THE ROLE OF CHOLINERGIC AND SEROTONERGIC NEOCORTICAL PROJECTIONS IN CONTROLLING SKILLED MOVEMENT IN RATS: EVALUATION OF A MODEL OF DEMENTIA

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GENERAL ABSTRACT

The ascending cholinergic and serotonergic projections are central to cortical activation and normal behavior. The objective of this thesis was to determine whether unilaterally damaging both of these systems would disrupt the production of skilled movements on the contralateral side of the body. Rats received unilateral damage to either the ascending cholinergic, or serotonergic, or both projections. The respective lesions reduced neocortical levels of acetylcholine and serotonin as assessed by acetylcholinesterase reactivity and immunohistochemical staining for serotonin. Subjects were assessed on a battery of sensorimotor tasks sensitive to neocortical integrity. The cholinergic lesion produced mild deficits on some tasks but damage to both together did not abolish skilled movement. The impairments are decreased in relation to the severe effects of bilateral lesions. The results show that the sensorimotor cortex remains functional following deafferentation of both cholinergic and serotonergic afferents.

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LIST OF FIGURES

CHAPTER ONE

General Introduction

Cholinergic and serotonergic afferents densely innervate the neocortex and have been implicated in a variety of behavioral functions. The cholinergic and serotonergic projections to the neocortex are associated with producing a low voltage fast activity (LVFA) pattern of the neocortical electroencephalogram (EEG) (Dringenberg, Vanderwolf, 1998; Vanderwolf, Robinson, & Pappas, 1980; Vanderwolf & Baker, 1986; Vanderwolf & Stewart, 1986). The ACh-related LVFA is associated with alert immobility while the 5-HT-related LVFA is associated with overt movement of the body and limbs. Surprisingly, damage to either system has little, if any, effect on behavior. Conjoint damage to both, however, produces severe impairments in learning and memory. Such animals have been described as displaying "no intelligent behavior" (Vanderwolf, 1987). Consequently, the combined blockade preparation has been proposed as a model of dementia.

One difficulty in studying animals with bilateral depletions of ACh and 5-HT is that the preparation is not practical for chronic experiments because subjects are unable to care for themselves. Unilateral lesions, however, have been used to study the effects of conditions such as stroke and Parkinson's disease, conditions in which combined lesions would be incapacitating (Miklyaeva, Castaneda, & Whishaw, 1994; Rose, Whishaw, & van Hof, 1992; Whishaw, Gorny, & Sarna, 1998; Whishaw, O'Connor, & Dunnett, 1986). Animals with unilateral damage have sensory and motor impairments mainly to the contralateral side of the body. Their ipsilateral side of the body, controlled

by the intact hemisphere, is sufficient for self-maintenance. Surprisingly, there has been no previous investigation of the role of these ascending systems on sensorimotor behavior in the rat, as studies have been limited to learning/memory function. There also has been no previous examination of the effects of unilateral lesions.

Thus, the principal concern in the present thesis was to assess the effects of unilateral depletion and conjoint unilateral depletion of the neocortical ACh and 5-HT projections on sensorimotor behavior. In the introduction, neurotransmitters will be briefly described. The organization of acetylcholine and serotonin in the brain will be outlined as well. This will be followed by a description of studies that examined the behavioral significance of acetylcholine and serotonin. The focus of this literature review will be on the significance of the two systems with respect to learning and memory because few studies have examined the two systems in light of motor behavior. Finally, the interaction between acetylcholine and serotonin will be discussed.

Neurotransmitters

Four standard criteria are used to confirm that an agent is a neurotransmitter. (1) The substance must be present within the presynaptic neuron where it is synthesized and packaged into vesicles, (2) the substance must be released in response to presynaptic electrical activity, (3) specific receptors for the substance must be present on the postsynaptic cell, (4) exogenous application of the suspected substance should mimic the effect of presynaptic stimulation on the postsynaptic cell. By the 1950's, the list of neurotransmitters had expanded substantially using the above criteria.

Neurotransmitters are chemical messengers that pass from one neuron to another cell. This triggers a cascade of events in the receptive neuron generating a postsynaptic electrical signal. A neurotransmitter that did not bind to a postsynaptic receptor is rapidly removed from the synaptic gap. This allows the postsynaptic cell to engage in another cycle of neurotransmitter release or binding. The neurotransmitter is normally removed by way of degradation by a specific enzyme; it may also be taken into nerve terminals or surrounding glial cells. This mechanism of signaling between neurons is the most common system of communication in the central nervous system.

It is useful to separate neurotransmitters into two categories based on size. Neuropeptides are large transmitter molecules composed of chains of amino acids. Small molecule neurotransmitters include individual amino acids, biogenic amines and acetylcholine. It is not uncommon for neurons to produce and release more than one type of neurotransmitter. Acetylcholine and serotonin are considered small molecule neurotransmitters.

The discovery of acetylcholine: Initially all synapses were thought to function by electrical transmission. The idea of chemical signaling between neurons was introduced through Otto Loewi's experiment in 1921. Loewi isolated and perfused the hearts of two frogs. He then stimulated the vagus nerve of one heart, which slowed its beating rate. The perfusate flowing through the heart was collected and introduced to the second heart. This caused the second heart to slow down as effectively. Thus, stimulating the vagus nerve released a chemical that slowed the heartbeat. The substance was originally called "vagus substance," but the active ingredient was later named

acetylcholine. Loewi's experiment demonstrated that neurons indeed release chemicals known as neurotransmitters to send signals to other cells.

Acetylcholine synthesis and distribution: Acetylcholine is synthesized in nerve terminals. It is produced from acetyl coenzyme A and choline in a reaction catalyzed by the enzyme choline acetyltranseferase. Acetylcholine is broken down in the synaptic gap by the enzyme acetylcholinesterase. Both choline acetyltransferase and acetylcholinesterase are often used as biomarkers for the presence of acetylcholine.

The distribution of cholinergic neurons throughout the central nervous system has been thoroughly described in rats (Fibiger, 1982; Mesulam, Mufson, Wainer, & Levey, 1983; Rye, Wainer, Mesulam, Mufson, & Saper, 1984; Wainer et al., 1984; Wainer et al., 1993; Woolf, 1991). The basal forebrain is mainly composed of cholinergic-neurons. These neurons encompass a number of anatomical regions: septal nuclei, diagonal band of Broca, and nucleus basalis magnocellularis. The septal nuclei provide cholinergic input to the hippocampus. The horizontal and vertical limbs of the diagonal band send projections to the hippocampus, olfactory bulbs and amygdala. In the ventromedial corner of the rat globus pallidus is a group of large, cholinesterase reactive neurons, referred to as the nucleus basalis or nucleus basalis magnocellularis. This anatomical region is believed to be homologous to the nucleus basalis of Meynert in human and non-human primates (Flicker, Dean, Watkins, Fisher, & Bartus, 1983; Johnston, McKinney, & Coyle, 1979; Johnston, McKinney, & Coyle, 1981).

Neurons of the nucleus basalis provide 80-90% of the cholinergic input of the ipsilateral neocortex (Mesulam et al., 1983; Rye et al., 1984). An acetylcholinesterase-reactive pathway originating in this region and terminating in the cerebral cortex was first

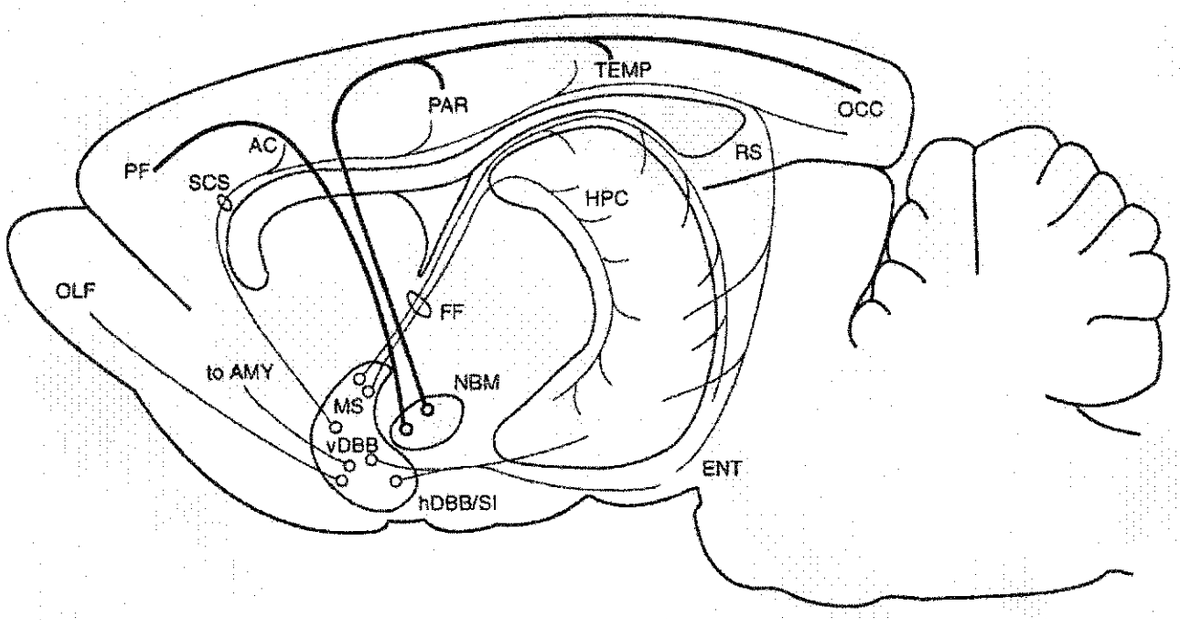
described by Shute and Lewis (1967). The projections are topographically organized; the anterior areas of the nucleus project to the frontal and temporal neocortex and the posterior regions project to the parietal and occipital neocortex. The nucleus basalis sends limited input to the olfactory bulbs and amygdala as well (Rye et al., 1984; Wenk, Bigl, & Meyer, 1980). The nucleus basalis has diffuse projections, has compact cells of origin, and utilizes acetylcholine. It provides the opportunity to study the relationship between cortical cholinergic projections and behavior and is therefore the structure of interest for the present experiments.

The discovery of serotonin: Serotonin has received attention since the mid-nineteenth century (Cooper, Bloom, & Roth, 1991). The neurotransmitter was first recognized as one that caused powerful contractions of smooth muscle organs and a cause of high blood pressure. 5-hydroxytryptamine (5-HT) was the active ingredient in serotonin isolated from various areas of the peripheral nervous system.

Twarog and Page (1953) were among the earliest to demonstrate serotonin in mammalian brains. They isolated serotonin from various brain structures of rats, rabbits, and dogs. The isolated substance was used in an experiment similar to that designed by Loewi (1921). The hearts of *Venus mercenaria*, which are also known as quahog or hard shell clam, were suspended in artificial seawater. The isolated substance was added to individual baths and caused an increase in the amplitude of the heartbeat. This discovery directed research towards the role of serotonin in the central nervous system.

Serotonin synthesis and distribution: Serotonin is synthesized from the amino acid tryptophan. This primary substrate is taken up into the neuron by a plasma membrane transporter. It is then hydroxylated in a reaction that is catalyzed by the

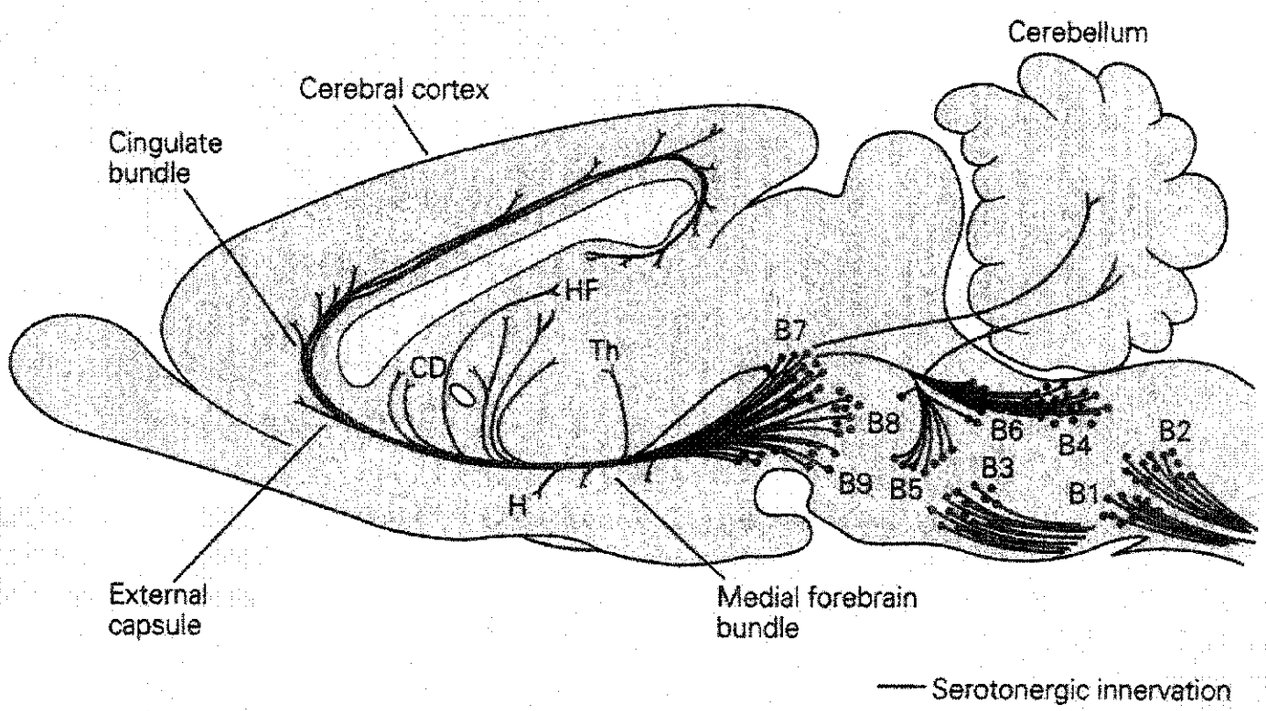
Figure 1.1: Schematic representation in the sagittal plane of the major subdivisions of the basal forebrain cholinergic system in the rat brain (modified from Leanza, 1996). AC, anterior cingulate cortex; AMY, amygdala; hDBB, horizontal limb of the diagonal band of Broca; vDBB, vertical limb of the diagonal band of Broca; ENT, entorhinal cortex; FF, fimbria fornix; HPC, hippocampus; MS, medial septum; NBM, nucleus basalis magnocellularis; OCC, occipital cortex; OLF, olfactory bulbs; PAR, parietal cortex; PF, prefrontal cortex; retrosplenial cortex; SCS, supracallosal striae; TEMP, temporal cortex.


