1994

Dopaminergic Modulation of Basal Forebrain Cholinergic Neurons

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DOPAMINERGIC MODULATION OF BASAL FOREBRAIN CHOLINERGIC NEURONS

by

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A Thesis Submitted to the Faculty of

the Graduate School of Loyola University, Chicago

in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Pharmacology

January, 1994

ACKNOWLEDGMENTS

To Dr. T. Celeste Napier I express my deepest appreciation for her guidance, encouragement, patience, and good humor throughout my graduate program. Sincere thanks also go to Dr. Israel Hanin and Dr George Battaglia for their interest and advice, without which this thesis never could have been completed. I also am grateful to the members of my laboratory, past and present, for their contributions: Renata Maslowski-Cobuzzi, Dr. James Chrobak, Dowan An, and Paul Grippo. Finally, a heartfelt thanks to my family-- Mom, Dad, John, Terry, and Kevin-- for their support and patience, and for never doubting that I would finish!

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LIST OF ABBREVIATIONS

ACh	Acetylcholine
AChE	Acetylcholinesterase
BNST	Bed Nucleus of the Stria Terminalis
ChAT	Choline Acetyltransferase
DA	Dopamine
DOPAC	3,4-dihydroxyphenylacetic acid
DβH	Dopamine-β-Hydroxylase
EM	Electron Microscopic
GABA	γ-Amino Butyric Acid
GAD	Glutamic Acid Decarboxylase
GP	Globus Pallidus
GP_{ep}	Entopeduncular Nucleus
HAChU	Sodium-Dependent High Affinity Choline Uptake
HDB	Nucleus of the Horizontal Limb of the Diagonal Band
5-HT	Serotonin
5-HIAA	5-Hydroxy Indoleacetic Acid
HRP	Horseradish Peroxidase
HVA	Homovanillic Acid
ic	Internal Capsule
IR	Immunoreactive (or Immunoreactivity)
LC	Locus Coeruleus

LM	Light Microscopic
MBN	Magnocellular Basal Nucleus
mfb	Medial Forebrain Bundle
MPO	Magnocellular Preoptic Area
MS	Medial Septal Nucleus
NA	Nucleus Accumbens
NB	Nucleus Basalis of Meynert
NT	Neurotensin
6-OHDA	6-Hydroxydopamine
OT	Olfactory Tubercle
PHA-L	*Phaseolus Vulgaris* Leucoagglutinin
SI	Substantia Innominata
SN	Substantia Nigra
SN_c	Substantia Nigra Pars Compacta
STR	Striatum
TH	Tyrosine Hydroxylase
VDB	Nucleus of the Vertical Limb of the Diagonal Band
vmGP	Ventromedial Globus Pallidus
VP	Ventral Pallidum
vSTR	Ventral Striatum
VTA	Ventral Tegmental Area
xac	Crossing of the Anterior Commissure

INTRODUCTION

The cholinergic and dopaminergic systems are essential for cognition and motor function. Studies examining the roles of these neurotransmitter systems have shown that they interact in a complex fashion (for review see Levin et al., 1990). Several sites of dopamine (DA)-acetylcholine (ACh) interaction already have been demonstrated, including the striatum (STR) and the septum.

Within the rat STR, DA fibers originating in the substantia nigra (SN) and ventral tegmental area (VTA) (Fallon and Moore, 1978) form close appositions with large, dendritic spine-free cells thought to represent cholinergic interneurons (Heimer et al., 1985; Lehmann and Langer, 1983). Electron microscopic (EM) analysis of double-labeled striatal sections revealed that tyrosine hydroxylase (TH)-immunoreactive (IR) terminals contact choline acetyltransferase (ChAT)-positive terminals of these interneurons (Chang 1988; Kubota et al., 1987). Furthermore, D1-like and D2-like receptor subtypes (Ariano et al., 1989; Camps et al., 1990; Contreras et al., 1987; Gehlert and Wamsley, 1985; Guela and Slevin, 1989; Mansour et al., 1990; Richfield et al., 1989), and their respective mRNA (Mansour et al., 1990; Meador-Woodruff et al., 1990; Mengod et al., 1991; Weiner et al., 1990), are prevalent in the STR. The D1-like receptor is positively coupled to adenylate cyclase and the D2-like receptor (henceforth these receptors are referred to as D1 and D2, respectively) is negatively coupled with the enzyme (Stoof and Kebabian, 1984).

In vivo data measuring ACh concentration and *in vitro* data measuring ACh release indicate that DA exerts an inhibitory effect on the striatal cholinergic cells, a phenomenon thought to be mediated by D2 receptors (Sherman et al., 1978; Scatton, 1982a;1982b; Stoof and Kebabian, 1982; Fage et al., 1984). Results concerning the involvement of D1 receptors are variable.

Experiments utilizing acute treatments with D1-selective agonists and antagonists do not support a D1-mediated inhibition of striatal cholinergic function. According to Scatton (1982b) and Stoof and Kebabian (1982), a pharmacological challenge with the D1 receptor agonist, SKF 38393 has no effect on striatal ACh concentration *in vivo* or ACh release from striatal slices. Other agents which increase cAMP content likewise do not change ACh release (Stoof and Kebabian, 1982). Contrasting data from Gorell et al. shows that application of either SKF 38393 or the D1 receptor antagonist, SCH 23390 causes a dose-dependent increase in ACh release that is not blocked by the D2 receptor antagonist, sulpiride. According to Gorell and associates, this indicates that the ACh release is mediated by receptors other than D1 or D2 (Gorell and Czarnecki, 1986; Gorell et al., 1986). Recent work has demonstrated that D1 receptors stimulate and D2 receptors inhibit striatal ACh output as measured by *in vivo* microdialysis (Damsma et al., 1991; DeBoer and Abercrombie, 1992). Furthermore, results also "suggest that an increased ACh release is mediated by a preferential stimulation of D1 receptors" (Imperato et al., 1992).

Published data concerning chronic DAergic drug treatment also are inconclusive. Rupniak et al. (1986) and Jenner and Marsden (1987) reported that 12 months'

administration of the atypical neuroleptic, clozapine to rats increases binding (B_{max}) of [3H]piflutixol to striatal D1 receptors, but does not influence binding of [3H]spiperone to striatal D2 sites. A concomitant increase in striatal ACh concentration also occurs, while striatal ChAT activity remains unchanged from control. A similar phenomenon occurs with chronic haloperidol (a D2 antagonist), which selectively increases striatal [3H]spiperone binding and ACh levels without affecting ChAT. Long-term sulpiride that does not induce D2 receptor upregulation in STR, also does not affect striatal ACh concentration or ChAT activity (Jenner and Marsden, 1987; Rupniak et al., 1986).

The septum, which contains D1 and D2 receptors (Camps et al., 1990; Contreras et al., 1987; Gehlert and Wamsley, 1985; Mansour et al., 1990; Richfield et al., 1989; Weiner et al., 1990), is another site of DA-ACh interaction. DA inputs originate entirely in the VTA and terminate primarily in the septum (Beckstead et al., 1979; Fallon and Moore, 1978; Onteniente et al., 1984; Palkovits and Brownstein, 1988; Robinson et al., 1979). In the septum, DA projections interact with ACh neurons whose cell bodies are located in the medial septum (MS) and whose axons terminate in the hippocampus (Bigl et al., 1982; Koliatsos et al., 1988; Saper, 1984; Woolf and Butcher, 1985; Woolf et al., 1984).

The DA axons appear to exert a tonic inhibitory influence on the septohippocampal cholinergic neurons. Intraseptal haloperidol selectively increases ACh turnover in hippocampus without altering ACh concentration (Robinson et al., 1979). Haloperidol also produces a long-lasting increase in hippocampal sodium-dependent high-affinity choline uptake (HAChU; Durkin et al., 1986). Local injection of 6-

hydroxydopamine (6-OHDA) into septum or VTA (area A10) likewise augments hippocampal ACh turnover without affecting ACh levels (Robinson et al., 1979). Intraperitoneal sulpiride increases HAChU and ACh release (Gilad et al., 1986), while low-dose apomorphine (s.c.), a nonspecific DA receptor agonist, reduces ACh turnover (Robinson et al., 1979) in hippocampus.

Evidence suggests that DA projections from the VTA and SN interact with cortically-projecting cholinergic cells in the nonseptal basal forebrain. This thesis focuses on the infracommissural extension of the dorsal globus pallidus (GP) known as the ventral pallidum (VP), and the caudal extension of the VP known as the sublenticular substantia innominata (SI; Heimer and Wilson, 1975). Data indicate that the VP/SI: 1.) receives inputs from dopaminergic nuclei of the brainstem (Beckstead et al.,1979; Haring and Wang, 1986; Martinez-Murillo et al., 1988; Russchen et al., 1985; Semba et al., 1988; Voorn et al., 1986); 2.) contains DA and its metabolites (Guela and Slevin 1989; Napier and Potter, 1989); 3.) is sensitive to local injections of DA and to systemically-administered DA agonists (Maslowski and Napier, 1989, 1991; Napier et al., 1991a; Napier and Breese, 1986; Napier and Maslowski, 1988; Napier and Potter, 1989); and 4.) contains D1 and D2 receptors (Beckstead et al., 1988; Contreras et al., 1987; Gehlert and Wamsley, 1985; Guela and Slevin, 1989; Napier et al., 1991b). Preliminary studies with tissue homogenates in our laboratory confirmed the presence of these DAergic receptors in VP/SI and demonstrated that DA uptake sites exist in this region (Napier et al., 1991b). The VP/SI also contains large, cortically-projecting cholinergic cells (Armstrong et al., 1983; Dinopoulos et al., 1988; Saper 1984) and anatomical studies

suggest that catecholaminergic terminals contact distal dendrites and somata of VP/SI cholinergic neurons (Martinez-Murillo et al, 1988; Zaborszky et al., in press; Zaborszky et al., 1991; Zaborszky, 1989). That a 6-OHDA-induced lesion of the SN or medial forebrain bundle (mfb) decreases DA concentration in the VP/SI and STR (Guela and Slevin, 1989; Napier and Potter, 1989) and reduces ChAT activity in the nucleus of the vertical limb of the diagonal band (VDB) and nucleus of the horizontal limb of the diagonal band (HDB) (Zaborszky et al., in press; Zaborszky 1989) suggests that basal forebrain cholinergic neurons are sensitive to Daergic influence.

