergic agents for the D_1 to the D_2 site) for SKF is 18 nM : 9300 nM (517 times greater for D_1); for SCH, it is 0.14 nM : 895 nM (6400 times greater for D_1); for QUIN, it is > 5000 nM : 720 nM (> 7 times greater for D_2); for SUL, it is > 10,000 nM : 70 nM (> 143 times greater for D_2); for APO, it is 87 nM : 98nM (1.1 times greater for D_1; Andersen and Jansen, 1990); and for *cis*-FLU, it is 0.32 nM : 0.34 nM (equipotent for D_1 and D_2) against the *in vitro* binding of the selective D_1 and D_2 radioligands, [^{3}H]SCH and [^{3}H]spiroperidol, respectively (Andersen, 1988). The results of these

binding studies demonstrate that SKF is selective for the D_1 receptor since it is a more potent inhibitor of the D_1 than the D_2 radioligand; QUIN and SUL are selective for the D_2 receptor since they more readily inhibit binding of the D_2 radioligand; and, APO and *cis*-FLU are nonselective since they display about equal inhibitory potency against both the D_1 and D_2 radioligands.

Although SCH may interact with the serotonin$_2$ binding site, the use of [^{3}H] SCH as an appropriate marker of D_1 binding sites has previously been established since this compound selectively binds the D_1 receptor both *in vitro* (Andersen *et al.*, 1985; Billard *et al.*, 1984) and *in vivo* (Andersen and Gronvald, 1986; Andersen and Nielsen, 1986) with a low equilibrium dissociation constant, or K_d value of about 0.5 nM in the rat STR (Billard *et al.*, 1984; Hess *et al.*, 1986; Schulz *et al.*, 1985). Although the affinity of SCH for serotonin$_2$ sites is in the nanomolar range (Bishchoff *et al.*, 1986), it is 20 times more potent in displacing a nonselective DA radioligand from striatal D_1 receptor sites (Hyttel, 1983). Similarly, when administered *in vivo*, doses of 1.5 mg/kg of SCH are required to inhibit 50% of [^{3}H]spiperone binding to serotonin ($5HT_2$) receptors in frontal cortex (Bishchoff *et al.*, 1986); this dose exceeds those required to inhibit DA-dependent behaviors ($\leq$ 0.1 mg/kg; Molloy and Waddington, 1984).

Comparison of pharmacological characteristics of *in vitro* [^{3}H]SCH and [^{3}H]spiroperidol binding in the rat STR with *in vivo* [^{3}H]SCH and [^{3}H]raclopride binding in the mouse brain indicates that compounds with selectivity *in vitro* retained this selectivity *in vivo* (Andersen, 1988). Similarly, in the monkey, [^{3}H]SCH labels a homogeneous and saturable high-affinity D_1 site (K_d = 0.35 nM; Madras *et al.*, 1988). Binding potencies for the D_1 or D_2 sites for select dopaminergic drugs are: SKF (125 times greater for D_1; SCH (5000 times greater for D_1); QUIN (> 3000 times greater for D_2); SUL (2500 times greater for D_2); APO (3 times greater for D_2); *cis*-FLU (2 times greater for D_2); DA (1.4 times greater for D_1; Madras *et al.*, 1988).

High and Low Affinity States of D_1 and D_2 Receptors. The D_1 and D_2 receptor sites are thought to exist in two interconvertible states exhibiting either high or low affinity for agonists, and are modulated by a guanine nucleotide-selective regulatory protein (Urwyler and Markstein, 1986). The high-affinity state can be converted into the low-affinity state by the addition of a guanine nucleotide. In the absence of exogenous guanine nucleotide, both receptors have similar high affinities for DA of about 40 nM (range 9-74 nM), and low affinities for DA of between 2 and 4μM (Richfield *et al.*, 1989). With the similarities in dissociation rate constants for DA at the two subtypes, neither receptor will predominate in its binding of endogenous DA, and if both receptors are found in the same dendritic area of a neuron, both receptors are likely to bind endogenously released DA (Richfield *et al.*, 1989). However, the D_1 receptor accounts for an average of 78% of the total number of DA receptors in most regions, but studies indicate that only 20% may be in the high affinity state (Richfield *et al.*, 1989). The D_2 receptor makes up the remaining 22% of the total number of DA receptors; but, in contrast to the D_1 receptor, 80-90% of the D_2 receptors may be in high affinity state *in vitro* (Richfield *et al.*, 1989). If these differences in affinity states for the D_1 and D_2 receptors exist *in vivo*, the proportions may influence the effects of DA and dopaminergic agents on individual brain regions.

Distribution of D_1 and D_2 DA Receptors

Location of D_1 and D_2 Radioligand Binding Sites. Autoradiographic studies have aided the localization of D_1 and D_2 DA binding sites in VP/SI (see Fig. 1). Binding of [^{3}H]SCH and [^{3}H]spiperone in the rat indicates that the D_1 and D_2 receptor densities in the VP/SI are 689 ± 26 fmol/mg protein and 70 ± 14 fmol/mg protein, respectively (Richfield *et al.*, 1989). Similarly, autoradiographic studies of cholinergic

basal forebrain regions in the rat indicate that absolute density of D_1 receptors is 440 ± 53 fmol/mg protein in the VP, and 292 ± 51 fmol/mg protein in the SI; whereas D_2 receptor densities are 30 ± 5 fmol/mg protein in the VP and 11 ± 3 fmol/mg protein in the SI (Zilles *et al.*, 1991). Compared with other cholinergic basal forebrain regions, D_1 receptors exhibit the highest density in the VP (Zilles *et al.*, 1991). In contrast to the density of D_1 receptors, [^{3}H]SUL binding to the D_2 receptor indicates that the density of these receptors in the VP/SI is one-tenth that found in the NA; this is about equal to the amount for the substantia nigra pars reticulata (SNr), and 1/3 of the amount found in the SNc (Gehlert and Wamsley, 1985). Thus, the autoradiographic and binding studies concur that the density of the D_1 subtype is high in the VP (Contreras *et al.*, 1987; Dawson *et al.*, 1988; Napier *et al.*, 1991a) and seems to prevail by as much as 10 times over the D_2 subtype (Boyson *et al.*, 1986; Geula and Slevin, 1989; Napier *et al.*, 1991a; Richfield *et al.*, 1989; Richfield *et al.*, 1987; Zilles *et al.*, 1991), and with an even greater difference in the SI (Zilles *et al.*, 1991).

Location of DARPP-32 Antibody-labeled Sites. Another applicable technique for visualizing D_1 receptors in the brain is through co-localization of [^{3}H]SCH binding with DARPP-32, which is a DA- and cyclic AMP-regulated phosphoprotein (Ouimet *et al.*, 1984). This co-localization does not appear to be coincidental, since stimulation of D_1 receptors *in vivo* in the rat STR by SKF increases phosphorylation of DARPP-32 (Lewis *et al.*, 1990). DARPP-32 appears to be concentrated in a subpopulation of dopaminoceptive neurons, namely those containing D_1 receptors, where it is localized in cell bodies, dendrites, axons, and nerve terminals (Hemmings Jr and Greengard, 1986; Ouimet *et al.*, 1984; Walaas and Greengard, 1984). Staining for DARPP-32 in the basal forebrain demonstrates that the VP and the GP display brightly fluorescent fibers and puncta (presumed nerve terminals), but that

neuronal cell bodies and dendrites are unstained (Ouimet *et al.*, 1984). The distant groups of DARPP-32-containing cell bodies (*e.g.*, from the NA and STR) are considered the source of these DARPP-32-labeled fibers and nerve terminals in the VP and GP, respectively (Ouimet *et al.*, 1984). Thus, the presence both of this receptor and of DARPP-32 within the VP (see Fig. 1) and the GP suggests that D_1 receptors may be located presynaptically on afferents to these regions.

Location of D_1 and D_2 mRNA-labeled Sites. Studies concur that the VP contains a higher concentration of D_1 receptors than the GP (Bardo and Hammer, 1991; Beckstead *et al.*, 1988; Boyson *et al.*, 1986; Dawson *et al.*, 1986a, 1986b; Mansour *et al.*, 1990; Napier *et al.*, 1991a; Richfield *et al.*, 1987; Savasta *et al.*, 1986). Additional evidence for this DA receptor diversity in the VP versus the GP is provided by *in situ* hybridization techniques demonstrating that the apparent density of D_2 receptor mRNA within VP is not as great as that observed in the GP (Mansour *et al.*, 1990). In contrast, D_1 DA receptor mRNA labels cells in the VP/SI (Fremeau *et al.*, 1991), whereas no specific hybridization signals were observed in the GP, suggesting that the D_1 receptor in the GP may be present on afferent nerve terminals originating in other brain regions (Fremeau *et al.*, 1991; Mengod *et al.*, 1991; Weiner *et al.*, 1991). Considering that DARPP-32 labeling of the VP and the GP (see above) indicates that D_1 receptors are located on afferents to these regions, and that D_1 mRNA labels VP neuronal cell bodies, the VP may have both pre- and post-synaptic D_1 receptors (Fig. 1). Moreover, since the VP postsynaptic receptors are not associated with DARPP-32 labeling, they may also be independent of ACy and cyclic AMP generation. Corroborating this theory are studies on the amygdala that demonstrate D1 receptor binding (Boyson *et al.*, 1986; Dawson *et al.*, 1986) without DA-stimulated ACy (Dawson *et al.*, 1986b; Kilts *et al.*, 1988; Mailman *et al.*, 1986). Thus, although the

VP/SI is morphologically similar to the GP (see anatomical description of VP/SI), the above evidence suggests that these two regions may have distinct responses to DA receptor activation.

Neurochemical, Behavioral and Electrophysiological Studies Involving Dopamine

Introduction

The studies of this dissertation examined whether separate D_1 or D_2 receptor activation within the VP/SI region is sufficient to alter VP/SI neuronal activity. The effects of D_1/D_2 nonselective agonists on the activity of VP/SI neurons were then compared to the results of selective DA agonists. In an attempt to formulate a model incorporating all the conceivable effects of selective D_1 and D_2 receptor stimulation, the functional interactions between D_1 and D_2 receptors on the neuronal activity of the VP/SI are considered below. The potential interaction between these receptors suggest three possibilities: independent actions of each receptor, oppositional interactions, and non-oppositional interactions.

The non-oppositional interactions of D_1 and D_2 receptor activation may involve enabling or synergistic actions. Enabling actions suggest that the activation of one subtype is necessary for drug actions on the other subtype. It is often suggested that stimulation of the D_1 receptor subtype by a selective D_1 agonist "enables" or permits the activation of the D_2 receptor subtype by a D_2 selective agonist to produce the same magnitude of effect as DA or as nonselective dopaminergic agents. The synergistic actions of D_1 and D_2 receptors imply that the response magnitude is less when either receptor subtype is activated alone versus the magnitude of concurrent activation of these receptors.

Potential Effects of D_1 and D_2 Receptor Activation

Independent Actions. In the STR, SKF has a dose-dependent effect that is similar to DA or APO on the induction of inositol phosphates (Undie and Friedman,

1990). SCH, but not SUL, blocks this agonist-induced response, whereas QUIN lacks effect on inositol phosphate accumulation (Undie and Friedman, 1990). DA-induced stimulation of the depolarization-induced release of [^{3}H]GABA from rat slices isolated from SNr, entopeduncular nucleus, GP, and caudate-putamen is blocked by SCH, suggesting that DA modulates the release of GABA via the D_1 receptor (Floran *et al.*, 1990). In contrast, D_2, but not D_1, receptor activation decreases DA release in the NA and caudate measured by microdialysis in freely-moving rats (Imperato *et al.*, 1988). Chronic treatment with SCH increases the density of striatal D_1 receptors, which are located postsynaptically on intrinsic neurons (Cross and Waddington, 1981; Filloux *et al.*, 1987; Leff *et al.*, 1981), without altering the D_2 receptor population (Creese and Chen, 1985).

The independent effects of D_1 or D_2 receptor activation likewise are expressed through specific behavioral responses. D_1 receptor activation in rats mediates non-stereotyped sniffing (Molloy and Waddington, 1985), and induces episodes of a specific grooming behavior that involves the snout being directed vigorously into the body (Dall'Olio *et al.*, 1988; Molloy and Waddington, 1984, 1985; Starr and Starr, 1986). These SKF-induced behaviors are blocked by SCH but not by a D_2 antagonist, suggesting selective activation of D_1 receptors (Molloy and Waddington, 1984, 1985). SKF also induces grooming in rats chronically pretreated with SCH (Dall'Olio *et al.*, 1988). Unilateral injection of SKF into the SNr results in contralateral rotation (Asin and Montana, 1988; Jackson and Kelly, 1983). D_1 receptors have been implicated in the modulation of: 1) rapid eye movement sleep (Trampus *et al.*, 1991), 2) bar pressing to receive rewarding VTA stimulation (Kurumiya and Nakajima, 1988; Nakajima and McKenzie, 1986), and 3) the duration of free-running rhythms of locomotor activity during constant dark conditions (Yamada and Martin-Iverson, 1991) in the rat. Mouthing movements that mimic oro-facial dyskinesia are produced

following intra-VP/SI injection of SKF, and are attenuated by local injection of SCH (Spooren *et al.*, 1991a). Similarly, injection of SKF82958 (a full D_1 agonist) within the VP induces prominent mouthing movements in addition to enhancing locomotion and rearing/wall climbing behaviors (Napier and Rehman, 1992).

In contrast to D_1-mediated effects, D_2 receptor stimulation has been implicated in the antipsychotic and anti-dopaminergic activity of classical neuroleptic agents (Creese *et al.*, 1976; Ellenbroek *et al.*, 1991). Activation of D_2 receptors in rats mediates locomotion, rotational behavior and some stereotypic sniffing and rearing behavior (for review see Clark and White, 1987). D_2 receptor agonists increase the amplitude, but not duration, of free-running rhythms of locomotor activity in rats maintained in a constant dark environment (Yamada and Martin-Iverson, 1991). Intra-NA injections of QUIN, but not SKF, reduces exploratory locomotion in a dose-dependent manner (Mogenson and Wu, 1991). QUIN injected within the GP increases locomotion and rearing/wall climbing behaviors (Napier and Rehman, 1992).

Electrophysiological studies concur that separate D_1 or D_2 receptor activation is sufficient to alter neuronal activity. White and Wang (1986) observed a heterogeneous population of NA neurons that respond with rate suppression to both D_1 and D_2 agonists, or to either agonist independently. Intrastriatal infusion of SKF, which mimics the effects of nonselective DA agonists (Groves *et al.*, 1981; Tepper *et al.*, 1984), decreases the ability of antidromic stimulation to initiate action potentials (*i.e.*, application of a D_1 agonist within the terminal region decreases the excitability of the terminals to antidromic stimulation). Thus, the terminal excitability of antidromically-identified nigrostriatal dopaminergic neurons is attenuated by D_1 receptor activation (Diana *et al.*, 1989). However, D_2 receptors are involved with enhancement of terminal excitability of hippocampal-NA neurons since microiontophoretic application of DA or QUIN within the NA mimics the effects of VTA stimulation on the excitability of

these hippocampal terminals (Yang and Mogenson, 1986). In contrast, the excitatory response of striatal neurons to cortical stimulation is attenuated by electrical stimulation of the nigrostriatal dopaminergic system via D_2 receptors (Vives and Mogenson, 1986b). Signal transmission from the hippocampus to the VP neurons that innervate the pedunculopontine nucleus is modulated by intra-NA application of QUIN (Yang and Mogenson, 1987). Thus, the possibility of independent actions of D_1 and D_2 receptor agonists is supported by neurochemical, behavioral and electrophysiological studies.

Opposing Actions of D_1 and D_2 Receptor Activation. Neurochemical studies have indicated that D_1 receptor agonists stimulate, and D_2 receptor agonists inhibit, ACy and cyclic AMP efflux and accumulation within the STR (Kelley and Nahorski, 1986; Pifl *et al.*, 1991; Setler *et al.*, 1978; Stoof and Verheijden, 1986). Recent studies reveal that D_1 receptors can be positively coupled to phospholipase C, leading to the production of phosphatidyl inositols and diacyl-glycerol; conversely, D_2 receptors are negatively linked to this enzyme (Enjalbert *et al.*, 1986; Pizzi *et al.*, 1988). SKF also antagonizes D_2 receptor-mediated inhibition of DA metabolism *in vivo* in rats (Saller and Salama, 1985). Furthermore, activation of the D_1 increases, whereas activation of the D_2 receptor decreases striatal acetylcholine release (Ajima *et al.*, 1990; Damsma *et al.*, 1990; Gorell and Czarnecki, 1986; Gorell *et al.*, 1986).

Behavioral and electrophysiological studies substantiate the opposing interaction of D_1 and D_2 receptors. The two receptors mediate opposite effects on: 1) oral movements (Johansson *et al.*, 1987; Koshikawa *et al.*, 1990b; Rosengarten *et al.*, 1986); 2) the convulsant effects of pilocarpine (Al-Tajir *et al.*, 1990); 3) body temperature (Costentin *et al.*, 1990); 4) the amount of area traversed during exploratory behavior (Eilam *et al.*, 1991); and, 5) the direction of rotation following injection of D_1 or D_2 selective agonists into the SNr (Asin and Montana, 1988; Jackson and Kelly,

1983) in rats. In addition, atypical jerking response is not induced by the D_2 agonist alone, but is dependent upon the removal of tonic D_1-mediated dopaminergic activity that would otherwise oppose its manifestation (Murray and Waddington, 1989). Similarly, D_1 receptor agonists enhance, whereas D_2 receptor agonists suppress event-related slow potentials recorded from the rat cortex (Pirch *et al.*, 1988), which are generated by VP/SI cholinergic neurons (Pirch *et al.*, 1986). Several neurochemical, behavioral and electrophysiological findings suggest that D_1 and D_2 receptor stimulation can express opposing influences on certain functions within the brain.

Non-oppositional Interactions between D_1 and D_2 Receptor Activation. Neurochemical studies reveal that D_1/D_2 receptor synergism is involved in: 1) the dopaminergic control of the electrically-evoked release of [^{3}H]GABA in rat prefrontal cortex (Retaux *et al.*, 1991) and, 2) DA-mediated inhibition of NA^+/K^+-dependent ATPase (Bertorello *et al.*, 1990).

A variety of behaviors also involve the cooperative effects of stimulating D_1 and D_2 receptors, and the expression of some behaviors require the activation of both receptor subtypes (for review see, Clark and White, 1987; Murray and Waddington, 1989; Waddington and O'Boyle, 1989; White *et al.*, 1988). Stimulation of both D_1 and D_2 postsynaptic receptors is necessary for the expression of stereotyped (Arnt *et al.*, 1987; Braun and Chase, 1986; Vasse *et al.*, 1988), and climbing (Moore and Axton, 1988; Vasse *et al.*, 1988) behaviors. Priming with QUIN is essential for SKF-mediated effects on contralateral turning in rats with unilateral 6-OH-DA lesions (Morelli *et al.*, 1990). In monkeys, the D_1 receptor has a permissive role in yawning induced by D_2 receptor activation (Code and Tang, 1991). D_1 receptor activation can also potentiate D_2 receptor-mediated motor responses (Arnt *et al.*, 1988; Barone *et al.*, 1986; Molloy *et al.*, 1988; Molloy and Waddington, 1985; Morelli *et al.*, 1987; Plaznik *et al.*, 1989;

Robertson and Robertson, 1986, 1987), stereotypies (Bordi and Meller, 1989; Mashurano and Waddington, 1986; Meller *et al.*, 1988), catalepsy (Dall'Olio *et al.*, 1988; Wanibuchi and Usuda, 1990), jaw movements (Koshikawa *et al.*, 1989, 1990a), and yawning (Longoni *et al.*, 1987; Spina *et al.*, 1989) in normal and DA-depleted rats. Furthermore, the synergistic effects of D_1 and D_2 agonists on rotational behavior may also be mediated through D_1 and D_2 receptor activation in separate brain regions, such as the effect of D_1 receptor agonists in the SNr, and D_2 receptor agonists in the STR (Robertson and Robertson, 1987).

In electrophysiological studies, synergistic interactions between D_1 and D_2 receptors: 1) increases the activity of GP neurons (Carlson *et al.*, 1987a; Walters *et al.*, 1987), 2) potentiates both excitatory and inhibitory effects of SNr neurons as compared to activation of individual receptor (Weick and Walters, 1987), and, 3) decreases the activity of NA neurons (White, 1987). In addition, VP neuronal activity is excited similarly by intra-NA application of DA, or SKF followed by QUIN, but not by either agonist alone (Yang and Mogenson, 1989). Much of the evidence supporting the synergistic effects of D_1 and D_2 receptor stimulation originates from studies of the systemic effects of DA agonists and antagonists, which may simultaneously activate D_1 and D_2 receptors in many brain regions.

Effects of Endogenously-released and Exogenously-applied DA

The sensitivity of evoked neuronal activity of several brain regions to DA and dopaminergic agonists and antagonists has been studied using similar methods to those proposed in this dissertation. The categorization of DA as either an excitatory or inhibitory neurotransmitter is controversial since these studies indicate that actions of DA depend on the brain region examined. Electrophysiologic studies of the well-defined SNc projection to the STR indicate that stimulation of the SNc has an excitatory

effect on these STR neurons (Frigyesi and Purpura, 1967; Fujimoto *et al.*, 1981; Hull *et al.*, 1970; Kitai *et al.*, 1975, 1976; Ohno *et al.*, 1985, 1986; York, 1967). Microiontophoretic application of DA (Ohno *et al.*, 1985, 1986; York, 1967) and DA D_2, but not D_1, receptor antagonists within the STR inhibits the excitatory SNc-evoked response, suggesting that the effects of SNc stimulation on STR neuronal activity is mediated by DA acting at D_2 receptors (Ohno *et al.*, 1985, 1986).

However, DA can have an inhibitory role since other studies reveal that VTA/SNc (Connor, 1970; Le Douarin *et al.*, 1986; Zarzecki *et al.*, 1976) or medial forebrain bundle (Akaoka *et al.*, 1987) stimulation usually evokes inhibitory STR responses (in 73% and 47% of the neurons tested in Akaoka *et al.*, 1987; Connor, 1970, respectively). The STR neurons with inhibitory responses to VTA/SNc (Connor, 1970; Le Douarin *et al.*, 1986; Zarzecki *et al.*, 1976) or medial forebrain bundle (Akaoka *et al.*, 1987) stimulation also are suppressed by microiontophoretic application of DA. The complexity of STR responses to SNc stimulation may result from the extensive innervation of the STR region by dopaminoceptive brain regions that are concurrently affected by SNc stimulation. Intracellular recording studies supporting this conclusion reveal that only long latency excitatory postsynaptic potentials (the long latency time frame is analogous to our short latency response described in Chapter V) remain after removal of all of the non-nigral response components by denervation of the cortical, and transection of the thalamic inputs (Wilson et al., 1982).

Stimulation of the sensory motor cortex also produces excitatory responses in STR neurons that are attenuated by preceding cortical stimulation with train stimulation of the SNc (Hirata *et al.*, 1984), or intra-STR application of DA (Hirata *et al.*, 1984; Johnson *et al.*, 1983). Intra-STR application of SUL, but not SCH, reversed the attenuating effect of SNc stimulation on the excitatory response of STR neurons to cortical stimulation (Vives and Mogenson, 1986a), suggesting the involvement of D_2

DA receptors in SNc-evoked STR responses to cortical stimulation. Stimulation of the SNc produced predominantly inhibitory, yet some excitatory affects on the activity of STR neurons; and microiontophoretic application of DA also inhibits glutamate-induced excitatory effects of STR neurons (Zarzecki *et al.*, 1977).

Microiontophoretic studies indicate that DA is generally a inhibitory neurotransmitter when applied locally within the brain. DA inhibits the glutamate-induced firing of the majority of NA neurons tested (Akaike *et al.*, 1983). Excitatory NA responses to hippocampal or AMN stimulation are attenuated: 1) by prior stimulation of the VTA with a train of pulses, or 2) by intra-NA application of DA, indicating that DA mediates the VTA-evoked attenuation of the excitatory NA responses (Yang and Mogenson, 1984; Yim and Mogenson, 1982). Likewise, excitatory NA responses to stimulation of the parafascicular nucleus of the thalamus are inhibited by: 1) VTA conditioning stimulation and iontophoretically applied DA (Akaike *et al.*, 1984), and 2) iontophoretically-applied selective D_1 or D_2 agonists (Hara *et al.*, 1989) within the NA. In addition, VTA stimulation alters the responses of NA neurons to sensory input (West and Michael, 1990).

The activity of GP neurons is inhibited by stimulating the sensory motor cortex, and these inhibitory responses are reduced when the SNc is activated with a train of pulses prior to cortical stimulation (Hirata and Mogenson, 1984). Stimulation of the hippocampus (Tsai *et al.*, 1989) or the AMN (Tsai *et al.*, 1989; Yim and Mogenson, 1983) evokes inhibitory and excitatory VP responses. The inhibitory VP responses to AMN stimulation are attenuated: 1) by DA released within the NA, or 2) by prior stimulation of the VTA with a train of pulses (Yim and Mogenson, 1983). Thus, orthodromic stimulation studies provide strong evidence that DA released from the nerve terminals of the VTA and the SNc produces receptor specific effects on neuronal activity of several brain regions. In addition, inhibitory actions of VTA/SNc stimulation on the

neuronal activity of target regions that appear to be modulate the effects of other afferents, are mimicked by microiontophoretically-applied DA.

Functional Relevance for DA within the VP/SI

Studies indicate that the VP/SI receives dopaminergic innervation from the midbrain, and that D_1 and D_2 receptors are located within this region (see anatomical review and distribution of D_1 and D_2 receptors above). Biochemical studies corroborate this anatomical evidence since DA and its major metabolites have been isolated from VP/SI tissue, and are significantly reduced following 6-OH-DA lesions of midbrain dopaminergic regions (Geula and Slevin, 1989; Napier and Potter, 1989). Recent behavioral studies demonstrate that intracerebral microinjections of DA directly into the VP/SI dose-dependently increase locomotion in an open field (Napier and Chrobak, 1992; Napier and Rehman, 1992), but do not alter working memory in rats previously trained on a 12 arm radial maze (Napier and Chrobak, 1992). Pretreatment by systemic administration of the D_1/D_2 antagonist, *cis*-FLU, attenuates the increase in locomotor activity (Napier and Chrobak, 1992). Intra-VP/SI injection of DA or a D_1 agonist, results in dose-related increases rearing and wall climbing, and the D_1 agonist also produced robust mouthing movements (Napier and Rehman, 1992). Similarly, intra-VP/SI injection of SKF in cats elicits oro-facial dyskinesia that are attenuated by local injection of SCH (Spooren et al., 1991a). Thus, intra-VP/SI DA application elicits readily discernible motoric and grooming behaviors, whereas assessment of cognitive behaviors await more discrete testing paradigms.