TEMP
PAR
OCC
AC
PF
SCS
RS
HPC
OLF
FF
to AMY
NBM
MS
vDBB
ENT
hDBB/SI

enzyme tryptophan-5-hydroxylase. This step produces 5-hydroxytryptophan, which is then decarboxylated to yield 5-hydroxytryptamine or serotonin.

Raphe nuclei are a collection of predominantly midline neurons within the mammalian brain stem (De Olmos & Heimer, 1980). Raphe neurons are rich with serotonin (Palkovits, Brownstein, & Saavedra, 1974) and are the main source of serotonergic projections to most of the central nervous system (Azmitia & Segal, 1978; Descarries, Beaudet, & Watkins, 1975; Moore, Halaris, & Jones, 1978; O'Hearn & Molliver, 1984; Sakanaka et al., 1980; Takagi, Shiosaka, Tohyama, Senba, & Sakanaka, 1980) (Fig. 1.2). The cell bodies of the serotonergic neurons have been grouped and labeled B_1-B_9 (Dahlstrom & Fuxe, 1964). The groups B_7, B_8, and B_9 are the most rostral of these neurons and provide most of the ascending serotonergic afferents (De Olmos & Heimer, 1980). Projections of these neurons converge in the ventral tegmental area. They pass through the medial forebrain bundle and then diverge and terminate in the hippocampus, basal forebrain, thalamus and cortex. The raphe projections to the neocortex are topographically organized. The laterality of the median (B_8) and dorsal (B_7) raphe nuclei is not certain. There are reports indicating that the projections from the median raphe are ipsilateral (Porrino & Goldman-Rakic, 1982) and others in favor of a contralateral projection (Jacobs, Foote, & Bloom, 1978). Most reports on the dorsal raphe, however, indicate that the projections are organized in a predominantly ipsilateral fashion (Jacobs et al., 1978; van der Kooy & Hattori, 1980).

Figure 1.2: raphé nuclei neurons along the midline of the brain stem (Kandel, Schwartz, & Jessell, 2000). CD, caudate nucleus; HF, hippocampal formation; H, hypothalamus; Th, thalamus.


Cerebellum
Cerebral cortex
Cingulate bundle
HF
Th
CD
B7
B8
B6
B4
B2
B3
B9
B5
B1
H
External capsule
Medial forebrain bundle
Serotonergic innervation

Functions of Acetylcholine

The functions of acetylcholine in the central nervous system have been debated. A number of proposals have been suggested, however, in the last few decades. The following section will describe: (1) its involvement in Alzheimer's disease, (2) learning and memory studies in animals, (3) its influence on brain electrical activity.

Alzheimer's disease: There are a number of abnormalities in the brains of Alzheimer's disease (AD) patients. Alzheimer, Stelzmann, Schnitzlein, and Murtagh (1907) were first to describe post mortem brain tissue of an AD patient. The authors first noted signs of atrophy or shrinkage of the tissue. This was obvious by the enlarged sulci and shrunken gyri. The brain was later sectioned and stained with the Bielschowsky silver method. It revealed changes in the cytoskeleton of neurons now known as neurofibrillary tangles, which is an abnormal form of tau protein (Wood, Mirra, Pollock, & Binder, 1986). In addition the neurons contained a deposition of a pathological metabolic substance. These are beta-amyloid protein deposits outside the neurons now known as neuritic plaques. Alzheimer suggested that the abnormalities were separate from the brain changes noted in any other psychiatric illness. A considerable correlation between the degree of dementia and the relative number of plaques and tangles was documented in later specimens (Alzheimer et al., 1907). Alzheimer could not detect other abnormalities in AD brains, however, because the neurochemical tools were not available at the time.

It was later discovered that acetylcholine is deficient in the brains of AD patients. Whitehouse et al. (Whitehouse, Price, Clark, Coyle, & DeLong, 1981; Whitehouse et al., 1982) studied post mortem brain tissue of AD subjects and discovered that there was a

significant decline in the levels of acetylcholine throughout the brain. They also report that the basal forebrain was severely damaged. The findings suggested that the loss of acetylcholine might be, in part, responsible for the learning and memory deficits seen in AD patients. The discovery stimulated research on the possible relationship between cholinergic mechanisms and cognitive processes. Bartus, Dean, Beer, and Lippa (Bartus, Dean, Beer, & Lippa, 1982) proposed the *cholinergic hypothesis* in which they suggest that acetylcholine is instrumental for memory processes. The suggestion was based on evidence of cholinergic dysfunction in age-related memory disturbances. An extensive body of animal studies later demonstrated that acetylcholine was also instrumental for learning. Thus, Bartus, Dean, Pontecorvo, and Flicker (Bartus, Dean, Pontecorvo, & Flicker, 1985) revisited the cholinergic hypothesis and expanded it to include learning as well as memory.

Animal studies: The interruption of acetylcholine transmission has been thoroughly tested in rats. This may be accomplished in a variety of ways. An acute method involves the administration of an agent, which blocks the cholinergic receptors. Atropine sulphate and scopolamine, for example, are commonly used to block the muscarinic receptors of acetylcholine. A chronic method requires damage to the cholinergic neurons. Infusion of an excitotoxin or the passage of electrical current through the neurons are the most common ways to produce such a lesion. Damaging the basal forebrain allows the experimenter to produce structure specific depletions of acetylcholine. For example, producing a lesion in the nucleus basalis will deplete cholinergic input to the neocortex, but damaging the septum or diagonal band will restrict the depletion to the hippocampus. Infusing a selective immunotoxin, such as IgG 192

Saporin, into the ventricles of the brain produces global depletions of acetylcholine (Wrenn & Wiley, 1998).

Bilateral damage to the basal forebrain has been shown to cause impairments in learning and memory (Berger-Sweeney et al., 1994; Waite, Chen, Wardlow, & Thal, 1994). The septal nuclei, for example, provide cholinergic input to the hippocampus. Damage in that region has been shown to impair performance in the water maze. This testing procedure involves placing an animal in a swimming pool that has a hidden refuge. Initially the animal finds the platform by chance. The subject learns the position of the platform in relation to various cues around the room in order to successfully locate the platform in future trials. The ability to learn this place response is lost in animals with damage to the basal forebrain.

Damage to the nucleus basalis has been shown to cause learning impairments in rats as well (Wenk, 1997). Bilateral damage of the region has been demonstrated to impair acquisition of the water maze task (Berger-Sweeney et al., 1994). Furthermore, bilateral damage to the nucleus basalis impairs subjects' ability to make simple associations. Flicker et al. (Flicker et al., 1983) for example, demonstrated that rats with a bilateral nucleus basalis lesion were impaired on the acquisition of a shock avoidance task. The subjects could not learn the association between a conditioned stimulus such as a tone or a light and an unconditioned stimulus such as a shock to the feet. Rats with bilateral nucleus basalis damage are also impaired on conditioned taste aversion tests (Gonzalez, Miranda, Gutierrez, Ormsby, & Bermudez-Rattoni, 2000; Lopez-Garcia, Fernandez-Ruiz, Escobar, Bermudez-Rattoni, & Tapia, 1993). Lithium chloride solution and saline are indistinguishable by rats. Lithium chloride, however, is aversive to rats,

and they quickly learn to consume less of any solution in case it is lithium chloride. Subjects with nucleus basalis damage did not show signs of leaning the aversive effects of lithium chloride solution. Furthermore, Dubios, Mayo, Agid, Le Moal, and Simon (Dubois, Mayo, Agid, Le Moal, & Simon, 1985) demonstrated that bilateral damage to the nucleus basalis disturbed species typical behavior as well as learning. In their study, rats did not hoard food as they normally would after a nucleus basalis lesion.

Blocking the transmission of acetylcholine also causes learning impairments. Buresova, Bolhuis, and Bures (Buresova, Bolhuis, & Bures, 1986) argue that the administration of scopolamine interferes with working memory, it blocked the acquisition of a place response in the water maze. The drug, however, did not impair animals that were pre-trained on the task. In other words, retention was unaffected. A major cholinergic pathway enters the hippocampal formation through the fimbria fornix. Nilsson, Shapiro, Gage, Olton, and Bjorklund (Nilsson, Shapiro, Gage, Olton, & Bjorklund, 1987) demonstrated that the bilateral transection of the fimbria fornix impaired the acquisition of a place response in the water maze. In addition, introducing grafts of cholinergic-rich fetal tissue into the fimbria fornix reversed the deficits, and atropine sulphate abolished the recovered place navigation in the grafted rats.

The *cholinergic hypothesis* has been debated, however. There is evidence that the selective disruption of cholinergic transmission does not abolish learning. Whishaw (Whishaw, 1985), for example, showed that some learning ability is preserved in rats administered atropine sulphate. The author suggests two problem-solving systems to be involved in the acquisition and retention of a place response in the water maze. The locale strategy, involves making rapid use of relational properties of distal cues and is

impaired in rats drugged with atropine sulphate. Taxon strategy, however, involves cue or position responses and is less dependent on cholinergic brain mechanisms. Some learning was, therefore, preserved in subjects drugged with atropine sulphate.

Selective lesions of cholinergic pathways do not impair learning and memory processes. Baxter and colleagues (Baxter et al., 1996; Baxter & Gallagher, 1996), for example, produced lesions of the medial septum and vertical limb of the diagonal band using a selective immunotoxin (IgG 192-Saporin). They demonstrated that this selective interruption of cholinergic input to the hippocampus did not impair the acquisition or retention of a place response in the water maze. Furthermore, Baxter et al. (Baxter et al., 1996) showed that the selective depletion of cholinergic input to both the hippocampus and the cortex did not affect spatial learning. The results seem to be species specific, however. Berger-Sweeney et al. (Berger-Sweeney et al., 2001) showed that the same protocol produced severe deficits in mice. The above suggests that lesion selectivity and specie are important factors in interpreting the significance of acetylcholine in learning and memory.

The *cholinergic hypothesis* does not account for the deficits seen in aged animals. The findings of Whishaw and colleagues (Whishaw, 1985; Whishaw & Petrie, 1988) in two separate studies demonstrate that blocking cholinergic neurotransmission only interrupts certain aspects of learning in spatial navigation. Gage, Bjorklund, Stenevi, Dunnett, and Kelly (Gage, Bjorklund, Stenevi, Dunnett, & Kelly, 1984a; Gage, Dunnett, & Bjorklund, 1984b) studied aged rodents. Comparing the findings of Whishaw et al. (1985, 1988) to those of Gage et al. (1984a,b) reveals differences between disturbing the cholinergic neurotransmission and the effects of aging on behavior. First, animals

drugged with atropine sulphate (Whishaw, 1985; Whishaw & Petrie, 1988) were much less impaired in learning than aged animals (Gage et al., 1984a; Gage et al., 1984b). Second, introducing cholinergic rich grafts into the hippocampal formation of aged rodents only restored some learning abilities but did not reverse the deficits (Gage et al., 1984a). This suggests additional deficits, possibly in another neurotransmitter system, in aged animals.

Electrical activity: Waves of electrical activity can be recorded from the brain of a freely moving animal. Vanderwolf recorded electrical activity or electrocorticogram (EEG) from the hippocampus of rats engaging in various behaviors (Vanderwolf, 1969). The author uses the terms rhythmical slow wave activity (RSA) (Vanderwolf, 1969) and low voltage fast wave activity (LVFA) (Vanderwolf, 1975) to refer to hippocampal and neocortical EEG recorded during voluntary behavior.

Vanderwolf demonstrated a correlation between electrical activity in the hippocampus and the neocortex with behavior. Vanderwolf and Pappas (Vanderwolf & Pappas, 1980) organized behavior into two main categories. Type 1 behavior involves voluntary behaviors, such as head turning, postural adjustment and forepaw use; and Type 2 behavior involves automatic behaviors such as immobility, whisker movements, grooming, gnawing. Both types of behavior are known to generate RSA and LVFA of different frequency ranges. Vanderwolf and Pappas (1980) discovered that the RSA and LVFA associated with Type 2 behavior are sensitive to atropine. These frequency ranges (4-6 Hz) were termed atropine-sensitive. This suggests that Type 2 behavior is more dependent on cholinergic brain mechanisms. Cholinergic blockers do not affect the RSA

and LVFA associated with Type 1 behavior. This suggests that the EEG of Type 2 behavior is activated by a neurotransmitter other than acetylcholine.

Cholinergic neurons do not work alone. Evidence from selective lesions (Baxter et al., 1996; Baxter & Gallagher, 1996; McMahan, Sobel, & Baxter, 1997), cholinergic blockade (Whishaw, 1985; Whishaw & Petrie, 1988), fetal grafting in aged animals (Gage et al., 1984a), and EEG (Stewart, MacFabe, & Vanderwolf, 1984; Vanderwolf & Stewart, 1986), suggest that a different neurotransmitter is able to behaviorally compensate for the decreased neurotransmission of acetylcholine. This has been demonstrated in humans as well. Attempts to treat AD patients using acetylcholine precursors or acetylcholinesterase inhibitors, which enhance cholinergic transmission, have been associated with modest success (Davis & Mohs, 1982; Peters & Levin, 1979). This supports Whitehouse, Maurer, and Ballenger's (Whitehouse, Maurer, & Ballenger, 2000) suggestion of damage to multiple neurotransmitter systems in AD. The cholinergic hypothesis may be considered too reductionistic. Others have suggested this criticism as well (Cassel & Jeltsch, 1995).

Functions of serotonin

The functions of serotonin in the central nervous system are not well understood. Evidence against the cholinergic hypothesis has suggested the involvement of another neurotransmitter in learning and memory. There is evidence that suggests that serotonin may be complementing the functions of acetylcholine in the brain. The idea of an interaction between acetylcholine and serotonin has received significant support recently (Cassel & Jeltsch, 1995; Decker & McGaugh, 1991; Steckler & Sahgal, 1995). The

following section will describe the interaction between the two neurotransmitters with respect to: (1) Alzheimer's disease, (2) learning and memory studies in animals, (3) its influence on brain electrical activity.

Alzheimer's disease: It is now well established that the serotonergic projections are compromised in AD. Post mortem examination of AD brains revealed a significant decline in levels of serotonin as well as acetylcholine (Bowen et al., 1983; Haroutunian, Santucci, & Davis, 1990; Mann & Yates, 1986). The role of serotonin in the cognitive decline, however, is not understood.