Thus, the research presented here explored the possibility that terminal regions of VP/SI cholinergic cells react to manipulations of the DA system. Rats were treated for 21 days with D1- or D2-receptor-specific antagonists. Radioligand binding of [3H]SCH 23390 and [3H]spiperone to VP/SI tissue homogenates obtained from these animals was employed to indicate whether D1 and/or D2 receptors, respectively, adapted to this pharmacological challenge. ChAT activity in cortex and amygdala served as a marker for changes in VP/SI cholinergic terminal function (Kuhar, 1976; Russchen et al., 1985; Watson et al., 1985). For comparison purposes these parameters also were examined in the STR and septohippocampal system, where DA-ACh interactions already have been established. The morphologic and electrophysiologic similarity of the GP to the VP (Heimer and Wilson, 1975; Napier et al., 1991a) prompted us to record receptor changes in the GP as well.

LITERATURE REVIEW

Recent evidence indicates that the nucleus basalis of Meynert (NB) of the basal forebrain is an important part of the motor and limbic systems in primates (Heimer and Wilson, 1975; Mitchell et al., 1987; Richardson and DeLong, 1988). As such, the NB participates in a multisynaptic pathway which channels neural information from core limbic structures to modality-specific cortical areas (Mesulam et al., 1986). Serving as a point of convergence, the basal forebrain has substantial influence in the pathologies of many neurological disorders (Hornykiewicz et al., 1984; Mesulam et al., 1983). Degeneration of the NB, for example, contributes to the cognitive deficits of Alzheimer's Disease (Durkin, 1989; Kordower et al., 1989; Mufson and Kordower, 1989; Richardson and DeLong, 1988; Salamone, 1986). Whether loss of neurotransmitter systems that project to NB contributes to the dementia of Alzheimer's or other neurological diseases is unknown. To better deduce the role of the NB, it is necessary to understand its anatomical location and communicative framework.

The NB is included in a collection of magnocellular cholinergic neurons spanning the entire human basal forebrain. The simian analogue of this nucleus consists of the cholinergic cells extending anteriorly from the level of the olfactory tubercle to the ansa peduncularis, posteriorly (Mesulam et al., 1983). The NB equivalent in rodent is not so easily defined.

Schwaber et al. (1987) used a digital microscopy system to construct a three-dimensional model of the distribution of cholinergic cells in rat basal forebrain. From a top view, the rostral cholinergic projection neurons lie along the midline within the MS and VDB. Slightly posterior to VDB, in the HDB, the distribution sweeps caudolaterally to the crossing of the anterior commissure (xac). As it progresses caudally, the cholinergic cell column spreads laterally into the magnocellular preoptic area (MPO), the VP, and SI. At the most posterior levels, the column narrows to "sheets of cells" along the lateral border of the internal capsule (ic) (Schwaber et al., 1987).

In attempting to determine the rat homologue of the primate NB, researchers studied forebrain sections ranging from the xac and the relevant nuclei at that level (i.e., HDB, MPO, VP), to the most posterior sections which included the SI, ventromedial globus pallidus (vmGP) and the peri-capsular cholinergic cells (actually labeled "basal nucleus of Meynert" in the atlas of Paxinos and Watson, [1986]). With some variation, the cortically-projecting, cholinergic cell population in this entire area often is referred to as the magnocellular basal nucleus (MBN). Unfortunately, much confusion in basal forebrain nomenclature has arisen from research to identify the rodent equivalent of the primate NB. The forebrain cholinergic column fails to coincide with any one structure. Instead, it is included in several of the cytoarchitecturally distinct nuclei within each brain region through which it passes (Armstrong et al., 1983; Luiten et al., 1987; Saper, 1984; Schwaber et al., 1987; Zaborszky, submitted; Zahm, 1989). Furthermore, "...authors have used different terms for the same [forebrain] structure, as well as the same term for completely different structures" (Butcher and Semba, 1989). Because the anatomical

connections (Bigl et al., 1982; Luiten et al., 1987; Saper, 1984; Woolf et al., 1984) and functional significance (Heimer and Alheid, 1991) of the anterior vs posterior MBN have been reported to differ, it is important to consider the MBN coordinates given when interpreting published data in this field.

Efferents

Anatomical evidence agrees that the primary efferents of the primate NB (Kohler et al., 1984; Mesulam et al., 1983; Mesulam et al., 1986) and the rodent MBN (Eckenstein et al., 1988; Houser et al., 1985; Luiten et al., 1987; Saper, 1984; Woolf and Butcher, 1985; Woolf et al., 1984) project ipsilaterally to the entire cortical mantle. Moderately-large, morphologically-similar, ChAT-positive cell bodies of the NB or MBN (Armstrong et al., 1983; Dinopoulos et al., 1988; Mesulam et al., 1983; Saper 1984) are the "single major source of cholinergic innervation" to the cortex (Mesulam, 1983). Studies have shown that 80-90% of all cortically-projecting axons arising from the basal nuclei are cholinergic, the highest percentage originating in the most caudal regions (Luiten et al., 1987; Mesulam et al., 1983; Rye et al, 1984). Biochemical data confirm these conclusions. MBN lesions in the VP/SI and in vmGP/ic produce significant decreases in cortical ChAT activity (30-70%) (Fibiger et al, 1988; Saper, 1984; Santos-Benito et al., 1988; Watson et al., 1985; Watson et al., 1985; Wenk et al., 1980) and reduce the B_{max} (45%) for HAChU carriers (Watson et al.,1985).

The organization of MBN axons is still unknown. Experiments in rat brain utilizing ChAT and/or acetylcholinesterase (AChE) histochemistry in conjunction with the anterograde tracer *phaseolus vulgaris* leucoagglutinin (PHA-L) (Luiten et al., 1987), or

with retrograde fluorescent label horseradish peroxidase (HRP) (Bigl et al., 1982; Koliatsos et al., 1988; Saper, 1984; Woolf and Butcher, 1985; Woolf et al., 1984), have indicated several trends. First, anterior MBN, but not posterior MBN, sends a moderate projection to limbic structures in mesocortex (i.e., infralimbic, prelimbic, anterior cingulate, retrosplenial, orbitofrontal, agranular insular, and perirhinal corticies) and allocortex (i.e., olfactory bulb, anterior olfactory nucleus, olfactory tubercle, lateral piriform, and entorhinal corticies). Second, the most caudal aspect of anterior MBN and the intermediate MBN innervate anterior neocortex, whereas only the posterior MBN substantially innervates posterior neocortex. The exception to this is the occipital cortex, which receives fibers from the most anterior MBN. All areas of the MBN still maintain their predominant output to frontal-parietal cortices in terms of the quantity of fibers. Also, all subdivisions of MBN send numerous axons to the amygdala. Third, there appears to be a loose dorsomedial-ventrolateral organization. Anterior MBN, the most medial section, projects to dorsomedial cortex, and posterior MBN, the most lateral section, to ventrolateral cortex. Similar patterns of innervation were observed in primates (Koliatsos et al., 1988; Mesulam et al., 1983, 1986). The heterogeneity of cholinergic markers across the cortical surface (Lehmann et al., 1984, Mesulam et al., 1986), or the differential branching between the neurons innervating the anterior and posterior cortex (McKinney et al., 1983; Price and Stern, 1983), might partially explain discrepancies in the literature.

The MBN traditionally has referred to the cholinergic neurons located in the ventral forebrain, as described previously. However, the nuclei containing the MBN also

contain non-cholinergic cells. These may contribute to the neurological pathologies involving the basal forebrain, either by their own projections or by their interaction with the cholinergic neurons. Anatomical evidence demonstrated that the cholinergic neurons represent only a small percentage of the total cell groups of the basal forebrain nuclei (e.g., Saper, 1984). Different neuron subpopulations also have been identified electrophysiologically (Aston-Jones, 1985; Griffith, 1988; Griffith and Matthews, 1986). Cholinergic and GABAergic projection neurons are intermingled throughout the basal forebrain and exhibit remarkable structural similarity (Fallon and Moore, 1978; Kohler et al., 1984; Zaborszky et al., 1986). Axons of both cell types follow similar trajectories to their targets, including the cortex (Woolf and Butcher, 1985; Woolf et al., 1986).

Non-cortical efferents from the basal forebrain region between the xac and vmGP have been demonstrated, although in many cases the neurotransmitters have not been identified. These efferents reach the reticular nucleus of the thalamus (Heimer et al., 1985; Levey et al., 1987) the mediodorsal thalamic nucleus (Heimer et al., 1982, 1985; Heimer and Wilson, 1975; Young et al., 1984; Zahm et al., 1987), the ventral striatum [vSTR (Haber et al., 1985; Heimer et al., 1985)], the entopeduncular nucleus (GP_{ep}; Heimer et al., 1985), the mesencephalic locomotor region (Mogenson and Wu, 1986), the substantia nigra (Haber et al., 1985; Woolf et al., 1984; Zahm, 1989), and the ventral tegmentum (Haber et al., 1985; Zahm, 1989). As indicated by its location and connections, the basal forebrain may integrate limbic and motor systems.