Likewise, electrophysiological studies reveal that VP/SI neurons are affected by: 1) systemic administration of APO, 2) microiontophoretic application of DA (Napier *et al.*, 1991b) and 3) electrical stimulation of the SNc (Napier *et al.*, 1991a). Systemic administration of APO often induces dose-dependent rate increases (fewer neurons demonstrate dose-dependent rate suppressions), that are attenuated by haloperidol, verifying that the actions of the agonist are mediated through DA receptors (Napier *et al.*, 1991a). Although intra-NA DA application also increases the firing rate of VP/SI

neurons (Yang and Mogenson, 1989), the excitatory effects of APO remains following pharmacological inactivation of the NA, indicating that APO-mediated effects on VP/SI neuronal activity may be independent of the NA (Napier, 1992a). In addition, intra-VP/SI application of DA mediates VP/SI rate excitations and inhibitions, with the latter observed more frequently (Napier *et al.*, 1991b). Systemic administration of haloperidol or SCH attenuate these effects, indicating that alterations in activity of VP/SI neurons involve both D_1 and D_2 receptors subtypes (Napier *et al.*, 1991b). Furthermore, preliminary data corroborates the inhibitory effects of DA within the VP/SI since electrical stimulation of the SNc evokes inhibitory VP/SI responses (Napier *et al.*, 1991a). Thus, the combined results from neurochemical, behavioral and electrophysiological studies indicate that DA is not only located within the VP/SI, but is also functionally relevant in this brain region.

Summary

This literature review has outlined 1) the afferent and efferent anatomy of the VP/SI; 2) the pharmacology of D_1 and D_2 dopaminergic agents, as well as evidence of the localization of D_1 and D_2 receptor subtypes within the VP/SI; 3) the possible interactions between D_1 and D_2 receptor activation; and, 4) the functional relevance of DA within the VP/SI. The hypotheses of this dissertation are: 1) that the D_1 and the D_2 DA receptor subtypes mediate DA-induced effects on VP/SI neuronal activity, and 2) that DA is a neuromodulatory transmitter within the VP/SI altering neuronal activity evoked in this brain region by electrical stimulation of afferents from the amygdaloid nuclei (AMN). To investigate these hypotheses, the following specific aims were proposed:

Specific Aim 1: **To characterize the DA receptor subtypes that mediate the responses of single VP/SI neurons to systemic administration of DA agonists.**

In vivo electrophysiological experiments were performed on anesthetized rats to investigate the responsiveness of single VP/SI neurons to systemic administration of the selective D_1 agonist, SKF, and the selective D_2 agonist, QUIN. Spontaneously active VP/SI neurons were characterized by action potential properties (configuration, amplitude and duration) and activity (firing rate and pattern). SKF or QUIN were injected intravenously in increasing doses. VP/SI neuronal activity was assessed as alterations in firing rate in response to increasing drug concentrations. If any significant rate changes occurred, the antagonist specific for the receptor subtype activated (i.e., SCH for the D_1, and SUL for the D_2 receptor) were injected to determine if the rate alterations were mediated by specific receptor subtypes. To determine whether activation of one receptor subtype was sufficient to mediate the actions of a nonselective DA agonist (*i.e.*, one that mimics the actions of endogenous DA within the brain), the

D_1 and D_2 DA agonist apomorphine was administered, and any effects induced were tested for receptor subtype specificity by administration of selective D_1 or D_2 antagonists.

Specific Aim 2: **To determine the DA receptor subtypes involved in the VP/SI responses evoked by endogenously-released DA during stimulation of midbrain dopaminergic regions. To characterize the VP/SI responses evoked by electrical activation of the AMN. To determine if endogenously-released, and exogenously-applied DA within the VP/SI modulate AMN-evoked responses of VP/SI neurons.**

In vivo electrophysiological experiments were used to describe the effects of orthodromic (*i.e.*, trans-synaptic) stimulation of the AMN and two midbrain dopaminergic regions, the ventral tegmental area and the substantia nigra pars compacta (VTA/SNc), on the activity of VP/SI neurons. To verify whether VTA/SNc stimulation results in the release of DA in the VP/SI the following criteria were used: 1) exogenously-applied DA via microiontophoresis should mimic the effects of electrical stimulation and 2) exogenously-applied SCH and/or SUL within the VP/SI should attenuate the effects of electrical stimulation on VP/SI neuronal firing rate. The effects of microiontophoretic application of SKF and QUIN were also assessed to determine the contribution of these subtypes to alterations of spontaneous activity of VP/SI neurons. In addition, possible modulatory effects of DA within the VP/SI on VP/SI responses evoked by AMN stimulation were examined to determine: 1) if electrical stimulation of the VTA/SNc (which presumably releases endogenous DA), prior to AMN stimulation alters the effects of AMN stimulation alone, and 2) exogenous application of DA mimics the modulatory effects of endogenously-released DA on VP/SI neuronal activity that resulted from AMN stimulation.

CHAPTER III

D_1 AND D_2 DOPAMINE AGONISTS INDUCE OPPOSITE CHANGES IN THE FIRING RATE OF VENTRAL PALLIDAL NEURONS

Abstract

Selective D_1 and D_2 dopamine (DA) agonists were used to determine the contributions of each receptor subtype in the modulation of firing rate of ventral pallidum/substantia innominata (VP/SI) neurons. Administration of cumulative doses of the D_2 agonist, quinpirole (QUIN), decreased activity in 59% of the VP/SI cells tested. The decrease in firing rate was dose-dependent between 0.002-0.2 mg/kg, i.v. and was blocked by the D_2 antagonist, sulpiride (SUL; 12.5 mg/kg, i.v.). In addition, the magnitude and the distribution of responses of VP/SI neurons was not changed following administration of QUIN as a single versus a divided cumulative dose of 0.1 mg/kg.

In contrast, administration of the D_1 agonist, SKF38393 (SKF), excited 69% of the neurons sampled. Similar maximal responses were observed following administration of either a single or a divided cumulative dose of 3.2 mg/kg of SKF. The D_1 receptor antagonist, SCH23390 (SCH; 0.1-0.4 mg/kg, i.v.) often attenuated the SKF-induced increases.

The results illustrate that, 1) VP/SI neurons are sensitive to systemically administered DA agonists, 2) D_1 or D_2 receptor activation is sufficient to change the

activity of these neurons, and 3) these selective agonists mediate opposite effects on VP/SI neuronal activity. These differential responses contrast with effects observed for other dopaminoceptive brain regions, and distinguish VP/SI neurons from morphologically related neurons of the dorsal globus pallidus.

Introduction

D_1 or D_2 DA receptors are classified based upon differential regulation of the enzyme, adenylate cyclase. D_1 receptor is coupled positively to adenylate cyclase (Kebabian and Calne, 1979), and D_2 receptor is negatively coupled to the enzyme (Stoof and Kebabian, 1981). The availability of agonists and antagonists selective for D_1 or D_2 receptors has provided the means to investigate the roles of DA receptor subtypes in the modulation of neuronal systems. Electrophysiologic studies of dopaminoceptive brain regions, including the striatum (STR), nucleus accumbens (NA), globus pallidus (GP), and amygdala (AMN), as well as the substantia nigra (SNc) and ventral tegmental area (VTA), have contributed significantly toward understanding the role of DA receptor subtypes on neuronal transmission.

Systemically administered selective D_2 agonists increase the firing rate of STR neurons (Hu and Wang, 1988), induce biphasic changes in NA activity (White and Wang, 1986), suppress SNc neurons innervating the STR (Kelland *et al.*, 1988), and increase GP neuronal activity (Carlson *et al.*, 1988). The D_1 selective agonist, SKF inhibits the firing rate of neurons in the STR (Hu and Wang, 1988) and NA (White and Wang, 1986). Under similar anesthetic conditions to the above studies, SNc neurons are not affected by systemic administration of this agonist alone (Carlson *et al.*, 1987b; Kelland *et al.*, 1988). High doses of SKF often increase GP neuronal activity (Carlson *et al.*, 1988), but behaviorally relevant doses (Meller *et al.*, 1988; Vasse *et al.*, 1988) are

not effective. Thus, DA receptor activation results in complex responses that may be distinctive for the brain region under investigation.

The ventral pallidum/substantia innominata (VP/SI) is a ventral extension of the GP (Heimer and Wilson, 1975), and is directly contacted by presumptive catecholaminergic neurons (for review see Zaborszky *et al.*, 1991). DA has been suggested as a possible neurotransmitter for this input (for review see Napier *et al.*, 1991a). DA and its metabolites are located within VP/SI and lesions of midbrain dopaminergic neurons yield significant depletion of DA within VP/SI tissue (Napier and Potter, 1989). In addition, VP/SI neurons are altered by microiontophoretic applications of DA, often exhibiting an inhibition of firing rate (Napier and Potter, 1989), but the receptor subtypes involved have not been determined. Autoradiographic studies demonstrate that the VP/SI contains D_1 and D_2 binding sites (Contreras *et al.*, 1987).

To evaluate the functional consequence of D_1 and of D_2 DA receptor stimulation on extracellularly recorded single unit activity of VP/SI neurons, the present *in vivo* electrophysiologic study characterized effects following systemic administration of the D_1 agonist, SKF, and the D_2 agonist, QUIN. Because responses to DA agonists differ among brain regions, initial experiments defined the dose-response relationships of the effects induced by these agonists in the VP/SI in order to compare among dopaminoceptive brain regions. Agonists were administered as multiple dose treatments emulating the protocol and dose range used in previous electrophysiologic studies on other brain regions (*e.g.*, Carlson *et al.*, 1987b). In a subsequent experiment, agonist doses that induced near maximal responses were injected as a single dose treatment to verify that the onset, magnitude, and duration of the effects of VP/SI neurons were similar to those observed after administration of the agonist in divided doses.

Surgical Preparation of Animals

Male Sprague-Dawley rats (270-350 g, Harlan Inc., Indianapolis, IN) were anesthetized with chloral hydrate (400 mg/kg, i.p.), and a lateral tail vein was cannulated for intravenous administration of treatment drugs and supplements of chloral hydrate. The rats were mounted in a stereotaxic apparatus (David Kopf, Tujunga, CA) with the nose piece set at 3.3 mm below the horizontal, and the skull was exposed. A burr hole was drilled in the skull 0.5 mm posterior to Bregma, and ± 2.5 mm lateral to the midline for recording in the VP/SI (Paxinos and Watson, 1986), and the dura was removed. During all experiments, body temperature was maintained at 37 ^{o}C with a thermostatically controlled heating pad (Fintronics, Inc., Orange, CT).

Extracellular Recording

Neuronal activity was recorded extracellularly through a single barrel micropipette pulled from 2.0 mm O.D. glass tubing (A-M Systems, Inc., Everett, WA), with a vertical electrode puller (Narishige PE-2, Tokyo, Japan). The tip was broken back to a diameter of approximately 2 μm, and the electrode was filled with a 2M sodium chloride solution saturated with fast green dye (Fisher Scientific Co., St. Louis, MO). The impedance of these electrodes was 4-8 megohms, measured *in vitro* at 165 Hz with a micro-electrode tester (Winston Electronics, San Francisco, CA). The electrode was lowered through the hole with a hydraulic microdrive (Trent Wells, South Gate, CA), within a VP/SI sampling distance of 7.5 - 8.5 mm below the dura.

Electrical signals recorded by the electrode were passed through a high-impedance amplifier (Fintronics, Inc., Orange, CT), filtered, and monitored on an oscilloscope (Tektronix, Inc., Beaverton, OR) and audiomonitor (Haer Inst., Inc.,

Brunswick, ME). The signals were relayed to a window discriminator (Fintronics, Inc.) with the digital output representing action potentials from single, spontaneously active neurons. The output was recorded by a computer (IBM-AT) that, with the aid of Brainstorm Systems Spikes to Stats software (Chapel Hill, NC), displayed rate histograms, and stored all data for future statistical analysis.

Drug Administration

After a 5 min period of stable activity was recorded for each VP/SI neuron, drugs were administered through the tail vein cannula. Stable baseline activity was reestablished following any chloral hydrate supplements, and the anesthetic was not administered during DA agent administration. The specific D_2 agonist, QUIN (Tsuruta *et al.*, 1981; 0.002 - 0.2 or 0.1 - 25.6 mg/kg) or the specific D_1 agonist, SKF (Setler *et al.*, 1978; 0.002 - 1.6 or 0.1 - 25.6 mg/kg) was injected at 2 min intervals such that each dose added to the previous dose. Control studies were conducted using volumes of vehicle solutions that were similar to those used to dissolve the agonists. Only one neuron was recorded from each animal.

The selective D_2 antagonist, SUL (O'Connor and Brown, 1982; 12.5 - 25 mg/kg, one neuron was tested also with 50 mg/kg) or the selective D_1 antagonist, SCH (Hyttel, 1983; 0.1 - 0.4 mg/kg) was administered routinely 5 min after the injection of the highest dose of QUIN or SKF, respectively. Antagonist doses were selected based on their capacity to block QUIN- and SKF-induced responses observed in other studies (Hu and Wang, 1988; Molloy and Waddington, 1984).

Single injection treatments of QUIN or SKF were tested to determine if responses with this protocol were similar to those observed with cumulative dose administration. For this experiment, baseline firing rate was monitored for 5 min, after which 0.1 mg/kg of QUIN or 3.2 mg/kg of SKF was administered and neuronal activity

was monitored for 10 min. In some studies, firing rate was monitored for an additional 20 min during which two successive antagonist vehicle injections (either tartaric acid or saline solution) were administered at 10 min intervals after the agonist. These studies verified that maximal agonist responses could be obtained within 2 min of injection and were maintained for at least 20 min. For the remaining neurons, SUL, then SCH, was administered at 10 min intervals to test for antagonism of the QUIN-induced responses, and the antagonists were injected in reverse order following SKF.

Neuronal activity following drug injections was converted to a percent of pretreatment control, determined by comparison of the firing rates averaged over the last 30 s interval of pre-drug baseline (considered as 100%) with that observed during the last 30 s interval following each injection. This interval was increased for neurons that exhibited a cyclic firing pattern lasting more than 30 s to accurately assess drug effects. Treatment rates that differed from pretreatment levels by greater than 20% (and maintained for at least two consecutive doses in the multiple dose experiments), were considered significant. Antagonism was defined as an attenuation of agonist-induced responses by at least 30%. A more stringent criterion than that for agonist-induced responses was select to assess antagonism since most of the antagonist effects exceeded the usual 20% criterion. Reversal was defined as a re-establishment of pretreatment rates. The number of neurons that satisfied these criteria for a given response direction were determined.

At the end of each experiment, the rat was overdosed with chloral hydrate and the location of the recording site was marked with fast green dye by passing anionic current for 10 - 30 s through the electrode. The brain was removed, mounted and frozen, then cut with a microtome to locate the fast green dye deposit. The site of the fast green deposit that denoted the recording site was recorded on standardized stereotaxic maps reproduced from Paxinos and Watson (1986).

Statistics

Cumulative log dose-response curves were constructed for the effects of repeated injections of DA agonists on the firing rate of VP/SI neurons. ED_{50}, defined as the dose required to induce half of the maximum response, was determined from the x-axis intercept of the linear portion of each curve using linear regression analysis of a double reciprocal plot of dose versus population response values. Emax, defined as the maximal observed effect, standardized as percent of baseline firing rate, was determined from the y-axis intercept. These data are presented as mean ± standard error of the mean.

To compare overlapping portions of the dose response curves for low and high dose administration of QUIN or SKF, a repeated measures analysis of variance (ANOVA) was used. In the experiment involving single injection treatments of SKF or QUIN, statistical differences among groups were determined using a repeated measures ANOVA and Tukey's test; and a *t* - test was used to compare mean rate responses to agonist administration between single and cumulative dose treatment. Chi-square analysis was used to detect changes in distribution of responses to the agonists. The criterion of significance for all statistical tests was $p < 0.05$.

Drugs

The drugs used in this study were chloral hydrate (Sigma Chem. Co., St. Louis, MO), quinpirole (LY171555; trans-(-)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-(or 2H)-pyrazolo-(3,4-g) quinoline monohydrochloride; Lilly Res. Lab., Indianapolis, IN), SCH23390 ((R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hemimaleate; Schering Corp., Bloomfield,NJ), SKF38393 (2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine-7,8-diol hydrochloride; Smith, Kline, and

French Labs., Philadelphia, PA), and sulpiride (5-(aminosulfonyl)-N-((1-ethyl-2-pyrrolidinyl) methyl)-2-methoxybenzamide; Sigma Chem Co.). Chloral hydrate, QUIN and concentrations of SKF below 3 mg/ml were dissolved in a saline solution. Higher concentrations of SKF were dissolved in water. SCH was dissolved in a 0.3% tartaric acid solution, and SUL was dissolved in a few drops of glacial acetic acid, then diluted with deionized water, and the pH adjusted with a 1M sodium hydroxide solution. Drug doses are expressed in terms of the weight of their bases, except for chloral hydrate and SCH, which are provided as the salt.

Results

Electrophysiologic Characteristics of Extracellularly Recorded Neurons in the VP/SI

One hundred twenty five histologically verified VP/SI neurons were investigated in the present study (Fig. 2). Of these spontaneously active VP/SI neurons, 114 (91%) displayed biphasic and 11 (9%) had triphasic action potential configurations. The mean duration of the action potentials was 1.3 ± 0.04 ms, and the peak to peak amplitude was 370 ± 22 μV. One hundred three recordings (82%) had initially negative action potential waveforms, and the remaining 22 (18%) had initially positive waveforms. VP/SI neurons demonstrated regular, irregular, or bursting firing patterns (Fig. 3) with an average firing frequency of 11.5 ± 0.74 spikes/s. The most frequently encountered

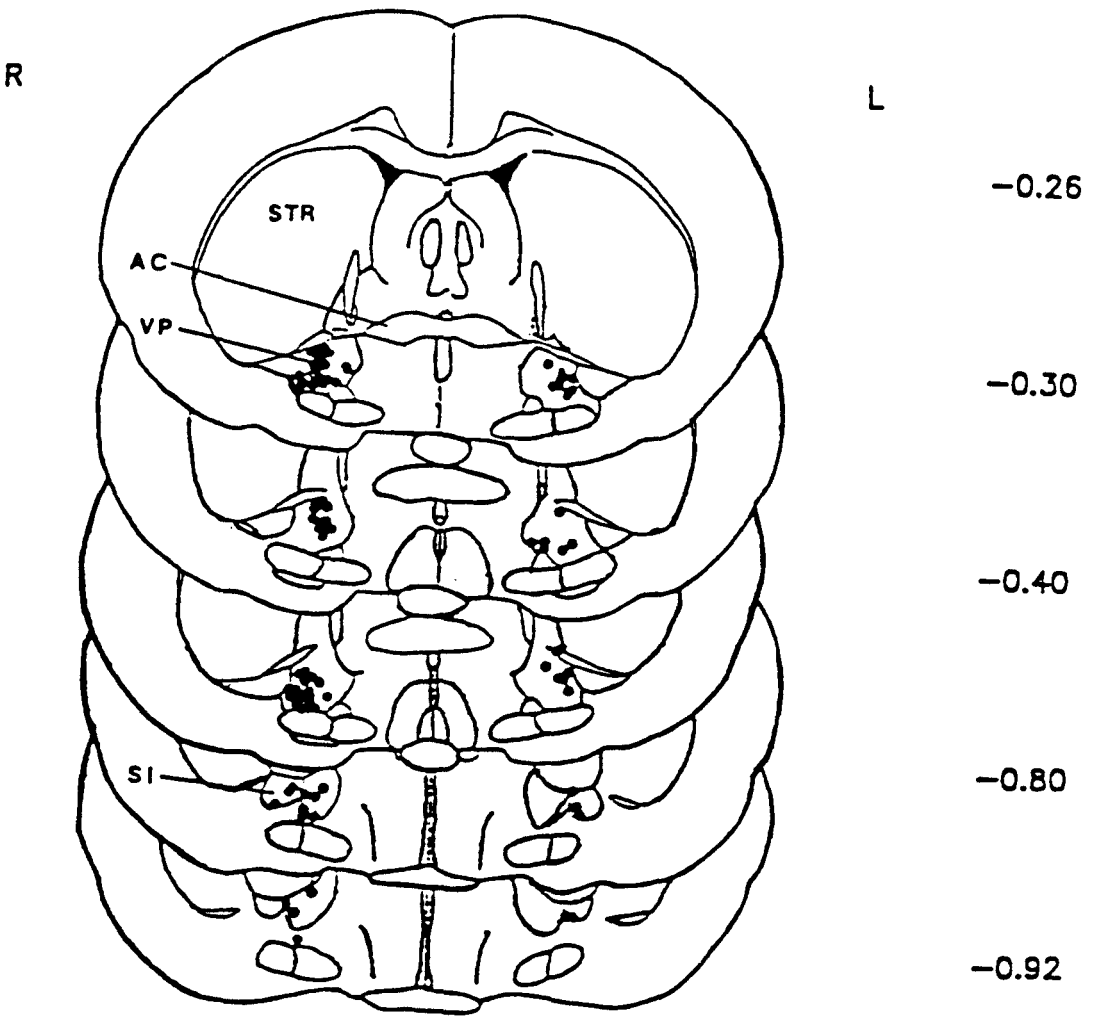

Fig. 2. STEREOTAXIC MAPS ILLUSTRATING THE HISTOLOGICAL LOCATIONS OF NEURONS WITHIN THE VP/SI FROM WHICH EXTRACELLULAR POTENTIALS WERE RECORDED (maps obtained from Paxinos and Watson, 1986). The side from which each neuron was recorded is indicated by L, left or R, right; and many recording sites overlapped. Anterior-posterior locations of brain sections are indicated in millimeters from Bregma by the number of each section. AC, anterior commissure; SI, substantia innominata; STR, striatum; VP, ventral pallidum.

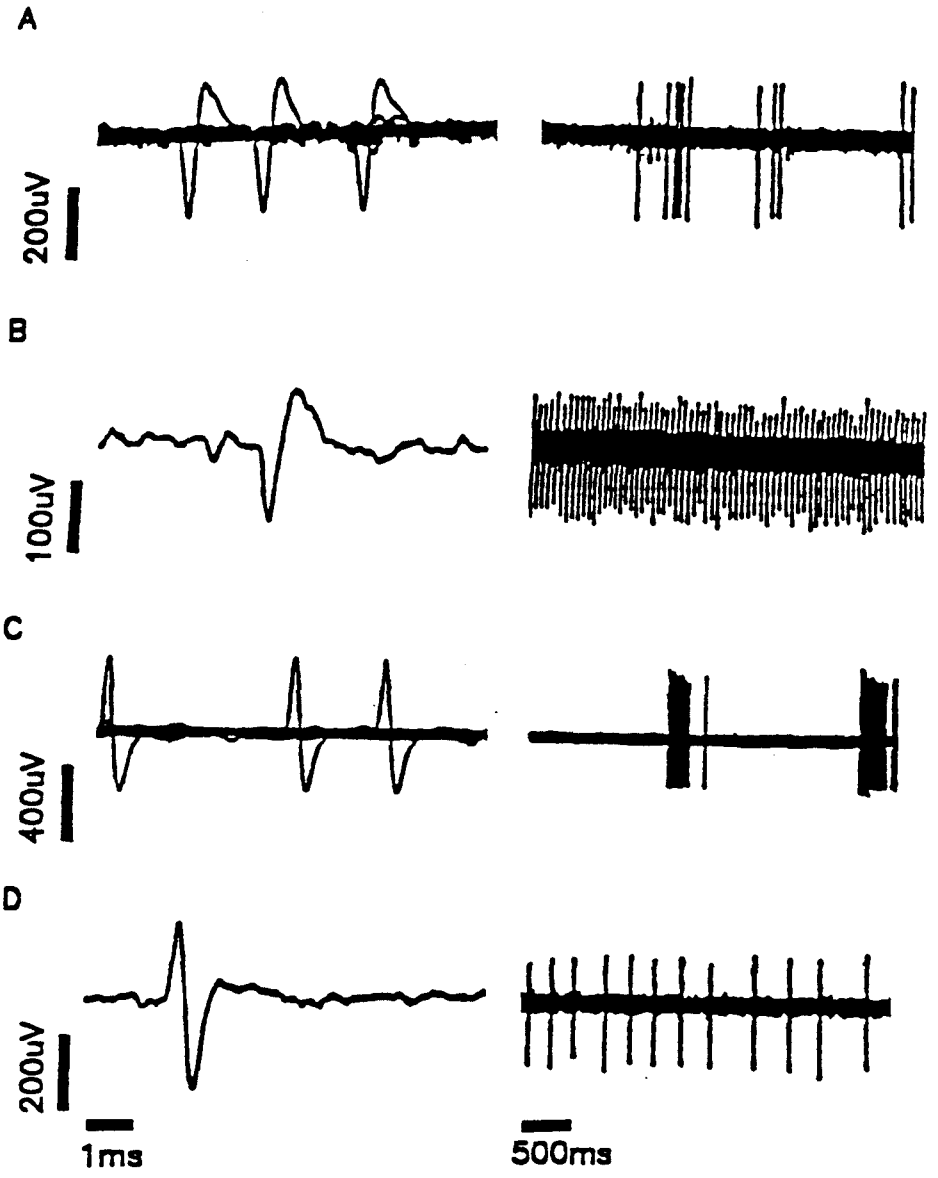

Fig. 3. OSCILLOSCOPE TRACES ILLUSTRATING THE CHARACTERISTICS OF ACTION POTENTIALS AND FIRING PATTERNS RECORDED FROM NEURONS LOCATED IN THE VP/SI. The most frequent recording encountered had an initially negative, biphasic action potential and a slow, irregular firing rate as illustrated in (A). Neurons with large, initially positive action potentials and bursting activity as illustrated in (C) were encountered least frequently. Up is positive.

recording displayed an irregular firing pattern often demonstrating slow cyclic increases and decreases in rate (illustrated by histograms in Fig. 6A-B).

Effect of Cumulative Doses of the Selective D_2 DA Receptor Agonist, Quinpirole, on the Activity of VP/SI Neurons

QUIN was administered to determine the effects of DA D_2 receptor activation on the firing rate of VP/SI neurons. Firing rates following injection of vehicle solutions in volumes similar to those for agonist treatments did not meet the criteria for a drug-induced rate change (N = 13 neurons). In contrast, injections of 0.1 - 25.6 mg/kg of QUIN decreased firing rate in 16 of 26 neurons tested (Table 1). The activity of 7 neurons ceased with the 0.2 mg/kg dose of QUIN, and thus, were not included in the dose-response curve presented in Fig. 4B. The magnitude of the rate suppression in the other 9 sensitive neurons at 0.1 mg/kg of QUIN was significant, and administration of higher doses of the agonist did not decrease firing rate further. Responses of the 10 remaining neurons tested with these doses of QUIN are summarized in Table 1.