Animal studies: The interruption of serotonin neurotransmission has been thoroughly tested in rats using various methods. An acute method involves the administration of a serotonin receptor blocker such as methothepin mesylate. A variety of blockers are now available; some can even selectively target a subset of the serotonergic receptors. Another acute method involves disturbing the presynaptic terminal. The administration of *p*-chlorophenylalanine, for example, inhibits the synthesis of serotonin in the presynaptic terminal. More chronic methods require damage to the serotonergic neurons originating in the brain stem. This may be achieved by infusing a neurotoxin selective for serotonin such as 5,7-dihydroxytryptamine directly into the raphe nuclei or in the ventricles. The neurotoxin destroys serotonergic nuclei in the brain stem depleting its projections to the rest of the brain.

The functions of serotonin in the brain have been debated. A number of studies report that the interruption of serotonin neurotransmission does not interfere with learning and memory processes (Altman, Ogren, Berman, & Normile, 1989; Asin, Wirtshafter, & Fibiger, 1985; Dringenberg & Zalan, 1999; Nilsson, Strecker, Daszuta, &

Bjorklund, 1988; Richter-Levin & Segal, 1991). Dringenberg and Zalan (Dringenberg & Zalan, 1999), for example, showed that the administration of non-specific serotonin receptor blockers or *p*-chlorophenylalanine, which inhibits the synthesis of serotonin, alone did not impair rats on the acquisition or retention of a place response in the water maze. Furthermore, Altman, Normile, Galloway, Ramirez, and Azmitia (Altman, Normile, Galloway, Ramirez, & Azmitia, 1990) demonstrated that damaging the serotonergic input to the hippocampus might improve learning. The number of trials to learning criterion in a T-maze was decreased following the infusions of 5,7-dihyroxytrptamine into the fimbria fornix and cingulum bundle.

Most studies examining the role of serotonin in learning employ a classical conditioning paradigm using aversive stimuli. In passive avoidance, for example, the subject learns an association between a tone or a light and an electric shock to the feet. The subject can avoid the shock simply by standing still on a rescue platform. The animal, therefore, has to learn not to jump off the platform to avoid the shock. Interpreting performance on such a task while manipulating the levels of serotonin has two predicaments. First, studies using this task often assess overall performance and do not distinguish between neural efficiency and motivational or arousal state. This may be misleading in interpreting results because it is not possible to attribute performance to learning or anxiety. Second, performance on this task is likely to be influenced by motivation and anxiety, and serotonin is known to have an effect on both. The role of serotonin in learning processes remains unclear due to narrowly focused testing procedures.

There are two potential explanations for the lack of understanding of serotonergic function in learning. One possible explanation is that serotonin may not be central to learning. This is unlikely, however, given its wide distribution throughout the cortex and its role in plasticity. An alternate explanation is that the serotonergic neurons do not work alone. This means that another neurotransmitter may behaviorally compensate for the interruption of serotonergic transmission. In other words, the interruption of two neurotransmitters would be necessary for deficits to reveal.

Electrical activity: The RSA and LVFA of Type 1 behavior are undisturbed by atropine sulphate. This electrical activity is, therefore, referred to as atropine-resistant. Vanderwolf, Robinson, and Pappas (Vanderwolf, Robinson, & Pappas, 1980) proposed that this type of electrical activity was dependent on a monamine. Administration of *p*-chlorophenylalanine, has been shown to change the EEG normally recorded during Type 2 behavior. Blockers of other neurotransmitters, however, did not have the same effect. This suggested that Type 2 behavior is more dependent on serotonergic neurons.

Functional interaction between acetylcholine and serotonin

The interaction between acetylcholine and serotonin has received significant attention over the past fifteen years. The interaction has been recognized as instrumental for the organization of behavior. Understanding it has, therefore, become an important endeavor in behavioral neuroscience. There are a number of factors that suggest an interaction between acetylcholine and serotonin. The following section will focus on: (1) anatomical, (2) behavioral, and (3) electrophysiological indications.

Anatomical indications: The layout of the projections of the cholinergic and serotonergic systems was described earlier. The focus here is to show that the anatomy is optimal for an interaction between the two systems.

Projections of various neurotransmitters may be anatomically linked to the cholinergic basal forebrain neurons. These include: the norepinephrine projections of the locus coreleus, the dopamine projections of the ventral tegmental area, and the GABA projections of the nucleus accumbens and lateral septal area. These neurons project to the cerebral cortex, which is, therefore, a common target for the cholinergic projections and the ones listed above. The serotonergic projections of the raphe nuclei hold a stronger interest, however, because they innervate the basal forebrain as well as send projections to the cortex.

The anatomical organization of the cholinergic and serotonergic neurons allows for two possible avenues for an interaction between both systems. A direct avenue would involve the serotonergic projections acting on the cholinergic neurons in the basal forebrain. There are relatively fewer serotonin receptors in the basal forebrain. This means that serotonin is not likely to act intrinsically on the cholinergic neurons of the nucleus basalis but would modulate ongoing synaptic activity in the region. In other words, it would act as a neuromodulator and control the amount of acetylcholine released from the basal forebrain. The indirect route involves the cholinergic and serotonergic projections converging onto a common target. The olfactory bulbs, the hippocampus, the amygdala and the neocortex receive both cholinergic and serotonergic input. Layers II/III of the cortex receive projections from the nucleus basalis and the dorsal raphe

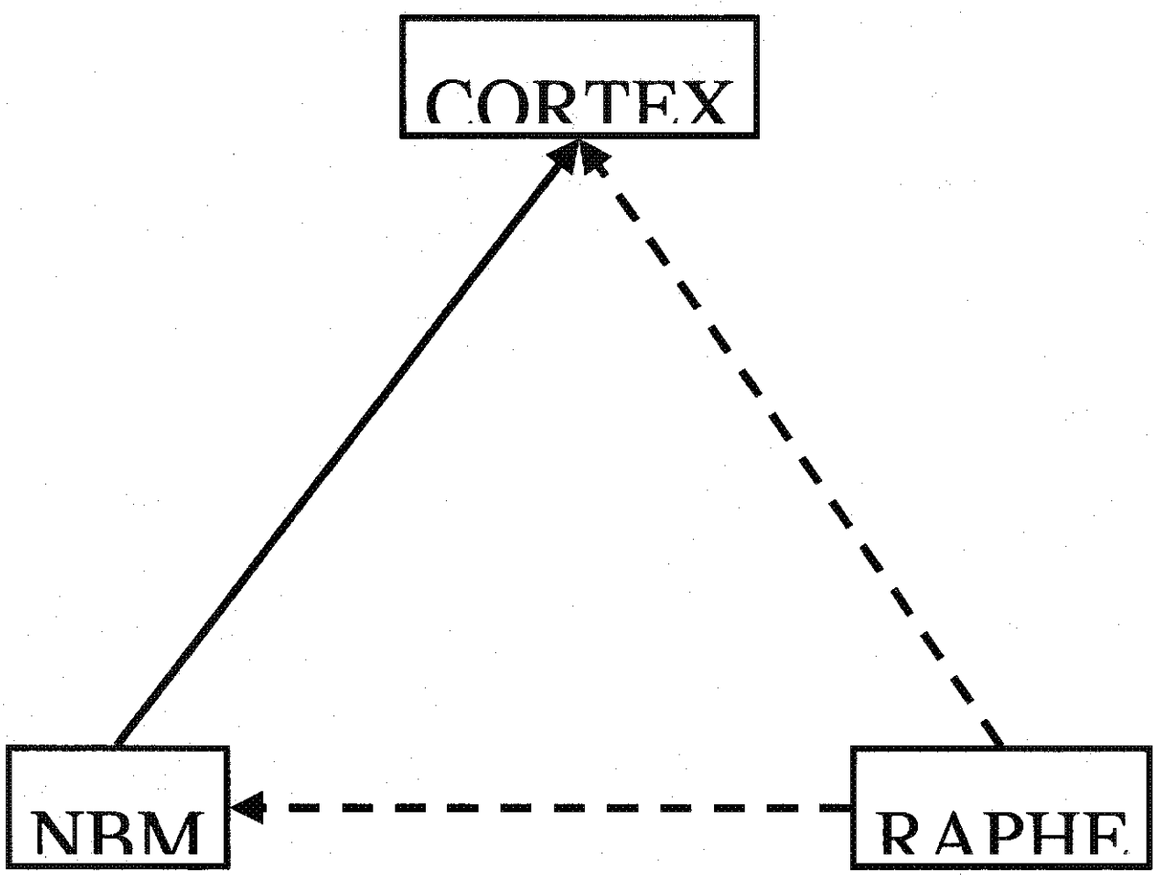
nuclei, which make a potential site for the interaction between acetylcholine and serotonin (Fig. 1.3).

Behavioral indications: The conjoint interruption of cholinergic and serotonergic neurotransmission has been thoroughly tested in rats. The interruption is often achieved using a combination of the methods described earlier. The administration of drugs that target both systems is an acute method of blocking transmission. Producing lesions in the basal forebrain and the raphe nuclei chronically depletes the cholinergic and serotonergic projections. It is also possible to deplete one system and temporarily block the other. For example, lesions may be produced in the brain stem, and atropine sulphate may be administered to produce the combined effect.

A number of reports have failed to demonstrate that the interaction between acetylcholine and serotonin is relevant to learning. The methods used for blockade of the neurotransmitters are questionable, however. Nakamura, Tani, Maezono, Ishihara, and Ohno (Nakamura, Tani, Maezono, Ishihara, & Ohno, 1992), for example, demonstrated negative results in the water maze task. The authors produced unilateral lesions to test the interaction between acetylcholine and serotonin (Hagan, Jansen, & Broekkamp, 1989; Hagan, Jansen, Nefkens, & de Boer, 1990). The intactness of the contralateral hemisphere may have been sufficient to produce normal behavior.

Other studies have shown antagonism between the two systems when observing behavior (Altman et al., 1990; Normile, Jenden, Kuhn, Wolf, & Altman, 1990). These studies showed that the interruption of neurotransmission in either system facilitated neurotransmission in the other. Serotonergic lesions in the fimbria fornix, for example, were shown to facilitate performance in maze learning. This was attributed to the

Figure 1.3: Schematic diagram demonstrating the ascending cholinergic projections of the nucleus basalis and the serotonergic projection of the raphe nuclei. The solid line represents the cholinergic projection, and the dotted lines represent the serotonergic projections.


CORTEX
NRM
RAPHE

decreased inhibition normally caused by the serotonergic neurons on the basal forebrain. The increase in cholinergic activity, therefore, facilitated learning (Normile et al., 1990).

The extensive body of evidence favors a synergistic interaction between acetylcholine and serotonin that is central to learning and memory (Lehmann et al., 2000; Nilsson et al., 1988; Riekkinen, Sirvio, & Riekkinen, 1990; Vanderwolf, 1987). Vanderwolf (Vanderwolf, 1987) was first to propose an interaction between the two neurotransmitters. Vanderwolf drugged rats with atropine and/or *p*-cholorophenylalanine and found that the administration of either agent alone had a mild effect on behavior. The combined treatment, however, caused severe behavioral deficits in tasks of learning and memory. Vanderwolf (1987) reports behavioral similarities between the combined treatment and the behavioral disorganization seen in decorticated animals (Kolb & Tees, 1990; Vanderwolf, Kolb, & Cooley, 1978; Whishaw, Nonneman, & Kolb, 1981).

Vanderwolf's (1987) findings were replicated in a number of labs (Beiko, Candusso, & Cain, 1997; Lehmann et al., 2000; Nilsson et al., 1988; Riekkinen et al., 1990). The results were consistent despite the use of different methods for both neurotransmission interruption and behavioral testing. Animals sustaining the combined depletion of acetylcholine and serotonin showed severe deficits on tests of learning and memory. In the water maze, for example, the combination of nucleus basalis and dorsal raphe lesions impaired acquisition more than either lesion alone (Riekkinen et al., 1990). Furthermore, medial septal lesions and intraventricular infusions of serotonin selective neurotoxins impaired place navigation more than either lesion alone (Nilsson et al., 1988; Richter-Levin, Greenberger, & Segal, 1993; Richter-Levin & Segal, 1991).

The cholinergic and serotonergic systems work together. The decreased neurotransmission of either agent is with little effect on type 1 behavior. This is likely due to the other neurotransmitter behaviorally compensating for the deficit. The blockade or depletion of both neurotransmitters, however, reveals the behavioral deficits.

Electrical activity indications: Vanderwolf (1987) demonstrated that animals may still engage in "intelligent behavior" if one type of RSA and LVFA is maintained. Multiple reports (Vanderwolf, 1987; Vanderwolf, 1988; Vanderwolf & Baker, 1986; Vanderwolf & Stewart, 1986) suggest that both the cholinergic and serotonergic neurons are active during Type 1 LVFA and RSA, and that damaging both the ascending cholinergic and serotonergic projections causes severe deficits on tasks that involve Type 1 behavior. Vanderwolf (1987) suggests that damaging the ascending cholinergic and serotonergic projections impairs the LVFA and behavior controlled by the neocortex.

Acetylcholine and serotonin are closely linked anatomically, behaviorally, and in EEG. The close connection between the two systems suggests a functional interaction between the two. Despite the growing body of literature on the topic, the experimental design adopted thus far has been narrowly focused. Previous studies have focused on testing the interaction in cognitive tasks such as maze learning. It remains unclear, however, whether the interaction is important for other behaviors as well, such as motor tasks and species typical behaviors. The structural specificity of the interaction remains mysterious as well. Most studies addressing the interaction have relied on global methods to interrupt the neurotransmission of acetylcholine and serotonin. It has not been possible to determine whether the interaction is more relevant to certain brain structures more than others.

Objectives of the present study

There are three main goals of this thesis: 1) to assess the effects of unilateral depletion of ascending cholinergic projections and global cholinergic receptor blockade on motor performance; 2) to assess the effects of unilateral depletion of ascending serotonergic projections and global serotonergic receptor blockade on motor performance; 3) determine whether a unilateral model of impairment may be produced due to the necortical loss of acetylcholine and serotonin.

Three main experiments were conducted to address the issues above. In the first experiment, rats were trained on a skilled reaching task for two weeks. A selective neurotoxin was then used to produce unilateral depletions of the ascending cortical-cholinergic projection. The objective was to determine whether depriving the neocortex of cholinergic input would affect the performance of skilled movements. The behavioral measures used are sensitive to neocortical intactness. The tests included: skilled reaching, rung walking, cylinder test, swimming test and adhesive dot removal. These tests have been used reliably to assess unilateral neurological models. They are described in detail in the following chapters. In the same experiment, control subjects were administered various doses of atropine sulphate to determine how the global blockade of cholinergic neurotransmission would affect performance on skilled motor tasks.