Functional Significance of Efferent Projections

The ventral striatopallidal system, in parallel with the dorsal striatopallidal system,

work together for the planning and initiation of movements (Heimer et al., 1982). Behavioral studies in primates have shown that the neurons in the SI/NB exhibit changes in activity preceding reinforced (rewarded) movements during a task (Mitchell et al., 1987; Richardson and DeLong, 1988). Therefore, a change in ACh released to the cortex is expected during reinforcement.

ACh has been suggested to contribute to many functions in the cortex, including state of arousal (Bradley and Elkes, 1957; Longo, 1955; Phillis, 1968), learning (Mandel et al., 1974), and memory (Davis et al., 1978). ffrench-Mullen et al. (1983) discovered that pyramidal neurons of rat prepyriform cortex increase firing rates in response to application of ACh. In cells which do not respond, ACh potentiates responses to glutamate and aspartate. Both excitation and potentiation by ACh are blocked by the muscarinic receptor antagonist, atropine but not by the nicotinic receptor antagonist, curare (ffrench-Mullen et al., 1983). Richardson and DeLong (1988) demonstrated that direct application of ACh onto cortical and hippocampal neurons causes a prolonged decrease in a Ca^{++}-activated potassium current with a consequent decrease in after-hyperpolarization. This alteration of the cell's responsiveness can last more than an hour in certain cortices (Richardson and DeLong, 1988). In a study combining electrophysiological and behavioral techniques in monkeys, Aou et al. (1983) observed that 90% of ACh-sensitive cells in orbitofrontal cortex change activity during a bar-press feeding task. Of these neurons, 67% respond to more than one phase of the task. Atropine antagonizes the excitatory response to ACh in this paradigm, similar to the result in ffrench-Mullen's experiment.

Pirch and associates have demonstrated in rats that the increase in VP/SI activity preceding reinforcement is essential for the normal development of cue-elicited slow potentials (Rigdon and Pirch, 1984; 1986). Slow potentials are recorded from rat frontal cortex in response to a reinforced stimulus. Pirch observed that these specific waveforms are suppressed by VP/SI lesioning, by VP/SI microinjection of procaine or GABA (to depress neuronal activity), or by cortical muscarinic receptor blockade (Pirch et al., 1986; Rigdon and Pirch, 1984; 1986). These results provide evidence that cortical responses are dependent upon cholinergic innervation from the MBN. They also demonstrate the possibility that the GABAergic cells intermingled with MBN may influence cortical function *via* modulation of the cholinergic efferents, and by a direct GABAergic projection to cortex.

ACh release from VP/SI terminals preceding reinforced movements may contribute to state of arousal or learning and memory, by producing increased responsiveness in cortical neurons (Richardson and DeLong, 1988). This hypothesis is supported by several researchers who reported that lesions of VP/SI cholinergic neurons, which decrease cortical ChAT by 30-40%, cause learning and memory deficits in rats (Altman et al., 1985; Chrobak et al., 1987; 1988; Friedman et al., 1983; Murray and Fibiger, 1985; Ueki and Miyoshi, 1989).

Afferents

While the efferents of the basal forebrain cholinergic system have long been investigated, information concerning the afferents to the nuclei containing the MBN is just beginning to accumulate. The complexity of this region indicates that interactions

between its various cell components play an important role in regulating cholinergic transmission in the cortex. Since removal of synaptic input causes up-regulation of receptors, disruption of cellular metabolism or even transsynaptic degeneration, knowledge of the afferent connections of the VP/SI/vmGP (rat) and SI/NB (primate) might unfold some pathophysiological mechanisms of Alzheimer's disease, Parkinson's disease, and other forms of dementia.

Inputs to the SI/NB are predominantly ipsilateral and originate from a variety of sources (Irle and Markowitsch, 1986; Semba et al., 1988). Studies in primates using HRP or PHA-L with AChE staining, or with tritiated amino acids, revealed a cortical innervation of SI/NB exclusively from limbic or paralimbic areas. Only the orbitofrontal, medial temporal, prepyriform, periamygdaloid, entorhinal, and anterior insular corticies are connected reciprocally with the SI/NB. The rest of the neocortex, i.e., posterior parietal, peristriate, lateral temporal and posterior insular, does not project to SI/NB (Irle and Markowitsch, 1986; Mesulam and Mufson, 1984; Russchen et al., 1985). Therefore, SI/NB receives information from the sensory modalities (except olfaction) only after extensive cortical processing.

The vSTR, nucleus accumbens (NA), and olfactory tubercle (OT) send substantial fibers to VP/SI (Bolam et al., 1986; Grove et al., 1986; Haber and Nauta, 1983; Heimer et al., 1985; Heimer and Wilson, 1975; Russchen et al., 1985; Zaborszky et al., 1982; 1986). This projection is thought to be analogous to that from dorsal STR to GP (Armstrong et al., 1983; Heimer et al., 1982; 1985). PHA-L filled axons from vSTR seem to selectively contact cell somata of ChAT-positive or AChE-positive VP/SI neurons

(Bolam et al., 1986), while non-cholinergic VP/SI cells are enmeshed by striatopallidal "woolly fibers", a common characteristic of striatopallidal connections (Grove et al., 1986; Haber and Nauta, 1983). Zahm and Heimer (1988) noted that medial VP is topographically related to medial vSTR in terms of neurotensin-immunoreactivity (NT-IR [a characteristic of neuronal fibers]). Because NT-IR also is associated with the (medially) adjacent bed nucleus of the stria terminalis (BNST), they suggested that this medial ventrostriatopallidal pathway is included in a larger basal forebrain circuit involved with emotional behavior and memory. This hypothetical system coincides with the "extended amygdala" (Heimer et al., 1985), consisting of "a continuum formed by the centromedian amygdala, sublenticular SI, and BNST with direct output channels to visceromotor and somatomotor centers in the brainstem and spinal cord and reciprocal relations with the hypothalamus." (Heimer and Alheid, 1991)

Immunohistochemical evidence demonstrates that GABAergic fibers of the NA project to VP/SI (Heimer et al., 1985) and neurochemical studies have shown that electrical stimulation of NA decreases cortical ACh turnover (Wood and McQuade, 1986). Many researchers have confirmed: the high concentration of glutamic acid decarboxylase (GAD) in the VP/SI region (Ingham et al., 1988), the dense GAD-immunoreactive terminals surrounding VP/SI cells (Ingham et al., 1988; Zaborszky et al., 1986), and that GABA axon terminals form synapses with VP/SI cholinergic neurons (Chang, 1989; Ingham et al., 1986; Ingham et al., 1988; Zaborszky et al., 1986). Unilateral injection of the GABA receptor agonist, muscimol into the VP decreases ACh output from the ipsilateral frontoparietal cortex. Interestingly, both the GABA receptor antagonist,

picrotoxin and the catecholamine releasor, amphetamine antagonize this effect (Casamenti et al., 1986). Microinfusion of muscimol into the SI decreases cortical HAChU (Wenk, 1984). Direct injections and parenteral muscimol decrease cortical ACh turnover, an effect suppressed by picrotoxin. Picrotoxin administered alone does not produce an effect, indicating that the GABAergic transmission influencing MBN is not tonically-active (Wood, 1986; Wood and McQuade, 1986; Wood and Richard, 1982).

Other important afferents to rat VP/SI or primate SI/NB originate in the amygdala. Heaviest projections are from the basal amygdala nuclei, especially the basolateral nucleus, and from the central nucleus. EM studies have shown that amygdalofugal fibers, likely glutamatergic, synapse on the basal forebrain ChAT-positive cells. MBN also returns a dense projection to the amygdala (Irle and Markowitsch, 1986; Russchen et al., 1985; Semba et al., 1988; Zaborszky and Cullinan, 1989; Zaborszky et al., 1984). The amygdala is connected to the olfactory system, the sensory association areas of the temporal and insular corticies, temporal pole, orbitofrontal cortex, perirhinal cortex, entorhinal cortex, and the subiculum. Furthermore, nuclei of the hypothalamus and brainstem provide the amygdala with autonomic and visceral information. The predominance of the amygdala projection to MBN demonstrates that polysensory and limbic convergence centers channel neural information to the basal forebrain (Mesulam and Mufson, 1984; Russchen et al., 1985).

The diencephalon also innervates the basal forebrain. Inputs include axons from the ventromedial hypothalamus and the lateral hypothalamus (Haring and Wang, 1986; Irle and Markowitsch, 1986; Russchen et al., 1985) which establish synaptic contact with

cholinergic projection cells of the VP/SI (Zaborszky and Cullinan, 1989). The area also is innervated by the thalamus, including the centromedian and parafascicular nuclei, and by the subthalamus--zona incerta (Haring and Wang, 1986; Irle and Markowitsch, 1986; Russchen et al., 1985; Zaborszky and Cullinan, 1989).

A variety of structures found in the brainstem project to VP/SI or SI/NB. These are: the dorsal raphe, the periaqueductal gray, the pedunculopontine nucleus, the parabrachial nuclei, the pontine and medullary reticular formation, the retrorubral area, the solitary nucleus and the locus coeruleus [LC, (Haring and Wang, 1986; Irle and Markowitsch, 1986; Russchen et al., 1985; Semba et al., 1988)].