Many VP/SI neurons responded to 0.1 mg/kg of QUIN, and additional doses did not induce further rate changes (Fig. 4B), suggesting that maximal responses were attained with this dose. Thus, 12 VP/SI neurons were tested with lower doses of quinpirole (0.002 - 0.2 mg/kg, i.v.) to determine if VP/SI neurons were sensitive to these doses, and if these changes in firing rate were dose-dependent. As illustrated in Fig. 4A-B, firing rate of 6 neurons decreased in a dose-dependent manner with a maximal decrease of 40% below control rates and an ED50 of 7.6 µg/kg. Responses of the remaining 6 neurons were equally distributed between a slight increase in firing rate (Fig. 4C) or an insensitivity to agonist administration (Table 1).

The decrease in activity of only half of the neurons tested with a total dose of 25.6 mg/kg of QUIN was abated after administration of the selective D_2 antagonist,

SUL (12.5 or 25.6 mg/kg, i.v. and 1 neuron was tested and antagonized with 50 mg/kg). SUL administration antagonized only 2 of the 6 cells that responded with QUIN-mediated increases, and 2 of 3 with biphasic rate changes. Furthermore, the average firing rate recorded after administration of 25.6 mg/kg of QUIN or after a subsequent injection of SUL was not different (Table 2). In contrast, SUL (12.5 mg/kg, i.v.) reversed the decrease in firing rate of all 6 neurons tested with a 0.2 mg/kg total dose of QUIN (Fig. 4A), as well as 1 of 3 cells excited by this dose of QUIN. The firing rate of responding neurons was different between QUIN plus SUL and the agonist alone at the lower agonist concentration (Table 2).

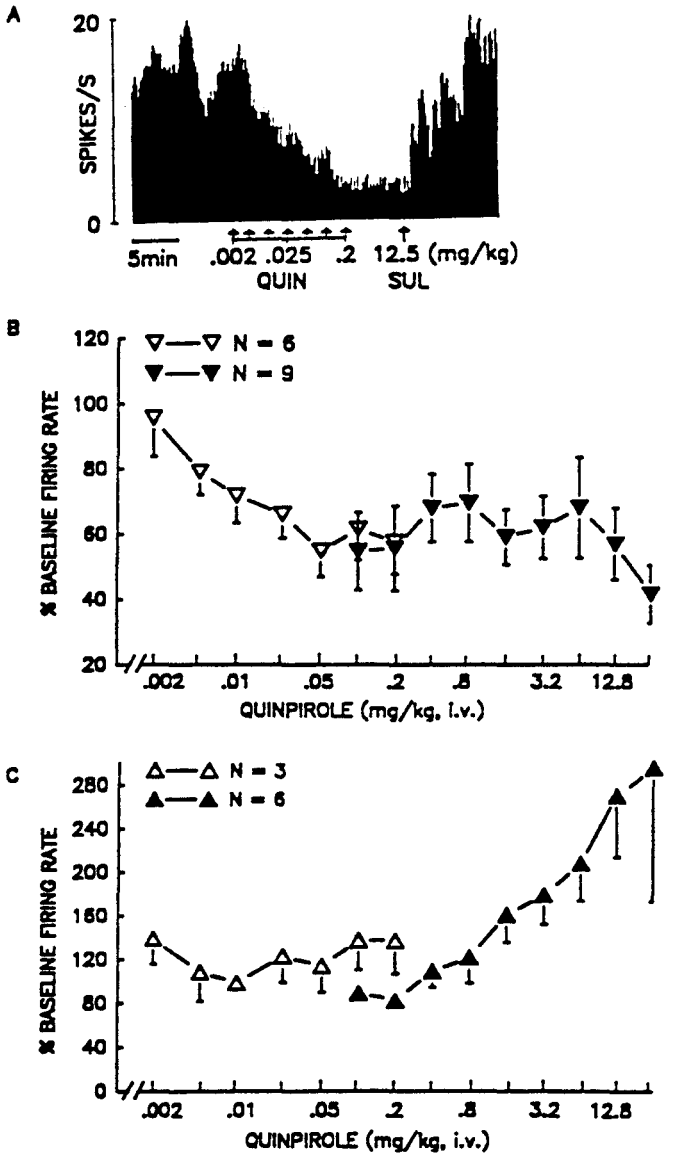

Fig. 4. RESPONSES OF VP/SI NEURONS TO CUMULATIVE DOSE ADMINISTRATION OF QUINPIROLE (QUIN). (A) A histogram illustrating the effects of dopamine D_2 receptor stimulation by low doses of QUIN. The decrease in firing rate mediated by QUIN was reversed by sulpiride (SUL). (B and C) Cumulative dose-response curves summarizing the effects of intravenous administration of QUIN on the spontaneous activity of VP/SI neurons. In (B), six of 12 cells tested with low doses of QUIN responded with dose-dependent decreases in activity (open triangles; $df = 5$, $r = -0.92$, $p < 0.01$). The decreases in firing rate of 9 neurons following administration of 0.1 - 25.6 mg/kg of QUIN did not correlate with dose ($df = 7$, $r = 0.08$, $p > 0.1$). (C) Increases in firing rate occurred in 3 neurons after administration of low doses; and 6 cells after high doses of QUIN that was dose related ($df = 7$, $r = 0.79$, $p < 0.05$). Three cells that demonstrated biphasic responses are not represented. N is the number of VP/SI neurons included in each curve.

TABLE 1

SUMMARY OF RESPONSES OF VP/SI NEURONS TO SYSTEMIC ADMINISTRATION OF SELECTIVE DOPAMINE RECEPTOR AGONISTS

In the experiments involving a dose range, dopamine agonists were administered at 2 min intervals such that each dose added to the previously administered dose. In the remaining experiments, quinpirole or SKF38393 was administered as a single injection of the dose indicated.

Agonist (doses)	Response[a]			
	Increase (%)	Decrease (%)	Biphasic (%)	No Effect (%)
Quinpirole				
(0.1 - 25.6 mg/kg)[b,c]	6/26 (23)	16/26 (61.5)	3/26 (11.5)	1/26 (4)
(0.002 - 0.2 mg/kg)	3/12 (25)	6/12 (50)	0/12 (0)	3/12 (25)
(0.1 mg/kg)	3/13 (23)	8/13 (62)	0/13 (0)	2/13 (15)
SKF38393				
(0.1 - 25.6 mg/kg)[b,c]	17/25 (68)	4/25 (16)	0/25 (0)	4/25 (16)
(0.002 - 1.6 mg/kg)	6/13 (46)	4/13 (31)	1/13 (8)	2/13 (15)
(3.2 mg/kg)	19/23 (83)	1/23 (4)	0/23 (0)	3/23 (13)

[a] The ratio of responding cells divided by total number tested. Chi-square analysis of the distribution of responses following the administration of SKF38393 versus that following quinpirole administration indicated that the number of cells that increased, decreased or were unaffected is dependent on the agonist administered ($df = 3$; $\chi^2 = 28.91$; $p < 0.01$).

[b] The distribution of responses following administration of high and low doses of quinpirole, or high and low doses of SKF38393 was not significant ($df = 3$; $\chi^2 = 5.08, 3.49$; $p > 0.1$, respectively).

[c] The distribution of responses following administration of cumulative versus single doses of quinpirole and SKF38393 also was not significant ($df = 6$; $\chi^2 = 6.42, 9.35$; $p > 0.1$, respectively).

TABLE 2

SUMMARY OF ANTAGONISM OF DA AGONIST-INDUCED EXCITATION OF VP/SI NEURONAL ACTIVITY

Neurons that were excited by quinpirole (total dose 0.2 or 25.6 mg/kg) were tested with sulpiride (SUL; 12.5 or 12.5-50 mg/kg, respectively). Neurons that were excited by SKF38393 (total dose 1.6 or 25.6 mg/kg) were tested with SCH (0.1-0.4 mg/kg). In addition, SCH (0.1 mg/kg) and SUL (12.5 mg/kg) were injected in 10 min intervals to 11 neurons that responded to a single dose of SKF38393 (3.2 mg/kg).

Agonist (doses)	Response % control	Antagonist Treatment No. 1	Treatment No. 2
	Increase	SUL	SUL + SCH
Quinpirole			
25.6mg/kg	296±123.9	292 ± 89.1	.....
0.2 mg/kg	137 ± 30.3	144 ± 17.6	.....
0.1 mg/kg	159 ± 21.7	120 ± 16.9[a]	.....
	Increase	SCH	SCH + SUL
SKF38393			
25.6mg/kg	162 ± 15.4	115 ± 16.1[b]	.....
1.6 mg/kg	180 ± 29.1	123 ± 19.2[c]	.....
3.2 mg/kg	179 ± 9.4	133 ± 15.3[d]	105 ± 12.9[d]

The mean percent response to agonist administration was different than the mean response after subsequent injection of antagonists: [a] $df = 5$, $F = 24.33$; [b] $df = 25$, $F = 4.76$; [c] $df = 11$; $F = 7.81$; $p < 0.05$.

[d] The mean percent response to SKF was different from the responses following SCH or subsequent injection of SUL (SCH + SUL); repeated measures ANOVA: $df = 29$, $F = 17.68$, and Tukey's HSD for SCH or SCH + SUL from SKF: $df = 18$, $p < 0.01$). However, SCH + SUL was not different from SCH ($df = 18$, $p > 0.05$).

TABLE 2 - Continued

SUMMARY OF ANTAGONISM OF DA AGONIST-INDUCED SUPPRESSION OF VP/SI NEURONAL ACTIVITY

Neurons that were inhibited by quinpirole (total dose 0.2 or 25.6 mg/kg) were tested with sulpiride (SUL; 12.5 or 12.5-50 mg/kg, respectively). In addition, SUL (12.5 mg/kg) was injected 10 min after quinpirole was administered as a single dose of 0.1 mg/kg, and SCH23390 (SCH; 0.1 mg/kg) was administered 10 min after SUL in 2 neurons. Neurons that were inhibited by SKF38393 (total dose 1.6 or 25.6 mg/kg) were tested with SCH (0.1-0.4 mg/kg). In addition, SCH (0.1 mg/kg) and SUL (12.5 mg/kg) were injected in 10 min intervals to 11 neurons that were inhibited by a single dose of SKF38393 (3.2 mg/kg).

Agonist (doses)	Response % control	Antagonist Treatment No. 1	Treatment No. 2
	Decrease	SUL	SUL + SCH
Quinpirole			
25.6mg/kg	42 ± 8.8	65 ± 15.0	.....
0.2 mg/kg	58 ± 9.9	99 ± 5.1[a]	.....
0.1 mg/kg	50 ± 6.1	74 ± 11.4[b]	50 ± 13.6
	Decrease	SCH	SCH + SUL
SKF38393			
25.6mg/kg	33 ± 26.3	57 ± 6.0	.....
1.6 mg/kg	52 ± 15.4	104 ± 14.1[c]	.....
3.2 mg/kg	6[d]	62	76

The mean percent response to agonist administration was different than the mean response after subsequent injection of antagonists: [a] df = 11, F = 10.49; [b] df = 15, F = 7.76; $p < 0.05$; [c] df = 7, F = 26.54, $p < 0.01$.

[d] Only one neuron was suppressed by administration of SKF38393.

Effect of Cumulative Doses of the Selective D_1 DA Receptor Agonist, SKF38393, on Activity of VP/SI Neurons

SKF was administered to investigate the responsiveness of VP/SI neurons to D_1 receptor stimulation. Cumulative dose administration of 0.1 - 25.6 mg/kg SKF altered the firing rate of 21 of 25 neurons tested (Fig. 5B-C). Seventeen neurons displayed dose-dependent increases in firing rate to agonist administration (Fig. 5A-B), and 4 neurons were suppressed (Fig. 5C).

Unexpectedly, many VP/SI neurons responded to low doses of SKF (*e.g.* 0.1 mg/kg) that were below those necessary to induce responses in other dopaminoceptive brain regions (*e.g.*, 20 mg/kg for the GP; Carlson *et al.*, 1987a), suggesting that VP/SI neurons may be more sensitive to this agonist. Thus, lower doses (0.002 - 1.6 mg/kg) of the agonist were tested on an additional 13 VP/SI neurons, and rate increases were observed in 6 of these (Fig. 5B). The responses illustrated in both the high and low dose SKF curves were dose-related. The ED_{50} of the SKF-induced increases, determined by combining the responses for 0.2 - 6.4 mg/kg doses in both cumulative dose studies, was 0.9 mg/kg, and the Emax was 162% of control firing rate. The responses of the remaining 7 neurons tested with low doses of SKF are illustrated in Fig. 5C and summarized in Table 1.

Administration of the selective D_1 antagonist, SCH (0.1 - 0.4 mg/kg, i.v.) antagonized the SKF-induced excitation of 10 of the 15 neurons tested with a total dose of 25.6 mg/kg, but only 1 of 4 suppressions (Fig. 5A). SCH injection antagonized 4 of the 6 neurons with increased rates, all 4 of the decreases, and the last phase of the biphasic response, for neurons tested with a total dose of 1.6 mg/kg of SKF. The responses to antagonist treatment differed from the SKF-induced excitations for both dose ranges, and for the neurons with decreases in rate after injection of a total dose of 1.6 mg/kg of SKF (Table 2).

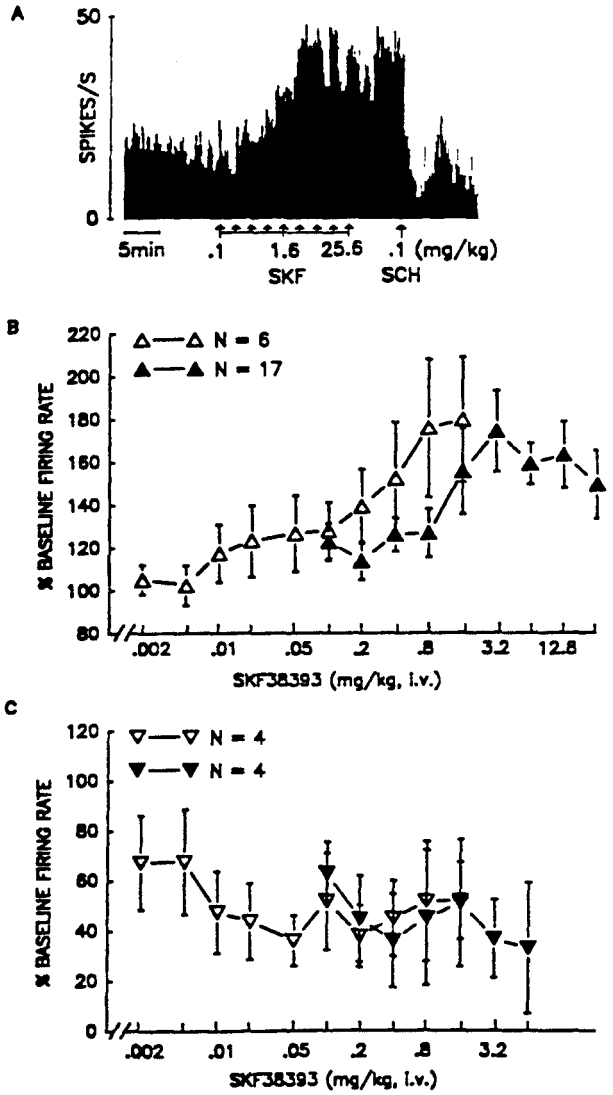

Fig. 5. VP/SI NEURONAL RESPONSES TO ADMINISTRATION OF CUMULATIVE DOSES OF THE D_1 RECEPTOR AGONIST, SKF38393 (SKF). (A) A histogram depicting a rate increase induced by SKF. The D_1 specific antagonist, SCH23390 (SCH) attenuated the response. (B and C) Cumulative dose-response curves illustrating the effects of SKF on single unit neuronal activity of the VP/SI. (B) Six neurons displayed dose-dependent increases in firing rate following injection of low doses of SKF ($df = 8$, $r = 0.69$, $p < 0.05$). An additional 17 of 25 neurons exhibited dose-dependent increases in firing rate after administration of higher doses of SKF ($df = 4$, $r = 0.87$, $p < 0.05$). Rate increases with 0.1 - 1.6 mg/kg of SKF38393 were not different in the two experiments ($df = 1, 20$, $F = 2.28$, $p > 0.1$). (C) Decreases in firing rate following administration of low doses of SKF occurred in 4 neurons, and also in 4 neurons that received high doses of the agonist. In addition, one neuron (not illustrated) demonstrated a SKF-mediated biphasic response, the last phase of which was blocked by SCH. N is the number of cells.

Effect of Single Injections of Dopaminergic Agonists on the Firing Rate of VP/SI Neurons

Electrophysiologic studies have demonstrated that DA agonist pretreatment can attenuate the effect of higher doses of the same agonist in GP neurons (Bergstrom *et al.*, 1984). To determine whether different schedules of administration influence the magnitude or direction of the responses of VP/SI neurons, effects of single injection treatments of QUIN and SKF were tested. Doses of the agonists selected were those that resulted in near Emax responses as determined from the above studies (*i.e.*, 0.1 mg/kg for QUIN and 3.2 mg/kg for SKF). The firing rate of 8 of 13 (62%) neurons tested with QUIN decreased, and 19 of 23 (83%) neurons tested with SKF increased (Fig. 6B-C). Eight of 9 neurons that demonstrated a D_1-mediated excitation were tested with vehicle solutions. Vehicle treatments did not affect the ongoing increase in firing rates, and SKF-induced rate increases lasted for at least 20 min (Fig. 6A). The average suppression induced by a single injection of QUIN was 50 ± 6.1%, which was not different from the response following a cumulative dose of 0.1 mg/kg QUIN (mean: 62 ± 10%; df = 12, t = -1.02, $p > 0.1$). The average increase in firing rate induced by administration of SKF38393 was 228 ± 37%, which was not different from the response after a 3.2 mg/kg cumulative dose of the same agonist (mean: 174 ± 19%; df = 34, t = 1.25, $p > 0.1$). Responses of the other neurons tested with QUIN or SKF are provided in Table 1.

SUL (12.5 mg/kg) injected 10 min after administration of QUIN attenuated the agonist-induced decreases in 5 of 8 (63%) neurons (Fig. 6C), and 1 of 2 remaining neurons tested was attenuated with a subsequent injection of 0.1 mg/kg of SCH. Firing rates following initial administration of QUIN were different from those observed with a subsequent injection of SUL (Table 2).

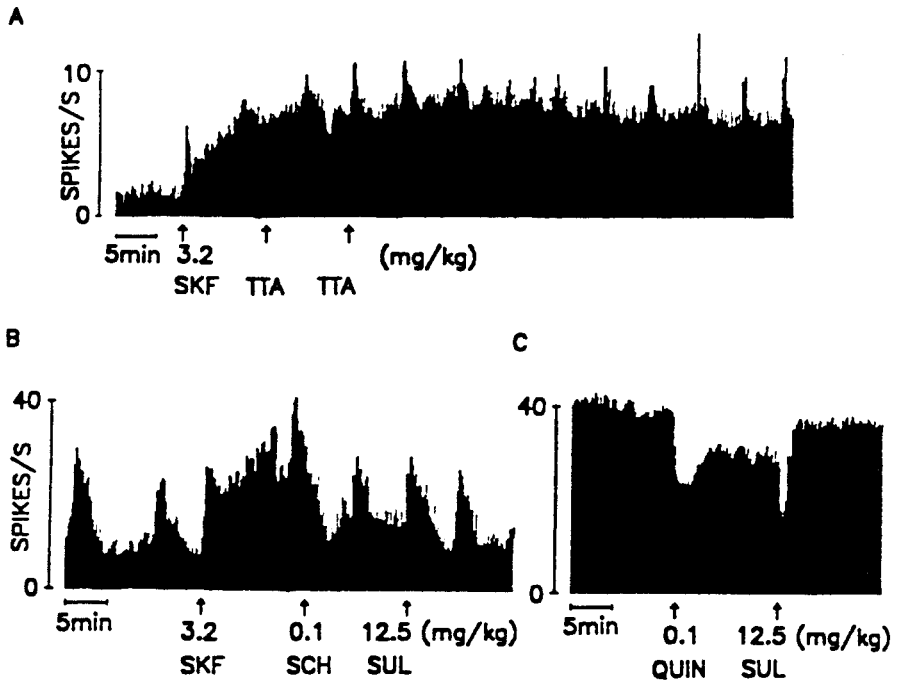

Fig. 6. VP/SI RESPONSES TO INJECTION OF SINGLE DOSES OF SKF38393 (SKF) AND QUINPIROLE (QUIN). (A and B) Rate histograms illustrating the increase in firing rate of VP/SI neurons after administration of single 3.2 mg/kg dose of SKF. (A) Administration of a 0.3% tartaric acid solution (TTA), the vehicle for SCH23390 (SCH) injected in volumes equal to those used for the antagonist, did not alter the SKF-induced rate increase. This recording depicted one of the more marked rate increases in response to SKF, but the duration of agonist induced excitation (more than 1 h) was similar to responses of other VP/SI neurons. (B) A dose of 0.1 mg/kg of SCH attenuated the SKF-induced increase, and a subsequent injection of sulpiride (SUL) reduced neuronal firing to pretreatment rates. (C) A histogram illustrating the rate decrease frequently observed following administration of 0.1 mg/kg of QUIN. Subsequent injection of SUL antagonized this D_2-mediated response.

Ten neurons demonstrating SKF-induced increases also were tested with SCH, and the response of 7 of these was attenuated by SCH. A subsequent injection of SUL reversed rates to pretreatment levels or below in these 7, as well as an additional 2 of the 10 neurons. The firing rate change induced by SKF was different than that after administration of SCH, as well as the subsequent SUL injection (Table 2).

Comparison of D_1- or D_2-mediated Responses

The distribution of responses to SKF (*i.e.* the number of neurons with increased or decreased activity, and the insensitive neurons) versus that following QUIN was conditional to the agonist administered (Table 1). QUIN- or SKF-induced responses were antagonized more often when lower doses rather than higher ones were administered (Table 2). SKF-induced increases in firing rate were attenuated by SCH administration at each dose ranges tested, suggesting that the response to SKF was selectively mediated by D_1 receptor activation even at a total dose of 25 mg/kg of the agonist (Table 2). In contrast to the selectivity of SKF for the D_1 receptor at high doses, QUIN-induced decreases were attenuated by SUL at the 0.2 mg/kg, but not at the 25.6 mg/kg total dose of the agonist (Table 2), suggesting that these high doses of QUIN may induce nonselective alteraction of VP/SI activity.

Discussion

The present experiments demonstrated that VP/SI neurons were sensitive to intravenous administration of either D_1 or D_2 agonists, and that the specific activation of the distinct receptor subtypes produced opposite effects on activity of these neurons. D_2 DA receptor stimulation altered the firing rate in 45 of 51 (88%) neurons tested with QUIN, and rate suppressions were observed in 30 of 51 (59%) neurons. Responses to

0.002 - 0.2 mg/kg of QUIN were usually attenuated, and the rate decreases were reversed completely by SUL administration verifying that the agonist-induced effects involved D_2 receptor activation for this dose range. The effects of QUIN in the present study occurred at doses that facilitate defense behaviors in cats (Sweidan *et al.*, 1990), indicating that the systemically administered doses used in the present study are behaviorally relevant.

D_1 DA receptor stimulation by SKF altered neuronal activity in 52 of 61 (85%) VP/SI neurons tested. In contrast to QUIN-induced responses, SKF increased firing rate in 42 of 61 (69%) neurons. The specific D_1 receptor antagonist, SCH was more effective in attenuating the responses to lower doses of SKF, but agonist-induced increases were attenuated in approximately 70% of all neurons tested. The agonist-induced effects in the present study occurred at doses that also induce grooming in rats (Meller *et al.*, 1988; Vasse *et al.*, 1988) indicating that these systemically doses are sufficient to influence neuronal activity and behavior. Thus, behaviorally relevant doses of QUIN and SKF administration altered VP/SI neuronal activity in opposite directions via stimulation of specific receptor subtypes.

Although the VP/SI is a ventral extension of the GP with similarities in cell morphology and neurotransmitter content, the present study revealed that the effects of D_1 or D_2 DA agonists on VP/SI neuronal activity contrasts with those previously demonstrated in the GP. For example, 71% of VP/SI neurons tested responded by the 0.2 mg/kg dose of QUIN, whereas 0.3 mg/kg of QUIN affects only 54% of GP neurons tested (Carlson *et al.*, 1988). The predominant response direction observed for the D_2 agonist also differs for each area; activity of VP/SI neurons decreased, but GP neurons respond with excitation (Carlson *et al.*, 1987a, 1988). D_1 receptor activation initiated responses in 52 of 61 (85%) VP/SI neurons tested with SKF doses that were less than 6.4 mg/kg. GP neurons are insensitive to these doses, and at 20 mg/kg SKF,

only 54% of the recorded neurons are affected (Carlson *et al.*, 1988). Furthermore, dopaminergic agonist pretreatment in the GP diminishes the effect of subsequent administration of higher doses of the same agonist (Bergstrom *et al.*, 1984); a phenomenon that was not observed for the VP/SI in the present study. Thus, changes in activity induced by systemically administered DA agonists discriminate between VP/SI and GP neurons.

To determine the contribution of DA receptor subtypes in agonist-induced responses of VP/SI neurons, both D_1 and D_2 antagonists were administered after agonist treatments if the first antagonist did not attenuate the agonist-induced response. Decreases induced by single dose treatment of QUIN usually were attenuated with SUL, and subsequent administration of SCH was tested on only two neurons. These results suggest that the effects induced by quinpirole were mediated through D_2 receptor activation without involving the D_1 receptor. In contrast, SCH attenuated the effects of SKF, although subsequent administration of SUL often reduced rates to or below control levels. These results imply that SKF-mediated effects of VP/SI neurons may involve the D_2 receptor. However, SUL administration alone has been observed to suppress VP/SI activity (unpublished results). Thus, SUL-induced rate suppression may not be due to antagonism of the response to SKF, but through a blockade of a tonically active dopaminergic input. This would explain the observation that SUL was able to induce rate decreases that surpassed pretreatment levels in the present study.