In the second experiment, rats were trained on a skilled reaching task for two weeks. The ascending serotonergic projections were then damaged unilaterally by infusing a selective neurotoxin into the medial forebrain bundle. The neurotoxin was supposed to be retrogradely taken up to destroy the raphe nuclei. The objective was to determine whether depriving the neocortex of serotonergic input would affect the

performance of skilled movements. The subjects were assessed using the same battery of tests mentioned above. In the same experiment, control subjects were administered various doses of methiothepin mesylate to determine how the global blockade of serotonergic neurotransmission would affect performance on skilled motor tasks.

In the third experiment, unilateral depletions of the ascending cholinergic and serotonergic projections were produced. The lesions were produced in two different manners. First, animals from the first two experiments received a second lesion to the intact system. In other words the nucleus basalis lesion group received a medial forebrain bundle lesion. In addition, the medial forebrain bundle lesion group received a nucleus basalis lesion. Second, the conjoint depletion was produced in a single operation. This was achieved in untrained subjects. The goal here is to determine whether the combined depletion produces a synergistic effect that is larger than the sum of either depletion alone. This will verify if Vanderwolf's two neurotransxmitter theory (Vanderwolf, 1987) may be applied to a unilateral animal model. Furthermore, the study will clarify whether the interaction between the two systems is instrumental for the performance and/or learning of skilled motor tasks.

The results and implication of the three major experiments are discussed in the final chapter. The unilateral combined depletion is discussed as a model of dementia as well.

CHAPTER TWO

Selective impairments in skilled reaching movements follow quisquilate neurotoxic lesions of basal forebrain cholinergic neurons in the rat

ABSTRACT

The cholinergic projection of the nucleus basalis magnocellularis (nbm) to the neocortex is proposed to be involved in synaptic plasticity, and thus should be central to skilled motor behavior that depends upon plastic changes in the motor cortex. Despite the possible importance of the nbm for skilled behavior, there has been no examination of the changes in skilled movements that follow cholinergic lesions or muscarinic receptor blockade. In the present study, the use of the contralateral limbs in skilled movements of rats with unilateral nbm lesions (quisqualate, 0.5 μg/μl) was compared with the ipsilateral limbs and with the limbs of control rats on tests of: limb use in reaching for food, limb placing while walking a horizontal ladder, limb use in support (cylinder test), limb use during swimming, and sensory responsiveness to contact (dot removal test). Whereas there were no quantitative impairments on any of the tests of either the forelimbs or hindlimbs, aiming and rotatory movements of pronation and supination when reaching with the contralateral limb were impaired. Similar results were obtained following the administration of atropine sulphate. Thus, whereas the neocortical cholinergic projection is not required for the more general use of the limbs in postural support, walking, swimming, tactile sensitivity, or in retrieving food, it is required for producing the rotatatory movements of the forelimb in reaching. The results suggest either that the nbm cholinergic projection facilitates the cortical plasticity necessary for skilled reaching or it

plays a role in the actual production of a subset of the movements used in skilled reaching.

INTRODUCTION

The nucleus basalis magnocellularis, a group of cholinergic neurons in the ventromedial corner of the globus pallidus of the rat, is believed to be homologous to the nucleus basalis of Meynert in primates (Flicker, Dean, Watkins, Fisher, & Bartus, 1983). It is the primary source of cholinergic afferents to the neocortex, the olfactory bulbs, and the amygdala (Rye, Wainer, Mesulam, Mufson, & Saper, 1984; Wenk, Bigl, & Meyer, 1980). There is evidence that the cholinergic projection of the nucleus basalis plays a role in activation of the cortical electroencephalogram (EEG) (Detari & Vanderwolf, 1987; Stewart, MacFabe, & Vanderwolf, 1984), facilitates the learning of conditioned motor responses (Richardson & DeLong, 1990), and enhances plastic processes such as those involved in compensatory responses to brain damage (Mesulam, 1998; Miranda, Lopez-Colome, & Bermudez-Rattoni, 1997; Russell, Escobar, Booth, & Bermudez-Rattoni, 1994). In addition, the ascending projection may also activate the cortical vasculature in order to enhance cerebral blood flow (Biesold, Inanami, Sato, & Sato, 1989; Sato & Sato, 1990; Uchida, Suzuki, Kagitani, & Hotta, 2000).

Given the extensive projection of the nucleus basalis to the sensorimotor cortex in the rat, and given the diverse functions of this projection, it might be expected that sensorimotor behavior in the rat would be impaired by loss of the nucleus basalis afferents. Surprisingly, there has been little methodical investigation of the role of this system in sensorimotor behavior. In many studies in which the projection has been damaged by selective neurotoxic lesions to the nucleus basalis cells, it has been observed that many sensorimotor functions, including orienting to sensory stimulation, climbing, swimming, and walking are seemingly unaffected by the lesions (Dunnett, Whishaw,

Jones, & Bunch, 1987; Jacobs & Juliano, 1995; Waite et al., 1995). In the main, however, these behavioral assessments are not sensitive to cortical injury because even animals with extensive neocortical ablations would perform such behaviors quite well. Therefore, in order to evaluate whether the nucleus basalis is important for normal cortical function, it is necessary to use testing methods that are sensitive to cortical integrity.

The cholinergic receptors are widely spread throughout the brain and are categorized as either nicotinic or muscarinic (Siegel & Agranoff, 1999). Several subtypes exist within each category. Generally, muscarinic receptors are responsible for post-ganglionic neurotransmission and their organization is well documented. The M1 subtype of the muscarinic receptors is of particular interest here because of its abundance in the neocortex as demonstrated using immunohistochemical techniques (Levey, Kitt, Simonds, Price, & Brann, 1991). In vitro quantitative autoradiography studies have also shown that the M1 receptor is diffusely observed in all the layers of the neocortex of the rat (Miyoshi, Kito, Shimizu, & Matsubayashi, 1987). Furthermore it is localized on the horizontal connection of layers II/III of the rat motor cortex (Hess & Krawczyk, 1996) and located presynaptically on glutamatergic terminals. The receptors are therefore located such that they would be expected to be central to cortical functioning.

The role of the M1 receptors in controlling behavior is unclear. There is evidence suggesting that the M1 receptors may contribute both to information processing and synaptic plasticity within the motor cortex (Hess & Krawczyk, 1996), which is instrumental in controlling skilled movements of the forelimbs (Kleim et al., 2002; Kleim, Barbay, & Nudo, 1998; Klintsova & Greenough, 1999).

For the current experiment, the nucleus basalis was damaged with the neurotoxin quisqualic acid, which produces extensive depletion of neocortical acetylcholine as assessed by acetylcholinesterase staining of postmortem tissue. Two weeks after surgery, the rats were given a series of sensorimotor tests, all of which have been demonstrated to be sensitive to sensorimotor cortex lesions. The assessment included tests of forelimb support (cylinder test), limb placing while traversing a horizontal ladder with variably spaced rungs, sensory responsivness to sensory contact (adhesive dot removal test), forelimb inhibition during swimming, and limb use in reaching for food. The performance of the rats was videotaped to assess the quantitative and qualitative performance of the animals. In addition to being compared to a control group, performance related to the limbs ipsilateral to the lesion was compared to performance of the limbs contralateral to the lesion with the expectation that the limbs contralateral to the lesion should be more affected, as typically occurs following frank cortical injury. A follow-up experiment was conducted to test the animals' ability to reach for food pellets (Whishaw & Pellis, 1990) while drugged with a central muscarinic receptor blocker such as atropine sulphate. A quantitative analysis of the performance was compared to each rat's own performance undrugged.

METHODS

Subjects

The subjects were 22 Long-Evans hooded female-adult rats, 120 days old and weighing 250-300g. They were born and raised in the University of Lethbridge

Vivarium. The animals were assigned to three different groups, control (n=7), lesion (n=6), atropine (n=9). The animals were housed in groups of three or four individuals in hanging wire mesh cages. The colony room was maintained on a 12/12h light/dark cycle (08:00-20:00 h).

Feeding

For the experiment, rats were food deprived but with *ad lib* water access. Three weeks prior to surgery, the rats were food deprived to 85% of their original body weight by providing 15g of solid chow per rat/per day to maintain body weight.

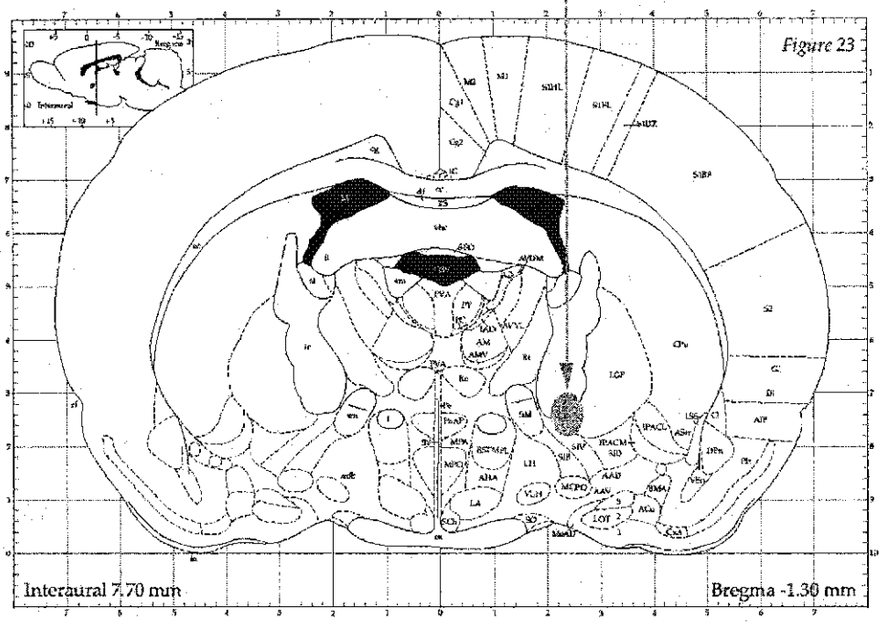
Surgery

Animals received an injection of atropine nitrate (0.1 mg/kg i.p.) (Sigma-Aldrich, St. Loius, MO) to facilitate respiration throughout surgery. Under 0.5 ml/kg sodium pentobarbital anaesthesia, each rat received stereotaxic infusions of 0.12 M Quisqualic Acid (Sigma-Aldrich, St. Loius, MO) via a 30-gauge cannula connected to a micro drive pump by a polythene tube. Two 0.5µl infusions were made unilaterally in the nucleus basalis. Each infusion was delivered over three min and an additional five min allowed for diffusion before the cannula was retracted. Stereotaxic coordinates anterior (A), lateral (L) and ventral (V) for the two infusions were A=0.2 mm, L=3.4 mm, V=7.0 mm (below dura) and; A=1.0 mm, L= 2.6 mm, V=7.3 mm (below dura), with the incisor bar set 5.0 mm above the interaural line (see Fig. 2.1). The lesions were made in the hemisphere contralateral to the subject's dominant paw as determined during the

Figure 2.1: Sections from Paxinos and Watson (1997) Rat Atlas; (A) coronal; (B) saggital. The diagram is representative of the two quisqualic acid infusion sights, at A=0.2 mm, L=3.4 mm, V=7.0 mm (below dura) and; A=1.0 mm, L= 2.6 mm, V=7.3 mm (below dura), with the incisor bar set 5.0 mm above the interaural line (modified from Paxinos & Watson, 1997).

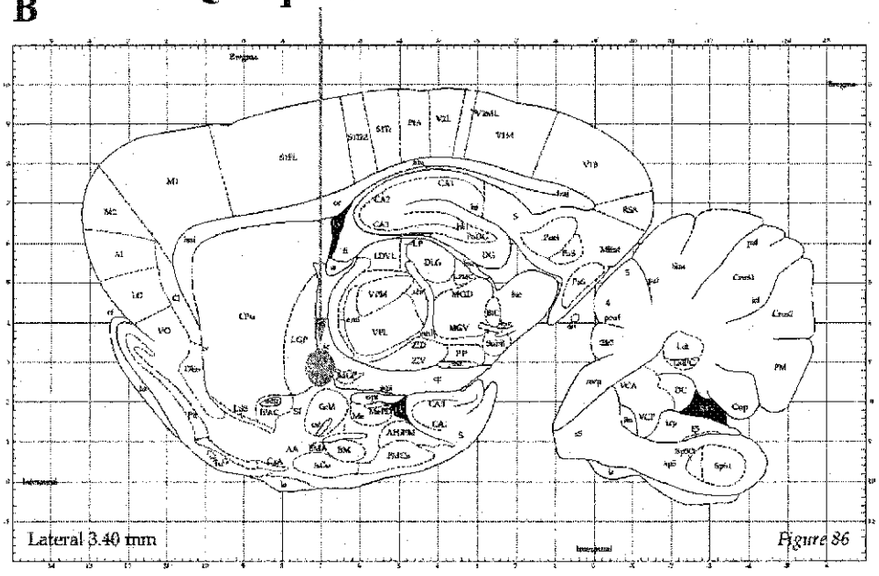
Quisqualic acid

A



Quisqualic acid

B



pretraining phase of single pellet reaching. Animals were allowed two weeks to recover before behavioral testing.

Atropine Sulphate

The drug atropine sulphate was administered to block the muscarinic receptors, thus interrupting the neurotransmission of acetylcholine. Three doses of atropine sulphate (Sigma-Aldrich, St. Loius, MO): 5,10, and 25 mg/kg were prepared in 0.9% sterile saline solution. A single dose was administered (i.p.) to the control group 20-30 min prior to testing on the single pellet reaching task, each dose was only used once. The drug doses were administered starting with the lowest dose to minimize tolerance effects. After each rat was injected with the drug, it was returned to its home cage until it was due for testing on the reaching task.

Behavioral Training and Test Analysis

Reaching boxes and training: All animals were pre-trained to reach through a slot for single pieces of food for two weeks prior to surgery or drug administration (Whishaw, 2000). Reaching boxes were made of clear Plexiglas. Each box was 45x14x35 cm high. In the center of each front wall was a 1 cm-wide slit which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm-wide shelf. Two indentations on the floor of the shelf were located 2 cm from the inside of the wall and were centered on the edges of the slit were rats could reach. Food pellets (45 mg Rodent Chow food pellets, Bioserve Inc.) were placed in the indentation contralateral to the limb with which the rat

reached (Whishaw & Pellis, 1990). Following each reach, a short pause preceded the presentation of the next pellet and an additional pellet could be dropped in the back of the box. This encouraged animals to return to the back of the box after each reach and so forced them to reposition themselves and prepare for the next reach. The animals were trained for ten minutes each day for the first week and were presented with 20 pellets each day for the second week. Reaching performance was assessed on two measures: "reaching success" = number of pellets retrieved and "reaches/pellet retrieved" = number of reaching attempts/successful retrieval. After the recovery period following surgery, the animals were tested every day for two weeks. They were presented with 20 pellets in each testing session.