Most relevant to this thesis is the evidence for a midbrain DA projection to the VP/SI and vmGP/ic. DA neurons of the substantia nigra pars compacta (SNc) are continuous with the DA cells located in the ventrolateral tegmental region near the caudal pole of the SN, and with those located dorsomedially in the VTA (Beckstead et al., 1988; Deutch et al., 1988; Fallon and Moore, 1978). HRP injections into VP/SI or vmGP/ic retrogradely label cells in the SNc and VTA, although sparsely (Haring and Wang, 1986; Russchen et al., 1985; Semba et al., 1988). Beckstead et al. (1979) observed that tritiated amino acid injections into VTA and medial SNc yield very light labeling of VP/SI; however, no VP/SI labeling occurs when only SNc is involved. Voorn et al. (1986) showed that the VP/SI is dispersed with bundles of DA-IR fibers which branch out and exhibit varicosities, indicating the VP/SI is a target for these fibers. By combining AChE histochemistry with TH immunostaining, Martinez-Murillo et al. (1988) demonstrated that large AChE-positive cell bodies are surrounded by dense TH-IR fibers. Many of the TH-

IR terminals come in close contact with AChE-positive cell bodies, suggesting synaptic contact. HRP injected into the vmGP/ic is retrogradely transported to ipsilateral SN and LC, *via* fibers making up approximately 1% of the SN and 1% of the LC TH-IR cells (Martinez-Murillo et al., 1988). Since LC contains noradrenergic cell bodies, these results indicate that the VP/SI receives a noradrenergic input comparable to the DAergic input.

The above reports are in agreement with the findings of Zaborszky and associates (Zaborszky et al., in press; Zaborszky et al., 1991; Zaborszky, 1989), who used light microscopy (LM) and EM to visualize ventral forebrain neuronal elements in sections double-labeled for ChAT and norepinephrine-specific, dopamine-β-hydroxylase (DβH), or TH. DβH-IR terminals closely appose cholinergic neurons throughout the basal forebrain, particularly within the SI. DßH-IR varicosities primarily are distributed around distal cholinergic dendrites and form assymmetric synapses. The distribution of putative synapses between TH-IR terminals and basal forebrain cholinergic neurons is similar to that of DßH-cholinergic contacts. Putative contacts in the vmGP and the caudal GP/ic region, however, exclusively are between TH-IR axons fibers and cholinergic neuronal elements. TH-cholinergic synapses are symmetric and primarily are located on proximal cholinergic dendrites or cell bodies.

Although TH immunostain also labels many DβH-IR axons, the different distribution, arborization, and synaptic specializations of TH- and DβH- cholinergic interactions suggests that DA-cholinergic contact does occur in the basal forebrain (Zaborszky et al., in press; Zaborszky et al., 1991; Zaborszky, 1989)

Gustafson et al. (1989) also reported that DβH- and TH-positive axons possess

different fiber characteristics and regional distributions. Thus, they surmised that at least some of "the TH-IR fibers...are DAergic and not noradrenergic." Furthermore, fibers positively labeled for DARPP-32 (a DA- and cyclic AMP-regulated phosphoprotein localized in dopaminoceptive brain regions) are "almost completely coextensive" with TH-IR fibers within the basal forebrain at the level of the xac. In particular, they observed that the VP contains a dense DARPP-32-IR terminal plexus (possibly originating in the OT) as well as moderate innervation by TH-IR axons from the SN/VTA (Gustafson et al., 1989).

Confirming Voorn and Zaborszky's observations, biochemical and electrophysiological data suggest that a DAergic input regulates VP/SI neurons. Guela and Slevin (1989) and Napier and Potter (1989) demonstrated that DA is present within the VP/SI and that bilateral 6-OHDA-induced destruction of the SN reduces DA and its major metabolites in the VP/SI. Napier and associates (Napier et al., 1986; 1991a; Napier and Potter, 1989) also reported that ~40% of VP/SI cells alter their firing rates in response to locally-applied DA. Further experimentation revealed dose-related increases in cell activity upon intravenous administration of apomorphine (Napier et al., 1991a) or SKF 38393, and dose-dependent decreases in firing rate in response to the D2 receptor-specific agonist, quinpirole (Maslowski and Napier, 1989; 1991).

Autoradiographic studies show variable amounts of ligand binding to D1 (Beckstead et al., 1988; Contreras et al., 1987; Mansour et al., 1990; Richfield et al., 1989; Weiner et al., 1990) and D2 (Beckstead et al., 1988; Contreras et al., 1987; Gehlert et al., 1985; Mansour et al., 1990; Richfield et al., 1989; Weiner et al., 1990) DAergic

receptors in VP/SI slices, (see Tables 1 and 2). The majority report moderate to dense binding to D1 but sparse or no binding to D2 receptors in this region. Conversely, D2 receptor mRNA has been visualized in VP/SI in moderate (Mansour et al., 1990) and low (Weiner et al., 1990) amounts while D1 receptor mRNA was not observed (Mengod et al., 1991; Weiner et al., 1990). A lesion of the SN which does not change binding of [3H]sulpiride to D2 receptors in VP/SI tissue homogenates (Guela and Slevin, 1989) decreases DA content in both VP/SI and STR (Guela and Slevin, 1989; Napier and Potter, 1989).

Casamenti et al. (1986) observed that DAergic inputs influence the activity of VP cholinergic neurons, resulting in changes of ACh release in the neocortex. Amphetamine (i.p. injection) dose-dependently increases cortical ACh output (measured *via* the cortical cup technique), an effect suppressed by electrolytic destruction of the VP and by a 6-OHDA-induced lesion of the SN. This phenomenon also occurs after a large dose of apomorphine (10 mg/kg i.p.) (Casamenti et al., 1986). Utilizing *in vivo* brain microdialysis and HPLC with electrochemical detection, Day and Fibiger (1992) measured a significant increase in cortical ACh release following administration of 1.0 mg/kg apomorphine. Pretreatment with SCH 23390 (0.3 mg/kg), but not raclopride (1.0 mg/kg), completely blocked the increase. The D1 receptor agonist CY 208-243 likewise increased cortical dialysate concentrations while D2 receptor agonists quinpirole and PHNO had no effect (Day and Fibiger, 1992). These data coincide with the increase in cell firing rate following systemic apomorphine (Napier et al., 1991a) and SKF 38393 (Maslowski and Napier, 1989; 1991).

In summary, DA fibers likely terminate in the VP/SI, DA and its metabolites exist in this region and are reduced following a 6-OHDA lesion of the SN, both receptor subtypes are present in the VP/SI, and VP/SI cells are sensitive to DA and DA agonists. These data support the hypothesis that the VP/SI is a dopaminoceptive brain region. The dense population of cortically-projecting cholinergic cells in the VP/SI is involved in learning and memory and appears to be critical for the development of reinforced behaviors. Amphetamine and apomorphine can increase ACh output from VP/SI neuron terminals. Therefore, the possibility that DA modulates cholinergic cells in the VP/SI warrants further investigation.

The present study was adopted: 1.) to investigate whether DA receptors in the basal forebrain, particularly the VP/SI, adapt to chronic DA receptor antagonist treatment; and 2.) to determine if cholinergic neurons in the VP/SI respond to the chronic antagonism by altering ChAT in their terminal regions. Rats were divided into three groups (30 rats/group), receiving either the D2 receptor-specific antagonist sulpiride (Jenner et al., 1978; Jenner and Marsden, 1982; Peselow and Stanley, 1982; Trabucchi et al., 1975; Worms, 1982), D1 specific antagonist SCH 23390 (Billard et al., 1984; Hyttel, 1983, 1984; Iorio et al., 1983; O'Boyle and Waddington, 1987), or vehicle injections for 21 days. (A treatment duration of 21 days commonly is used to induce physiological conditions associated with chronic drug administration [Gandolfi et al., 1988; Grebb et al., 1990; Memo et al., 1987; Satoh et al., 1987].) On day 23, the rats were killed and their brains removed, hand-dissected, and frozen (-70°). ChAT activity, thought to reflect changes in cholinergic neuron activity (Kuhar, 1976; Watson et al.,

1985), was measured in cortical tissues. Changes in VP/SI DA receptor number were measured with the D1-selective ligand [3H]SCH 23390 (Creese and Chen, 1985) and the D2-selective ligand [3H]spiperone (Seeman 1980). The above parameters also were measured in the STR and septohippocampal system, where DA-ACh interactions are well-characterized.

Another study also was undertaken to ascertain that acute DA antagonist treatment did not down-regulate DA receptors. A separate group of 18 rats received single injections of either SCH- 23390, sulpiride, or vehicle at the same dose and volume as that given during the chronic study. Radiolabeling of D1 or D2 sites subsequently was performed on striatal tissue taken from these animals 48 hours after injection.

Table 1. REGIONAL D1 DOPAMINE RECEPTOR BINDING

AUTHOR	SPECIES	METHOD	LIGAND	CAUDATE; PUTAMEN	VP/SI	GP	GP[ep]	SEPTUM
Camps et al., 1990	Mouse	Quant	[3H]SCH 23390, 1nM	757.13±77.36	ND	132.35±25.27	292.63±36.07	60.0±3.5
	Rat			527.3±52.0	ND	184.2±17.8	358.3±107.6	86.6±2.3
	Guinea pig			612.4±41.15	ND	106.3±22.3	289.6±7.3	82.1±19.2
	Cat			597.0±60.0; 509.4±66.0	ND	64.9±60.0	349.3±157.0	100.7±65.1
	Monkey			420.6±12.34; 372.3±41.5	ND	78.5±49.1	206.2±77.8	ND
	Human			267.5±21.8; 251±21.3	ND	27.8±10.5	102.9±18.4	ND
Mansour et al., 1990	Rat	Qual	[3H]SCH 23390, 4.4nM	Dense	Dense	Moderate	ND	Light
Weiner et al., 1990	Rat	Qual	D1 specific	ND	None	Considerable	ND	Light
Richfield et al., 1989	Rat	Quant	[3H]SCH 23390	1342±44	689±26	192±33	600±48	79±34
Beckstead et al., 1988*	Cat	Quant	[3H]SCH 23390, 2nM	279-362; 219-330	135±15	0	217±6	ND
Contreras et al., 1987	Rat	Qual	[3H]SCH 23390, 10nM	Very dense	Dense	Light	ND	Light

Data are represented as the mean±S.E.M. in fmol/mg protein or *fmol/mg tissue. Quant, quantitative autoradiography; Qual, qualitative autoradiography; ND, not determined.