An additional interpretation involves the possibility that endogenous DA is released by SKF, and the neurotransmitter acts at the D_2 receptor to contribute to the SKF-mediated response. Carlson *et al.* (1988) observed that the electrophysiologic effects of high doses of SKF in the GP are attenuated by removal of endogenous DA. However, pharmacologic inactivation of central dopaminergic systems did not eliminate SKF-induced rate increases in the VP/SI (Maslowski *et al.*, 1990), suggesting that the

expression of SKF-induced responses may involve, but do not require, dopaminergic systems. Further studies are necessary to determine if removal of endogenous DA eliminates the ability of SUL to influence SKF-induced responses in the VP/SI.

The present experiments suggest that D_1 and D_2 receptors fulfill oppositional roles in mediating VP/SI neuronal activity. In addition, the VP/SI generates cue-elicited, event-related potentials in the cortex (Pirch *et al.*, 1986; Rigdon and Pirch, 1986), and these slow potentials respond in opposite directions to D_1 and D_2 agonists (Pirch *et al.*, 1988). These observations concur with the opposite effects of these agonists in the modulation of adenylate cyclase (Stoof and Kebabian, 1981). In contrast, many studies of other dopaminoceptive brain regions demonstrate that responses to D_1 or D_2 receptor activation are in the same direction (*e.g.*, Carlson *et al.*, 1988). Thus, the VP/SI may be unique in its responses to separate administration of DA agonists selective for receptor subtypes.

The location of DA receptor subtypes that mediate agonist-induced alterations in firing rate of VP/SI neurons has not been determined. Receptors located within the VP/SI as well as inputs from other dopaminoceptive brain regions may contribute to responses observed after systemic administration of DA agonists. Previous studies indicate that approximately 40% of the VP/SI neurons sampled are sensitive to microiontophoretic application of DA, and rate suppression was observed more often than excitation (Napier *et al.*, 1991b). Thus, inhibitory effects of the DA agonists demonstrated in the present study may reflect receptor activation within the VP/SI.

Although NA inputs to VP/SI are substantial (for review see, Heimer *et al.*, 1985), and the dopaminergic modulation of this input increases activity in the VP/SI, microinjection of QUIN or SKF individually in the NA does not alter VP/SI activity (for review see, Mogenson and Yang, 1991). In addition, inactivating the NA with procaine microinjections does not eliminate responses of VP/SI neurons to systemic

administration of QUIN or SKF (Napier, 1990). Thus, the responses of VP/SI neurons in the present study may be independent of NA inputs.

Alteration of dopaminergic projections from the midbrain is another means that VP/SI activity could be changed by systemic administration of DA agonists. However, the contribution of this input may be modest since pharmacological suppression of midbrain dopaminergic neuronal activity by systemic pretreatment with gamma-butyrolactone, did not eliminate the effects induced by systemic administration of either QUIN or SKF (Maslowski *et al.*, 1990).

AMN neurons also project to the VP/SI (for review see Zaborszky *et al.*, 1991). In addition, the AMN receives dopaminergic inputs from the midbrain (for review see, De Olmos *et al.*, 1985). The activity of AMN neurons is altered by systemic administration (Bashore *et al.*, 1978) and local application (Nakano *et al.*, 1987) of DA agonists. Preliminary evidence suggests that inactivation of the AMN by local injection of procaine, attenuated the responses to systemically administered SKF (Napier, unpublished results). Thus, the effects of systemic administration of dopaminergic agents may involve the summated activation of DA receptors within VP/SI, as well as indirect influences from the AMN afferents to the VP/SI.

Dopaminergic modulation of neuronal activity may propagate changes in the transmission of VP/SI efferents. The VP/SI is located between projection neurons of extrapyramidal motor (for review see, Mogenson and Yang, 1991) and limbic systems (Haber *et al.*, 1985) which may permit the VP/SI the capacity to integrate motoric, cognitive, and motivational processes. For example, neuronal activity is enhanced in VP/SI when an animal performs task-related movements and during appropriate responses to rewarded stimuli (for review see Richardson and DeLong, 1991). Recent findings indicate that the VP/SI is involved in alterations of specific behaviors in the rat, including cocaine self-administration (Hubner and Koob, 1990). Changes in firing rate

of VP/SI neurons in response to DA activation of D_1 or D_2 receptors may mediate these behaviors, thus underscoring the importance of continued investigation of the dopaminergic influence on VP/SI neurotransmission.

CHAPTER IV

EFFECTS OF D_1 AND D_2 ANTAGONISTS ON APOMORPHINE-INDUCED RESPONSES OF VENTRAL PALLIDAL NEURONS

Abstract

The ventral pallidum and adjacent substantia innominata (VP/SI) is innervated by dopaminergic terminals and contains D_1 and D_2 DA receptors. Repeated systemic injections of the DA agonist, apomorphine (APO), induce dose-dependent alterations in VP/SI neuronal activity. The present studies evaluated the contribution of D_1 and D_2 receptor subtypes to APO-induced alterations in extracellularly recorded VP/SI neuronal activity. Both sulpiride (SUL; D_2 antagonist) and SCH23390 (SCH; D_1 antagonist) attenuated many of these responses; however, pretreatment with either antagonist did not alter the number of responding neurons, or the maximal effect induced by APO. Thus, activation of either receptor subtype by APO is sufficient to initiate the observed responses, and both may be involve in dopaminergic modulation of VP/SI neurons.

Introduction

The ventral pallidum and substantia innominata (VP/SI) are basal forebrain regions involved with the processing and transmission of motor, cognitive and motivational functions. Dopaminergic modulation of these behaviors may occur since

the activity of VP/SI neurons is affected by manipulation of dopaminergic systems. The VP/SI is directly innervated by dopaminergic inputs (Voorn *et al.*, 1986), and both D_1 and D_2 DA receptor subtypes are present in this area (Contreras *et al.*, 1987). VP/SI neurons demonstrate dose-dependent changes in firing rate after intravenous administration of the D_1/D_2 DA agonist, APO (Napier *et al.*, 1991b). In the dorsal globus pallidus (GP), which is morphologically similar to the VP/SI, APO stimulates D_1 and D_2 receptor subtypes, and both are necessary for maximal responding (Carlson *et al.*, 1987a). GP neuronal responses to APO also are attenuated when the agonist dose is administered in multiple injections, a phenomenon the authors termed a "priming effect" (Bergstrom *et al.*, 1982).

The present electrophysiologic studies evaluated the contribution of DA receptor subtypes to APO-induced effects in the VP/SI by determining (1) whether antagonists selective for either the D_1 or D_2 receptor influenced the effects of APO, and (2) whether pretreatment with either antagonist precluded the agonist-induced effects. In addition, a single dose of APO was tested to determine if the agonist-induced rate changes with this protocol were higher than those obtained previously in this laboratory for the VP/SI using the same dose administered in multiple injections (Napier *et al.*, 1991b).

Methods

Extracellular activity of single VP/SI neurons was monitored in chloral hydrate (400 mg/kg, i.p.) anesthetized, male Sprague-Dawley rats (270-330 g). Chloral hydrate supplements were intravenously administered to maintain surgical levels of anesthesia. Supplementation was discontinued prior to test treatments with DA agents. Only one neuron per animal was tested. Action potentials (spikes) were recorded with a glass pipette containing a 2 M sodium chloride solution saturated with fast green dye.

Standard recording techniques were used as described elsewhere (Maslowski and Napier, 1991a). VP/SI neurons were recorded within the stereotaxic coordinates 0.5 mm P from Bregma, ± 2.5 mm L from the midline and 7.5-8.5 mm below the dura (according to Paxinos and Watson , 1986). Stable baseline firing was obtained for 5 min, and treatments were then injected in 10 min intervals through a tail vein cannula.

The following vehicle solutions were tested in equal volumes to the respective drug treatments: saline (0.12 ml) and 0.3% tartaric acid (0.19 ml), which were vehicles for APO and SCH (D_1 specific antagonist), respectively; and buffered acetic acid (0.19 ml; pH = 5), which was the vehicle for both SUL (D_2 specific antagonist) and haloperidol (D_1/D_2 nonselective antagonist). The dose of APO selected (0.5 mg/kg, i.v.) is known to produce near maximal responding in VP/SI neurons (Napier *et al.*, 1991b), and the duration of APO-induced rate changes persists for longer than 20 min (Napier *et al.*, 1986). APO was administered as a single dose, followed at 10 min post-injection by SUL (12.5 mg/kg) or SCH (0.1 mg/kg). In 10 neurons tested with SCH, haloperidol (0.5 mg/kg) was also administered to evaluate the possible role of D_2 receptors in APO-induced effects. The antagonist doses selected are sufficient to reverse APO-induced behavioral changes in rats (Kendler *et al.*, 1982; Martres *et al.*, 1984; Molloy and Waddington, 1985).

In additional experiments, a 10 min pretreatment of SUL or SCH preceded APO administration. These neurons were also tested with a saline vehicle before the antagonist pretreatment. Neurons that remained sensitive to APO after competitive antagonism of one receptor subtype, subsequently were tested with the specific antagonist for the other subtype. Responses were considered significant if baseline firing was altered by greater than 20%. Antagonism was defined as an attenuation of agonist-induced responses by at least 30%. A more stringent criterion than that for agonist-induced responses was select to assess antagonism since most of the antagonist

effects exceeded the usual 20% criterion. At the end of each experiment, the rat was overdosed with chloral hydrate, and the recording site was determined by the histological location of a fast green dye deposit from the electrode tip (Maslowski and Napier, 1991a).

Neuronal activity after drug injections is presented as mean percent of pretreatment activity ± S.E.M., determined by comparing the firing rate averaged over the last minute of pre-drug period (considered as 100%) with that observed during the last minute interval following each injection. Alterations in mean firing rate among different drug treatments or time intervals were evaluated with repeated measures, ANOVA and *t* - test. Differences in the distribution of effects were examined with Chi-square analysis. The criterion of significance for all statistical tests was $p < 0.05$.

Results

Sixty-five neurons were recorded within the VP/SI (Fig. 7). These VP/SI neurons displayed biphasic, and infrequently triphasic, action potentials with a mean duration of 1.4 ± 0.04 ms, and peak to peak amplitude of 350 ± 20 μV. VP/SI neurons demonstrated regular, irregular, or bursting firing patterns with an average firing frequency of 10.5 ± 1.0 spikes/s. APO induced responses in 28 of 35 (80%) neurons tested (*eg.*, Fig. 8), and 7 were insensitive (data not shown). The average rate suppression (N=15, 64 ± 8% below baseline rate; baseline = 100%) and excitation (N=13, 105 ± 29% above baseline) induced by this single injection of APO were similar to responses previously observed after a cumulative dose of 0.5 mg/kg administered in multiple injections (95 ± 5% and 96 ± 12%, respectively Napier *et al.*, 1991). Neuronal activity was not affected by vehicle solutions in all 7 neurons tested using the protocol

for drug treatments, nor by saline injected prior to antagonist pretreatments in 20 of 23 neurons tested.

The magnitude of the difference between APO-induced rate suppression compared to excitation was not statistically significant ($df = 26$, $t = 1.45$, $p > 0.1$), and the D_1 or D_2 DA receptor-selective antagonists demonstrated a similar capability to attenuate APO-induced effects regardless of the agonist-induced response direction. Thus, the data were combined omitting direction of responses (Table 3). SUL attenuated the effects of APO in 5 of 12 neurons tested, and SCH antagonized 11 of 16 neurons tested (*eg.*, Fig. 8; Table 3). In 10 neurons, haloperidol was administered after SCH, and did not potentiate the SCH-induced antagonism in 8 neurons, but attenuated the APO-induced responses in 2 of 4 neurons that were not affected by SCH (data not shown).

APO effects also were evaluated after pretreatment with either SUL or SCH to determine whether functional removal of either receptor subtype would eliminate APO-induced effects. Four of 12 (33%) neurons pretreated with SUL, and 1 of 11 (9%) pretreated with SCH were insensitive to APO. The APO-induced effects of these 5 VP/SI neurons appear to be mediated specifically through one receptor subtype. However, APO mediated responses through either receptor subtype in the majority of neurons tested. Thus, the proportion of APO sensitive compared to insensitive neurons was unaltered by antagonist pretreatment ($df = 2$; $x^2 = 2.08$; $p > 0.1$). The magnitude of APO-induced effects was also unchanged ($df = 2, 43$; $F = 0.96$; $p > 0.1$).

Table 3 illustrates the effects of antagonist pre- and post-treatment on APO-induced responses in VP/SI neurons. The ratios indicate the number of neurons responding to a treatment compared to the number tested. For both SUL and SCH post-treatment groups, this is the number of APO-sensitive neurons whose agonist-induced response was attenuated by greater than 30% by the antagonists, compared to the

number of APO-sensitive neurons tested with the antagonists. Following pretreatment with SCH or SUL, the proportion of neurons that were sensitive to APO and subsequently antagonized by SUL or SCH post-treatment ($df = 1$, $x^2 = 1.02$; $x^2 = 1.07$, $p > 0.1$, respectively), was not different from neurons tested without antagonist pretreatment.

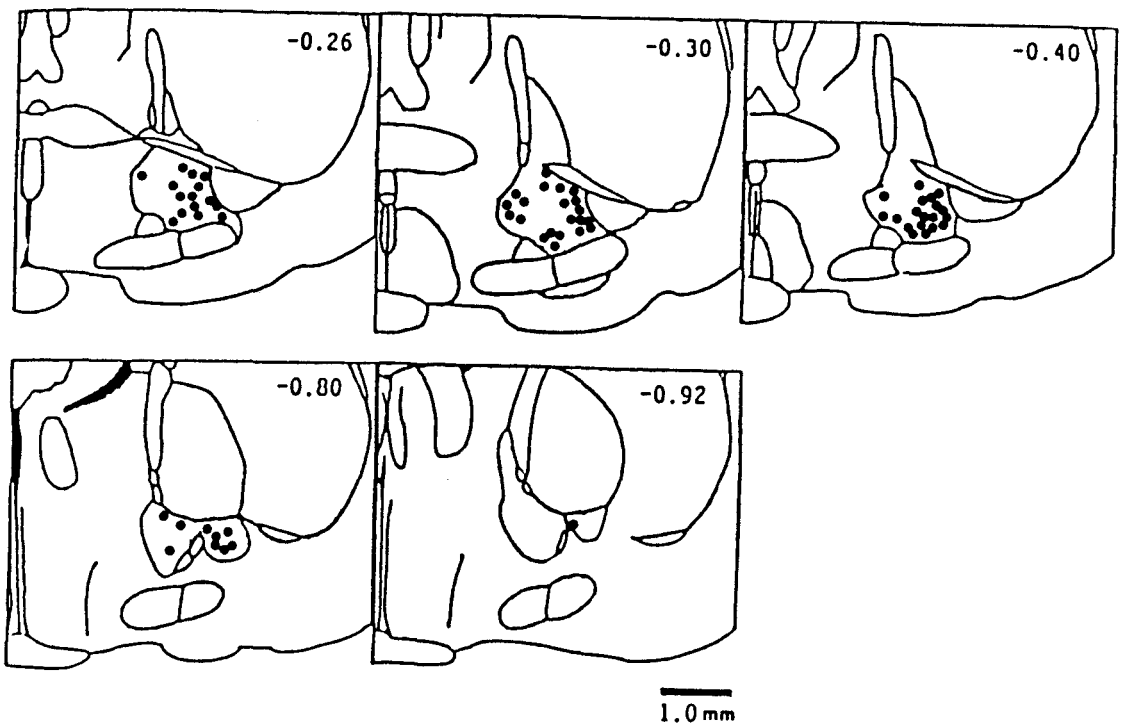

Fig. 7. STEREOTAXIC MAPS ILLUSTRATING THE HISTOLOGICAL LOCATIONS OF EXTRACELLULAR RECORDING SITES WITHIN THE VENTRAL PALLIDUM (maps obtained from Paxinos and Watson, 1986. Reproduced with kind permission from: The Rat Brain in Stereotaxic Coordinates. 2nd edition; copyright 1986, Academic Press, Orlando, Florida). Three recording sites were also located (in the section labeled - 0.80) medial to the ventral pallidum within the substantia innominata. The number on each map indicates the distance posterior to Bregma in millimeters.

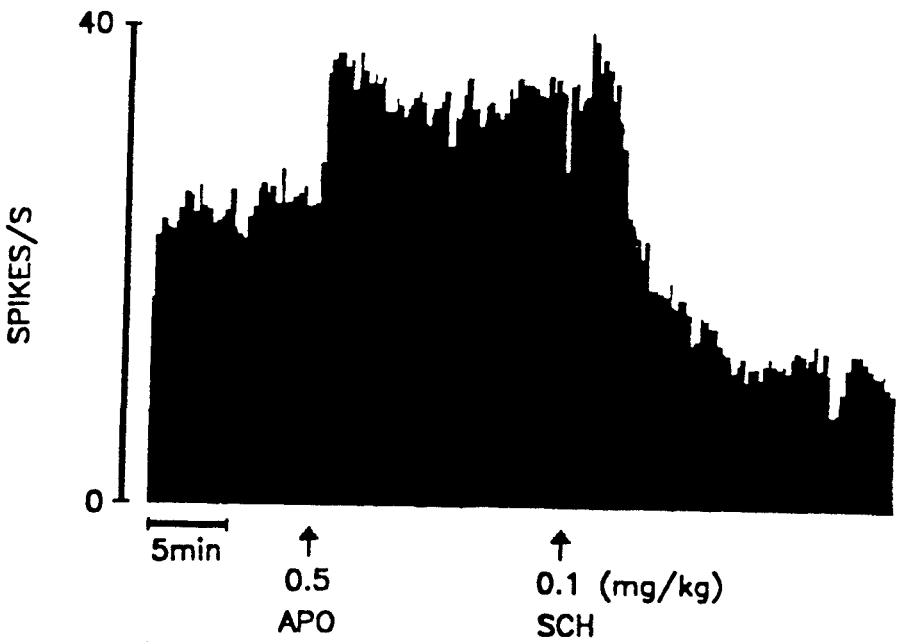

Fig. 8. A REAL-TIME FREQUENCY HISTOGRAM DEPICTING A RATE INCREASE FOLLOWING ADMINISTRATION OF APOMORPHINE (0.5 mg/kg, i.v.). Subsequent administration of the D_1 DA receptor antagonist, SCH23390 (0.1 mg/kg, i.v.) attenuated the response below pretreatment rate.

TABLE 3

EFFECT OF ANTAGONIST PRE- AND POST-TREATMENT ON APOMORPHINE-INDUCED RESPONSES BY VP/SI NEURONS

Antagonist Post-treatment[b]	Antagonist Pretreatment[a]		
	None n (%)	Sulpiride n (%)	SCH23390 n (%)
Sulpiride	5/12 (42)	.....	6/8 (75)
SCH23390	11/16 (69)	4/10 (40)	.....

[a] Animals receiving an antagonist pretreatment were injected with sulpiride (12.5 mg/kg, i.v.) or SCH23390 (0.1 mg/kg, i.v.) 10 min before the administration of apomorphine (0.5 mg/kg, i.v.).

[b] Animals receiving antagonist post-treatment 10 min after the administration of apomorphine.

Discussion

The present study revealed that activation of either the D_1 or D_2 receptor subtype by APO was sufficient to induce neuronal rate changes in the VP/SI. The magnitude and distribution of responses was similar among neurons receiving APO alone and after either receptor subtype was blocked by pretreatment with specific antagonists. This suggests that the receptor subtype that was not blocked by the antagonist is adequate to maintain the response to APO. In agreement, previous experiments demonstrated that stimulation of D_2 receptors by the specific D_2 agonist, quinpirole, or D_1 receptor

activation by the specific D_1 agonist, SKF38393, is sufficient to alter VP/SI neuronal activity (Maslowski and Napier, 1991a).

The present study determined that the response magnitude of VP/SI neurons after single dose of the APO was similar to that observed when the dose is achieved through multiple injections (Napier *et al.*, 1991b). This contrasts with results reported for the GP, where prior APO injection at a dose that does not induce a response, attenuates the response to an effective dose of the agonist (termed a priming effect by the authors; Bergstrom *et al.*, 1982). APO-induced responses of substantia nigra pars reticulata neurons do not display a priming effect (Waszczak *et al.*, 1984), and additional studies in the VP/SI revealed that responses to quinpirole or to SKF38393 also do not exhibit priming (Maslowski and Napier, 1991a). Thus, absence of the priming effect of APO may differentiate the VP/SI from the GP, but is not unique for the VP/SI.

These studies illustrate that regional variations exist with regard to dopaminergic influences, and underscore the necessity of obtaining pharmacological profiles for the individual dopaminoceptive brain regions. The results demonstrate that stimulation of one DA receptor subtype can alter neuronal activity within the VP/SI, which concurs with previous experiments using agonists selective DA receptor subtypes (Maslowski and Napier, 1991a). Further studies are needed to determine if the responses involve endogenous DA, and if these effects are mediated within the VP/SI or through other dopaminoceptive regions that impinge upon the VP/SI.

Conclusion

The present study indicates that activation of either the D_1 or D_2 DA receptor subtype is sufficient to initiate changes in VP/SI neuronal firing, and further supports the independence of function for the subtypes.

CHAPTER V

DOPAMINE WITHIN THE VENTRAL PALLIDUM MODULATES NEURONAL ACTIVITY THROUGH D_1 AND D_2 RECEPTORS, AND ATTENUATES VENTRAL PALLIDAL RESPONSES TO AMYGDALA STIMULATION

Abstract

The ventral pallidum and adjacent substantia innominata (VP/SI) receive dopaminergic afferents, demonstrate binding for D_1 and D_2 dopamine (DA) receptors, and intra-VP/SI DA application alters the firing rate of these neurons. The present studies evaluated the effects of stimulating the ventral tegmental area (VTA) or the substantia nigra pars compacta (SNc), which presumably releases endogenous DA within the VP/SI, and of microiontophoretic application of DA within the VP/SI on the spontaneous and amygdala-evoked activity of VP/SI neurons. The contribution of VP/SI D_1 and D_2 DA receptor subtypes to DA-mediated effects also was examined. VP/SI neurons often responded to VTA/SNc stimulation with short latency ($\leq$ 12 ms) inhibition, indicative of monosynaptically-mediated events. Intra-VP/SI application of SCH23390 (a D_1 antagonist) or sulpiride (a D_2 antagonist) attenuated this response. VTA/SNc-evoked VP/SI responses were mimicked by DA applied in the VP/SI. Intra-VP/SI application of the D_1 agonist SKF38393 or the D_2 agonist quinpirole modified VP/SI activity, confirming that either receptor subtype can modulate VP/SI neuronal activity. Thus, endogenously-released DA or exogenously-applied dopaminergic

agonists alter(s) VP/SI neuronal activity via D_1 or D_2 receptors. Stimulation of amygdaloid nuclei (AMN) evoked short and long (> 12 ms) latency VP/SI responses, and the effects of AMN and VTA/SNc stimulation converged extensively within the VP/SI. Attenuation of AMN-evoked VP/SI responses was observed by: 1) prior stimulation of the VTA/SNc, and 2) intra-VP/SI DA application. The results suggest that activation of VTA/SNc releases DA within the VP/SI where it can act as a physiological antagonist of AMN-evoked VP/SI responses. Thus, DA displays neuromodulatory effects on the activity of the ventral striatopallidal system at the level of the VP/SI.

Introduction

The ventral pallidum (VP/SI), defined as the ventral subcommissural extension of the globus pallidus, and the adjacent substantia innominata (Heimer and Wilson, 1975), receives ventral striatal and nucleus accumbens (NA) projections that transmit limbic information from cortical and basolateral amygdaloid inputs (for review see, Heimer and Alheid, 1991). The VP/SI can integrate and transmit this limbic information to medial dorsal thalamic (Young *et al.*, 1984) and brainstem regions (Swanson *et al.*, 1984) that govern motoric behaviors (Brudzynski and Mogenson, 1985; Swerdlow and Koob, 1987). Thus, the VP/SI, as a component of the ventral striatopallidal system, "must participate in the execution and modulation of motor responses resulting from various sensory and cognitive activities in the cerebral cortex" (Heimer and Alheid, 1991).

Midbrain dopaminergic neurons can influence the ventral striatopallidal system via a dopamine-induced suppression of NA neuronal firing (Yim and Mogenson, 1982) that may alter the activity of NA efferents to the VP/SI and result in VP/SI rate increases

(Yang and Mogenson, 1989). Considerable evidence demonstrates that DA within the NA can mediate locomotor activity via the VP/SI (Austin and Kalivas, 1991; Jones and Mogenson, 1980; Mogenson and Nielson, 1983; Swerdlow *et al.*, 1984). However, recent studies indicate that dopamine (DA) within the VP/SI is sufficient to influence VP/SI neuronal activity (Napier and Potter, 1989; Napier *et al.*, 1991b) and locomotion (Napier and Chrobak, 1992).

The VP/SI is innervated by midbrain dopaminergic neurons (for review see, Napier *et al.*, 1991a; Zaborszky *et al.*, 1991) that originate from both the ventral tegmental area (VTA; Grove, 1988; Haring and Wang, 1986; Jones and Cuello, 1989; Russchen *et al.*, 1985; Semba *et al.*, 1988; Zaborszky, 1989) and the substantia nigra pars compacta (SNc; Fallon and Moore, 1978; Haring and Wang, 1986; Jones and Cuello, 1989; Martinez-Murillo *et al.*, 1988; Russchen *et al.*, 1985; Semba *et al.*, 1988; Zaborszky, 1989), regions often associated with mesolimbic and extrapyramidal motoric functions, respectively. Biochemical studies demonstrate the presence of DA and its metabolites within the VP/SI (Geula and Slevin, 1989; Napier and Potter, 1989). VP/SI neuronal activity frequently exhibits short latency inhibitory responses, suggestive of monosynaptic effects, to the electrical stimulation of the SNc (Napier *et al.*, 1991a), and often is inhibited by microiontophoretic applications of DA within the VP/SI (Napier and Potter, 1989; Napier *et al.*, 1991b).