For a qualitative analysis of reaching, a reach was subdivided into ten components (Whishaw, Pellis, Gorny, Kolb, & Tetzlaff, 1993). (1) Limb lift: the limb is lifted from the floor with the upper arm and the digits adducted to the midline of the body. (2) Digits close: as the limb is lifted, the digits are semiflexed and the paw is supinated so that the palm faces the midline of the body. (3) Aim: using the upper arm, the elbow is adducted so that the forearm is aligned along the midline of the body, with the paw located just under the mouth. This movement involves fixation of the distal portion of the limb, so that digits remain aligned with the midline of the body. This is likely produced by a movement around the elbow that reverses the direction of movement of the paw to compensate for the adduction of the elbow. (4) Advance: the head is lifted and the limb is advanced directly forward above and beyond the food pellet. (5) Digits open: as the limb is advanced the digits are extended and opened. (6) Pronate: using a movement of the upper arm, the elbow is abducted, pronating the paw over the food. Full pronation of

the paw onto the food is aided by a movement of the paw around the wrist. (7) Grasp: as the pads of the palm or the digits touch the food, the food is grasped by closure of the digits. This can occur as an independent movement or the grasp can occur as the paw is withdrawn. (8) Supination I: as the limb is withdrawn, the paw is dorsiflexed and is supinated 90^0 by a movement around the wrist and by adduction of the elbow. These movements can occur as soon as the food is grasped or can occur as the limb is withdrawn. (9) Supination II: as the rat sits back with food held in the paw, the paw is further supinated by 90^0 and ventroflexted to present the food to the mouth. (10) Release: the digits are opened and the food transferred to the mouth.

Five successful reaches were analyzed frame-by-frame on the video tapes. Each movement was rated on a three-point scale. If the movement appeared normal, it was given a score of "0", if it appeared slightly abnormal but recognizable it was given a score of "1", and a score of "2" was assigned if the movement was absent or completely unrecognizable.

Rung Walking: The runway consisted of a straight section 1m in length with walls 19 cm high and a square goal box at one end in which food was located (Metz & Whishaw, 2002). The width of the alley was adjusted to the size of the animal allowing 1 cm on either side of the animal to prevent it from turning around. The floor of the runway was made of a readily changeable arrangement of horizontal steel rods 3 mm in diameter. An irregular but unchanged rung pattern was maintained throughout all trials, gap sizes varied from 1 cm to 5 cm. A high-8mm camera was positioned at a slight

ventral angle, so that the positions of all four limbs can be filmed simultaneously from a ventral view.

The novel foot-fault scoring system (Metz & Whishaw, 2002) was modified and used to assess forelimb and hindlimb qualitative placement. Each step was rated on a five-point scale: if the foot placement appeared normal where the midportion of the palm was placed on the rung, it was given a score of "0"; if placement on the rung was done using the wrist or digits of the forelimb or the heel or toes of the hindlimb, it was given a score of "1"; if a limb was placed on a rung and slipped off during weight shifting without disturbing balance, it was given a score of "2"; if a limb was placed on a rung and slipped off during weight shifting causing a fall, it was given a score of "3"; and if a limb missed the targeted rung completely and fell through the gap compromising body posture and balance, it was given a score of "4". Animals received three trials during each testing day. The asymmetry score, which is a ratio of foot faults committed by both sides of the body, was calculated for each group; this is: contralateral limb faults/ipsilateral limb faults.

Cylinder test: Forelimb use for weight support during explorative activity was examined by placing rats in a transparent cylinder 20 cm in diameter and 30 cm high for four minutes (Schallert, Kozlowski, Humm, & Cocke, 1997). A mirror was placed underneath the cylinder at an angle to allow the experimenter to videotape the animal's activity from a ventral view. The cylindrical shape encouraged vertical exploration of the walls with the forelimbs. The cylinder was high enough so that animals could not reach the top and was wide enough to allow 2 cm between either end of the animal and the

walls. Forelimb use was measured during vertical exploration following rearing. Independent use of each forelimb during wall contact was scored during weight shift initiation or to regain center of gravity while moving laterally in a vertical posture. The asymmetry score of forelimb use in wall exploration was calculated for each group, this is: contralateral forelimb wall contact/ipsilateral forelimb wall contact.

Adhesive Dot Removal: Procedures for this task have been described previously by Schallert et al. (1982). Animals were removed from their home cages and their forelimbs were washed with 50% ethanol solution, then wiped with cotton gauze and allowed to dry. Two parallel creases were formed in adhesive paper stimuli (113 mm^2, manufactured by Avery International) to facilitate wrapping them around the forelimb. The stimuli were attached to the distal-radial aspect of both forelimbs. Immediately after, the experimenter firmly touched both forelimbs simultaneously and placed the animal in a clear Plexiglas tub (45x26x20 cm) without bedding for ease of recording. A stainless steel lid was used to cover the tub and contain the rat. The fine forelimb hair was not pulled out in the process, however, the stimuli were sticky enough that they rarely fell off when the animal moved around, groomed or shook its forelimb. Trials in which either stimulus fell off spontaneously were disregarded and repeated. The order of stimulus attachment to the contralateral and ipsilateral forelimbs was counterbalanced for all animals. Subjects contacted and attempted to remove the adhesive paper. The order and latency of removal was recorded for each forelimb for four trials. Each trial was ended after both labels were removed or after three minutes. The asymmetry score of latency of

dot removal was calculated for each group contralateral forelimb latency/ipsilateral forelimb latency.

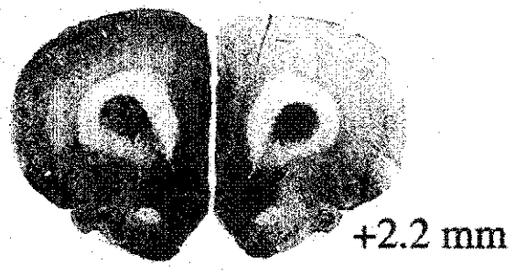
Swimming Test: Video recordings were made in a large rectangular aquarium (120x43x50 cm) as described by Whishaw, Nonneman, and Kolb (Whishaw, Nonneman, & Kolb, 1981). Water was high enough to prevent animals from touching the bottom of the aquarium but at the same time low enough to prevent them from escaping to the edge of the pool, temperature was maintained at 21^0C. At one end of the pool was an escape wire mesh platform onto which the animals could climb. The platform was visible to the animals at all times. During the training phase, animals were released close to the platform, after they learned to swim and climb onto the platform, they were released at progressively longer distances until they swam directly from the opposite end of the tank. Initially, most animals used all four limbs to stroke, rapidly changed direction, and sometimes swam aimlessly. Once animals learned to swim directly to the platform and were more familiar with the task, they held their forelimbs immobile under their chins and only used their hindlimbs to propel through the water. Each animal performed four trials during which they had to swim directly to the platform. Animals were dried and returned to their home cages after completing four trials. Disruption to the normal swim pattern was quantified by counting the number of strokes by each forelimb. The asymmetry score of forelimb inhibition was calculated for each group, this is: contralateral forelimb strokes/ipsilateral forelimb wall strokes.

All subjects were tested on the rung walking, cylinder, swimming, and adhesive dot removal tests once a week for four weeks after surgery.

Histological procedures

After six weeks of behavioral testing, lesion and control groups were sacrificed using a lethal dose of sodium pentobarbital. They were intracardially perfused, first with saline in PBS followed by 4% paraformaldehyde in PBS. The brains were removed and placed in a cryoprotectant solution of 30% sucrose in 4% paraformaldehyde for three days. All brains were then cut into 40 μm sections using a cryostat (2800 Frigocut, Reichert-Jung). Sections were mounted onto glass slides and stained for acetylcholinesterase using a procedure modified from Karnovsky and Roots (1964), to assess the extent to which acetylcholine was depleted from the neocortex (Fig. 2.2).

Figure 2.2: Representative photomicrographs from the nucleus basalis lesion group of coronal sections of the motor cortex stained for acetylcholinesterase. The left hemisphere is contralateral to the lesion and appears darker than the ipsilateral hemisphere indicating the presence of more acetylcholinesterase.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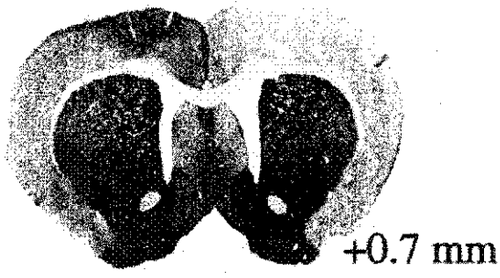
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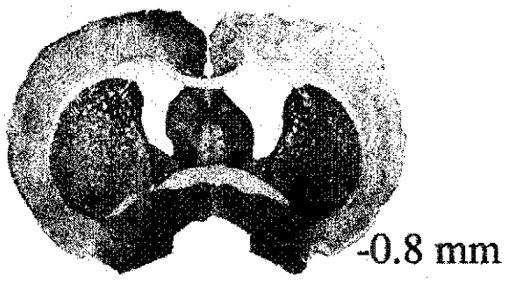
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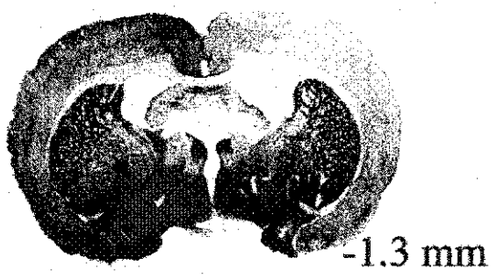

-1.3 mm

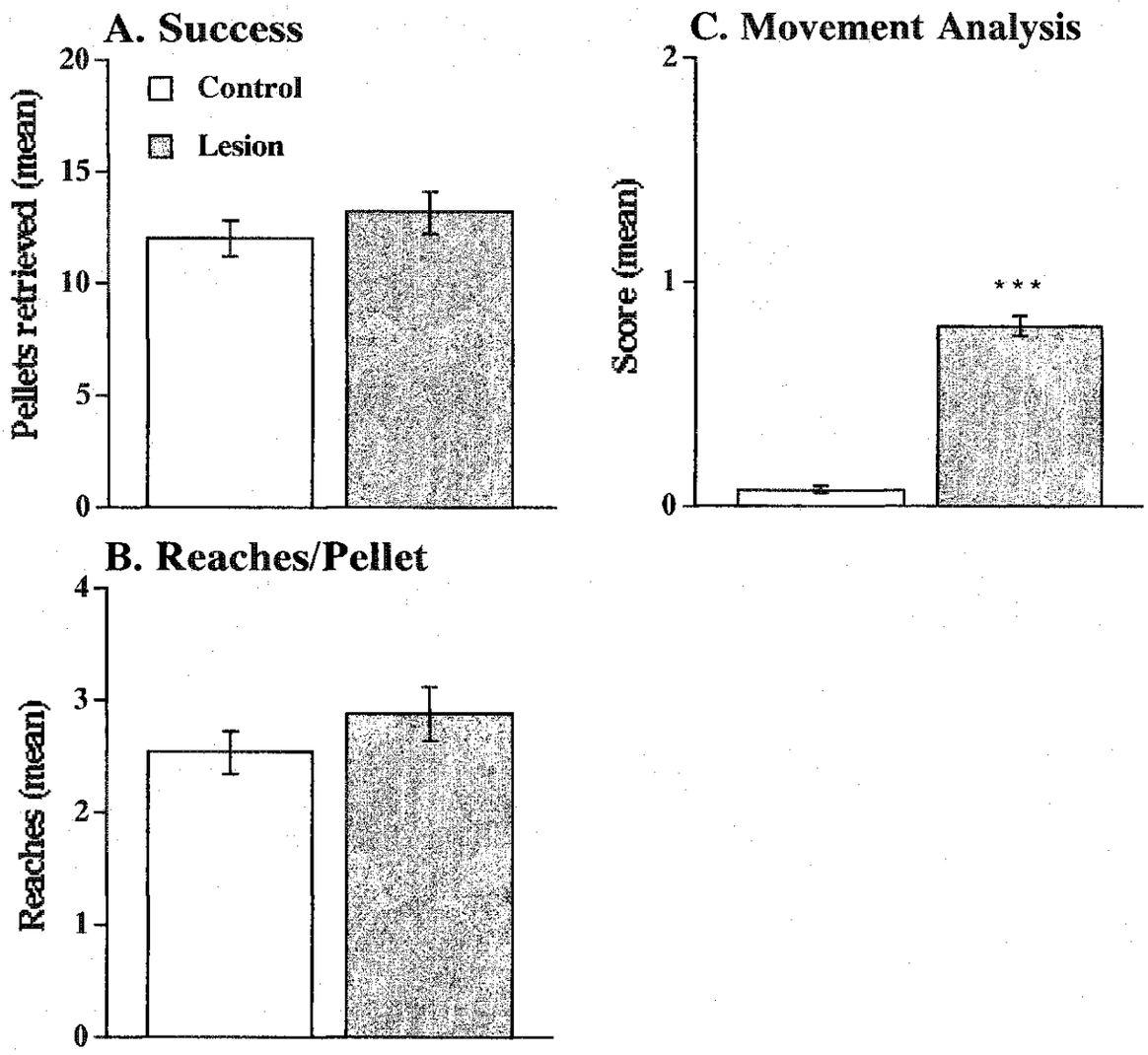
RESULTS

Behavioral Observations

Single pellet reaching (nucleus basalis lesion): The ability of lesion and control groups to use their forelimbs was assessed in a skilled-reaching task in which animals reached through a narrow slot onto an elevated shelf to retrieve a food reward. The lesion group reached for pellets with the same forelimb used prior to surgery, that is the contralateral forelimb. The control group continued to use their contralateral forelimb as well. A mean success score was calculated for all animals and compared across all groups. A simple ANOVA revealed no significant main effect of treatment on success score ($F(1,11) = 0.703$; $p=0.413$) (Fig. 2.3A). A mean reaches/pellet retrieved rate was calculated for all animals as well and compared across all groups. A simple ANOVA revealed no significant main effect of treatment on the number of reaches/pellet ($F(1,11) = 1.329$; $p=0.273$) (Fig. 2.3B). Thus, the lesions did not affect skilled reaching.