Table 2. REGIONAL D2 DOPAMINE RECEPTOR BINDING

AUTHOR	SPECIES	METHOD	LIGAND	CAUDATE; PUTAMEN	VP/SI	GP	GP[ep]	SEPTUM
Camps et al., 1990	Mouse	Quant	[3H]CV 205502, 1nM	477.41±42.13	ND	67.5±27.5	ND	52.0±16.5
	Rat			282.11±15.2	ND	35.72±5.01	25.22±9.41	56.21±10.4
	Guinea pig			213.54±17.37	ND	31.36±6.1	20.2	38.21±6.13
	Cat			335.08±26.07; 326.8±11.49	ND	126.63±38.45	55.35±.62	50.83±2.84
	Monkey			255.66±7.37; 272.66±13.89	ND	17.18±1.29	14.33±9.79	ND
	Human			151.4±11.6; 163.8±13.3	ND	44.1±9.9	31.6±6.5	ND
Mansour et al., 1990	Rat	Qual	[3H]raclopride, 5.7nM	Dense	None	Light	ND	Light
Weiner et al., 1990	Rat	Qual	D2 specific	ND	None	Light	ND	Light
Guela & Slevin, 1989	Rat	Homog	[3H]sulpiride, 1nM	ND	58.2	ND	ND	ND
			[3H]sulpiride, 15nM	ND	547.2±80.1	ND	ND	ND
Richfield et al., 1989	Rat	Quant	[3H]spiperone	562±18	70±14	28±8	19±14	59±11
Beckstead et al., 1988*	Cat	Quant	[3H]spiperone, 2nM	154-248; 190-222	0	80±8	35±4	ND
Contreras et al., 1987	Rat	Qual	[3H]sulpiride, 10nM	Very dense	Light	Light	ND	Light
Gehlert & Wamsley 1985*	Rat	Quant	[3H]sulpiride, 15nM	17.3±0.50	1.18±0.07	ND	ND	1.15±.04

Data are represented as the mean±S.E.M. in fmol/mg protein or *fmol/mg tissue. Quant, quantitative autoradiography; Qual, qualitative autoradiography; Homog, tissue homogenate; ND, not determined.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (Harlan Laboratories) weighed approximately 220 grams at the outset of the study and reached approximately 300 grams at the termination of the treatment. The animals were housed in groups of 2-3 per cage, with food (Wayne Lab Blox rat chow) and water provided *ad libitum*. The facility was maintained at 23-25°C with 12:12 hour light:dark cycles (0700 on, 1900 off). Upon arrival to the laboratory, the rats were acclimated for at least one week prior to experimental use.

Chronic Drug Treatment

At a constant time in the afternoon (between 1:00-3:00 p.m.), 90 rats received a subcutanous injection of 0.25 mg/kg SCH 23390 (Schering), SCH 23390 vehicle, 12.5 mg/kg sulpiride (Sigma), or sulpiride vehicle, once daily, for 21 days. No injections were given on day 22. Following the 48-hour washout, animals were sacrificed by decapitation, the brains removed, and the appropriate tissues dissected on ice and frozen at -70°C for future analysis.

The injection volume for all treatments was 1 ml/kg. Drugs were prepared in advance as follows: a sufficient quantity of SCH 23390 (as salt) was dissolved in a solution of 0.3% tartaric acid and 1.5% ethyl alcohol, such that the final concentration was 0.25 mg/ml. A sufficient quantity of sulpiride was dissolved in a drop of glacial

acetic acid plus distilled water such that the final drug concentration was 12.5 mg/ml. The sulpiride solution next was buffered to physiological pH with 1-2 drops of 12N sodium hydroxide. The pH of all drug and all vehicle solutions ranged from 7.0 to 7.5.

The SCH 23390 and sulpiride doses chosen were those which blocked respectively SKF38393- and quinpirole-induced changes in VP/SI neuron firing rates during electrophysiologic studies in our laboratory (Maslowski and Napier, 1991; Napier, 1991). These doses also are sufficient to reverse apomorphine-induced hyperactivity in rats (Iorio et al., 1983; Köhler et al., 1979; Ögren et al., 1986).

Acute Drug Treatment

D1 and D2 receptor binding following acute drug treatment and 48-hour washout additionally was performed in STR. A total of eighteen rats received one subcutaneous injection of either SCH 23390 (n=6), sulpiride (n=6), or vehicle (n=6) at the same dose and volume as used in the chronic study. Forty-eight hours later, the animals were decapitated and the brains dissected and frozen. [3H]SCH 23390 and [3H]spiperone binding each were determined in three rats per group. This was done to verify that a single dose did not down-regulate DA receptors and that the tissue washes employed in the assay protocol adequately removed any residual drug.

Tissue Dissection

Brain regions are described in terms of coordinates given in the atlas of Paxinos and Watson (1986) and by structural landmarks. **Frontoparietal cortex**, **anterior cingulate**, **septum**, and **STR** were taken from a coronal section between 1.7 and 0.26mm

posterior to bregma. In relation to the ventral surface of the brain, the anterior cut occurred in the center of the olfactory tubercles and the posterior cut just before the optic chiasm, exposing the xac.

The next section caudally extended approximately 1.5mm from xac, ending immediately behind the widest part of the optic chiasm (about 1.3mm posterior to bregma). From this slice, several regions were obtained: **VP/SI**; small squares of tissue were taken from beneath the xac, from 1-4mm lateral to midline. The knife was slanted to accommodate the lateral shift of this nucleus. **GP**; the rounded tissue was peeled from the area above the xac and between the ic and STR. The dissection was such that more tissue was obtained from the posterior side of the slice, reflecting the GP's increase in size at more caudal positions. A similar dissection was described by Geula and Slevin (1989).

The next tissue block caudally extended about 1mm from the previous cut and contained the **amygdala**. At this point the hippocampus is present although not fully formed. The region where the ventrolateral aspect of the brain folds under temporal cortex was dissected.

The **hippocampus** was removed from the remaining brain.

Radioligand Binding in STR, Septum, VP/SI, and GP

D1 receptor binding with [3H]SCH 23390

The D1 receptors were labeled using the D1-selective ligand [3H]SCH 23390 (Creese and Chen, 1985), according to the method of Hess et al. (1986). In brief, tissue from STR, septum, VP/SI and GP was homogenized using a Polytron homogenizer

(setting 7, 10 seconds) in 40 volumes of ice-cold 50mM Tris-HCl buffer (pH 7.7 at 25°C). Samples subsequently were centrifuged (48,000 x gravity, 15 minutes, 4°C) and the pellets resuspended and washed three times. The final resuspension was into assay buffer consisting of 50mM Tris-HCl, 5mM $MgSO_4$, 0.5mM ethylenediaminetetra-acetic acid (EDTA), in 0.02% ascorbic acid at a tissue concentration of 10.0mg wet weight/ml buffer. Incubations were initiated by adding tissue homogenate (0.10ml) to quadruplicate tubes containing [3H]SCH 23390 (*Acute study*: 2.8nM; *Chronic study*: STR and VP/SI, 2.3nM; GP and septum, 1.9nM) in the absence and presence of (+)-butaclamol (10^{-6}M) to define specific binding. All tubes contained 40nM ketanserin to preclude binding of radioligand to $5HT_2$ receptors, yielding a 3ml final assay volume. Tubes were incubated for 30 minutes at 37°C, filtered over Whatman GF/C filters under vacuum using a Brandell cell harvester, and then washed rapidly three times with 5ml of Tris buffer (15ml total). Radioactivity trapped on the filters was measured by liquid scintillation spectroscopy (Beckman) at an efficiency of approximately 50%. Ligand binding is reported as fmol ligand bound/mg protein.

D2 receptor binding with [3H]Spiperone

The D2 receptors were labeled using the D2-selective ligand [3H]spiperone (Seeman 1980), as previously described by Norman et al. (1987). Again, STR, septum, VP/SI, and GP tissues were homogenized, washed, and resuspended in assay buffer at the concentration of 10.0mg wet weight tissue/ml buffer. Incubation began by the addition of tissue homogenate (0.10ml) to quadruplicate tubes containing [3H]spiperone (*Acute study*: 0.67nM; *Chronic study*: STR, 0.5nM; VP/SI, 1.5nM; GP and septum, 0.78nM) and

40nM ketanserin (to preclude binding of radioligand to $5HT_2$ receptors) at a total assay volume of 3ml. (+)-Butaclamol (10^{-6}M) was used to determine specific binding. Tubes were incubated for 40 minutes at 37°C, filtered over Whatman GF/C filters, and washed 3 times with 5ml Tris buffer (15ml total). Radioactivity trapped on the filters was measured by liquid scintillation spectroscopy at an efficiency of approximately 50%. [3H]spiperone binding is reported as fmol ligand bound/mg protein.

Protein content of each homogenate was determined by the method of Lowry et al. (1951).

ChAT Activity in Cortex, Hippocampus, Amygdala, and Striatum

ChAT activity was estimated according to Fonnum (1969) with modifications made in our laboratory.

Several solutions were prepared in advance. 1.) Sodium phosphate buffer (75mM, pH 7.4): Sodium phosphate monobasic solution (5.850g/L) was added to sodium phosphate dibasic solution (10.647g/L) until the buffer reached a pH of 7.4. 2.) Buffer substrate: Into 10ml sodium phosphate buffer was added NaCl 0.351g, $MgCl_2 \cdot 6H_2O$ 0.0813g, physostigmine 0.0083g, AcetylCoenzymeA (AcCoA) 0.008g, choline iodide 0.0231g, and bovine serum albumin 0.005g. The solution was frozen and stored at -20°C in 1ml aliquots. 3.) Heptanone solution: 75mg sodium tetraphenylboron was dissolved per ml 3-heptanone. This was prepared the day of the assay.