Recent studies using molecular biological techniques reveal the existence of several DA receptor subtypes within the brain (for review see, Civelli *et al.*, 1991). The effects of activating the D_1 and/or the D_2 receptor subtypes within the VP/SI were examined in the present study. The D_1 receptor subtype within the VP/SI was defined pharmacologically by the ability of the selective D_1 agonist, SKF38393 (SKF) to alter VP/SI neuronal activity, and the attenuation of the agonist-induced effects by the selective D_1 antagonist, SCH23390. Likewise, the D_2 receptor subtype within the

VP/SI was characterized by the actions of the selective D_2 agonist, quinpirole (QUIN), and the attenuation of agonist-mediated effects by the selective D_2 antagonist, sulpiride (SUL). Biochemical studies, using radioligands that selectively bind the D_1 or D_2 receptor subtype confirm the presence of both receptor subtypes within the VP/SI (Contreras *et al.*, 1987; Beckstead *et al.*, 1988; Napier *et al.*, 1991a). Similarly, DA-induced alterations of VP/SI neuronal activity involve both receptor subtypes, since systemic administration of SCH or haloperidol (a D_2 antagonist) attenuates the responses to systemic treatments of apomorphine (Maslowski and Napier, 1991b) and microiontophoretic applications of DA (Napier *et al.*, 1991b). Previous studies indicate that <u>systemic</u> activation of D_1 or D_2 receptor is sufficient to alter the spontaneously activity of VP/SI neurons (Maslowski and Napier, 1991a). However, it has not been determined whether specific activation of D_1 or D_2 receptor subtypes <u>within</u> the VP/SI can alter VP/SI neuronal activity. The involvement of these receptor subtypes in the responses of VP/SI neurons to endogenously-released DA via electrical stimulation of midbrain dopaminergic neurons is also unknown.

In addition to NA and midbrain dopaminergic inputs, amygdaloid nuclei (AMN) provide limbic influences on the ventral striatopallidal system and subsequently affect the activity of VP/SI efferents to brain regions involved with motoric behaviors. The AMN, which is innervated by midbrain dopaminergic afferents (Fallon *et al.*, 1978; Fallon, 1981) and is influenced by administration of dopaminergic agents (Anderson and Rebec, 1988; Bashore *et al.*, 1978; Nakano *et al.*, 1987), impinges directly onto VP/SI neurons (Krettek and Price, 1978; Zaborszky *et al.*, 1984; Price, 1986), as well as providing afferents to the NA (Krettek and Price, 1978; Price, 1986). Evidence from combined anatomical and histochemical studies indicate that AMN projections contain the excitatory neurotransmitter, glutamate (or aspartate) (Fuller *et al.*, 1987). Electrical stimulation of the AMN usually excites NA neurons (Yim and Mogenson,

1982), which presumably activates NA inhibitory efferents to the VP/SI to produce the observed late onset (polysynaptic) inhibition of VP/SI activity (Yim and Mogenson, 1983). However, AMN stimulation also evokes short onset inhibitory and excitatory effects on VP/SI neuronal activity that are independent of the NA (Yim and Mogenson, 1983), and may involve to some extent the direct innervation of the VP/SI by the AMN.

VTA stimulation (presumably releasing endogenous DA), attenuates the monosynaptically-mediated, excitatory NA responses (Yim and Mogenson, 1982), as well as the polysynaptically-mediated, inhibitory VP/SI evoked by AMN stimulation (Yim and Mogenson, 1983). The modulatory effects of VTA stimulation are mimicked by exogenously-applied DA within the NA. However, only a portion of the VTA-induced modulation of the AMN-evoked VP/SI responses is eliminated through procaine-induced inactivation of the NA (Yim and Mogenson, 1983), suggesting other pathways for DA modulation AMN-evoked VP/SI responses. Since both midbrain dopaminergic and AMN afferents converge within the VP/SI, DA influences on AMN inputs may occur within the VP/SI, providing additional processing of the information transmitted to brainstem locomotor regions.

The present electrophysiological study was designed to address the following questions: 1) Are the effects of intra-VP/SI DA application mediated through D_1 or D_2 receptor subtypes within the VP/SI? 2) Are selective D_1 or D_2 DA receptor agonists within the VP/SI sufficient to induce rate changes in VP/SI neurons? 3) Does stimulation of the VTA evoke similar responses in VP/SI neurons as SNc stimulation? 4) If the VTA/SNc influence VP/SI neuronal activity, are the evoked responses mediated through D_1 and/or D_2 receptor subtypes within the VP/SI? 5) Does VTA/SNc stimulation modulate AMN-evoked activity of VP/SI neurons? 6) Does exogenously-applied DA within the VP/SI mimic the effects of VTA/SNc stimulation on VP/SI neuronal activity?

Surgical Preparation of Animals

Male Sprague-Dawley rats weighing 250-350 g were anesthetized with chloral hydrate (400 mg/kg, administered intraperitoneally; Sigma Chem. Co, St. Louis, MO). A lateral tail vein was cannulated for intravenous injection of anesthetic supplements to maintain surgical levels of anesthesia. The animals were then placed into a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the nose piece set at 3.3 mm below the horizontal, and the skull exposed. Coordinates used for recording the VP/SI were 0.5 mm posterior to Bregma (P), 2.5 mm lateral to the midline (L) and 7.5-8.5 mm below dura (V). Those for the VTA/SNc were 5.7 mm P, 1.0 mm L for VTA and 1.6-2.0 mm L for SNc, 7.8-8.0 mm and 7.6-7.8 mm V for VTA and SNc, respectively. Coordinates for the AMN were 2.8 mm P, 4.8 mm L and 7.2-7.5 mm V. Rectal temperature of the animals was monitored throughout the experiments, and maintained at 35-37°C with a thermostatically controlled heating pad (Fintronics Inc., Orange, CT).

Electrical Stimulation

Stainless steel concentric bipolar electrodes (NEX-100, 0.5 mm shaft diameter and 0.5 mm tip separation; David Kopf Instruments) were used for delivery of electrical stimulation, generated by Grass S88 stimulators, each coupled to a Grass stimulation isolation unit (SIU 5) and a Grass constant current unit (CCU 1; Grass Instrument Co., Quincy, MA). For experiments involving single pulse stimulation to the AMN or VTA/SNc, monophasic pulses of 0.1 ms duration were applied at a frequency of 1 Hz and with a current range of 0.05-1.5 mA (see Fig. 9). For experiments in which the interaction of VTA/SNc with AMN on the activity of VP/SI neurons was examined, the stimulation was sequenced as follows: 1) the VTA/SNc was stimulated with a train

consisting of 10 pulses, each 0.1 ms in duration and the pulses within the train occurred at a frequency of 10 Hz. 2) Following a delay of 100 ms, a single pulse of 0.1 ms duration was delivered to the AMN. 3) The effect of this stimulation on VP/SI neuronal activity was recorded for approximately 900 ms. 4) This entire VTA/SNc train - AMN single pulse sequence was repeated at a rate of once every 2 s for 128 stimulation epochs.

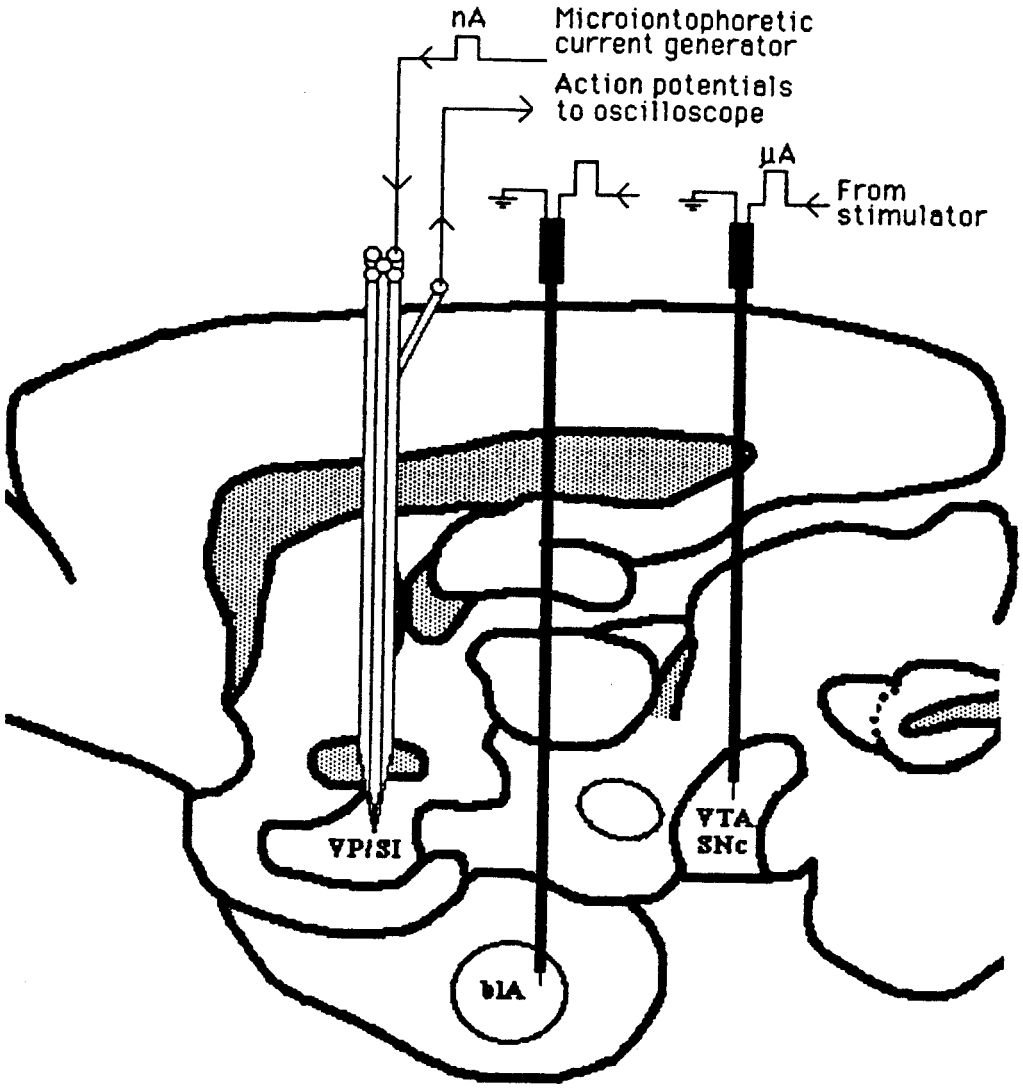

Fig. 9. SCHEMATIC DRAWING ILLUSTRATING THE ORIENTATION OF THE MICROIONTOPHORETIC RECORDING ELECTRODE IN THE VP/SI AND THE STIMULATING ELECTRODES IN THE AMYGDALA AND VENTRAL TEGMENTAL AREA (VTA) OR THE SUBSTANTIA NIGRA, PARS COMPACTA (SNC). A single glass pipette used for recording action potentials within the VP/SI was attached to five-barrel glass pipette that was used for microiontophoresis of dopaminergic agonists and antagonists. Bipolar electrodes were used for: 1) single pulse, orthodromic stimulation of the amygdaloid nuclei, often within the basolateral nucleus of the amygdala (blA), and 2) either single pulses or a train of pulses to orthodromically stimulate the VTA or SNc. See text for detailed description of methods. nA - nanoAmps of current used to eject drugs from the microiontophoretic electrode; µA - microAmps of current used for orthodromic stimulation of blA or VTA/SNc.

Extracellular Single-Neuron Recordings and Microiontophoresis

Standard extracellular recording of single neurons and microiontophoresis techniques were employed as described previously (Fig. 9; Napier *et al.*, 1991b). Pre-assembled five-barrel glass pipettes (A-M Systems, Inc., Everett, WA) were pulled with a vertical electrode puller (Narishige PE-2, Tokyo, Japan) and the tips broken back to 8-12 μm. A single barrel pipette, with a tip diameter of approximately 2 μm, was then glued in parallel to the five barrel assembly such that the single barrel was positioned below the five barrels by 8-15 μm. The single barrel, filled with 2 M NaCl saturated with fast green dye (Fisher Scientific Co., St. Louis, MO), served as the recording electrode. The *in vitro* impedance of the recording barrel was 3-6 MΩ, measured at 165 Hz with a micro-electrode tester (Winston Electronics, San Francisco, CA). The center barrel of the five-barrel pipette also was filled with 2 M NaCl saturated with fast green dye, and was using for automatic current balancing of the other barrels.

The remaining four barrels each contained one of the following drugs: DA hydrochloride (0.2 M, pH 4; Sigma), quinpirole (QUIN; LY171555; trans-(-)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H (or 2H)-pyrazolo-(3,4-g) quinoline monochloride; 0.01-0.02 M, pH 4; Lilly Research Lab., Indianapolis, IN), SCH23390 (SCH; (R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hemimaleate; 0.01 M, pH 4; Schering Corp., Bloomfield, NJ), SKF38393 (SKF; 2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine-7,8-diol hydrochloride; 0.01 M, pH 4; Research Biochemicals Inc., Natick, MA), and sulpiride (SUL; 5-(aminosulfonyl)-N-((1-ethyl-2-pyrrolidinyl) methyl)-2-methoxybenzamide; 0.02 M, pH 4; Sigma). The high concentration of agonists and antagonists used were selected to allow the passage of ionized drug through high resistance (20-60MΩ) barrels of the microiontophoretic pipette. Microiontophoresis of these agents (some of which have low solubility)

necessitates the use of saturable concentrations to achieve satisfactory conductance (Duggan, 1983; Salmoiraghi and Weight, 1967), and to minimize electrical noise on drug ejection (Duggan, 1983). All drugs were dissolved in sterile deionized water, but SCH and SUL were solubilized with HCl before being diluted to the final volume with deionized water, and then 10N NaOH was added until a pH of 4 attained.

In some experiments, the D_1/D_2 dopamine receptor antagonist, flupentixol (4-[3-[2-(trifluoromethyl)-9H-thioxanthen-9-ylidene] propyl]-1-piperazine ethanol dihydrochloride; Research Biochemical Inc.) was injected through a lateral tail vein cannula at a dose of 0.5 mg/kg. A concentration of 1 mg/ml of flupentixol was dissolved in sterile water, and a pH of 4 was established by addition of 10N NaOH.

For recording VP/SI neurons, the electrode assembly was lowered to 7.0 mm V, and a six-channel current generator and programmer (Fintronics, Inc.) was used to apply maximal ejection currents to each barrel for at least 30 min to concentrate the drugs at the pipette tips. Drugs were ejected with cationic currents, and negative retaining currents of 10 nA were applied to drug barrels between ejection periods. Action potentials were sampled from the VP/SI (7.5-8.5 mm V) using a hydraulic microdrive (Trent Wells, South Gate, CA), were filtered (200 Hz and 2 KHz) via a high-impedance amplifier (Fintronics, Inc.), and monitored on an oscilloscope (Tektronix Inc., Beaverton, OR) and audiomonitor (Grass Instruments Co.). To quantify the neuronal activity, the digital output from the window discriminator (Fintronics, Inc.) was transmitted to an IBM AT compatible computer, and Brainstorm Systems Spikes to Stats software (Chapel Hill, NC) was used to display rate histograms and to store and analyze data.

Data Collection and Analysis

For experiment 1, it was determined if VP/SI neuronal responses to microiontophoretically-applied DA were mediated through the D_1 and/or the D_2 DA receptor subtype within the VP/SI by assessing DA response attenuation produced by locally applied SCH (a D_1 antagonist) and/or SUL (a D_2 dopamine antagonist). Whether the DA-mediated effects could be mimicked by the D_1 DA receptor agonist, SKF, and/or the D_2 dopamine receptor agonist, QUIN also was examined. The following protocol was used: 1) A spontaneously active VP/SI neuron was isolated and stable pretreatment activity was obtained. 2) The agonist often was applied with an ejection current of 100 or 120 nA for at least 1 min. 3) If this current produced a rate change of greater than 20% of pretreatment activity that was reversible and reproducible, the agonist was considered effective. When the current tested affected VP/SI neuronal activity, often lower ejection currents were tested to determine if a current-response relationship existed. If the maximal ejection current did not produce a rate change of greater than 20% of control rates after being tested three consecutive times, the neuron was considered insensitive to agonist. 4) After an agonist response was characterized, either SCH or SUL was applied for at least 5 min prior to, and during the ejection of the agonist. 5) An attenuation of the agonist-induced response of greater than 20% was considered antagonism. The other antagonist was tested to verify that the agonist response was receptor subtype specific.

To evaluate the responses of VP/SI neurons subsequent to electrical activation of the VTA/SNc and/or the AMN, the following protocol was used: 1) A spontaneously active VP/SI neuron was isolated and pre-stimulation activity was monitored. 2) VTA/SNc or AMN stimulation-evoked effects on VP/SI activity were determined using a stimulation current that was shown in preliminary stimulation experiments to be effective for these regions (*i.e.*, 0.3 and 0.5 mA for VTA/SNc and AMN, respectively).

3) If an evoked response occurred in the VP/SI neuron (criteria for evoked response is defined below), the procedure was repeated at lower stimulation current to determine the response threshold. In contrast, if the VP/SI neuron was insensitive, currents up to 1.5 mA were used, after which an unresponsive neuron was considered insensitive, and was not studied further. 4) Often the VP/SI neuron was assessed for sensitivity to stimulation of the other brain region (for example, VTA/SNc stimulation would then be followed by AMN stimulation) to determine if this VP/SI neuron expressed convergent effects from these two brain regions. 5) DA was microiontophoretically-applied as described above. If VP/SI neuronal activity was altered by DA, the SCH or SUL were co-applied with DA. The antagonist ejection current necessary to attenuate the effects of DA was used to evaluate the effectiveness on the VTA/SNc evoked VP/SI response. If DA either had no effect on VP/SI activity or the antagonist (s) did not attenuate the DA-induced effect, the maximal ejection current that did not produce nonspecific membrane effects (*i.e.*, without reducing the amplitude or widening the duration of the action potential) was used for antagonist ejection during the period of VTA/SNc stimulation. 6) During antagonist application, VTA/SNc stimulation was repeated using a stimulation current that previously evoked a response on VP/SI neuronal activity that was clearly above threshold but not the maximum effect observed (i.e., a stimulation current producing approximately 50% of the maximal evoked response over the range of stimulation currents tested for that neuron, or ECu_{50}).

For experiment 2, which characterized the effects of VTA/SNc stimulation and DA application on the AMN-evoked responses of VP/SI neurons, the following protocol was used. 1) A spontaneously active VP/SI neuron was determined to be sensitive to both VTA/SNc and AMN stimulation. 2) DA was microiontophoretically-applied during AMN stimulation using an ECu_{50} for the AMN. 3) Subsequently, a train of 10 pulses was delivered to the VTA/SNc, at stimulation currents at or below ECu_{50},

100 ms prior to AMN stimulation to examine whether VTA/SNc stimulation could attenuate the AMN-evoked VP/SI responses. A train of pulses was used for activating VTA/SNc neurons, even though dopaminergic neurons in these regions fire with either single spikes or in bursts of up to 10 pulses (Grace and Bunney, 1984a,b), since biochemical studies indicate that the latter firing pattern elicits higher concentrations of extracellular DA (Gonon, 1988; Gonon and Buda, 1985; Manley *et al.*, 1992). In addition, Yim and Mogenson (1982) observed that while both single or train stimulation of the VTA is sufficient to evoke NA responses, only train stimulation is capable of attenuating the NA excitatory responses to AMN stimulation.

A peristimulus rate histogram was generated for each sample of 128 stimulation epochs, using a bin width of 2 ms. To test for significance in the evoked responses, the mean number of action potentials (or counts) per bin occurring 80-100 ms before the stimulation was determined and considered as the control interstimulus baseline. The onset of an evoked response was delineated by the first of three consecutive bins with counts that were greater than 1 standard deviation from the control mean, and the offset was similarly defined by the first of three consecutive bins with a count within 1 standard deviation of the control mean (similar to methods used in Yim and Mogenson, 1983). Counts occurring within this time period were compared to the number of counts occurring within this same time period after antagonist administration (or, in experiment 2, the counts observed after AMN stimulation was compared for the same boundary after dopamine application or VTA/SNc stimulation).

In some VP/SI neurons, drug application and/or stimulation of the VTA/SNc prior to AMN stimulation (considered "treatment") altered the interstimulus baseline. To compensate for any influence this baseline change might have had on action potential occurrence during the evoked response period, a more stringent criterion was determined by calculating an "expected value" (E) as described previously (Napier *et al.*,

1983). E was defined as the number of action potentials (or counts) which would be predicted to occur in the evoked period if the treatment did not alter interstimulus baseline. To calculate E, the mean counts/bin occurring during the 80-100 ms period prior to stimulation in the treatment sample (*i.e.*, mean counts/bin of the interstimulus baseline for the treatment) was divided by the mean counts/bin during the same period of the interstimulus baseline in the control sample; this value was multiplied by the number of counts occurring during the evoked period in the control sample (*i.e.*, (mean counts/bin for the treatment interstimulus ÷ mean counts/bin for the control interstimulus) X (mean counts/bin for the evoked response during control stimulation)). E then was compared to the mean counts/bin actually observed during the evoked period in the treatment sample. If the ratio of the observed counts to E was altered by greater than 25%, the treatment was considered effective.

Statistics

The magnitude of agonist-induced alterations of VP/SI firing rate is presented as mean ± S.E.M. Comparisons of the magnitude of agonist-induced effects on VP/SI neuronal activity for different DA agonists was assessed by an analysis of variance (ANOVA) followed by a post-hoc Newman Keuls test. Paired *t* -tests are used to assess differences in VP/SI neuronal activity induced by agonist application versus agonist-induced effects with concomitant application of antagonists. Chi-square analysis was used to detect changes in: 1) the distribution of VP/SI responses to microiontophoretically-applied agonists, 2) the number of VTA/SNc-evoked VP/SI responses that were sensitive or insensitive to the different antagonist treatments, and 3) the number of AMN-evoked VP/SI responses that were attenuated or insensitive to DA application versus VTA/SNc stimulation. The criterion of significance for all statistical tests was $p < 0.05$.

Histological Procedures

At the end of each experiment, the rat received an overdose of chloral hydrate intravenously. The location of the last recording site was marked with fast green dye by passing anionic current through the recording barrel of the electrode for 10-20 min. The brain was removed, mounted, frozen and then cut with a microtome to locate the electrode tracks as well as the fast green dye deposit. The brain sections were stained with cresyl violet and the location of each recording site was determined using the fast green spot and electrode tracks as reference points. The location of the stimulation sites were assessed from the position of stimulation electrode tracks and reconstructed on stereotaxic maps (according to Paxinos and Watson, 1986).

Results

Characteristics of Spontaneously Active VP/SI neurons

The data for this study were assessed for recordings of 233 neurons that were histologically verified to be within the ventral pallidum/substantia innominata (VP/SI; e.g., Fig. 10). All of the VP/SI neurons monitored were spontaneously active with a mean discharge rate of 12.2 ± 0.63 spikes per second. The most frequently encountered recording demonstrated a firing pattern with a broad interspike interval histogram denoting an irregular distribution of action potential occurrence intervals. Of the total recorded neurons, 211 (91%) displayed biphasic and 22 (9%) had triphasic action potentials. The mean duration of the action potentials was 1.2 ± 0.02 ms, with a

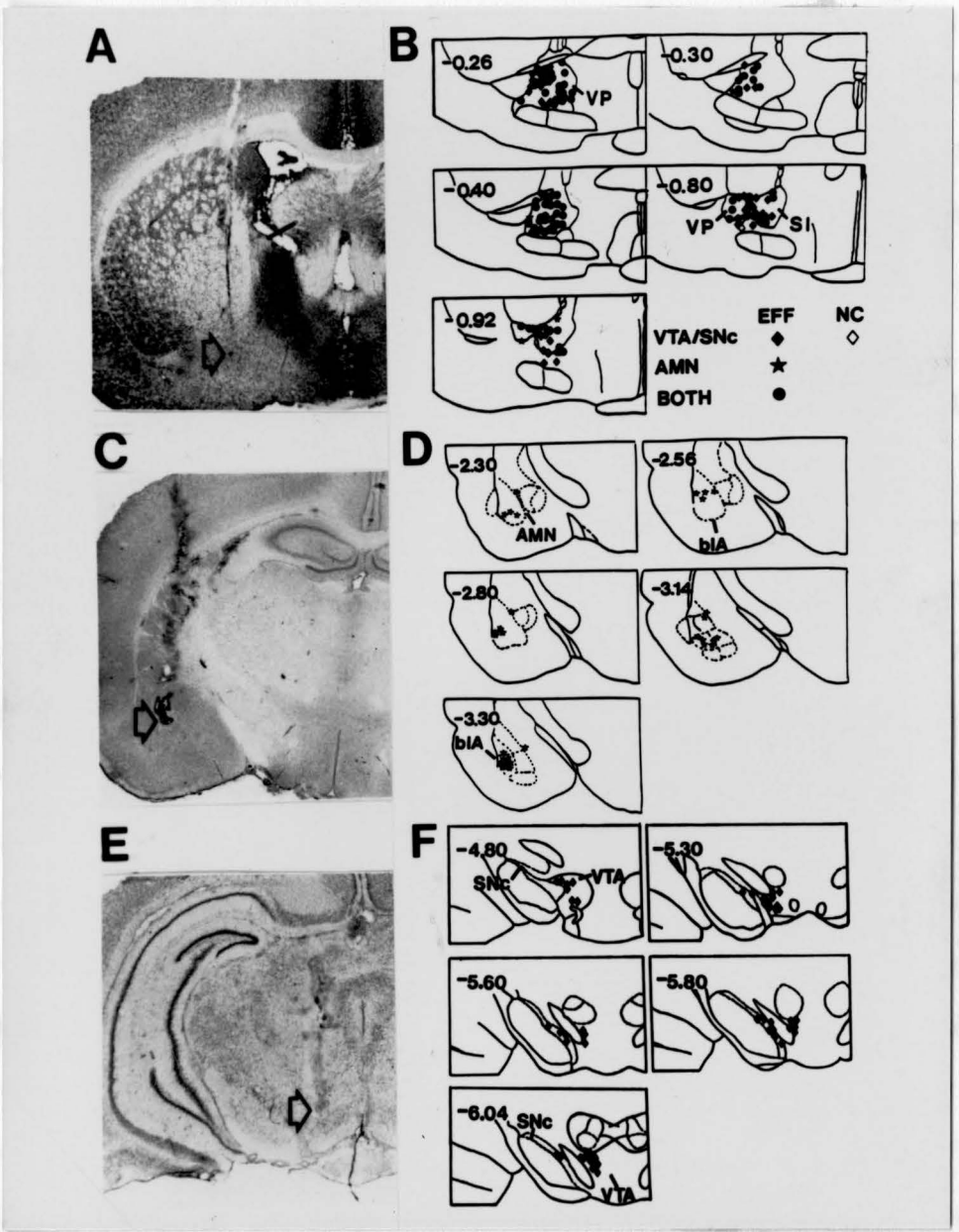

Fig. 10. LOCATION OF RECORDING SITES IN THE VP OR THE SI, AND STIMULATION SITES WITHIN THE AMN AND THE VTA OR SNc. (A, C, E) are cresyl violet stained coronal sections that are representative photomicrographs of the recording and stimulation sites. The arrows: in (A) indicates the position of the fast green dye spot, in (B, E) indicate the position of the stimulating electrode. The right column denotes individual recording and stimulation sites on maps redrawn from Paxinos and Watson (1986). Many recording sites overlapped. Numbers in the left corner indicate the distance (mm) from Bregma. Sections in (A, C, E) are denoted in the - 0.80, - 2.56, and - 5.30 maps in (B, D, F), respectively. In (B), filled symbols indicate effective (EFF) and clear symbols indicate no change (NC) after stimulation of VTA/SNc (diamonds), of AMN (stars), or convergence (circles). All VP/SI neurons responded to at least one of the AMN stimulation currents tested.

peak to peak amplitude of 338 ± 22 μV. One hundred ninety-eight recordings (85%) had initially negative action potential waveforms, and the remaining 35 (15%) had initially positive waveforms. The recordings obtained in this study had similar characteristics to those from previous studies on the effects of dopaminergic agents on VP/SI firing rate (Maslowski and Napier, 1991a,b; Napier *et al.*, 1991b; Napier, 1992).