To assess the extent to which reaching elements were changed in the lesion group, relative to the control group, the ten-element reaching data were subjected to a repeated measures ANOVA. The analysis revealed a significant main effect of treatment on movement component score ($F(1,11)= 158.709$; $p=0.0001$) (Fig. 3C). The lesion group had a significantly higher score, which means that they had more movement abnormalities than the control group. An interaction of movement x treatment showed that the high impairment score for the cholinergic depleted group was significant for certain components ($F(1,9) = 32.298$; $p=0.0001$). A follow up LSD post hoc analysis ($p<0.05$) showed that the lesion group was impaired on the elbow aim, advance,

Figure 2.3: Single pellet reaching scores (mean and standard error) in control and nucleus basalis lesion groups. (A) success, number of pellets retrieved out of 20; (B) reaches/pellet, number of reaches performed for each successfully retrieved pellet; (C) qualitative movement error scores of five representative reaches in control and lesioned animals, ***p=0.001.


A. Success
Control
Lesion
Pellets retrieved (mean)
20
15
10
5
0
B. Reaches/Pellet
Reaches (mean)
4
3
2
1
0
C. Movement Analysis
Score (mean)
2
1
0
* * *

pronation, supination I, supination II, and release components of the reaching movement (Fig. 2.4). This demonstrated qualitatively that the lesion affected skilled reaching.

Single pellet reaching (atropine sulphate administration): All animals continued to use their contralateral forelimb to reach for food items following the administration of atropine sulphate. The various doses caused different degrees of reaching impairments, however (see Fig. 2.5). A repeated measures ANOVA showed that the atropine sulphate treatment had a significant effect on the reaching success score ($F(3,8) = 32.549$; $p=0.0001$). A follow up LSD post hoc analysis ($p<0.05$) showed that the success rate at the highest dose was significantly different than the other doses. The lowest dose (5 mg/kg) did not have any effect on reaching. The medium dose (10 mg/kg) did not have a significant quantitative effect on reaching success, but it did cause qualitative impairments on some of the movement components of reaching. Movements associated with aiming such as the alignment of the elbow and the digits with the midline of the body and the advancing of the paw through the slot, were the most affected by the administration of atropine sulphate. The abnormalities in these movements often caused the animals to produce a reach that was too short and on occasion was reason to knock the pellet off the shelf instead of grasping it successfully. Most animals did not reach under the high dose of atropine (25 mg/kg), the testing session was terminated after 15 minutes of being placed in the box. Animals drugged with the high dose of atropine sulphate often appeared drowsy and did not show interest in reaching for the pellet and were unsuccessful when they did. Their behavior did not seem to have the same organization or goal orientation as under control conditions. A repeated measures

Figure 2.4: Reaching movement components (mean and standard error) on the 10 movement components of reaching for control and nucleus basalis lesion groups. Each of the movement components was rated on a 3-point scale, with 0=normal and 2=absent. *$p<0.05$

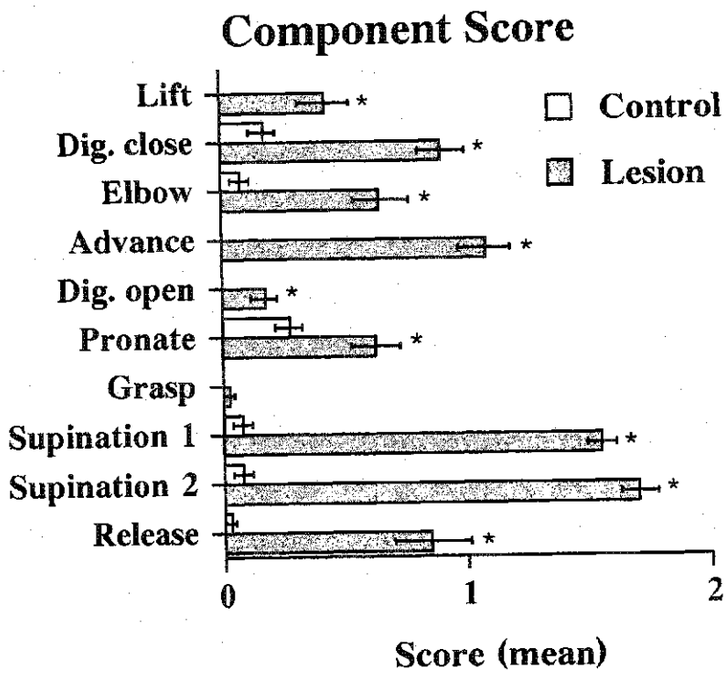
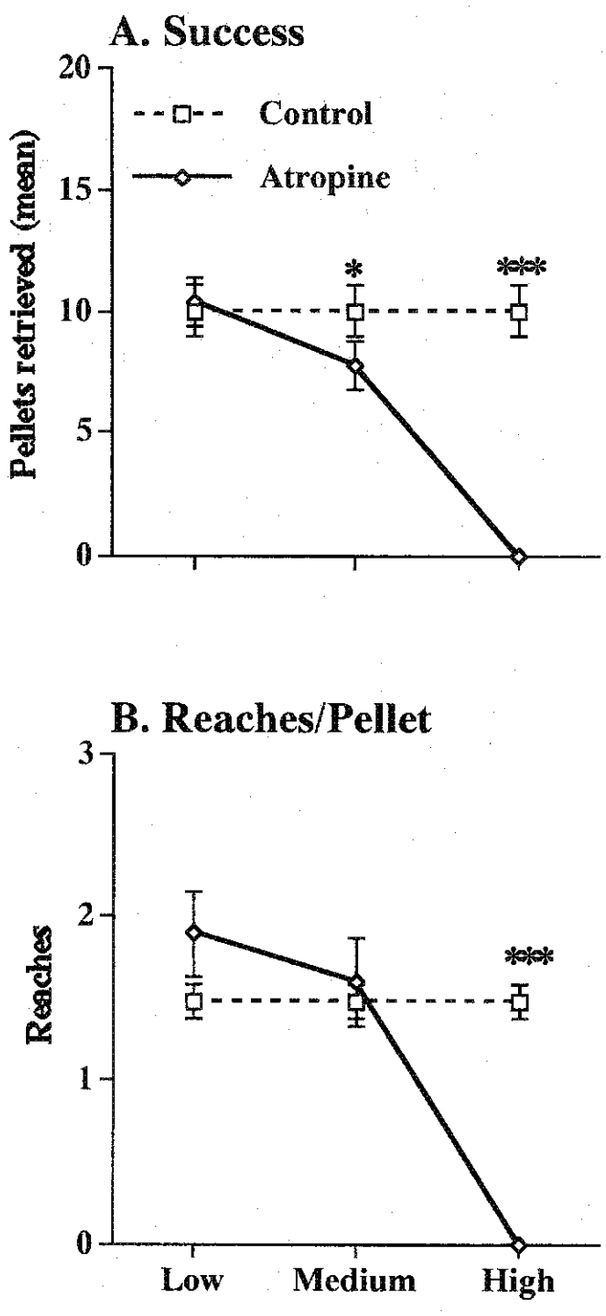

Component Score
Lift
Dig. close
Elbow
Advance
Dig. open
Pronate
Grasp
Supination 1
Supination 2
Release
Control
Lesion
0
1
2
Score (mean)

Figure 2.5: Single pellet reaching score (mean and standard error) in control animals and following the administration (i.p.) of one of three doses of atropine sulphate: low = 5 mg/kg; medium = 10 mg/kg; high = 25 mg/kg. (A) Success, number of pellets retrieved out of 20; (B) reaches/pellet, number of reaches performed for each successfully retrieved pellet

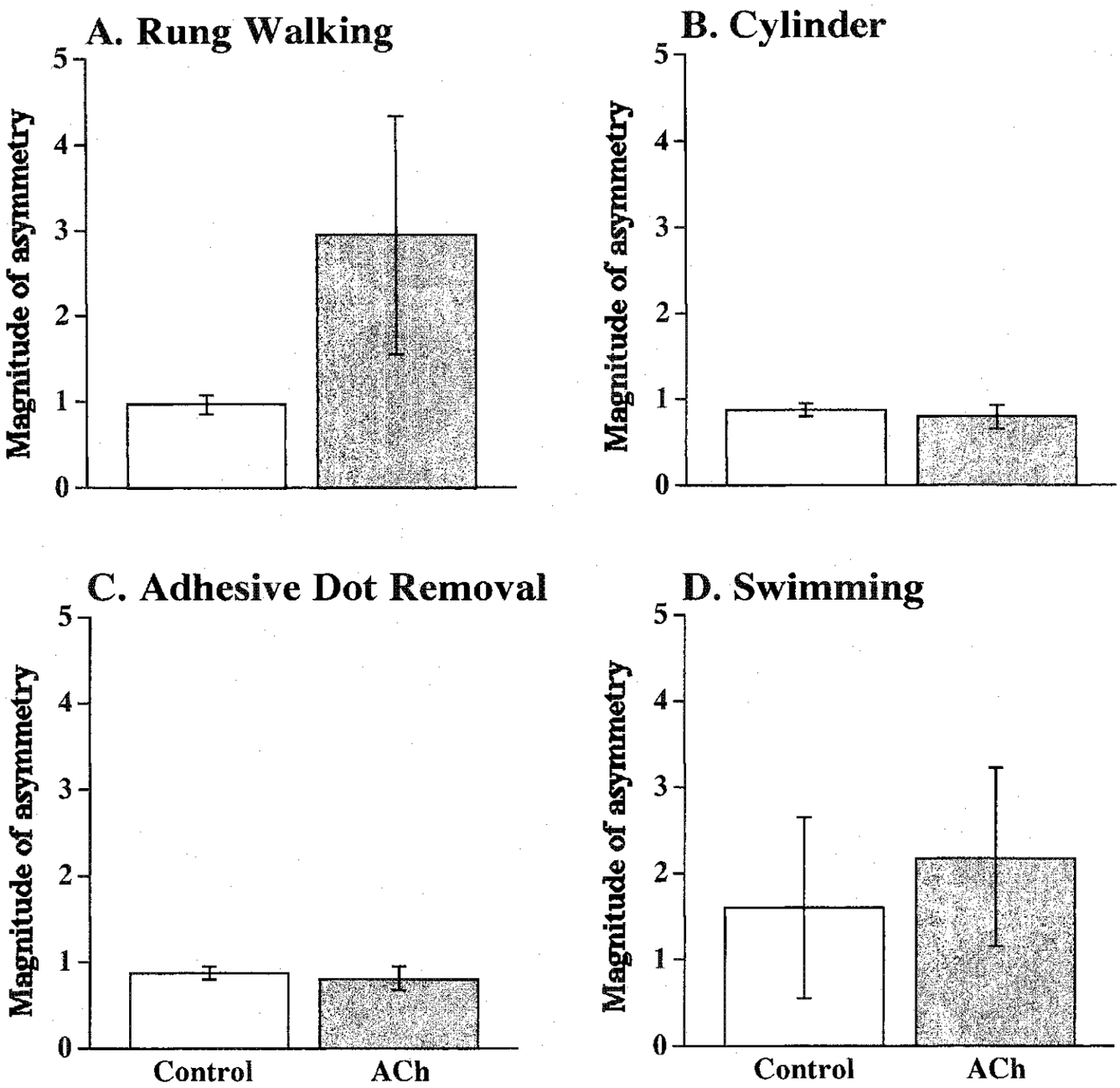

A. Success
Pellets retrieved (mean)
20
15
10
5
0
Control
Atropine
*
***
B. Reaches/Pellet
Reaches
3
2
1
0
***
Low
Medium
High

ANOVA showed that the atropine sulphate treatment affected the reaches/pellet retrieved significantly ($F(3,8) = 18.980$; $p=0.0001$). A follow up LSD post hoc analysis ($p<0.05$) showed that the reaches/pellet retrieved score at the low and medium doses was not different than control but was different at the highest dose.

Rung Walking: The ability of lesion and control groups to cross a horizontal ladder with randomly spaced bars was assessed by counting the number of foot faults. Animals from both groups walked across the horizontal ladder equally well. Both control and lesion groups committed a number of foot faults with both forelimbs and both hindlimbs. The scores on the five-point scale were summed for each group, and a total number of foot faults (contralateral+ipsilateral), including both forelimbs and hindlimbs, was calculated for each group. A simple ANOVA found no significant main effect of treatment between groups ($F(1,11) = 0.885$; $p= 0.3617$). The ratio of foot faults committed by both forelimbs and hindlimbs was calculated for the contralateral and ipsilateral sides of the body. A simple ANOVA found no significant main effect of treatment on foot fault asymmetry ($F(1,11) = 1.368$; $p=0.2605$) (Fig. 2.6A).

Cylinder Test: The ability of lesion and control groups to use their forelimbs during spontaneous exploration was assessed by comparing the number of wall contacts in a cylinder using the ipsilateral and contralateral forelimbs. Animals from both groups actively explored the cylinder; they reared and supported their body against the walls with their forelimbs. The total number of wall contact (contralateral+ipsilateral) was calculated for each group, and a simple ANOVA showed a significant effect of treatment

Figure 2.6: Asymmetry score (mean and standard error) for control and nucleus basalis lesion groups of contralateral/ipsilateral limbs on (A) rung walking, (B) cylinder test, (C) adhesive dot removal, (D) swimming. Note, there are no group differences.


A. Rung Walking
B. Cylinder
C. Adhesive Dot Removal
D. Swimming
Magnitude of asymmetry
0
1
2
3
4
5
Control
ACh

($F(1,11) = 7.056$; $p = 0.0180$). The control group was more active and contacted the walls of the cylinder more than the lesion group. The ratio of contralateral forelimb use to ipsilateral forelimb use was calculated for both groups, and a simple ANOVA revealed no significant main effect of treatment in forelimb use asymmetry ($F(1,11) = 0.157$; $p = 0.6977$) (Fig. 2.6B).

Adhesive Dot Removal: The ability of control and lesion groups to attend to sensory stimuli was assessed by comparing the latency to remove adhesive paper from their forelimbs. All animals successfully removed the adhesive paper from both paws within the three-minute time limit. Animals often removed the stimulus from their contralateral forelimbs, then proceeded to remove the stimulus from their ipsilateral forelimb. A simple ANOVA revealed no significant main effect of treatment on the overall latency (contralateral+ipsilateral) to remove both stimuli ($F(1,11) = 3.387$; $p = 0.0856$). A simple ANOVA revealed no significant main effect of treatment on asymmetry of dot removal latency ($F(1,11) = 0.009$; $p = 0.9255$) (Fig. 2.6C).

Swimming Task: The ability of control and lesion groups to inhibit their forelimbs while swimming was assessed by counting the number of strokes with each forelimb in a straight swim to a visible platform. On testing days, all animals swam directly to the platform and successfully climbed onto it. Animals from both groups showed no signs of swimming impairment by holding their forelimbs still under their chins and only using the hindlimbs to propel through the water. A simple ANOVA revealed no significant main effect of treatment on the overall (contralateral+ipsilateral)

number of strokes ($F(1,11) = 0.356$; $p=0.5598$) and no significant main effect of treatment on asymmetry of forelimb inhibition ($F(1,11) =0.245$; $p=0.6277$) (Fig. 2.6D).