Tissue from frontoparietal cortex, anterior cingulate, STR, hippocampus, and amygdala was homogenized in 20 volumes (40 volumes for STR) of sodium phosphate buffer. Next, homogenates (10µl) were pipetted into the bottom of triplicate microfuge

tubes kept on ice. Buffer substrate was thawed and spiked with [3H]AcCoA (20µl [3H]AcCoA per ml buffer substrate). Ten µl of the [3H]buffer substrate was added to each tube. Tubes were tapped to ensure adequate mixing and then incubated for 30 minutes in a 37°C water bath with constant shaking. Incubation was terminated by addition of 150 µl heptanone solution to each tube and subsequent centrifugation to extract the organic phase. Radioactivity in 100 µl of the organic phase was counted by liquid scintillation spectroscopy. ChAT activity is reported as nmol ACh formed/mg protein/minute.

Protein content of the tissue homogenate was determined according to the method of Lowry et al. (1951).

Statistics

For the radioligand binding experiments, significant differences between the mean fmol binding/mg protein for each drug treatment group and vehicle control was determined by one-way analysis of variance (ANOVA) and *post hoc* Student-Neuman-Keul's test. These analyses also were used to find significant differences between the mean ChAT activity (in nmoles ACh formed/mg protein/hour) of each treatment group and vehicle control. The minimum criterion for significance was $P<0.05$.

RESULTS

Radioligand Binding

Preliminary Experiments

To establish the nature of [3H]SCH 23390 and [3H]spiperone binding in our laboratory, preliminary experiments were performed (data not shown) on untreated rat STR according to the protocols described in "Materials and Methods". Binding was evaluated as a function of radioligand concentration. Saturation of specific sites was achieved while nonspecific binding increased linearly over the concentration ranges tested. Scatchard analysis indicated the presence of a single binding site for [3H]SCH 23390 with a k_D of 0.9 nM and a B_{max} of 448 fmol/mg protein [r=-0.98]. This is consistent with other reports in which the k_D ranges from 0.3 to 1.1 nM (Billard et al., 1984; Creese and Chen, 1985; Hess et al., 1986; Lappalainen et al., 1990; McGonigle et al., 1989) and the B_{max} ranges from <500 to 1538 fmol/mg protein (Billard et al., 1984; Camps et al., 1990; Hess et al., 1986; Lappalainen et al., 1990; Richfield et al., 1989). [3H]Spiperone also demonstrated binding to one site with a k_D of 0.18 nM and a B_{max} of 276 fmol/mg protein [r=-0.94]. Reported k_D values for this ligand range from 0.03 to 1.3 nM while B_{max} ranges from 135 to 600 fmol/mg protein. (Memo et al., 1980; Norman et al, 1987; Seeman 1980). Because our numbers generally fell within the ranges observed by others, we proceeded with the experiments described in this thesis.

Acute Studies

D1 and D2 receptor binding was performed on rat STR taken 48 hours after one subcutaneous injection of vehicle, SCH 23390 (0.25 mg/kg, s.c.), or sulpiride (12.5 mg/kg, s.c.). This was done to verify that a single injection of drug did not down-regulate DA receptors and that any residual drug present in basal forebrain tissue was adequately removed by repeated tissue washings (as described in "Materials and Methods"). Results are summarized in Table 3.

Striatal [3H]SCH 23390 sites

As shown in Table 3, a single injection of SCH 23390 did not alter D1 receptor binding from vehicle control. This indicates that a single treatment does not down-regulate D1 receptors and that 48 hours is an adequate drug washout period. This is consistent with the findings of Lappalainen et al. (1990), who reported that residual SCH 23390 was absent from the brain tissue of rats 16 hours after the last chronic treatment with the drug. A two-day washout also is compatible with the short plasma half-life (20-30 minutes) of SCH 23390 (Kilts et al., 1985).

Congruent with sulpiride's selectivity for the D2 receptor, (Jenner et al., 1978; Jenner and Marsden, 1982; Peselow and Stanley, 1982; Trabucchi et al., 1975; Worms, 1982) sulpiride had no effect on [3H]SCH 23390 binding to D1 receptors.

Striatal [3H]spiperone sites

D2 receptor binding performed 48 hours after acute sulpiride treatment likewise did not differ from vehicle control. Since the plasma elimination half-life of sulpiride is

relatively short, approximately 1-2 hours (Yamada et al., 1990), these data suggest that the residual sulpiride concentration is negligible 48 hours after a single injection. Thus, a 2-day washout period appears sufficient to prevent sulpiride interference with [3H]spiperone binding to D2 receptors. These data furthermore indicate that a single dose of sulpiride does not upregulate D2 receptors.

Consistent with its D1 selectivity, (Billard et al., 1984; Hyttel, 1983, 1984; Iorio, 1983; O'Boyle and Waddington, 1987) SCH 23390 did not significantly alter [3H]spiperone binding to striatal homogenates (Table 3).

Chronic Studies

Rats received daily injections of either SCH 23390 (0.25 mg/kg, s.c.), sulpiride (12.5 mg/kg s.c.), or vehicle for 21 days. Following a two-day washout, the animals were killed and appropriate brain regions were removed and frozen (-70°C) for future analysis. Thirty rats were assigned to each group: 24 rats provided brain tissue for the previously described experiments while the brain tissue of the remaining 6 rats in each group served as a back-up in the event that: 1.) a test had to be repeated; or 2.) opportunities to perform additional experiments arose. D1 receptors were labeled with [3H]SCH 23390 and D2 receptors with [3H]spiperone. Results are summarized in Tables 4 and 5, respectively. The data obtained from STR of rats subjected to chronic vehicle injection respectively are within the ranges of B_{max} values reported for these radioligands in untreated STR (Billard et al., 1984; Creese and Chen, 1985; Hess et al., 1986; Lappalainen et al., 1990; Norman et al., 1987; Seeman 1980). Our data from GP and septum are comparable to the corresponding values (Tables 1 and 2) reported by Camps

et al. (1990). This indicates that: 1.) the radioligand assay worked adequately; and 2.) in terms of receptor number, the experimental method itself did not induce a response. Consequently, we used the DA receptor concentrations present after vehicle treatment as the standard by which treatment responses could be identified.

Chronic SCH 23390 treatment

Chronic SCH 23390 (0.25 mg/kg/day) treatment significantly increased (~40%) the binding of [3H]SCH 23390 to D1 receptors in STR [$F_{(2,15)}$=5.31, P=0.018, one-way ANOVA; q=4.296, *post hoc* Student-Neuman-Keul's test] (Table 4). This is in agreement with several authors who reported selective upregulation of striatal D1 receptors after 12-21 days of SCH 23390 followed by 2-8 days withdrawal (Barone et al., 1988; Creese and Chen, 1985; Grebb et al., 1990; Memo et al., 1987). D1 binding in septum, VP/SI, and GP was not significantly changed from control levels, however. Acute studies by Zhu et al. (1990) similarly showed that rat STR, but not septum, responds to D1 receptor blockade by SCH 23390 (Zhu et al., 1990).

During the present research, in contrast, there was no upregulation of [3H]spiperone sites in STR or any basal forebrain region after 21-day treatment with SCH 23390 (Table 5).

Chronic sulpiride treatment

Chronic sulpiride (12.5 mg/kg/day) did not alter [3H]spiperone binding to D2 receptors in any brain region tested (Table 5). Striatal insensitivity to chronic sulpiride administration has been described by other researchers. Rupniak et al. (1984) and Jenner and Marsden (1987) reported that [3H]spiperone binding in rat STR is unchanged after animals receive 102-109 mg/kg sulpiride daily, for 12 months, *via* drinking water. Satoh et al. (1987) observed that when rats are injected i.p., daily sulpiride at 100 mg/kg (21 days) significantly increases the B_{max} for [3H]spiperone-labeled sites; but doses of 1 or 10 mg/kg/day are ineffective. Missale et al. (1990) likewise observed that chronic sulpiride in low doses (4 mg/kg) does not induce a change in striatal DA recognition sites after 21 days (Missale et al., 1990). Striatal D2 sites similarly are insensitive to chronic treatment with low doses of the sulpiride analog, raclopride (See et al., 1990).

Likewise, following chronic sulpiride, the number of [3H]SCH 23390 sites in each region was not significantly different from that in the corresponding control (Table 4).

ChAT Activity Measurement

In conjunction with the radioligand binding experiments performed on cholinergic cell body regions (i.e., MS, VP/SI, GP, and STR), ChAT activity in terminal regions (i.e., frontoparietal cortex, anterior cingulate, amygdala, hippocampus, and STR) was measured for its response to chronic antagonist treatment. Tissue obtained from animals that received vehicle for 21 days yielded ChAT activity values similar to those previously reported for the corresponding brain areas from untreated rats (Rupniak et al., 1986; Chrobak et al., 1987; 1988). As with the receptor binding data, this verifies the reliability of the assay and indicates that ChAT was unaffected by the experimental protocol.

The vehicle group served as the control to which the results from drug-treated rats were compared. The ChAT responses to chronic treatment are given in Table 6.

Chronic SCH 23390 treatment significantly increased ChAT activity in frontoparietal cortex [$F_{(2,65)}$=5.11, P=0.009, one-way ANOVA; q=4.521, *post hoc* Student-Newman-Keul's test] and amygdala [$F_{(2,65)}$=11.57, P=0.000, one-way ANOVA; q=6.723, *post hoc* Student-Neuman-Keul's test] but did not affect ChAT levels in anterior cingulate or hippocampus. Striatal ChAT appeared to be reduced, but did not reach significance [$F_{(1,10)}$=4.31, P=0.065, one-way ANOVA; q=2.937, *post hoc* Student-Neuman-Keul's test].