Effects of D_1 and/or D_2 DA Receptor Activation on VP/SI Neuronal Firing Rate

In agreement with our previous studies (Napier and Potter, 1989; Napier *et al.*, 1991b), approximately half of the VP/SI neurons tested were sensitive to microiontophoretic application of DA; responding with both rate suppressions and excitations (Fig. 11 and Table 4). Since DA can mediate responses through both D_1 and D_2 receptor subtypes, the D_1 DA agonist, SKF and the D_2 DA agonist, QUIN, were applied onto VP/SI neurons to determine whether separate activation of either receptor subtype was sufficient to affect VP/SI neuronal firing rate. VP/SI neurons responded to SKF with rate suppressions more often and with a greater magnitude than to either DA or QUIN (Table 4 and Table 5). Of the VP/SI neurons tested, 40% were inhibited by SKF but only 15% were inhibited by QUIN. The reverse was observed for VP/SI neuronal excitations, which occurred in only 7% of the neurons tested with SKF, but in 24% of the neurons tested with QUIN. In addition, of the 20 VP/SI neurons tested with both agonists, 1 each demonstrated inhibition, excitation, or opposite effects to both agonists; 5 were inhibited and 1 was excited only by SKF; 3 were excited only by QUIN, and 8 were not affected by either of these compounds.

VP/SI neurons often were insensitive to QUIN, even when the concentration of QUIN was twofold that of SKF (0.02 M versus 0.01 M, respectively). Higher QUIN ejection currents used for unresponsive neurons frequently produced action potential

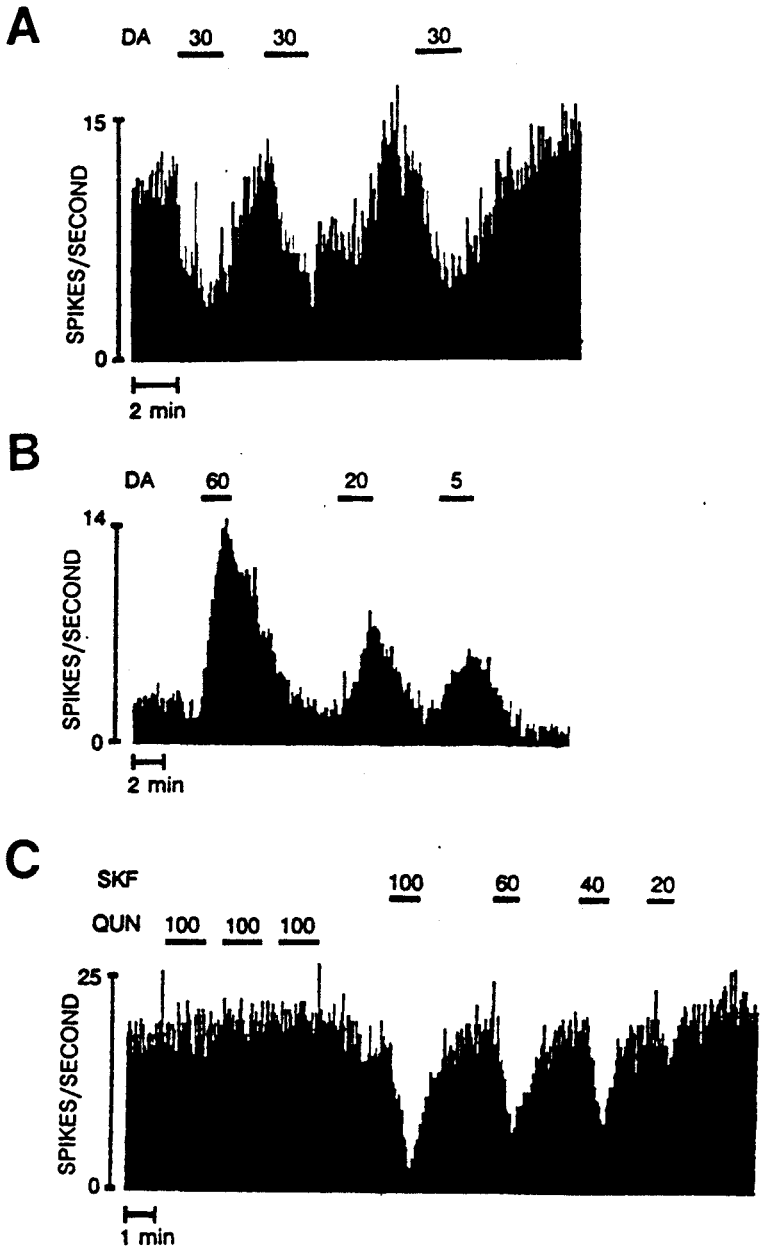

Fig. 11. CUMULATIVE RATE HISTOGRAMS ILLUSTRATING THE EFFECTS OF MICROIONTOPHORETIC APPLICATION OF DA, SKF AND QUN ON THE FIRING RATE OF VP/SI NEURONS. DA application induced both rate suppression (A) or rate excitation (B). SKF application typically induced a decrease in VP/SI firing rate that was current-dependent, but QUN application often did not alter activity (C). Drugs were applied during the time indicated by the horizontal bars above the histograms, and the magnitude of the ejection current is represented by number (in nanoamps) above the bar.

TABLE 4

DISTRIBUTION[a] OF AGONIST-INDUCED RESPONSES[b] OF VP/SI NEURONS

Agonist	Response Category		
	Decrease	Increase	No Effect
Dopamine (0.2 M; 5 - 120 nA)	21/93 (23 %)	26/93 (28 %)	46/93 (49 %)
SKF38393[c] (0.01 M; 10 - 120 nA)	12/30 (40 %)	2/30 (7 %)	16/30 (53 %)
Quinpirole (0.01 - 0.02 M; 10 - 120 nA)	5/33 (15 %)	8/33 (24 %)	20/33 (61 %)

[a] The ratio of the number of neurons affected by the agonist divided by the number tested.

[b] A significant decrease or increase in ventral pallidal firing rate was defined as a change of greater than 20% of baseline activity. No effect was determine as a change of less than 20% of baseline activity with agonist ejection currents ≥ 100 nA.

[c] Chi-square analysis of the number of neurons in each response category following the application of SKF38393 differed from that after dopamine or quinpirole application ($df = 2$; $\chi^2 = 7.15$ or 6.80, respectively; $p < 0.05$).

changes indicative of nonspecific membrane effects. In addition, the onset of SKF-induced inhibitory responses of VP/SI neurons occurred with significantly lower ejection currents (N = 12, mean current: 40 ± 7 nA) than QUIN-induced excitations (N = 8, mean current: 76 ± 15 nA; $df = 18$, $t = -2.42$, $p < 0.05$) or suppressions (N = 5, mean current: 92 ± 14; $df = 15$, $t = 3.75$, $p < 0.01$).

Subsequent applications of the D_1 receptor antagonist, SCH, or the D_2 receptor antagonist, SUL, were used to assess whether the effects of DA on VP/SI neuronal activity were mediated through D_1 and/or D_2 receptors, as well as the receptor specificity of SKF- and QUIN-mediated effects on VP/SI firing rate (Fig. 12).

TABLE 5

MAGNITUDE OF AGONIST-INDUCED RESPONSES OF VENTRAL PALLIDAL NEURONS

Agonist (100 or 120 nA) [a]	Response Category (% of control) [b]	
	Decrease	Increase
Dopamine (0.2 M)	59.79 ± 4.03 (N = 16)	145.22 ± 6.81 (N = 16)
SKF38393 (0.01 M)	38.77 ± 6.79 [c] (N = 11)	147.30 (N = 1)
Quinpirole (0.01-0.02 M) (N = 16)	69.63 ± 4.81 (N = 4)	133.70 ± 2.16 (N = 4)

[a] Data include only the response of neurons that were tested with the agonist at maximum ejection current.

[b] Control (pretreatment) rate was standardized to 100%.

[c] The magnitude of the SKF38393-induced decrease differs from both dopamine and quinpirole (ANOVA, df = 30; F = 6.15; $p < 0.01$, and significant Newman Keul's post-hoc analysis, df = 1; $q = 4.18$ and 4.13; $p < 0.05$).

Suppression of VP neuronal activity by DA or SKF was attenuated by SCH (Fig. 12A). In addition, SCH attenuated DA-induced excitations (Fig 12B). SKF-induced rate excitations (N = 2) were not tested with antagonists. SCH did not antagonize QUIN-induced rate inhibitions or excitations (Fig. 12A-B). SUL attenuated DA-induced rate suppressions (Fig. 12C) but, in contrast to SCH, SUL application was not sufficient to attenuate DA-mediated increases (Fig. 12D). Excitations induced by QUIN were antagonized by SUL (Fig. 12D). Thus, DA-induced effects on VP/SI neuronal activity were mediated through either D_1 or D_2 receptor subtypes, but SKF was specific for D_1 receptor-mediated rate suppressions and QUIN was specific for D_2 receptor-mediated

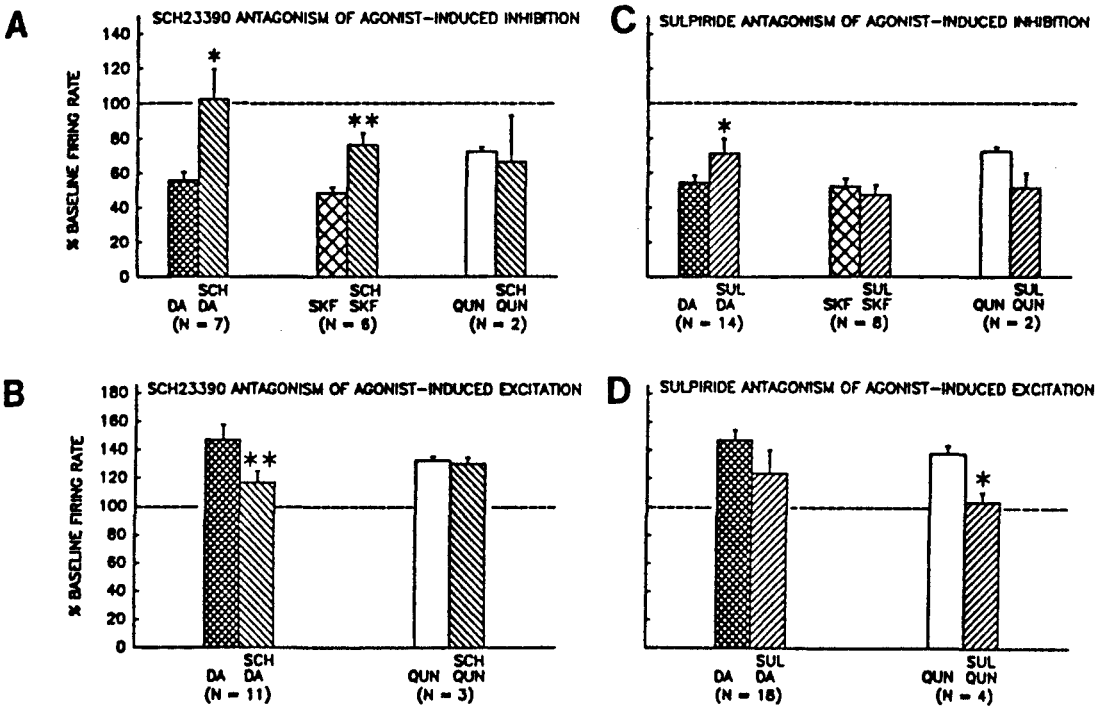

Fig. 12. BAR GRAPHS DEPICTING THE MAGNITUDE OF ATTENUATION OF DA-, SKF- AND QUN-MEDIATED EFFECTS ON FIRING RATE OF VP/SI NEURONS BY CONCOMITANT APPLICATION OF SCH OR SUL. Only those VP/SI neurons tested with both agonist and antagonist are included. DA- (0.2 M; 5-120 nA) and SKF- (0.01 M; 10-120 nA) induced VP/SI rate suppressions (A) were attenuated by SCH (0.01 M; 10-100 nA) application (Paired t - test, df = 6, t = -2.60, $p < 0.05$ for DA vs. SCH; and df = 5, t = -5.02, $p < 0.01$ for SKF vs. SCH). (B) SCH also attenuated DA- but not QUN-mediated VP/SI rate excitations (df = 10, t = 4.28, $p < 0.01$ for DA vs. SCH). SKF induced rate excitations in only 2 VP/SI neurons that were not subsequently tested with antagonists. (C) SUL (0.02 M, 10-120 nA) attenuated DA-induced rate suppression of VP/SI neurons (df = 13, t = -2.13 , $p < 0.05$). (D) QUN-mediated excitations were antagonized by SUL (df = 3, t = 5.49, $p < 0.05$). Horizontal lines indicate control level of neuronal activity (considered as 100%).

rate excitations. Microiontophoretic application of vehicle solutions for agonists (as well as antagonists) did not alter the firing rate of VP/SI neurons (N = 10; data not shown).

Responses of VP/SI Neurons to Single Pulse Stimulation of the Midbrain Dopaminergic Region

Figure 10 illustrates the location of the VP/SI neurons assessed for sensitivity to stimulation of VTA/SNc (Fig. 10A,B), and the stimulation sites within these midbrain dopaminergic regions (Fig. 10E,F). Of 138 VP/SI neurons tested, 135 were sensitive to midbrain stimulation of the ventral tegmental area VTA (N = 82) or the substantia nigra pars compacta SNc (N = 53). Stimulation of the VTA or the SNc produced similar number and types (i.e., inhibition or excitation) of evoked VP/SI responses, and the distribution onset latencies of each response type overlapped. Therefore, the results of activating these brain regions were combined. Although some VP/SI neurons responded to VTA/SNc stimulation with simple inhibition (Figs. 13A-C, 14, 15, 16) or excitation, 60% the sensitive VP/SI neurons exhibited a complex sequence of evoked responses (Fig. 13D-F and Table 6). The distribution of the latencies of the inhibitory responses was bimodal, with one peak in the range of 4 to 6 ms, and the other from 15 to 21 ms. The distribution of the latencies of the excitatory responses was also bimodal, with one peak in the range of 4 to 6 ms, and the other from 28 to 30 ms. The most frequently observed response was an inhibition with a range of onset latencies from 2 to 12 ms (Fig. 13A-C and Table 6). Since these distributions may indicate the involvement of distinct midbrain efferents to the VP/SI for the evoked response observed, the response were categorized into short ($\leq$ 12 ms) or long ($>$ 12 ms) latency, as well as inhibitions or excitations (Table 6).

The effects of VTA/SNc stimulation on VP/SI neuronal activity were assessed further in 111 VP/SI neurons to determine if there was a relationship between the characteristics of the evoked response and increasing stimulation intensity to the VTA/SNc. The duration of the VP/SI inhibitory responses was augmented by increasing the stimulation intensity to the VTA/SNc (Fig. 14A-C) suggesting the recruitment of additional inhibitory inputs. Similarly, VP/SI neurons that displayed an initial excitatory evoked response, often exhibited a secondary inhibition whose duration was augmented by increasing the stimulation currents. In 7 VP/SI neurons tested with VTA/SNc stimulation, currents up to 0.8 mA appeared to be restricted to a distance of 1 mm, since VP/SI evoked responses were terminated when the stimulating electrode was moved 1 mm from the "active site", and greatly attenuated when moved 0.5 mm away (Fig. 15).

DA Receptor Subtypes Involved with VP/SI Responses to VTA/SNc Stimulation

VP/SI responses to VTA/SNc stimulation often were mimicked by the effects of microiontophoretic application of DA on the firing rate of VP/SI neurons. Of the VP/SI neurons that responded to both VTA/SNc stimulation and microiontophoretic DA, 100% of the VTA/SNc-evoked inhibitory VP/SI responses also demonstrated DA-induced rate suppressions (N = 13), and 60% of VTA/SNc-evoked excitatory VP/SI responses, demonstrated DA-induced rate excitations (N = 15), and the remaining 40% displayed DA-induced rate inhibitions.

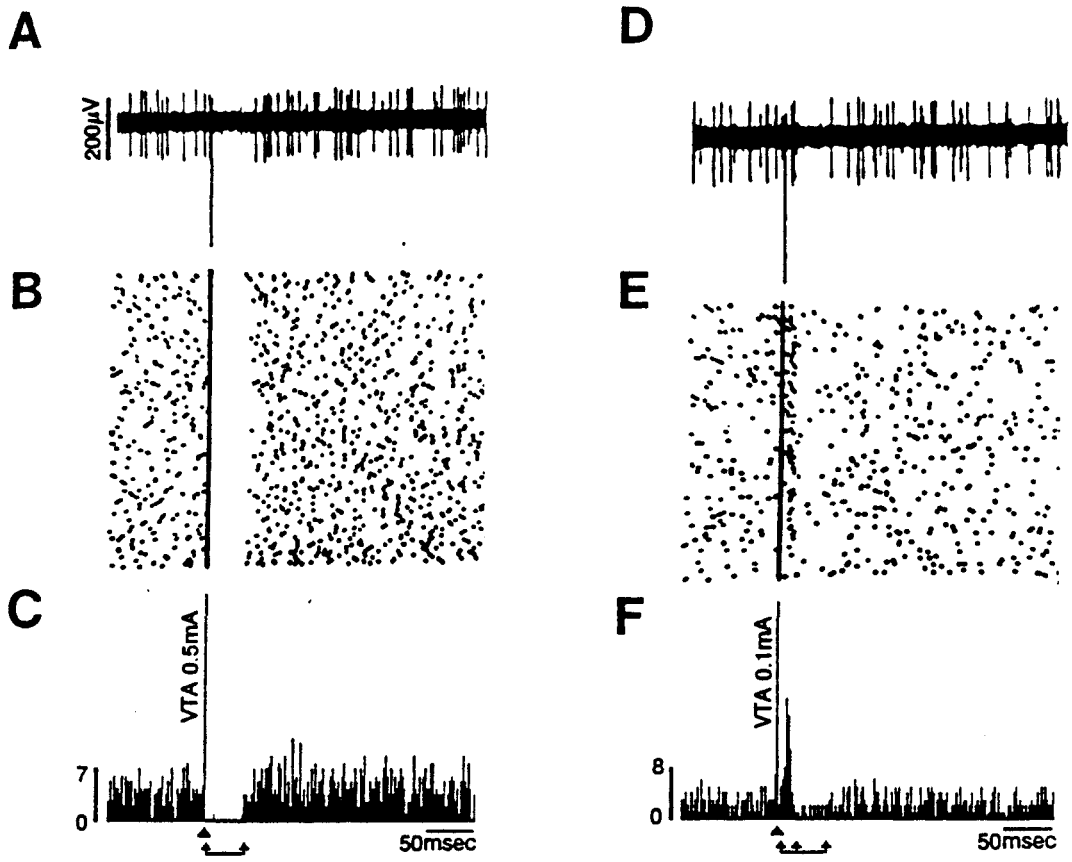

Fig. 13. EXAMPLES OF VP/SI RESPONSES TO SINGLE PULSE STIMULATION OF THE VTA. The most frequent response to VTA/SNc stimulation was a short latency inhibition depicted by a VP/SI neuron in (A-C). VTA stimulation also evoked short latency excitation in the activity of second VP/SI neuron in (D-F). Oscilloscope traces, composed of 10 superimposed sweeps (A, D); raster stepper recordings composed of 128 consecutive sweeps (B, E); and peristimulus rate histograms (C, F) of the same 128 sweeps as in B and E, respectively. Triangle indicates the stimulus artifact. Connected arrows indicate the onset and offset of VP/SI evoked responses. Vertical bars in (C and F) indicate the number of action potentials per 2 ms time bin. Stimulation parameters: 0.1 ms; 1 Hz; A-C. 0.5 mA; D-F. 0.1 mA.

Table 6

SUMMARY OF VENTRAL PALLIDAL EVOKED RESPONSES TO STIMULATION OF THE VENTRAL TEGMENTAL AREA/SUBSTANTIA NIGRA PARS COMPACTA AND AMYGDALA

Region Stimulated	Number of Neurons Sensitive to Stimulation [b]	Response Category [a]			
		Short Latency Inhibition (≤ 12 ms)	Short Latency Excitation (≤ 12 ms)	Long Latency Inhibition (> 12 ms)	Long Latency Excitation (>12 ms)
VTA/SNc	135/138	80/135 (59%)	41/135 (30%)	70/135 (52%)	70/135 (52%)
AMN	86/ 86	39/ 86 (45%)	42/ 86 (49%)	67/ 86 (78%)	52/ 86 (60%)

[a] The response categories were defined by the latency in ms to the onset of a particular evoked response, as described in the Methods.

[b] Only 3 VP/SI neurons were insensitive to VTA/SNc stimulation (stimulation current range: 0.5 to 1 mA) and were not included in the table. Sixty percent of VP/SI neurons responding to VTA/SNc stimulation and 80% responding to AMN stimulation exhibited a complex response sequence consisting of more than one evoked response. Thus, the sum of the percents is greater than 100, since the occurrence of each response is indicated in the numerator and each neuron tested is indicated in the denominator.

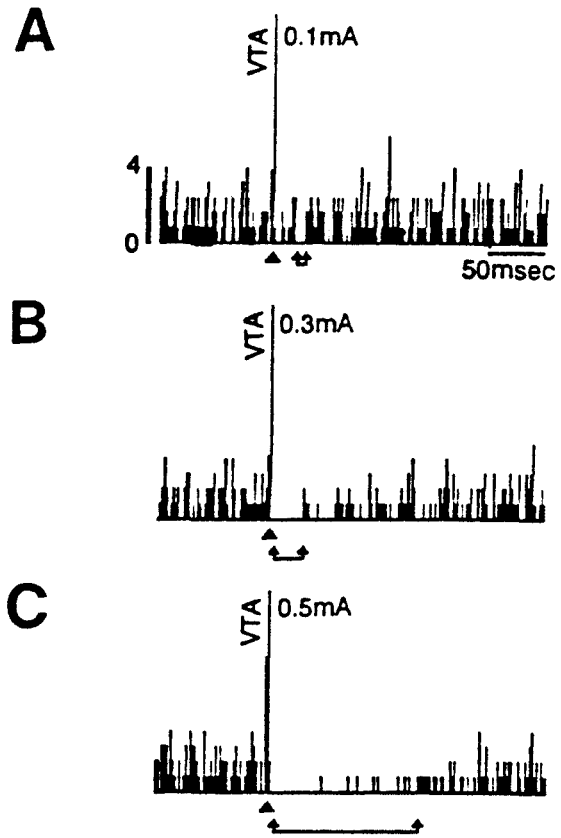

Fig. 14. AN EXAMPLE OF A VTA STIMULATION CURRENT-DEPENDENT INHIBITORY RESPONSE IN THE SAME VP/SI NEURON. As the current used to stimulate the VTA (0.1 ms, 1 Hz) was increased from 0.1 mA to 0.3 mA, a short latency inhibitory response was evoked in the VP/SI neuronal activity (A-B). With an increase of stimulation current to 0.5 mA the duration of the short latency inhibitory response increased to greater than 100 ms (C). Triangles indicate the stimulus artifact, the arrows depict the onset and offset of the VP/SI evoked responses. The vertical bar indicates the number of action potentials per 2 ms time bin.

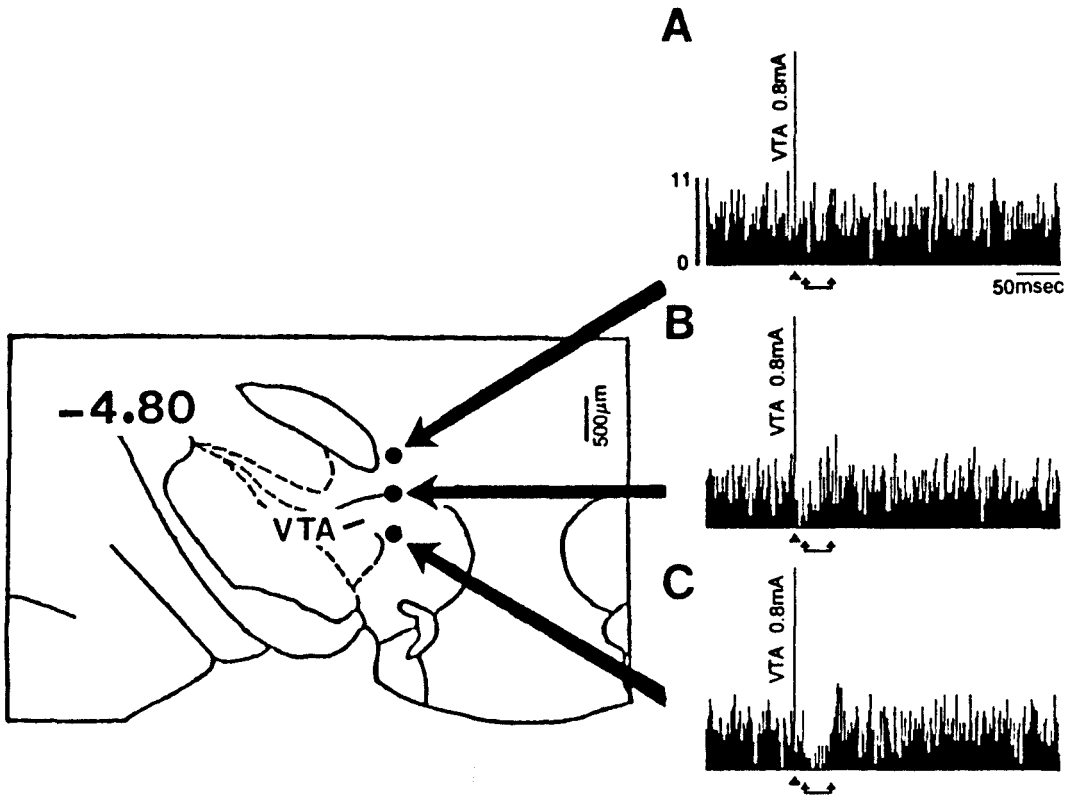

Fig. 15. RELATIONSHIP BETWEEN THE LOCATION OF THE MIDBRAIN STIMULATING ELECTRODE AND THE EVOKED VP/SI RESPONSE. VTA stimulation did not alter firing when the stimulating electrode was 1 mm above the active site within the VTA (A), but produced a slight inhibitory response when the stimulating electrode was located within 0.5 mm of this site on the periphery of the VTA (B). When the stimulating electrode was placed near the center of the VTA a VP/SI long latency inhibitory response of about 30 ms duration was evoked (C). The number in the upper left corner of the stereotaxic map represents the distance from Bregma. Triangles indicate the stimulus artifact, and the connected arrows depict the onset and offset of the inhibitory response to stimulation of the center of the VTA area (C). The vertical bar on the peristimulus histograms indicates the number of action potentials per 2 ms bin. Stimulation parameters were 0.1 ms; 1 Hz; 0.8 mA.