DISCUSSION

This study is the first detailed analysis of the effects of either selective acetylcholine depletion in the neocortex or central muscarinic receptor blockade on motor behavior. Interrupting the cholinergic neurotransmission only produced a mild deficit in skilled reaching but did not affect performance on any of the other behavioral measures. Nevertheless, an examination of high-speed video records of the rats' performance with nucleus basalis lesions revealed impairments in the advancement of the elbow and pronation and supination of the paw.

Among the movement components that were impaired by the lesion were pronating the wrist onto the pellet, supinating the wrist after grasping the pellet and supinating the wrist to bring the pellet to the mouth. These are movements that are primarily subject to an intact motor cortex (Whishaw, Pellis, Gorny, & Pellis, 1991) and involve significant cortical reorganization, which is dependent on synaptic plasticity in the neocortex (Kleim et al., 2002; Kleim et al., 1998; Klintsova & Greenough, 1999). Depleting the cholinergic neurons projecting to the cortex produced impairments in these reaching components similar to those seen following focal damage to the motor cortex. The results suggest that the cholinergic innervation of the neocortex is central to the neocortical plasticity required for the production of normal reaching patterns.

The neural basis of the movement deficits observed following damage to the nucleus basalis could be explained in light of the significance of acetylcholine in the

cortical network plasticity. Metherate, Tremblay, and Dykes (Metherate, Tremblay, & Dykes, 1988), for example, demonstrated that iontophoretic administration of acetylcholine facilitated responsiveness of somatosensory cortical neurons in cats by enhancing their responsiveness to somatic stimuli, or increasing their firing rate, or increasing their receptive field. In addition, Tremblay, Warren, and Dykes (Tremblay, Warren, & Dykes, 1990) demonstrated that stimulation of the basal forebrain, which increases the release of acetylcholine in the somatosensory cortex (Rasmusson, Clow, & Szerb, 1992), produced similar facilitation of neuronal activity as iontophoretically administered acetylcholine. The effect was diminished by atropine treatment. Both studies (Metherate et al., 1988; Tremblay et al., 1990) demonstrated that acetylcholine modifies the excitability of single neurons in the cortex. Webster, Hanisch, Dykes, and Biesold (Webster, Hanisch, Dykes, & Biesold, 1991) provided a good example that could relate the cholinergic modulation of single-unit responses to long-term cortical plasticity. They demonstrated that the reorganization of the somatosensory cortex is dependent on neocortical acetylcholine in rats. Transection of the sciatic nerve caused a reorganization of the hindlimb-map in the somatosensory cortex, but damaging the nucleus basalis with a neurotoxin prevented such plasticity. This result was confirmed in a similar study in which pairing a tone with nucleus basalis stimulation resulted in an expansion of the auditory cortical representation of the paired tone. Where as damaging the nucleus basalis prevented such reorganization in the auditory cortex (Kilgard & Merzenich, 1998). There is sufficient evidence implicating acetylcholine in cortical plasticity; it follows that acetylcholine may be involved in motor cortex plasticity as well. This may

explain the deficits observed in skilled reaching following quisqualate lesions of the nucleus basalis.

There are two explanations for the lack of impairment on any of the behavioral measures except skilled reaching. First, the cortex may have been activated by other means. Studies have indicated that low voltage fast activity (LVFA) in the neocortex and rhythmical slow activity (RSA) in the hippocampus can result from activity in either (or both) the cholinergic projections from the basal forebrain and the serotonergic projections from the brainstem raphe (Dickson & Vanderwolf, 1990; Dringenberg & Vanderwolf, 1998; Vanderwolf & Stewart, 1986; Vanderwolf, 1987; Vanderwolf, Harvey, Leung, 1987; Vanderwolf, 1988). These inputs appear to give rise to atropine-resistant LVFA and RSA, which correlate with Type 1 behavior. It is possible that the undisturbed ascending serotonergic projections may have maintained proper cortical activation and compensated for the functions of the destroyed cholinergic neurons.

Another potential explanation for the intact behavior is that the depletion levels were not large enough to produce impairments. Damage to the nucleus basalis using quisqualic acid has been reported to reduce choline acetyltransferase levels in the neocortex by 70-75% (Dunnett et al., 1987). Wenk, Stoehr, Quintana, Mobley, and Wiley (Wenk, Stoehr, Quintana, Mobley, & Wiley, 1994) reported no deficits on measures of learning and memory following a 50% destruction of the cholinergic neurons of the nucleus basalis. Baxter, Bucci, Gorman, Wiley, and Gallagher (Baxter, Bucci, Gorman, Wiley, & Gallagher, 1995) and Berger-Sweeney et al. (Berger-Sweeney et al., 1994), achieved a decline of up to 63% and 88%, respectively, of cortical levels of choline-acetyltransferase without behavioral impairment. In addition, Waite et al. (Waite

et al., 1995) reported that a reduction of 90% of cholinergic biomarkers is necessary for behavioral impairments to manifest. Although there was a reduction in acetylcholinesterase levels in the present experiment (Fig. 2.2), traces of the enzyme were still present in the neocortex, indicative of the presence of acetylcholine, which could potentially be sufficient for proper functioning of the motor cortex.

Previous investigations into the functional outcome of acetylcholine loss have emphasized the effects of bilaterally depleting or blocking cholinergic cortical projections on learning and memory. The present results are the first to describe the effects of depleting cholinergic cortical input using a comprehensive battery of motor assessment tests. The effects were isolated using a unilateral depletion model of the nucleus basalis. The findings here demonstrate that a near loss of cholinergic input into the cortex only impaired certain qualitative aspects in skilled reaching. The rat retains a remarkable ability to compensate for damage to major fibers. The importance of using detailed behavioral analyses to accompany endpoint measurement is critical for detecting subtle impairments in performance. These behavioral and high-speed filming methods can be usefully applied to the study of functional recovery. The present model could provide a useful tool in studies of motor behavior in neurodegenerative disorders.

CHAPTER THREE

5,7-Dihydroxytryptamine neurotoxic lesions of medial forebrain bundle do not impair skilled movements in the rat

ABSTRACT

The serotonergic projections of raphe nuclei that travel through the medial forebrain bundle to the cerebral cortex are thought to be involved in synaptic plasticity and thus should be central to skilled motor behavior that depends on plastic changes in the motor cortex. Despite the potential importance of these projections for skilled movements, there has been no systematic investigation of the changes in skilled motor behavior following restricted serotonergic lesions. In the present study, the use of the contralateral limbs in skilled movements of rats with unilateral medial forebrain bundle lesions (5,7-dihydroxtryptamine, 5 μg/4 μl) were compared with the ipsilateral limbs and with the limbs of control rats on tests of: limb use in support (cylinder test), limb placing while walking a horizontal ladder, sensory responsiveness to contact (adhesive dot removal test), forelimb inhibition during swimming, and limb use in reaching for food. There were no quantitative or qualitative impairments on any of the tests following the lesion. The results were further confirmed as animals were not impaired on the skilled reaching task after the administration of the serotonin receptor blocker methiothepin mesylate. The results suggest that either the serotonergic projection is not necessary for facilitating cortical plasticity associated with skilled movement or a compensatory mechanism exists in the brain where another neurotransmitter may produce the cortical changes for skilled movements.

INTRODUCTION

The principal ascending serotonergic fibers to the cortex arise from cell bodies located in the brain stem. The major ascending pathway from the dorsal raphé nucleus and the median raphe nucleus passes through the ventral tegmental area and joins the medial forebrain bundle (Moore, Halaris, & Jones, 1978; O'Hearn & Molliver, 1984). The most rostral of these projections terminate in the frontal lobes supplying the motor cortex with dense serotonergic input. Thus, the serotonergic fibers appear to be in a position to exert an important influence on the activity of the neocortex. The function of the ascending serotonergic input to the cortical neurons remains unclear, however.

There is evidence that serotonin is instrumental in certain aspects of plasticity in the central nervous system. Osterheld-Haas, Van der Loos, and Hornung (1994), for example, demonstrated that it is central to compensatory plastic responses after injury. Depleting serotonin also retards neurogenisis in the adult brain following injury (Brezun & Daszuta, 1999). Immunohistochemical studies demonstrated an increased sprouting of serotonergic fibers following lesions of the cingulate cortex and the hippocampus (Ueda, Sano, & Kawata, 1991). Serotonin is also instrumental in regulating brain development; it controls the release of astroglial proteins that are key to proper synapse formation (Mazer et al., 1997). Furthermore, it is thought that serotonin is important for normal EEG patterns and cortical activation (Vanderwolf, 1987a). A number of behavioral abnormalities largely attributed to motor deficits have been demonstrated following the near loss of serotonin (Vanderwolf, 1989). Type 1 behaviors such as walking, changing posture, and turning or raising the head, for example, appear disorganized and erratic (Vanderwolf, 1989; Vanderwolf, Kolb, & Cooley, 1978). Behavioral studies have also

shown that the serotonergic pathway produces some of the inhibition necessary to dampen behavioral responsivity, hyperactivity, and startle response (Geyer, Puerto, Menkes, Segal, & Mandell, 1976). Nonetheless, despite the extensive serotonergic projections to the neocortex, the role of serotonin in behavioral modulation has only been described in general terms, and the details of its role remain unclear.

Serotonin receptors are widely spread throughout the brain. Seven serotonin receptor families have so far been identified. Families 1,2,5 have subtypes within them. The receptor of interest here is the 5-HT_1 receptor family, mainly because of its wide distribution throughout the cerebral cortex and its high affinity for serotonin (Siegel & Agranoff, 1999 for review). Dringenberg and Zalan (1999) have shown that an acute blockade of serotonergic receptors produces the same behavioral effects as seen following the global depletion of serotonin. This suggestion was used in the current experiment to confirm the effects of damaging the ascending serotonergic projections.

For the current experiment, the serotonin-selective neurotoxin 5,7-dihydroxytryptamine was infused into the medial forebrain bundle in order to retrogradely destroy the raphé nuclei of the brain stem (Giambalvo & Snodgrass, 1978). This lesion method allowed the localization of damage to one hemisphere and left the descending serotonergic projections intact. After two weeks of recovery, the rats were given a series of sensorimotor tests, all of which have been demonstrated to be sensitive to sensorimotor lesions. The assessment included: tests of forelimb support (cylinder test), limb placing while traversing a horizontal ladder with variably spaced rungs, sensory responsivness to sensory contact (adhesive dot removal test), forelimb inhibition during swimming, and limb use in reaching for food. The performance of the rats was

videotaped, and the tapes were scored in order to assess the quantitative and qualitative performance of the animals. In addition to being compared to a control group, performance related to the limbs ipsilateral to the lesion was compared to performance of the limbs contralateral to the lesion with the expectation that the limbs contralateral to the lesion should be more affected as typically occurs following frank cortical injury. A different group of rats received an injection of methiothepin mesylate prior to reaching for food pellets (Whishaw & Pellis, 1990). A quantitative and qualitative analysis of the performance was compared to their own performance in the absence of the drug.

METHODS

Subjects

The subjects were 19 Long-Evans hooded female-adult rats, 120 days old and weighing 250-300g. They were raised in the University of Lethbridge Vivarium. The rats were divided into three groups, control (n=7), lesion (n=6) and methiothepin (n=6). The animals were housed in groups of three or four individuals in hanging wire mesh cages. The colony room was maintained on a 12/12h light/dark cycle (08:00-20:00 h).

Feeding

For the experiment, rats were food deprived but with *ad lib* water access. Three weeks prior to surgery, rats were food deprived to 85% of their original body weight by providing 15g of solid chow per rat/per day to maintain body weight.

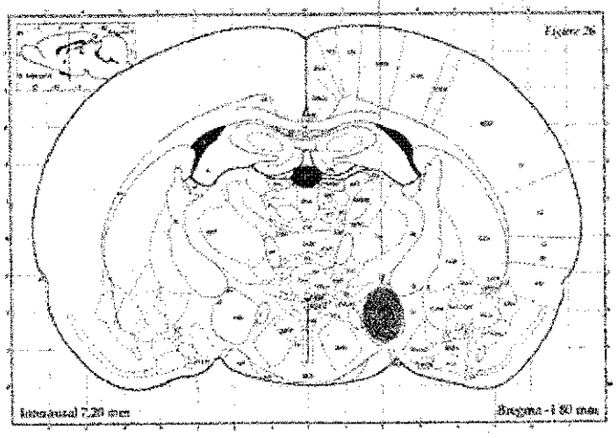
Surgery

Each animal received an injection of desipramine HCl (25 mg/kg i.p.) (Sigma-Aldrich, St. Loius, MO), a norepinephrine reuptake inhibitor 30 min prior to neurotoxin infusions. Animals then received an injection of atropine nitrate (0.1 mg/kg i.p.) (Sigma-Aldrich, St. Loius, MO) to facilitate respiration throughout surgery. Under sodium pentobarbital anaesthesia (0.5 ml/kg, i.p.) (Sigma-Aldrich, St. Loius, MO), each rat received stereotaxic infusions of 5,7-dihydroxytrptamine (5 μg/4 μl, Sigma-Aldrich, St. Loius, MO) via a 30-gauge cannula connected to a micro drive pump by a polythene tube. Two 2.0 μl infusions were made unilaterally in the medial forebrain bundle. Each infusion was delivered over five min and an additional five min allowed for diffusion before the cannula was retracted. Stereotaxic coordinates anterior (A), lateral (L) and ventral (V) for the two injections were A=-2.0 mm, L=1.5 mm, V=9.5 mm (below skull) and; A=-1.5 mm, L=1.5 mm, V=9.5 mm (below skull) (see Fig. 3.1). The lesion group was allowed two weeks to recover before behavioral testing.

Figure 3.1: Sections from Paxinos and Watson (1997) Rat Atlas; (A) coronal; (B) saggital. The diagram is representative of the two 5,7-dihydroxytryptamine infusion sights, at A= -2.0 mm, L=1.5 mm, V=9.5 mm (below skull) and; A= -1.5 mm, L= 1.5 mm, V=9.5 mm (below skull).