In contrast, ChAT in the anterior cingulate and hippocampus responded to long-term sulpiride administration. Respectively, those regions demonstrated increased [$F_{(2,64)}$=3.39, P=0.040, one-way ANOVA; q=4.26, *post hoc* Student-Neuman-Keul's test] and decreased [$F_{(2,67)}$=4.13, P=0.020, one-way ANOVA; q=4.037, *post hoc* Student-Neuman-Keul's test] activity compared to vehicle control. Amygdala ChAT also

appeared to be reduced following chronic sulpiride, but a significant response was not achieved [$F_{(1,43)}$=3.15, P=0.08, one-way ANOVA; q=2.51, *post hoc* Student-Newman-Keul's test]. ChAT in frontoparietal cortex and striatum was unaffected by sulpiride treatment (Table 6).

Table 3. STRIATAL D1 AND D2 SITES FOLLOWING SINGLE INJECTIONS OF DA RECEPTOR-SELECTIVE ANTAGONISTS: COMPARISON TO VEHICLE CONTROL

		Treatment	
Ligand	Vehicle	SCH 23390	Sulpiride
[3H]SCH 23390 (2.8 nM)	486±53 n=3	560±89 n=3	464±179 n=3
[3H]Spiperone (0.67 nM)	219±20 n=3	298±50 n=3	247±68 n=3

Data are represented as the mean±S.E.M. in fmoles/mg protein. No significant differences from vehicle control were detected. [3H]SCH 23390: $F_{(2,6)}$=0.18, P=0.842, one-way ANOVA; q=0.803, *post hoc* Student-Newman-Keul's test. [3H]Spiperone: $F_{(2,6)}$=0.065, P=0.555, one-way ANOVA; q=1.592, *post hoc* Student-Newman-Keul's test. n indicates the number of tissues per group.

Table 4. [3H]SCH 23390 BINDING TO D1 RECEPTORS: RESPONSE TO CHRONIC ANTAGONISM

		Treatment	
Tissue	Vehicle	SCH 23390	Sulpiride
Striatum	475±38.4 n=6	*667±50.9 n=6	507±43.9 n=6
Septum	64.2±14.6 n=6	50.9±16.5 n=5	62.1±3.60 n=5
Ventral Pallidum/Substantia Innominata	193±33.1 n=6	232±30.1 n=6	170±30.2 n=6
Globus Pallidus	226±25.4 n=6	216±40.9 n=6	200±40.0 n=6

Data are represented as the mean±S.E.M. in fmoles/mg protein. * indicates significant difference from vehicle control. Striatum: $F_{(2,15)}$=5.31, P=0.018, one-way ANOVA; q=4.296, *post hoc* Student-Neuman-Keul's test. Septum: $F_{(2,14)}$=0.29, P=0.756, one-way ANOVA; q=1.002, *post hoc* Student-Newman-Keul's test. VP/SI: $F_{(2,15)}$=1.01, P=0.389, one-way ANOVA; q=1.985, *post hoc* Student-Newman-Keul's test. GP: $F_{(2,15)}$=0.13, F=0.878, one-way ANOVA; q=0.717, *post hoc* Student-Newman-Keul's test. n indicates the number of tissues per group.

Table 5. [3H]SPIPERONE BINDING TO D2 RECEPTORS: RESPONSE TO CHRONIC ANTAGONISM

		Treatment	
Tissue	Vehicle	SCH 23390	Sulpiride
Striatum	203±22.3 n=6	205±34.8 n=6	207±18.9 n=6
Septum	18±3.6 n=10	16±1.5 n=11	24±3.6 n=12
Ventral Pallidum/Substantia Innominata	77±18 n=10	82±19 n=10	68±19 n=8
Globus Pallidus	40±6.5 n=11	40±16 n=10	34±4.3 n=9

Data are represented as the mean±S.E.M. in fmoles/mg protein. Striatum: $F_{(2,15)}$=0.01, P=0.995, one-way ANOVA; q=0.140, *post hoc* Student-Newman-Keul's test. Septum: $F_{(2,30)}$=2.12, P=0.138, one-way ANOVA; q=2.710, *post hoc* Student-Newman-Keul's test. VP/SI: $F_{(2,25)}$=0.14, P=0.868, one-way ANOVA; q=0.750, *post hoc* Student-Newman-Keul's test. GP: $F_{(2,27)}$=0.09, P=0.911, one-way ANOVA; q=0.549, *post hoc* Student-Newman-Keul's test. n indicates the number of tissues per group.

Table 6. ChAT RESPONSE TO CHRONIC DOPAMINE RECEPTOR ANTAGONISM

		Treatment	
Tissue	Vehicle	SCH 23390	Sulpiride
Frontoparietal Cortex	72.8±3.14 n=23	*87.4±3.65 n=22	79.7±2.87 n=23
Amygdala	126.8±5.08 n=22	*181.3±7.80 n=23	147.4±10.3 n=23
Anterior Cingulate	133.6±5.40 n=22	131.3±3.13 n=23	*145.8±3.92 n=22
Hippocampus	49.1±2.08 n=24	44.3±2.42 n=23	*40.9±1.50 n=23
Striatum	164.5±8.98 n=6	141.3±6.62 n=6	156.5±7.91 n=6

Data are represented as the mean±S.E.M. in nmoles ACh formed/hour·mg protein. * indicates significant difference from vehicle control. Frontoparietal Cortex: $F_{(2,65)}$=5.11, P=0.009, one-way ANOVA; q=4.521, *post hoc* Student-Newman-Keul's test. Amygdala: $F_{(2,65)}$=11.57, P=0.000, one-way ANOVA; q=6.723, *post hoc* Student-Neuman-Keul's test. Anterior Cingulate: $F_{(2,64)}$=3.39, P=0.040, one-way ANOVA; q=4.26, *post hoc* Student-Neuman-Keul's test. Hippocampus: $F_{(2,67)}$=4.13, P=0.020, one-way ANOVA; q=4.037, *post hoc* Student-Neuman-Keul's test. Striatum: $F_{(2,15)}$=2.22, P=0.143, one-way ANOVA; q=2.935, *post hoc* Student-Newman-Keul's test. n indicates the number of tissues per group.

DISCUSSION

The outcome of the present research indicates that terminal regions of basal forebrain cholinergic neurons (i.e., anterior cingulate, frontoparietal cortex, amygdala, and hippocampus) respond to chronic DA receptor antagonism with changes in ChAT activity. The inhibitory effect on the septohippocampal pathway and the stimulatory effect on the VP/SI-cortical projection appears to distinguish the two systems. Coincident changes in the number of DA receptors located in the cholinergic cell body regions (i.e., septum, VP/SI, GP) were not detected.

The present data additionally suggest that striatal interneurons react differently to chronic DA antagonist treatment than do cholinergic projection neurons of the basal forebrain. The STR responded to D1 antagonism with an increase of [3H]SCH 23390 sites. In contrast, no changes in [3H]spiperone sites occurred following chronic sulpiride. This lack of response to sulpiride has been reported by other researchers (Jenner and Marsden, 1987; Missale et al., 1990; Rupniak et al., 1986; Satoh et al., 1987). Neither drug treatment altered striatal ChAT levels, which may have been a consequence of the small sample size available for striatal ChAT measurement.

Tissue supersensitivity after long-term exposure to a DA antagonist can be characterized by an increase in receptor number and enhanced electrophysiological, enzyme, or behavioral responses to DA or DA receptor agonists (Bloom et al., 1981;

Creese and Sibley, 1980; Fage et al., 1984; Gnegy and Costa, 1980; Jenner and Marsden, 1987; Memo et al., 1987). Thus, the selective upregulation of striatal [3H]SCH 233390 sites after 21-days' SCH 23390 administration likely represents a supersensitive response. No changes in receptor number occurred in septum, VP/SI, or GP after chronic SCH 23390 or sulpiride, demonstrating that these areas are regulated differently than STR. It is possible that the small number of basal forebrain DA receptors precluded accurate detection of quantitative changes. It also is possible that the amounts of antagonist reaching these regions were insufficient to induce receptor upregulation, but were adequate to alter DAergic influence.

Despite the negative radioligand binding results, chronic DA antagonist treatment appeared to make basal forebrain cholinergic terminals more sensitive to endogenous DA. ChAT activity in frontoparietal cortex and amygdala increased following chronic SCH 23390 treatment while chronic sulpiride augmented ChAT in anterior cingulate. Conversely, chronic sulpiride treatment reduced hippocampal ChAT activity.

Several research groups also have observed DA's apparent stimulatory effect on telencephalic cholinergic terminals. Ho and Loh (1972) demonstrated that daily intracisternal injections of DA given to rats over four days significantly increase ChAT activity in both the frontal cortex and the rest of the brain. Casamenti et al. (1986) reported that intraperitoneal amphetamine dose-dependently increases cortical ACh output (measured *via* the cortical cup technique), an effect suppressed by electrolytic destruction of the VP and by a 6-OHDA-induced lesion of the SN. They also observed this phenomenon with a large dose of apomorphine (10 mg/kg; Casamenti et al., 1986).

During preliminary experiments, Day and Fibiger (1991) similarly observed that amphetamine (2 mg/kg) increased frontal cortical ACh output by 180% (measured by *in vivo* brain microdialysis). This increase was completely blocked by SCH 23390 administration (0.3 mg/kg) but only partially attenuated by haloperidol (0.15 mg/kg). Interestingly, local application of amphetamine (10^{-5}M) did not alter ACh release (Day and Fibiger, 1991). Day and Fibiger (1992) also noted that apomorphine elicited an increase in ACh release (measured by *in vivo* brain microdialysis and HPLC with electrochemical detection), but at a smaller dose than that used by Casamenti and associates: Cortical ACh output increased 120% following a 1.0 mg/kg dose. The apomorphine response was completely blocked by SCH 23390 (0.3 mg/kg) but not by raclopride (1.0 mg/kg). The D1 receptor agonist CY 208-243 likewise increased cortical dialysate concentrations of ACh by 80% while D2 receptor agonists quinpirole and PHNO had no effect on cortical ACh release (Day and Fibiger, 1992).