To determine the receptor subtype (s) involved in VP/SI responses to VTA/SNc stimulation, SCH or SUL was microiontophoretically applied within the local milieu of the recorded VP/SI neuron during the period of VTA/SNc stimulation. Figure 16 illustrates a typical VP/SI response to VTA/SNc stimulation (short latency inhibition), an attenuation by SCH, as well as the less frequently observed, and more modest attenuation by SUL. Seventeen of 20 evoked responses attenuated by SUL also were antagonized by SCH, but simultaneous application of the antagonists did not induce a greater magnitude of attenuation. Evoked responses that were not attenuated by either antagonist alone could not be attenuated by concurrent application of the antagonists (Table 7). However, intravenous administration of the D_1/D_2 DA antagonist, flupentixol was sufficient to attenuate the VP/SI short latency inhibitory responses in all 4 neurons tested (Table 7). In addition to the short latency inhibitions, both short and long latency excitations evoked in the VP/SI were attenuated by SCH or by SUL (Table 7).

Effects of VP/SI Neurons to Single Pulse Stimulation of the AMN

Figure 10 illustrates the location of VP/SI neurons affected by stimulating the AMN (Fig. 10A,B). Most of the AMN stimulation sites were within the basolateral nucleus (Fig. 10C,D), which evoked VP/SI responses with the lowest stimulation intensity. The evoked responses were not antidromically mediated since the criteria of constant onset latency and ability to follow pulse-pair stimulation at high frequency was not fulfilled (Lipiski, 1981). The distribution of latencies of evoked VP/SI inhibitory responses was bimodal, with one peak between 2 to 6 ms, and the other from 16 to 18 ms. The distribution of latencies of evoked VP/SI excitatory responses also was bimodal, with one peak occurring between 4 to 12 ms, and the other from 16 to 18 ms.

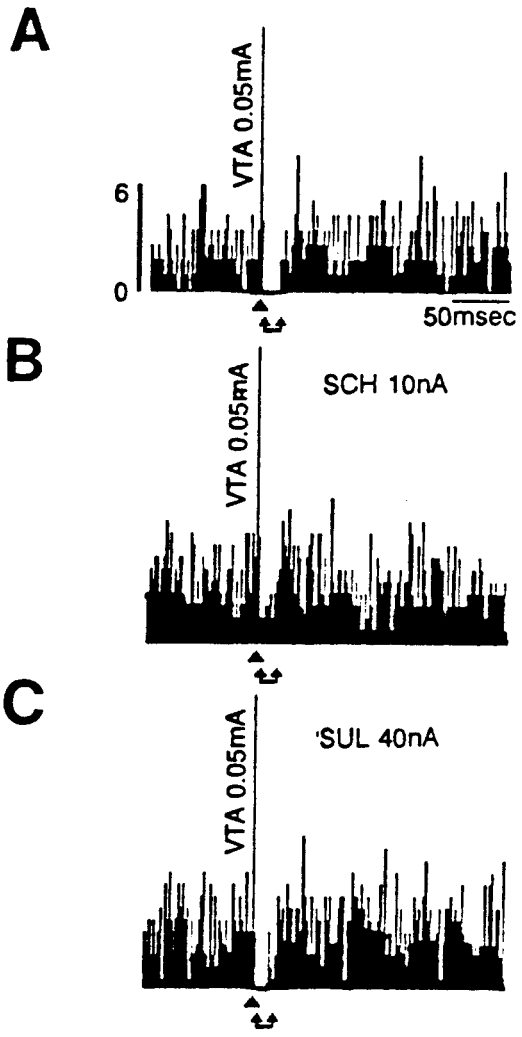

Fig. 16. PERISTIMULUS HISTOGRAMS OF A SHORT LATENCY INHIBITORY RESPONSE IN A VP/SI NEURON TO VTA STIMULATION (0.1 ms, 1 Hz), AND THE ATTENUATING EFFECTS OF CONCURRENT MICROIONTOPHORETIC APPLICATION OF SCH OR SUL. (A) VTA stimulation produced an inhibitory response of 2 ms latency and 14 ms duration for this VP/SI neuron (indicated by arrows). SCH antagonized this inhibitory response (B), while SUL application only produced a slight attenuation (C). The vertical bar in (A) indicates the number of action potentials per 2 ms bin, and triangles indicate the stimulus artifact.

Table 7

DOPAMINE ANTAGONIST-INDUCED ATTENUATION OF VENTRAL PALLIDAL RESPONSES EVOKED BY STIMULATION OF THE VENTRAL TEGMENTAL AREA/SUBSTANTIA NIGRA PARS COMPACTA

Antagonist	Response Category [a]			
	Short Latency Inhibition (≤ 12 ms)	Short Latency Excitation (≤ 12 ms)	Long Latency Inhibition (> 12 ms)	Long Latency Excitation (> 12 ms)
SCH23390 (10-100 nA)	16/23 (70%)	3/ 8 (38%)	6/10 (60%)	7/10 (70%)
Sulpiride (5-100 nA)	10/22 (45%)	3/11 (27%)	9/14 (64%)	7/13 (54%)
SCH23390/Sulpiride (10-100 nA)	3/ 8 [b] (38%)	.....	.....	.....
Flupentixol (0.05-4 mg/kg, i.v.) [c]	4/ 4 (100%)	.....	.....	.....

[a] The response category was defined as the ratio of the evoked responses displaying a 20% attenuation to the number of responses tested with the antagonist.

[b] These 3 evoked responses also were attenuated by individually applied sulpiride or SCH23390.

[c] Of the 4 neurons tested, 1 responded to dose of 0.05, another to 2.0, and the remaining 2 neurons responded to 4 mg/kg, i.v. of the antagonist.

Thus, the VP/SI responses (both excitatory and inhibitory) were categorized as short (≤ 12 ms) or long (> 12 ms) latency (Table 7). Although some VP/SI neurons displayed only one type of evoked response (Fig. 17A-C), the most frequent effect of AMN stimulation was a complex sequence of evoked VP/SI responses (Figs. 17D-F, 18, 19B). The most often observed AMN-evoked VP/SI response category was a long latency inhibition (Fig. 18, 19B, 20A; Table 7). For many VP/SI neurons tested, increasing the stimulation intensity evoked more complex response patterns (Fig. 18), rather than an increase in response duration observed with VTA/SNc activation.

Modulation of VP/SI Responses to AMN Stimulation by Activating the VTA/SNc and Microiontophoretically Applying DA

The majority of VP/SI neurons that responded to AMN stimulation also responded to activation of the VTA/SNc (Fig. 10B). Of sixty-one VP/SI neurons that displayed short latency effects to AMN stimulation, 92% also demonstrated short latency VP/SI responses to VTA/SNc, suggesting extensive convergence of these to inputs within the VP/SI (*e.g.*, Fig. 19). Similarly, of 48 VP/SI neurons with long latency effects evoked by AMN stimulation, 79% also exhibited short latency effects to VTA/SNc stimulation, suggesting that VTA/SNc may modulate, at the level of the VP/SI, the polysynaptically-mediated effects of AMN stimulation. To determine if VTA/SNc can influence AMN-evoked VP/SI neuronal activity, the effects of both endogenous DA (presumably released through VTA/SNc stimulation) and exogenously-applied DA (microiontophoretic) were examined. To assess the modulatory effects of activating the VTA/SNc with a train of 10 pulses on AMN-evoked responses of VP/SI neurons, a VTA/SNc stimulation current that minimally evoked (*e.g.*, Fig. 19C-D), or did not evoke (Fig. 20B-C), a VP/SI response was employed (see Methods).

Figures 19 and 20 illustrate DA-mediated attenuation of a VP/SI long latency inhibitory response to AMN stimulation by prior stimulation of the VTA and by microiontophoretically-applied DA (Fig. 19E). The long latency excitatory response of this neuron also was diminished by VTA stimulation and DA (Fig. 19C-E). Modulation of the AMN-evoked VP/SI response by prior VTA/SNc activation was observed even when VTA/SNc stimulation did not evoke a VP/SI response (Fig. 20B-C). Likewise, microiontophoretic application of DA, even at ejection currents that did not affect baseline interstimulus rate (Fig. 19B, E), effectively modified the AMN-evoked VP/SI response similar to the attenuation produced by VTA/SNc stimulation (Fig. 19D, E).

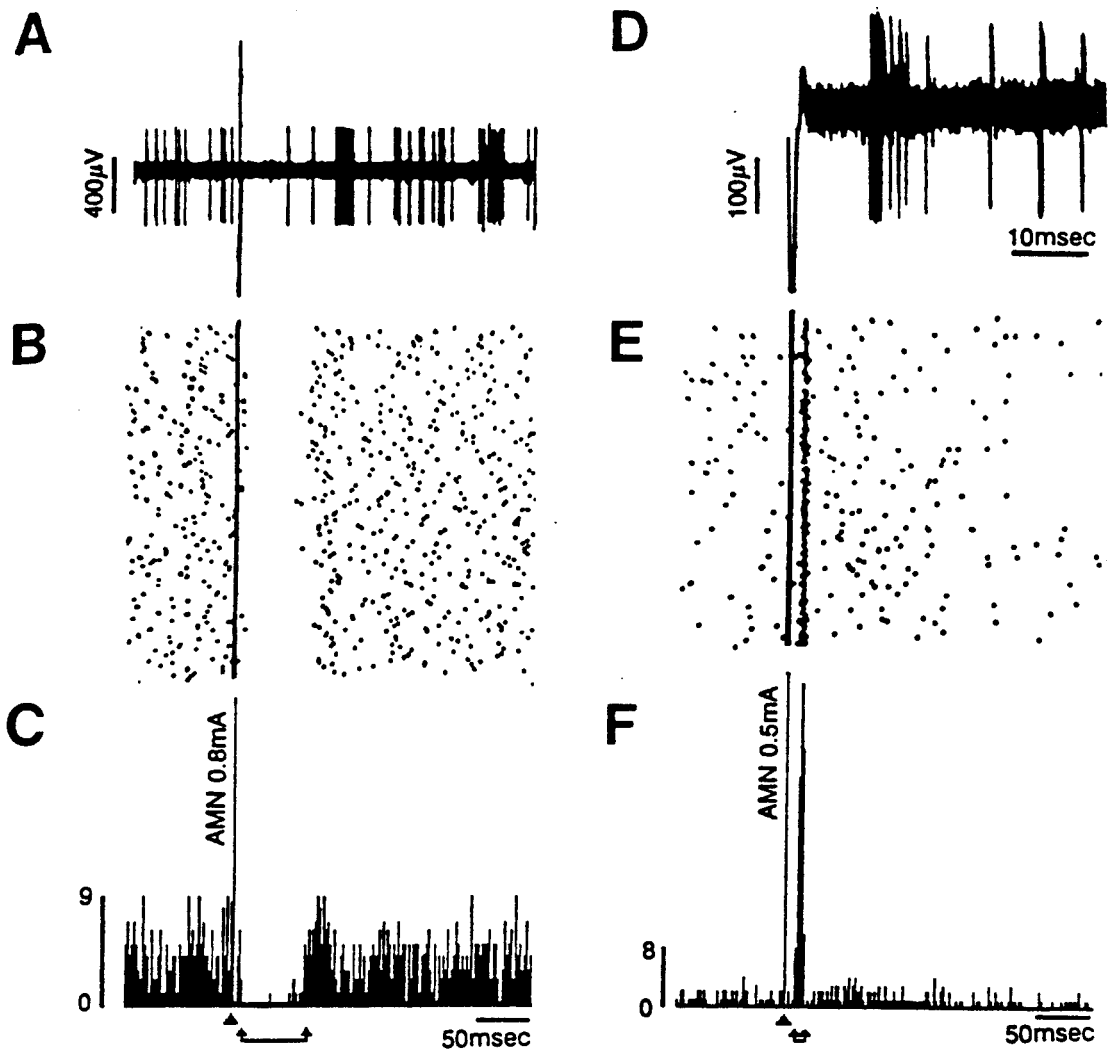

Fig. 17. TWO VP/SI NEURONAL RESPONSE SEQUENCES EVOKED BY SINGLE PULSE STIMULATION OF THE AMN (0.1 ms, 1 Hz). A short latency inhibition is illustrated by one VP/SI neuron in (A-C), and a short latency excitation represented by another VP/SI neuron in (D-F) Oscilloscope traces (A, D), composed of 10 superimposed sweeps, illustrating the VP/SI evoked responses. A faster sweep speed was used in (D) to more clearly illustrate the onset of the short latency excitation. Raster stepper recordings in (B, E) are composed of 128 consecutive sweeps of evoked VP/SI activity;. peristimulus rate histograms in (C, F) are of the same 128 sweeps as in B and E, respectively. The vertical bar in (A) indicates the number of action potentials per 2 ms bin; triangles indicate the stimulus artifact and connected arrows depicted the onset and offset of VP/SI evoked responses.

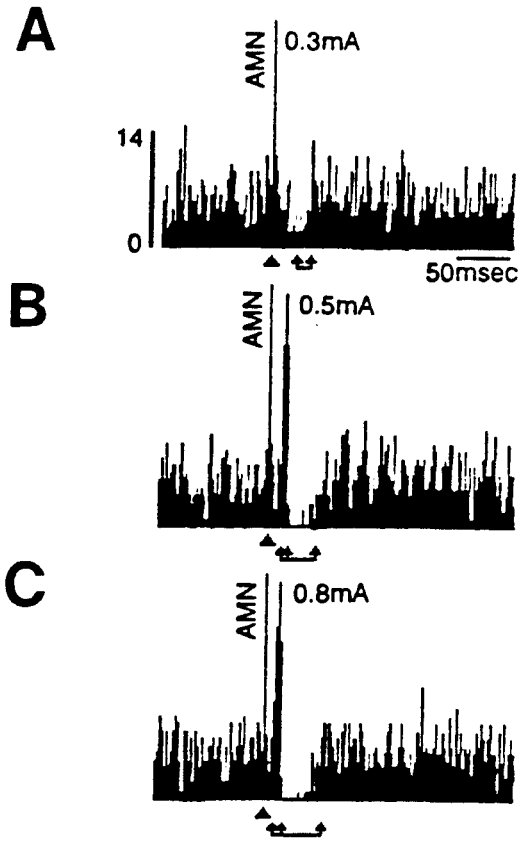

Fig. 18. AMN STIMULATION CURRENT-DEPENDENT INCREASE OF THE COMPLEXITY OF RESPONSES EVOKED IN A VP/SI NEURON. As the current used to stimulate the AMN (0.1 ms, 1 Hz) was increased from 0.3 mA to 0.5 mA, the long latency inhibitory response evoked in the VP/SI neuronal activity was preceded by a short latency excitation (A-B). With a further increase of stimulation current to 0.8 mA, the duration of the long latency inhibitory response increased from 28 to about 40 ms (C). Triangles indicate the stimulus artifact, the connected arrows depict the onset and offset of the VP/SI evoked responses. The vertical bar indicates the number of action potentials per 2 ms time bins.

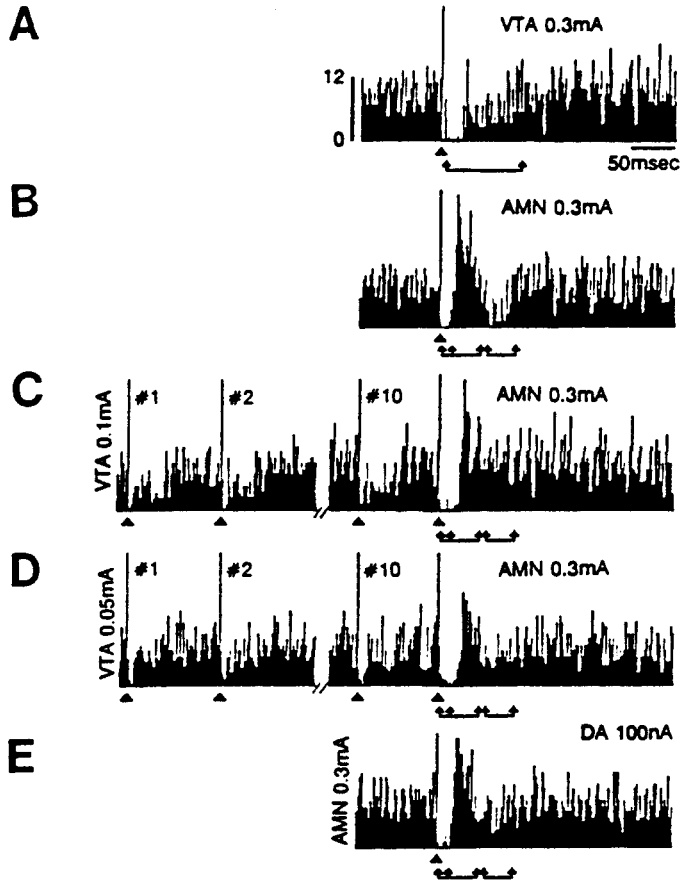

Fig. 19. CONVERGENCE OF VTA AND AMN INFLUENCES WITHIN THE VP/SI, AND MODULATION OF AMN STIMULATION-INDUCED VP/SI RESPONSES BY VTA STIMULATION AND BY DA. VTA stimulation (0.1 ms, 1 Hz) produced a short latency inhibitory response (A). AMN stimulation (0.1 ms, 1 Hz) produced a short latency inhibitory, a long latency excitatory and a long latency inhibitory response on the same neuron (B). A train of 10 pulses (0.1 ms, 0.1 or 0.05 mA) at 10 Hz to the VTA evoked a VP/SI short latency inhibitory response that attenuated the long latency excitatory and inhibitory responses to AMN stimulation (C,D). The short latency inhibition evoked by the AMN was potentiated by VTA stimulation in this neuron, although this phenomenon was not typically observed. Microiontophorectically-applied DA (0.2 M; in E) mimicked the attenuation of AMN-evoked VP/SI responses produced by the 0.05 mA VTA stimulation current in (D). Triangles indicate the stimulus artifacts, and connected arrows indicate the onset and offset of the evoked responses. The vertical bar represents the number of action potentials per 2 ms bin. In (C,D) the numbers 1, 2, 10 indicate the stimulus artifact for the first, second and last pulses of a 10 pulse train delivered to the VTA.

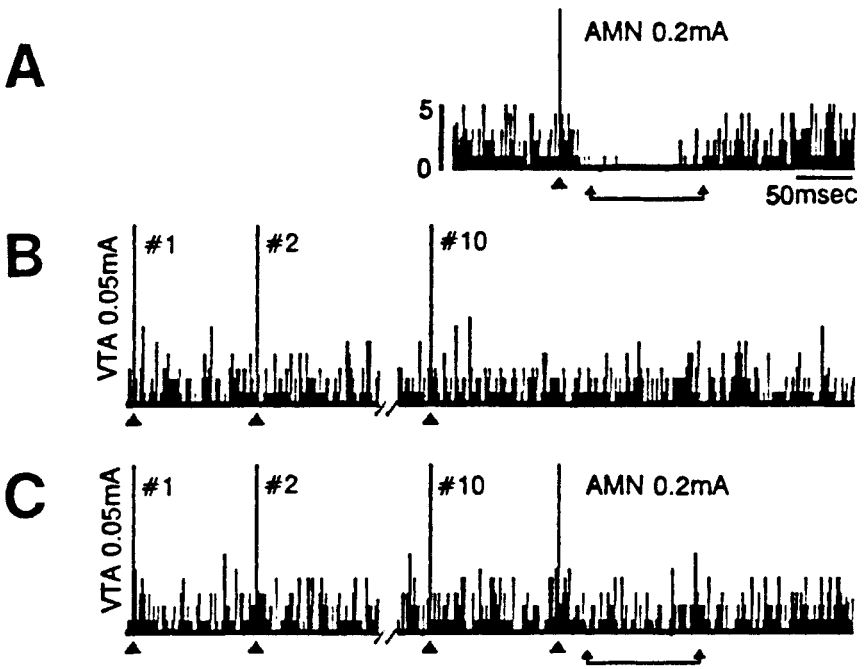

Fig. 20. MODULATION OF AN AMN-EVOKED VP/SI RESPONSE BY PRIOR STIMULATION OF THE VTA WITH A SUBTHRESHOLD CURRENT. AMN stimulation (0.1 ms, 1 Hz) induced a long latency inhibitory VP/SI response (A). Stimulation of the VTA with a train of 10 pulses at 10 Hz was below the threshold for evoking activity of this VP/SI neuron (B). However, train stimulation of the VTA 100 ms prior to AMN stimulation was sufficient to attenuate the AMN-evoked VP/SI long latency inhibitory response. Triangles indicate the stimulus artifacts, and connected arrows indicate the onset and offset of the VP/SI response evoked by AMN stimulation as shown in (A). The vertical bar represents the number of action potentials per 2 ms bin.

This example concurs with the similar effects of DA application and VTA/SNc stimulation on each of the AMN-evoked VP/SI response categories (Table 8). Thus, the modulatory effects of VTA/SNc stimulation on AMN-induced VP/SI responses may involve DA.

Table 8

ATTENUATION OF VENTRAL PALLIDAL RESPONSES TO AMYGDALA STIMULATION BY EXOGENOUS OR ENDOGENOUS DOPAMINE

Physiological Antagonist	Response Category [a]			
	Short Latency Inhibition (≤ 12 ms)	Short Latency Excitation (≤ 12 ms)	Long Latency Inhibition (> 12 ms)	Long Latency Excitation (> 12 ms)
Dopamine (5-120 nA)	7/11 (64%)	7/15 (47%)	20/25 (80%)	5/13 (38%)
VTA/SNc Stimulation (0.05-0.8 mA)	11/13 (85%)	10/18 (56%)	26/33 (78%)	11/19 (58%)

[a] Indicates the number of VP/SI evoked responses to amygdala stimulation that were attenuated by the physiological antagonist divided by the number of amygdala-evoked responses tested. Chi-square analysis comparing the response to dopamine versus VTA/SNc stimulation for each response category was not significantly different.

Discussion

Contribution of DA Receptor Subtypes to DA-mediated Effects on VP/SI Neuronal Activity

The present study demonstrated that microiontophoresis of DA alters the firing rate of VP/SI neurons, producing both inhibitions and excitations. Previous studies also indicate that DA can suppress or excite VP/SI neuronal activity, and collectively it appears that the predominant effect of DA is a current-dependent decrease in firing rate (Napier and Potter, 1989; Napier *et al.*, 1991b). DA-induced changes in the activity of

VP/SI neurons are antagonized by systemically administered SCH or haloperidol (Napier *et al.*, 1991b), suggesting an involvement of both D_1 and D_2 receptor subtypes. Similar results are observed following the systemic administration of the D_1/D_2 agonist, apomorphine, which also induces rate suppressions and excitations that are sensitive to haloperidol (Napier *et al.*, 1991b), SCH or SUL (Maslowski and Napier, 1991b). The present data concur that the DA-induced inhibitions involve the activation of both D_1 and D_2 DA receptors within the VP/SI, since locally-applied SCH or SUL antagonized the inhibitions. However, DA-induced rate excitations were antagonized by SCH but not by SUL, suggesting that the excitations were mediated through activation of the D_1 receptor subtype within the VP/SI. When the selective D_1 and D_2 antagonists were used to determine the contribution of D_1 and D_2 receptors to DA-induced changes of VP/SI neuronal activity, inhibitory effects of DA were mediated by D_1 or D_2 receptor activation within the VP/SI; moreover, D_1 receptor activation also mediated the excitatory effects.

The present results also demonstrated that activation of either the D_1 or the D_2 receptor within the VP/SI was sufficient to induce VP/SI rate changes. Forty-seven percent of VP/SI neurons tested were sensitive to SKF application, and the activity in 86% of these neurons was suppressed. This effect was attenuated by SCH but not by SUL, confirming that SKF-induced rate suppressions were mediated through the activation of the D_1 receptor subtype. In contrast, QUIN induced slightly more rate excitations than rate inhibitions. The excitations were antagonized by SUL but not by SCH, verifying the involvement of the D_2 receptor in this response. D_2-mediated increases in VP/SI activity induced by QUIN appear to be independent of DA-induced excitations, since the latter were not antagonized by SUL. These results suggest that D_1 and D_2 agonist applications within the VP/SI have opposing effects on VP/SI firing rate, since activating D_1 plus D_2 receptors with DA induces rate suppressions whose magnitude is less than activating the D_1 receptor with SKF.

Oppositional VP/SI responses also are obtained with systemic administration of SKF and QUIN, however, the direction of the responses is reversed such that intravenous SKF increases and QUIN decreases firing rate (Maslowski and Napier, 1991a). This data, together with the observation from the present study that polysynaptically-mediated effects were produced by VTA/SNc stimulation, suggest the possibility that VP/SI responses to systemically administered DA agonists reflect changes in the activity of dopaminoceptive regions that are afferent to the VP/SI. Previous studies support this hypothesis since inactivation of the AMN produced by procaine microinjections reduce the number of VP/SI neurons sensitive to systemically administered SKF (Napier, 1992).

Similarities between the Effects of Endogenous and Exogenous DA on the Spontaneous Activity of VP/SI Neurons

With the demonstration that VTA/SNc stimulation alters the activity of VP/SI neurons, the present results provide a function for anatomical reports of dopaminergic innervation of the VP/SI from the VTA (Grove, 1988; Haring and Wang, 1986; Jones and Cuello, 1989; Russchen *et al.*, 1985; Semba *et al.*, 1988; Zaborszky, 1989), and the SNc (Fallon and Moore, 1978; Haring and Wang, 1986; Jones and Cuello, 1989; Martinez-Murillo *et al.*, 1988; Russchen *et al.*, 1985; Semba *et al.*, 1988; Zaborszky, 1989) to the VP/SI, and concurs with previous findings that VP/SI neurons often are inhibited by SNc stimulation (Napier *et al.*, 1991a). VP/SI neuronal activity was similarly altered by VTA and SNc stimulation, which implies that limbic (VTA) and extrapyramidal motor (SNc) pathways both influence the output of VP/SI neurons.

Many of the VTA/SNc evoked responses of VP/SI neurons exhibited latencies less than 12 ms. The distance from the VTA/SNc to the VP/SI (as calculated from stereotaxic coordinates) is approximately 5-6 mm. With a conduction velocity of

dopaminergic fibers of approximately 0.5 m/s (Guyenet and Aghajanian, 1978; Yim and Mogenson, 1980) and a synaptic delay accounting for another 0.5 ms (Kuffler *et al.*, 1984), the onset of monosynaptic DA-mediated effects can occur within 12 ms. In VP/SI neurons with slower firing rates, the onset of evoked responses appears to occur immediately after the stimulus artifact because the interspike interval of the spontaneous activity was longer than the delay for orthodromic conduction, whereas neurons with faster firing rates displayed action potentials prior to the evoked response that were not driven by the stimulus. Some of the short latency VP/SI responses may be mediated by dopaminergic neurons from the VTA/SNc. This conclusion is supported by evidence that these short latency responses were attenuated by intra-VP/SI application of DA antagonists. Thus, the results indicate that some of the VTA/SNc inputs to the VP/SI are monosynaptic and dopaminergic.

According to criteria established by Werman (1966) for potential neurotransmitters, exogenously-applied DA should mimic the response to evoking the endogenous system. The present results fulfill this criterion, since all of the inhibitory, and 60% of the excitatory VP/SI responses produced by VTA/SNc stimulation were mimicked by microiontophoretic applications of DA within the VP/SI. In addition, SCH or SUL applied within the VP/SI antagonized the evoked responses, as well as DA-induced VP/SI rate suppressions. Thus, endogenous DA is released within the VP/SI during electrical stimulation of the VTA/SNc, and DA then alters the spontaneous activity of this region through the activation of D_1 or D_2 receptor subtypes.

VTA/SNc stimulation also evoked VP/SI responses that exhibited long onset latencies characteristic of polysynaptic transmission. These polysynaptic events may be mediated through interneurons within the VP/SI or through afferents to this brain region. Afferents to the VP/SI, including the NA and AMN, receive midbrain dopaminergic innervation (Fallon and Moore, 1978; Fallon *et al.*, 1978). Thus,

VTA/SNc evoked responses of the VP/SI may be mediated indirectly through alterations in neuronal activity of these systems. NA neurons display a variety of responses to VTA stimulation, and 50% of the responses are attenuated by systemic administration of the DA antagonist, haloperidol (Yim and Mogenson, 1982). Since microinjections of DA into the NA excites VP/SI neurons (Yang and Mogenson, 1989), it is possible that VTA stimulation-induced, endogenous release of DA affects VP/SI neuronal activity indirectly through altered output from NA afferents. The effects on VP/SI neuronal activity of DA receptor stimulation within the AMN are unknown. Since long latency responses of VP/SI neurons to VTA/SNc stimulation were readily attenuated by intra-VP/SI application of D_1 or D_2 antagonists, VTA/SNc stimulation-evoked responses of VP/SI neurons, regardless of their monosynaptic or polysynaptic nature, are under DA modulatory control at the level of the VP/SI.

Effects of Endogenous and Exogenous DA on AMN-evoked Responses of VP/SI Neurons

VP/SI is site of convergence for midbrain and AMN inputs, where 56 of 61 VP/SI neurons tested (92%) displayed short latency (monosynaptic) evoked responses to stimulation of both regions. This extensive convergence may allow the VTA/SNc to modulate AMN-evoked VP/SI responses, since 85% of the AMN-evoked short latency inhibitory, and 56% of the short latency excitatory, VP/SI responses were attenuated by prior stimulation of the VTA/SNc. Activation of these midbrain areas can function as a physiological antagonist of the monosynaptic AMN input to the VP/SI. Similarly, intra-VP/SI DA application attenuated 64% of the inhibitory, and 47% of the excitatory, short latency VP/SI responses to AMN stimulation, suggesting that DA may be the transmitter involved. Thus, VTA/SNc dopaminergic efferents to the VP/SI modulate the output of AMN efferents to the VP/SI.

AMN stimulation also evoked long latency inhibitory and excitatory VP/SI responses, the long latency inhibitions being the most frequently observed of all AMN-evoked effects. Long latency inhibitory effects can be produced via the NA, since Yim and Mogenson (1983) observed that procaine-induced inactivation of the NA attenuates 54% of the AMN-evoked long latency inhibitions of VP/SI neurons. The AMN-evoked long latency inhibitory responses of VP/SI neurons also are attenuated by the VTA stimulation (Yim and Mogenson, 1983; present study). DA release within the NA is thought to mediate the VTA stimulation-induced attenuation of AMN-evoked VP/SI responses, since d-amphetamine injected within the NA mimics this effect in 54% of the VP/SI neurons (Yim and Mogenson, 1983). The present study indicates that activating the VTA/SNc can modify the AMN-evoked long latency responses (either inhibitions or excitations) within the VP/SI, since 38 of 48 VP/SI neurons (79%) with AMN-evoked long latency responses, also were affected monosynaptically by VTA/SNc stimulation. Likewise, 80% of the long latency inhibitory, and 38% of the long latency excitatory VP/SI responses evoked by AMN stimulation were attenuated by intra-VP/SI DA application. Thus, the attenuating effects of VTA/SNc stimulation on long latency inhibitions (and excitations) of VP/SI activity evoked by AMN stimulation may be mediated through DA release within the VP/SI.

Functional Significance of the Convergence of VTA/SNc Dopaminergic and AMN Limbic Influences within the VP/SI

The AMN has been implicated in a number of functions, including modulation of hormonal secretion and autonomic activity, as well as defensive behaviors (Carlsen, 1989; Gloor, 1978; Nakano *et al.*, 1987). Electrical stimulation of the AMN alters the activity of VP/SI neurons (Tsai *et al.*, 1989; Yim and Mogenson, 1983; and the present study). VP/SI responses to AMN stimulation may involve transmission of limbic

influences from the AMN to the VP/SI. With the changes in VP/SI neuronal activity, the output of these neurons to its targets also may be altered. This hypothesis is supported by the finding that more than two-thirds of the VP/SI neurons that are antidromically activated by stimulation of the pedunculopontine nucleus exhibit AMN-evoked excitatory responses (Tsai *et al.*, 1989). The excitatory effects of AMN stimulation on the VP/SI can be transmitted to the pedunculopontine nucleus altering the activity of this nucleus. Thus, AMN afferents to the VP/SI may contribute limbic influences on the VP/SI efferents to brainstem regions associated with locomotor activity. The attenuation of AMN-evoked VP/SI neuronal responses by VTA/SNc and DA suggests that DA modulates the effectiveness of AMN limbic influences on VP/SI activity.

The role of the VP/SI in the ventral striatopallidal system proposed in previous studies (for review see Mogenson and Yang, 1991) was as an intermediary between NA outputs, that are extensively modified by limbic inputs from the AMN and hippocampus, and the mesencephalic locomotor region. This system is modulated further by midbrain dopaminergic neurons to the NA which can activate VP/SI neurons, since 1) intra-NA DA application increases the spontaneous activity of VP/SI neurons (Yang and Mogenson, 1989), and 2) intra-NA d-amphetamine attenuates the VP/SI inhibitory responses to AMN stimulation (Yim and Mogenson, 1983). A significant finding of the present study is that the VP/SI is affected monosynaptically by VTA/SNc stimulation, suggesting the potential for modulation "downstream" of NA disinhibitory effects on VP/SI activity. AMN-evoked VP/SI activity is also modulated at the level of the VP/SI by VTA/SNc stimulation and DA. These results expand the functions of the VP/SI from a relay site for NA output to an active processing center of AMN and VTA/SNc inputs beyond the level of the NA.

In conclusion, DA appears to be intimately involved in modulating processes mediated by the ventral striatopallidal system. Extensive findings indicate that the ventral striatopallidal pathway participates in consolidating the information from the limbic system and the extrapyramidal motor system (Mogenson *et al.*, 1980, 1988; Heimer *et al.*, 1982; Heimer and Alheid, 1991; Mogenson and Yang, 1991). These reviews are complemented by recent studies revealing that the VP/SI "may be an important site in the processing of the reinforcing effects of drugs" and that the NA to VP/SI innervation "may be a common pathway for both stimulant and opiate reinforcement" (Koob *et al.*, 1991). Considering the multifarious effects of DA on neuronal activity and behavior mediated through many brain regions, including the ventral striatopallidal system, the actions of DA are currently undergoing reevaluation (Alexander and Crutcher, 1990; Graybiel, 1990; Smith and Bolam, 1990; Le Moal and Simon, 1991). DA is thought to act as a neuromodulator to set the gain, or level of output rather than activating or inactivating the ventral striatopallidal system (Graybiel, 1990). The affects of DA within a brain region may be determined by the neuronal activity and functions encompassed by that brain region (Le Moal and Simon, 1991). Previous studies demonstrate that DA within the NA attenuates the effects of AMN stimulation on the neuronal activity of the NA (Yim and Mogenson, 1982). In the present study, DA application within the VP/SI modulated the effectiveness of AMN stimulation to alter the activity of VP/SI neurons. Thus, dopaminergic innervation at each level of the circuit could then serve as a dynamic modulator of specific behaviors elicited by ventral striatopallidal system via its efferent innervation.

CHAPTER VI

GENERAL DISCUSSION

Role of the D_1 and the D_2 Receptor Subtypes

Introduction

Previous studies have provided adequate anatomical, biochemical and functional evidence that the VP/SI is a dopaminoceptive brain region. The results presented in this dissertation indicate that the effects of DA on VP/SI activity can be mediated through separate stimulation of the D_1 or the D_2 receptor subtype. In the first experiment, systemic administration of selective D_1 or D_2 agonists was sufficient to elicit changes in VP/SI neuronal activity. These changes were subsequently attenuated by administration of the antagonist that is selective for the stimulated receptor subtype. It was also demonstrated that individual activation of the D_1 or the D_2 receptor subtypes had opposing influences on the neuronal activity of the VP/SI.

The opposite effects of D_1 and D_2 receptor activation on VP/SI neuronal activity differentiates this brain region from the morphologically similar GP (Heimer and Wilson, 1975). VP/SI neurons appear to be more sensitive than GP neurons to systemic administration of either QUIN or SKF, since: 1) more VP/SI neurons were responsive, and 2) responses were observed at lower doses of these agonists (compare results of Chapter III with Carlson *et al.*, 1988). However, the magnitude of the rate increases induced by SKF in either brain region is similar (Chapter III; Carlson *et al.*, 1988). In contrast to the effects of D_1 receptor activation in VP/SI, the predominant

response to D_2 receptor activation was suppression of VP/SI activity (Chapter III), and stimulation of GP activity (Carlson *et al.*, 1987a; 1988).

The difference between the VP/SI and the GP is obvious in the independent effects (and oppositional effects) of SKF and QUIN on VP/SI activity versus the synergistic effects of these same agonists on GP activity. The predominant difference between these brain regions is their sensitivity to D_1 receptor activation, which may be explained, in part, by studies demonstrating greater numbers of D_1 binding sites within the VP/SI as compared to the GP (Bardo and Hammer, 1991; Beckstead *et al.*, 1988; Boyson *et al.*, 1986; Dawson *et al.*, 1986a, 1986b; Mansour *et al.*, 1990; Napier *et al.*, 1991a; Richfield *et al.*, 1987; Savasta *et al.*, 1986). Recent studies using polyclonal antbodies against the D_1 receptor indicate that D_1 receptors are heavily concentrated within limbic regions including the VP/SI (Huang *et al.*, 1992).

There is also evidence from molecular biological studies that the VP/SI has post-synaptic (Fremeau *et al.*, 1991; Mengod *et al.*, 1991; Weiner *et al.*, 1991), whereas the GP does not appear to have post-synaptic D_1 receptors. In addition, the D1 post-synaptic receptors within the VP/SI may also be independent of ACy and cyclic AMP generation. Thus, DA or D_1 agonists can induce D_1-mediated responses within the VP/SI by activating both pre- and post-synaptic receptors, but only by activating pre-synaptic receptors within the GP. The differences in the amount and location of D_1 receptors, along with the possibility of activating alternative second messenger systems, may be responsible for the disparity of DA agonist effects on VP/SI versus GP activity.

The second set of experiments confirmed that the actions of the nonselective DA agonist, APO, were mediated through both D_1 and D_2 receptor subtypes, and that stimulation of either subtype is sufficient to influence the neuronal activity of the VP/SI. Alterations of VP/SI neuronal activity to a single dose of APO did not exhibit a predominant direction; approximately 40% of the tested neurons were excited, another

40% were inhibited, and the rest were insensitive to the agonist. These results are similar to the APO-induced effects on the neuronal activity of the SNr in rats that have not been treated with the catecholaminergic toxin, 6-OH-DA (Waszczak *et al.*, 1984). The interpretation of the effects of APO are complicated since the VP/SI is extensively innervated by other dopaminoceptive brain regions (also true for the SNr). Thus, the effects of a systemically administered, nonselective DA agonist on VP/SI neuronal activity may reflect the summated stimulation of all dopaminoceptive afferents to this brain region, as well as the simultaneous activation of the D_1 and D_2 receptor subtypes within the VP/SI.

The results of the third series of experiments demonstrated that DA receptors within the VP/SI mediated the effects of selective D_1 or D_2 DA agonists, and that stimulation of either receptor within this brain region was sufficient to alter the activity of VP/SI neurons. Furthermore, locally-applied DA often inhibited VP/SI activity, confirming the results of earlier studies (Napier and Potter, 1989; Napier *et al.*, 1991b). Likewise, attenuation of these effects by intra-VP/SI application of selective D_1 or D_2 receptor antagonists concur with previous results of systemically administered D_1 or D_2 receptor antagonists (Napier *et al.*, 1991b). Interestingly, the inhibitions induced by intra-VP/SI application of SKF occurred more often, and to a greater extent than those induced by DA. Since intra-VP/SI application of QUIN often excited VP/SI neurons, the effects of DA may reflect the summation of opposing activation of D_1 and D_2 receptors within this brain region. However, most of the VP/SI neurons that were tested with both D_1 and D_2 DA agonists were sensitive to only one agonist, suggesting that the opposing effects of D_1 and D_2 receptor activation may be mediated by different neurons.

Stimulation of the VTA/SNc also evoked inhibitory and excitatory responses of VP/SI neurons. Most of the VP/SI neurons that were tested with VTA/SNc stimulation

and intra-VP/SI microiontophoretic application of DA demonstrated similar effects to both treatments. This suggests that endogenous release of DA within the VP/SI may mediate the VTA/SNc-evoked VP/SI responses. Likewise, the effects of stimulating the VTA/SNc were attenuated by either application of SCH or SUL within the VP/SI, verifying that the effects are DA-mediated, and indicating that activation of the either receptor subtype is sufficient for inducing these changes in VP/SI neuronal activity. Thus, 1) separate D_1 or D_2 receptor stimulation is sufficient to alter the activity of VP/SI neurons, 2) D_1 receptor activation is more effective than D_2 receptor activation within the VP/SI, and, 3) since both subtypes are present within the VP/SI, DA can mediate its effects on the VP/SI through either receptor subtype.

Modulatory Effects of DA

Current reviews suggest that the role of DA within the brain may be to act as a neuromodulator, regulating and integrating functions of the neuronal systems that receive dopaminergic innervation (Bunney *et al.*, 1991; Graybiel, 1990; Le Moal and Simon, 1991; Smith and Bolam, 1990). This neuromodulatory role of DA takes into account the variety of behavioral responses attributed to activation or inactivation of the DA system (Le Moal and Simon, 1991), and suggests that DA acts as a gain-control system, adjusting the amount of influence that each brain region has on the final behavioral output (Graybiel, 1990; Le Moal and Simon, 1991). Moreover, studies of DA replacement in animals with DA-depleting lesions and humans suffering from Parkinson's disease support a neuromodulatory role for DA. Loss of dopaminergic neurons in Parkinson's disease or DA-depleting lesions results in the impaired ability of initiate movement. However, the anatomical substrate for motoric behavior is not eliminated by dopaminergic cell loss, since these behaviors are restored by the exogenous replacement of DA (Le Moal and Simon, 1991). Thus, it appears that the

normal function of DA is to enable the initation of appropriate movement to environmental or internal stimuli.

Similarly, both intracellular and extracellular recording studies support the neuromodulatory actions of DA. Intracellular recording studies in the STR indicate that DA acts as a neuromodulator, attenuating both excitatory (glutamate-induced) and inhibitory (GABA-induced) effects on the activity of STR neurons (Bernardi *et al.*, 1984; Mercuri *et al.*, 1985). DA acts as a functional antagonist of excitatory STR neurons evoked by local stimulation of the STR by activating D_1 receptors, which induces a depressant action on the postsynaptic membrane, and limits the excitability of STR neurons to a depolarizing event (Calabresi *et al.*, 1987, 1988). Prior stimulation of midbrain dopaminergic regions attenuates the extracellularly recorded: 1) excitatory STR responses to cortical stimulation (Hirata *et al.*, 1984), 2) excitatory NA responses to stimulation of the hippocampus, AMN (Yang and Mogenson, 1984; Yim and Mogenson, 1982) or parafascicular nucleus of the thalamus (Akaike *et al.*, 1984), 3) inhibitory GP responses to cortical stimulation (Hirata and Mogenson, 1984), and 4) excitatory (Chapter V) and inhibitory (Yim and Mogenson, 1983; Chapter V) VP/SI responses to AMN stimulation.

The results from the third series of experiments (Chapter V) confirmed that the convergence of midbrain dopaminergic and AMN inputs within the VP/SI allowed for dopaminergic modulation of VP/SI responses evoked by electrical stimulation of this AMN input. Electrical stimulation of the VTA/SNc, which presumably involves the release of endogenous DA, or exogenously-applied DA within the VP/SI attenuated the VP/SI responses to AMN stimulation. Historically, the effects of DA on the spontaneous and AMN-evoked activity of the VP/SI are thought to occur at the level of the NA (Mogenson and Yang, 1991; Yang and Mogenson, 1989; Yim and Mogenson, 1983). The present results suggest that the VP/SI is also a site of the modulatory effects

of DA on the monosynaptically- and polysynaptically-mediated VP/SI responses to AMN stimulation. Furthermore, the VP/SI long latency inhibitory responses to AMN stimulation appeared to be more sensitive to DA than the AMN-evoked excitatory responses of VP/SI neurons, suggesting that certain limbic information from the AMN which excites the VP/SI may bypass DA modulation. Thus, future studies should investigate whether the modulatory role of DA is selective for certain alterations of VP/SI neuronal activity (*i.e.*, are other inhibitory influences on VP/SI activity, such as those from the NA, also attenuated more readily by DA).

Potential Significance of DA within the VP/SI

Locomotor Behavior. Studies indicate that the VP/SI, with its innervation to the pedunculopontine nucleus (Swanson *et al.*, 1984), is involved in the initiation of locomotor behavior (Mogenson and Nielson, 1983, 1984; Mogenson *et al.*, 1985; Mogenson and Wu, 1986; Mogenson and Yang, 1991). The input from the NA to the VP/SI, and the dopaminergic modulation of this NA afferent system, has been similarly implicated in the induction of locomotor activity (Austin and Kalivas, 1991; Jones and Mogenson, 1980; Kalivas *et al.*, 1991; Mogenson and Nielson, 1983; Mogenson and Yang, 1991). Anatomical and electrophysiological studies concur that the VP/SI is dopaminoceptive and its neuronal activity can be altered by DA (Napier *et al.*, 1991a). The present studies confirmed that electrical stimulation of the ascending dopaminergic projection from the VTA/SNc evoked responses in VP/SI neurons. Recent evidence suggests the VP/SI is also a site for DA-mediated activation of locomotor behavior (Napier, 1992b; Napier and Chrobak, 1992). Thus, DA-mediated changes in the activity (and thus, the output) of VP/SI neurons may be reflected by an initiation of locomotor behavior.

Reward, Arousal and Drug Reinforcement. The VP/SI may also be involved with the increased arousal due to reinforced stimuli since changes in activity of many of these neurons occur in response to rewards and reward-associated stimuli (for review see, Richardson and DeLong, 1991). DeLong (1971) observed that some SI neurons in rhesus monkeys exhibit consistent alterations in discharge when a juice reward is delivered. These neurons also respond to the sight and taste of food, as well as to the satiety level of the animal (Rolls *et al.*, 1980). VP/SI neurons are highly responsive to events that precede a reward or movements made to obtain a reward (Richardson and DeLong, 1986). However, the VP/SI neurons that respond to appetitive stimuli also respond to aversive stimuli, suggesting that this region may be influenced by the arousing nature, and the behavioral significance, of these stimuli (Richardson and DeLong, 1991). Furthermore, the VP/SI is also an important site for mediating the reinforcing effects of cocaine (Hubner and Koob, 1990; Robledo and Koob, 1992) and heroin (Hubner and Koob, 1990). Although studies indicate that dopaminergic transmission within the NA contributes to some aspects of drug self-administration (Koob *et al.*, 1991), the contribution of DA within the VP/SI to motivated behavior and drug reinforcement should also be investigated. Since motivated behaviors and the reinforcing properties of drugs are both processed through the VP/SI in the rat, the circuitry involved with the former may assist in the discovery of the anatomical substrate for drug dependence in humans (Koob *et al.*, 1991).

VP/SI Cholinergic Neurons. Previous electrophysiological studies have demonstrated that the VP/SI provides a substantial cholinergic innervation of the cortex (Aston-Jones *et al.*, 1984, 1985; Lamour *et al.*, 1986; Reiner *et al.*, 1987) and that these cholinergic neurons exhibit heterogeneous physiological properties (Aston-Jones *et al.*, 1984, 1985; Reiner *et al.*, 1987). Cholinergic neurons that comprise the VP/SI cortical

projection display a variety of impulse amplitudes and waveforms, and have discharge rates of 0-40 Hz (Aston-Jones *et al.*, 1984). Since the VP/SI neurons studied in this dissertation have similar characteristics to these cholinergic neurons, and the recording sites overlap with those of the previous studies, it is likely that the present data included a portion of these VP/SI cholinergic neurons. Preliminary intracellular electrophysiology studies of VP/SI neurons in an *in vitro* slice preparation (Maslowski *et al.*, 1991) also suggest that some VP/SI neurons may be cholinergic since they displayed similar characteristics to septum/diagonal band cholinergic neurons (Griffith and Matthews, 1986; Griffith *et al.*, 1991).

The VP/SI may influence cognitive function through its cholinergic projection to the cortex. Electrical stimulation of the VP/SI has been shown to affect the firing rate of cortical neurons (Rigdon and Pirch, 1984), and kainic acid-induced lesions in the VP/SI result in a 60% reduction in cortical ChAT activity (Pirch *et al.*, 1985). In addition, frontal cortex neurons in unanesthetized rats trained to associate a light cue with medial forebrain bundle stimulation exhibit cue-elicited rate changes which are attenuated by kainic acid-induced lesions of VP/SI (Pirch *et al.*, 1986, 1991). These conditioned responses of the frontal cortex are associated with cognitive processes (Pirch *et al.*, 1985). Amphetamine produces a dose related depression of these potentials that is subsequently blocked by haloperidol (Pirch and Corbus, 1983). Systemic administration of QUIN mimics the effects of amphetamine whereas SKF has the opposite effect on the response of cortical neurons to the conditioning stimulus (Pirch *et al.*, 1988). Cognitive processes associated with cue-elicited changes in cortical activity involve the dopaminergic modulation of VP/SI cholinergic neurons (Pirch and Corbus, 1983; Pirch *et al.*, 1988), even though working memory tested on a 12 arm radial maze was not altered by intra VP/SI injections of DA (Napier and Chrobak, 1992). The effects of dopaminergic agents within the VP/SI on the conditioned potentials, and the

assessment of cognitive behaviors with testing paradigms other than the radial arm maze requires further investigation.

Future Directions of VP/SI Research

Potential experiments may include identifying VP/SI neurons through their efferent projection by antidromic stimulation, and determining the effects of dopaminergic agents within the VP/SI on these antidromically-activated VP/SI neurons. Similarly, the VP/SI responses to orthodromic stimulation of the VTA/SNc (which presumably releases endogenous DA) can be assessed for VP/SI neurons whose efferent targets have been identified. Previous studies have demonstrated that VP/SI neurons projecting to the pedunculopontine nucleus are differentially affected by AMN or hippocampal stimulation (Tsai *et al.*, 1989). Thus, differences in the effects of DA may be revealed for VP/SI neurons which project to the pedunculopontine nucleus versus those innervating the AMN and cortex.

Another electrophysiological technique that may assist in characterizing the different types of VP/SI neurons is intracellular recordings of VP/SI slices. As described above, preliminary studies indicated that at least three different cell type exist in this region (Maslowski *et al.*, 1991), and that one type exhibits similar characteristics to cholinergic neurons of the medial nucleus and the nucleus of the diagonal band (Griffith and Matthews, 1986; Griffith *et al.*, 1991). Future studies using intracellular fluorescent dyes in combination with ChAT staining may confirm these VP/SI neurons as cholinergic. In addition, inactivation of afferent input to the VP/SI, which can be done readily in this *in vitro* preparation, can determine the direct effects of dopaminergic agents on VP/SI neurons and may explain the multifarious effects of DA *in vivo*.

The VP/SI in Alzheimer's Disease and Parkinson's Disease

This dissertation involved the study of normal brain activity as a basis for the initial understanding of the clinical manifestations of Alzheimer's disease and Parkinson's disease. Anatomical studies have demonstrated that the midbrain

dopaminergic system, which is primarily involved in Parkinson's disease, projects to the cholinergic and non-cholinergic neurons of the VP/SI (Zaborszky *et al.*, 1991). The results presented in this dissertation indicate that stimulation of the VTA/SNc alters the activity of VP/SI neurons. Since location of the cholinergic and non-cholinergic neurons within the VP/SI overlap, the VP/SI may be an integrative site for dopaminergic afferents with both VP/SI cholinergic and non-cholinergic neurons. Thus, degeneration of dopaminergic afferents to the VP/SI in Parkinson's disease may seriously affect the normal neuronal activity of the VP/SI. In addition, loss of VP/SI cholinergic neurons in Alzheimer's disease (Lehericy *et al.*, 1991) may result in transneuronal degeneration of dopaminergic neurons. The association of the VP/SI with motoric and cognitive behavior suggests that altered activity of this brain region due to disease or drug intervention may result in aberrant movement and thought processes.

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ABSTRACTS

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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