DA's apparent inhibitory effect on hippocampal ChAT following chronic sulpiride agrees with earlier reports that DA exerts a tonic inhibitory influence on septohippocampal cholinergic neurons (Durkin et al., 1986; Gilad et al., 1986; Robinson et al., 1979). Acute DA blockade with intraseptal haloperidol, or disruption of septal DA innervation with 6-OHDA, increases ACh turnover in hippocampus (Robinson et al., 1979). DA receptor antagonists (e.g., haloperidol and sulpiride) also increase hippocampal HAChU (Durkin et al., 1986; Gilad et al., 1986), while DA receptor agonists (e.g., apomorphine) reduce hippocampal ACh turnover (Robinson et al., 1979).

The present data indicate that chronic DA receptor blockade can alter DA's

influence on basal forebrain neuron terminals in the absence of DA receptor upregulation. Similar phenomena have been reported elsewhere. For example, twelve months' sulpiride administration increases striatal cyclic AMP formation but does not alter the number of striatal [3H]spiperone sites (Jenner and Marsden, 1987). Limbic area and striatal homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) levels, but not [3H]spiperone sites, are sensitive to 21 days' sulpiride treatment (1, 10, or 100 mg/kg; Satoh et al., 1987). Because so few chronic studies have examined receptor number in tandem with other measures of cell function, the necessity of an increase in receptor number for the manifestation of tissue supersensitivity is unclear.

The involvement of other neurotransmitter systems in the observed alterations in ChAT activity also must be considered. SCH 23390 possesses marginal affinity for $\alpha 1$ and $\alpha 2$ adrenergic, muscarinic, histaminergic, and 5-HT_{1C} serotonergic receptors. The affinity of SCH 23390 for 5-HT_2 sites, however, is in the nanomolar range (Clark and White, 1987). As such, the possibility exists that the observed effects on cortical and amygdala ChAT activity following SCH 23390 treatment were due, in part, to changes in 5-HT transmission. For example, Bijak and Smialowski (1987) observed that repeated SCH 23390 administration (0.5 mg/kg) induces functional supersensitivity of central 5-HT receptors (increases quipazine-induced head twitches; Bijak and Smialowski, 1987). In contrast, investigators who measured 5-HT and 5-hydroxy indoleacetic acid (5-HIAA) levels in prefrontal cortex, frontal cortex, dorsal and medial raphé, VTA, SN, caudate, and NA reported that no changes occur after chronic SCH 23390 administration (Gandolfi et al., 1988; Lappalainen et al., 1990).

It also is unlikely that the changes in anterior cingulate and hippocampal ChAT after chronic sulpiride were induced by an interaction between sulpiride and non-DAergic receptors. Studies have shown that competition between sulpiride and radioligands labeling norepinephrine, 5-HT, ACh, or histamine receptors is negligible, with k_i values for sulpiride ranging from 1,000 - >10,000 nM (Jenner and Marsden et al., 1982). GAD activity and [3H]flunitrazepam binding to GABA receptors in STR and SN, appear unchanged by sulpiride treatment, as does [3H]quinuclidinylbenzilate ([3H]QNB) binding to striatal muscarinic receptors (Jenner and Marsden, 1987). Sulpiride does not alter norepinephrine or 5-HT turnover, nor does it block tryptophan-induced myoclonic activity in guinea pigs, (for review see Jenner and Marsden, 1982). Further investigation is necessary to determine the contributions of other neurotransmitters and peptides to DA-ACH interactions in the basal forebrain.

Because D1 receptors exist in rat frontoparietal cortex and amygdala (Camps et al., 1990; Contreras et al., 1987; Mansour et al., 1990; Nisoli et al., 1988; Weiner et al., 1990) as well as other cortical regions (Mansour et al., 1990), the possibility that SCH 23390 acts exclusively on these areas rather than *via* D1 receptors located on or near cholinergic cell bodies in the basal forebrain cannot be excluded; however, evidence that chronic SCH 23390 does not affect cortical DA systems has been reported. Memo et al. (1987) observed that in rats, 60-days' SCH 23390 administration enhances the ability of SKF 38393 to stimulate adenylate cyclase ($\uparrow V_{max}$) in STR but not in cortex or hippocampus. Lappalainen et al. (1990) found that HVA concentration in the caudate is reduced while that in prefrontal cortex is unchanged when rats receive SCH 23390 for 18

days. DA levels in both regions remain unaffected by the treatment (Lappalainen et al., 1990).

The possibility also exists that sulpiride acts exclusively on D2 receptors in the anterior cingulate and hippocampus (Camps et al., 1990; Contreras et al., 1987; Gehlert and Wamsley, 1985; Mansour et al., 1990); however there is evidence to suggest otherwise. Moghaddam and Bunney (1990) demonstrated that acute sulpiride doses ranging from 20 to 100 mg/kg i.v. do not affect DA outflow in rat medial prefrontal cortex (measured by *in vivo* microdialysis) but enhance DA outflow in NA and STR (Moghaddam and Bunney 1990). Other researchers demonstrated that sulpiride (40 mg/kg) inhibits [3H]spiperone binding in several subcortical brain regions but not from sites in frontal cortex (Jenner and Marsden, 1982; Köhler et al., 1979; Ögren et al., 1986) or hippocampus (Jenner and Marsden, 1982). Furthermore, 60 days' sulpiride administration enhances the bromocriptine (D2 agonist)-induced inhibition of adenylate cyclase in STR, but not in cortex or hippocampus (Memo et al., 1987).

Two trends in ChAT activity emerge from this research. The first trend indicates that the ChAT response to endogenous DA following chronic DA receptor blockade is related to the presence or absence of DAergic innervation. The regions that demonstrated increased ChAT activity, that is, frontoparietal cortex, anterior cingulate, and amygdala, receive significant mfb input. The hippocampus is not a **direct** target of this fiber pathway (Beckstead et al., 1979; Fallon and Moore, 1978; Palkovits and Brownstein, 1988) and demonstrated reduced ChAT levels. Thus, these results suggest that DA's influence on ChAT in VP/SI neuron terminals may be the net effect of mfb influence on

the cortex (i.e., mfb input to the cholinergic terminals themselves) and the VP/SI (i.e., input to cholinergic cell bodies). DA's influence on hippocampal ChAT may be limited to mfb input to the septum.

The second trend involves the probable location of the cholinergic cell bodies along the cholinergic column. The anterior cingulate and hippocampus, whose primary cholinergic innervation originates in anterior portion of the column, selectively responded to chronic treatment with sulpiride. Conversely, frontoparietal cortex receives terminals from the posterior section and selectively responded to SCH 23390. Although projections from cholinergic neurons along the entire length of the column reach the amygdala, it only was sensitive to SCH 23390. It is possible that this disparity is related to the participation of the more posterior basal forebrain (including the SI) in the extended amygdala system (Heimer and Alheid, 1990).

The results of this work also provide some insight to the functional status of the cholinergic column. Although cortex and hippocampus are innervated by different sections of the column, "... a nuclear organization would predict that drugs that affect [cholinergic parameters] in the hippocampus should have the same effect in the cortex" (Schwaber et al., 1986). Conversely, if the MBN is composed of contiguous nuclei with different afferent connections, cholinergic parameters "...would often be dissociable in the different terminal projections of these nuclei" (Schwaber et al., 1986). The different ChAT responses to chronic DA receptor antagonism indicate that the neurons forming the basal forebrain cholinergic column are not a single functional unit. This agrees with some earlier findings. Robinson et al. (1978; 1979) observed that ACh turnover in

hippocampus, but not cortex, is reduced after acute apomorphine (a dose sufficient to induce stereotypy). Wood and Cheney (1978) reported that acute doses of antimuscarinic drugs (i.e., benztropine, scopolamine, trihexyphenidyl) increase hippocampal and decrease cortical ACh turnover rates.

The present data also demonstrate that chronic DA receptor antagonism does not mimic the conditions in degenerative neural diseases. When ChAT activity is reduced in human disease states such as Parkinson's disease (Ruberg et al., 1987) and Alzheimer's disease (Gulya et al., 1986), the deficit occurs across the cortex and hippocampus. This unidirectional change reflects cell loss, whereas the regional increases and decreases in ChAT that occur after drug treatment demonstrate that the cholinergic cells are functional but are subject to altered neurotransmitter influences.

The significance of the pattern of reactivity observed during this experiment needs to be further explored. Understanding how neuroleptic drugs manipulate DAergic and cholinergic function bears clinical relevance to the long term treatment of psychoses and to resulting side effects such as tardive dyskinesia.

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VITA

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Ms. Muench began her graduate education in the Department of Pharmacology at Loyola University of Chicago in the summer of 1987, at which time she was awarded a basic science fellowship. Under the supervision of Dr. T. Celeste Napier, the author focused her work on dopamine-acetylcholine interactions in the basal forebrain. Significant portions of her research were completed in the laboratories of Dr. Israel Hanin and Dr. George Battaglia. Ms. Muench is a student member of the Society for Neuroscience and a member of the American Medical Writers Association. She has been employed as a Medical Writer at a pharmaceutical consulting firm for the last two years and presently works as an independent contractor with the same company.

The thesis submitted by Mary Elizabeth Muench has been read and approved by the following committee:

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The final copies have been examined by the Director and the signature that appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science in Pharmacology.

09/13/93 — T. Celeste Napier Ph.D.

Date — Director's Signature