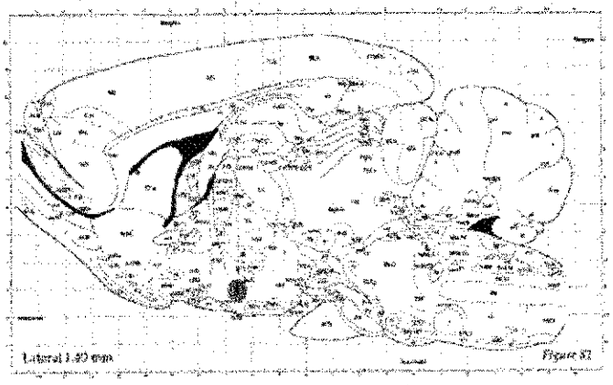
A

5,7-DHT



B

5,7-DHT



Methiothepin mesylate

This drug blocks the five subtypes of the 5-HT_1 receptor family. Five doses of methiothepin mesylate (Sigma-Aldrich, St. Loius, MO) 0.1, 0.15, 0.2, 0.25, 0.3 mg/kg were administered to the control group once per testing session and was only tested once. The animals were injected (i.p.) with the drug 20-30 min prior to testing in the single pellet reaching task. The drug doses were administered in systematic increments starting with the lowest dose to minimize tolerance effects. After each rat was injected with the drug, it was returned to its home cage until it was due for testing.

Behavioral Training and Test Analysis

Reaching boxes and training: All animals were pre-trained to reach through a slot for single pieces of food for two weeks prior to surgery or drug administration (Whishaw, 2000). Reaching boxes were made of clear Plexiglas. Each box was 45x14x35 cm high. In the center of each front wall was a 1 cm-wide slit which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm-wide shelf. Two indentations on the floor of the shelf were located 2 cm from the inside of the wall and were centered on the edges of the slit were rats could reach. Food pellets (45 mg Rodent Chow food pellets, Bioserve Inc.) were placed in the indentation contralateral to the limb with which the rat reached (Whishaw & Pellis, 1990). Following each reach, a short pause preceded the presentation of the next pellet and an additional pellet could be dropped in the back of the box. This encouraged animals to return to the back of the box after each reach and so forced them to reposition themselves and prepare for the next reach. The animals were

trained for ten minutes each day for the first week and were presented with 20 pellets each day for the second week. Reaching performance was assessed on two measures: "reaching success" = number of pellets retrieved and "reaches/pellet retrieved" = number of reaching attempts/successful retrieval. After the recovery period following surgery, the animals were tested every day for two weeks. They were presented with 20 pellets in each testing session.

For a qualitative analysis of reaching, a reach was subdivided into ten components (Whishaw, Pellis, Gorny, Kolb, & Tetzlaff, 1993). (1) Limb lift: the limb is lifted from the floor with the upper arm and the digits adducted to the midline of the body. (2) Digits close: as the limb is lifted, the digits are semiflexed and the paw is supinated so that the palm faces the midline of the body. (3) Aim: using the upper arm, the elbow is adducted so that the forearm is aligned along the midline of the body, with the paw located just under the mouth. This movement involves fixation of the distal portion of the limb, so that digits remain aligned with the midline of the body. This is likely produced by a movement around the elbow that reverses the direction of movement of the paw to compensate for the adduction of the elbow. (4) Advance: the head is lifted and the limb is advanced directly forward above and beyond the food pellet. (5) Digits open: as the limb is advanced the digits are extended and opened. (6) Pronate: using a movement of the upper arm, the elbow is abducted, pronating the paw over the food. Full pronation of the paw onto the food is aided by a movement of the paw around the wrist. (7) Grasp: as the pads of the palm or the digits touch the food, the food is grasped by closure of the digits. This can occur as an independent movement or the grasp can occur as the paw is withdrawn. (8) Supination I: as the limb is withdrawn, the paw is dorsiflexed and is

supinated 90^0 by a movement around the wrist and by adduction of the elbow. These movements can occur as soon as the food is grasped or can occur as the limb is withdrawn. (9) Supination II: as the rat sits back with food held in the paw, the paw is further supinated by 90^0 and ventroflexted to present the food to the mouth. (10) Release: the digits are opened and the food transferred to the mouth.

Five successful reaches were analyzed frame-by-frame on the video tapes. Each movement was rated on a three-point scale. If the movement appeared normal, it was given a score of "0", if it appeared slightly abnormal but recognizable it was given a score of "1", and a score of "2" was assigned if the movement was absent or completely unrecognizable.

Rung Walking: The runway consisted of a straight section 1m in length with walls 19 cm high and a square goal box at one end in which food was located (Metz & Whishaw, 2002). The width of the alley was adjusted to the size of the animal allowing 1 cm on either side of the animal to prevent it from turning around. The floor of the runway was made of a readily changeable arrangement of horizontal steel rods 3 mm in diameter. An irregular but unchanged rung pattern was maintained throughout all trials, gap sizes varied from 1 cm to 5 cm. A high-8mm camera was positioned at a slight ventral angle, so that the positions of all four limbs can be filmed simultaneously from a ventral view.

The novel foot-fault scoring system (Metz & Whishaw, 2002) was modified and used to assess forelimb and hindlimb qualitative placement. Each step was rated on a five-point scale: if the foot placement appeared normal where the midportion of the palm

was placed on the rung, it was given a score of "0"; if placement on the rung was done using the wrist or digits of the forelimb or the heel or toes of the hindlimb, it was given a score of "1"; if a limb was placed on a rung and slipped off during weight shifting without disturbing balance, it was given a score of "2"; if a limb was placed on a rung and slipped off during weight shifting causing a fall, it was given a score of "3"; and if a limb missed the targeted rung completely and fell through the gap compromising body posture and balance, it was given a score of "4". Animals received three trials during each testing day. The asymmetry score, which is a ratio of foot faults committed by both sides of the body, was calculated for each group; this is: contralateral limb faults/ipsilateral limb faults.

Cylinder test: Forelimb use for weight support during explorative activity was examined by placing rats in a transparent cylinder 20 cm in diameter and 30 cm high for four minutes (Schallert, Kozlowski, Humm, & Cocke, 1997). A mirror was placed underneath the cylinder at an angle to allow the experimenter to videotape the animal's activity from a ventral view. The cylindrical shape encouraged vertical exploration of the walls with the forelimbs. The cylinder was high enough so that animals could not reach the top and was wide enough to allow 2 cm between either end of the animal and the walls. Forelimb use was measured during vertical exploration following rearing. Independent use of each forelimb during wall contact was scored during weight shift initiation or to regain center of gravity while moving laterally in a vertical posture. The asymmetry score of forelimb use in wall exploration was calculated for each group, this is: contralateral forelimb wall contact/ipsilateral forelimb wall contact.

Adhesive Dot Removal: Procedures for this task have been described previously by Schallert et al. (1982). Animals were removed from their home cages and their forelimbs were washed with 50% ethanol solution, then wiped with cotton gauze and allowed to dry. Two parallel creases were formed in adhesive paper stimuli (113 mm^2, manufactured by Avery International) to facilitate wrapping them around the forelimb. The stimuli were attached to the distal-radial aspect of both forelimbs. Immediately after, the experimenter firmly touched both forelimbs simultaneously and placed the animal in a clear Plexiglas tub (45x26x20 cm) without bedding for ease of recording. A stainless steel lid was used to cover the tub and contain the rat. The fine forelimb hair was not pulled out in the process, however, the stimuli were sticky enough that they rarely fell off when the animal moved around, groomed or shook its forelimb. Trials in which either stimulus fell off spontaneously were disregarded and repeated. The order of stimulus attachment to the contralateral and ipsilateral forelimbs was counterbalanced for all animals. Subjects contacted and attempted to remove the adhesive paper. The order and latency of removal was recorded for each forelimb for four trials. Each trial was ended after both labels were removed or after three minutes. The asymmetry score of latency of dot removal was calculated for each group contralateral forelimb latency/ipsilateral forelimb latency.

Swimming Test: Video recordings were made in a large rectangular aquarium (120x43x50 cm) as described by Whishaw, Nonneman, and Kolb (Whishaw, Nonneman, & Kolb, 1981). Water was high enough to prevent animals from touching the bottom of

the aquarium but at the same time low enough to prevent them from escaping to the edge of the pool, temperature was maintained at 21^0C. At one end of the pool was an escape wire mesh platform onto which the animals could climb. The platform was visible to the animals at all times. During the training phase, animals were released close to the platform, after they learned to swim and climb onto the platform, they were released at progressively longer distances until they swam directly from the opposite end of the tank. Initially, most animals used all four limbs to stroke, rapidly changed direction, and sometimes swam aimlessly. Once animals learned to swim directly to the platform and were more familiar with the task, they held their forelimbs immobile under their chins and only used their hindlimbs to propel through the water. Each animal performed four trials during which they had to swim directly to the platform. Animals were dried and returned to their home cages after completing four trials. Disruption to the normal swim pattern was quantified by counting the number of strokes by each forelimb. The asymmetry score of forelimb inhibition was calculated for each group, this is: contralateral forelimb strokes/ipsilateral forelimb wall strokes.

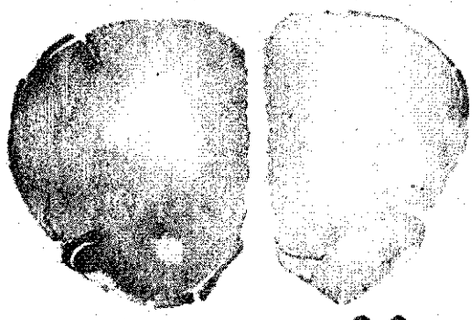
All subjects were tested on the rung walking, cylinder, swimming, and adhesive dot removal tests once a week for four weeks after surgery.

Histological procedures

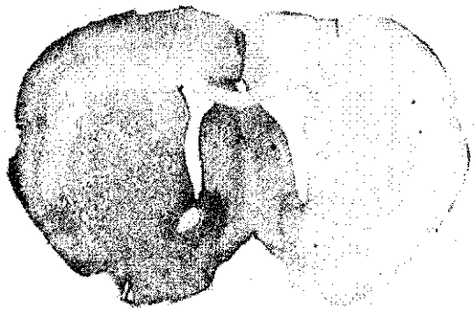
After six weeks of behavioral testing, all animals were sacrificed using a lethal dose of sodium pentobarbital. They were intracardially perfused, first with saline in 0.1 M PBS followed by 4% paraformaldehyde in 0.1 M PBS. The brains were removed, postfixed and cryoprotected in a solution of 30% sucrose in 4% paraformaldehyde

solution for three days in 4°C. All brains were then sectioned into 40 μm using a cryostat (2800 Frigocut, Reichert-Jung) and stored in 0.1 M PBS solution. The following day, free-floating sections from control and lesion groups were incubated for 15 min in a quench solution, 20 ml of 3% H_2O_2 in 180 ml 0.1 M PBS, to reduce background staining. The sections were then washed three times with 0.1 M PBS. The tissue was then incubated in a primary antibody solution, 15 ml 0.1 M PBS, 3 drops goat serum, 100 _l 3% Triton-X, 20 _l of serotonin antibody (donated by Dr. Richard Dyck), bovine serum albumin 150mg was added to reduce background staining. Six sections per centrifuge tube were rotated at 40 rpm while refrigerated at 4°C for 20 hrs. All sections were washed three times with 0.1 M PBS and incubated in a secondary antibody solution, 10 ml 0.1 M PBS, 3 drops goat serum and 1 drop anti-rabbit IgG (Vector Laboratories, Burlingame, CA). The sections were rotated at 40 rpm at 4°C for 1 hr. The tissue was washed three times with 0.1 M PBS and then incubated in an AB complex solution (Vector Laboratories, Burlingame, CA), 5 ml 0.1 M PBS, 2 drops solution A, 2 drops solution B and centrifuged at 40 rpm at 4°C for 30 min. The tissue was washed three times with 0.1 M PBS and then dipped into a solution containing: 5 ml distilled H_2O, 2 drops 7.5 M PBS, 4 drops D amino benzodine (DAB), 2 drops H_2O_2, and 2 drops Ni^{2+} (Vector Laboratories, Burlingame, CA). All sections were finally rinsed three times with 0.1 M PBS, mounted onto slides and cover slips were placed on top (see Fig. 3.2).

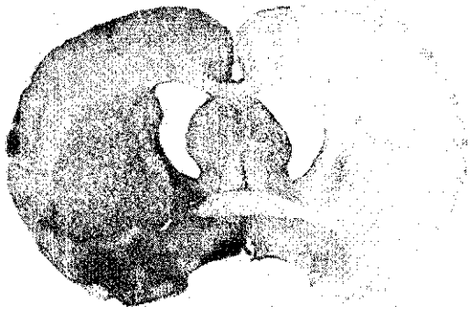
Figure 3.2: Representative photographs from the medial forebrain bundle lesion group of coronal sections of the motor cortex stained using an immunohistochemical technique for serotonin. The left hemisphere is contralateral to the lesion and appears darker than the ipsilateral hemisphere indicating the presence of more serotonin.



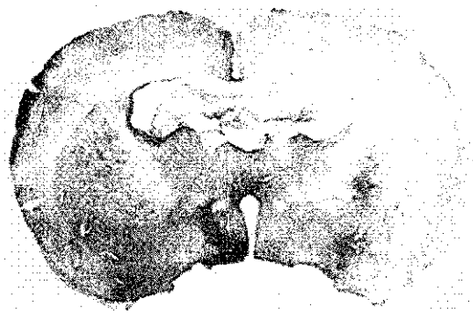
+2.2 mm



+0.7 mm



-0.8 mm



-1.3 mm

RESULTS

Behavioral Observations

Single pellet reaching (medial forebrain bundle lesion): The ability of lesion and control groups to use their forelimbs was assessed in a skilled reaching task where animals reached through a narrow slot onto an elevated shelf to retrieve a food reward. Both the lesion and the control groups continued to use their contralateral forelimbs for reaching as they did before surgery. A mean success score was calculated for all animals and compared across all groups. A simple ANOVA revealed no significant main effect of treatment on success score ($F(1,11) = 0.073$; p=0.7923) (Fig. 3.3A). A mean reaches/pellet retrieved rate was calculated for all animals as well and compared across all groups. A simple ANOVA revealed no significant main effect of treatment on the number of reaches/pellet ($F(1,11) = 0.819$; p=0.3848) (Fig. 3.3B). The success score and reaches/pellet retrieved score demonstrate quantitatively that the lesion did not affect skilled reaching. To assess the extent to which the 10-elements of reaching were changed in the lesion group relative to the control group, the overall component scores were analyzed and showed no significant differences between the control and the lesion groups (Fig. 3.3C). In addition there were no significant differences in the individual movement components between groups (Fig. 3.4).

Figure 3.3: Single pellet reaching scores (mean and standard error) in control and medial forebrain bundle lesion groups. (A) success, number of pellets retrieved out of 20; (B) reaches/pellet, number of reaches performed for each successfully retrieved pellet; (C) The qualitative movement error scores of five representative reaches in control and lesioned animals. Note, there are no group differences.

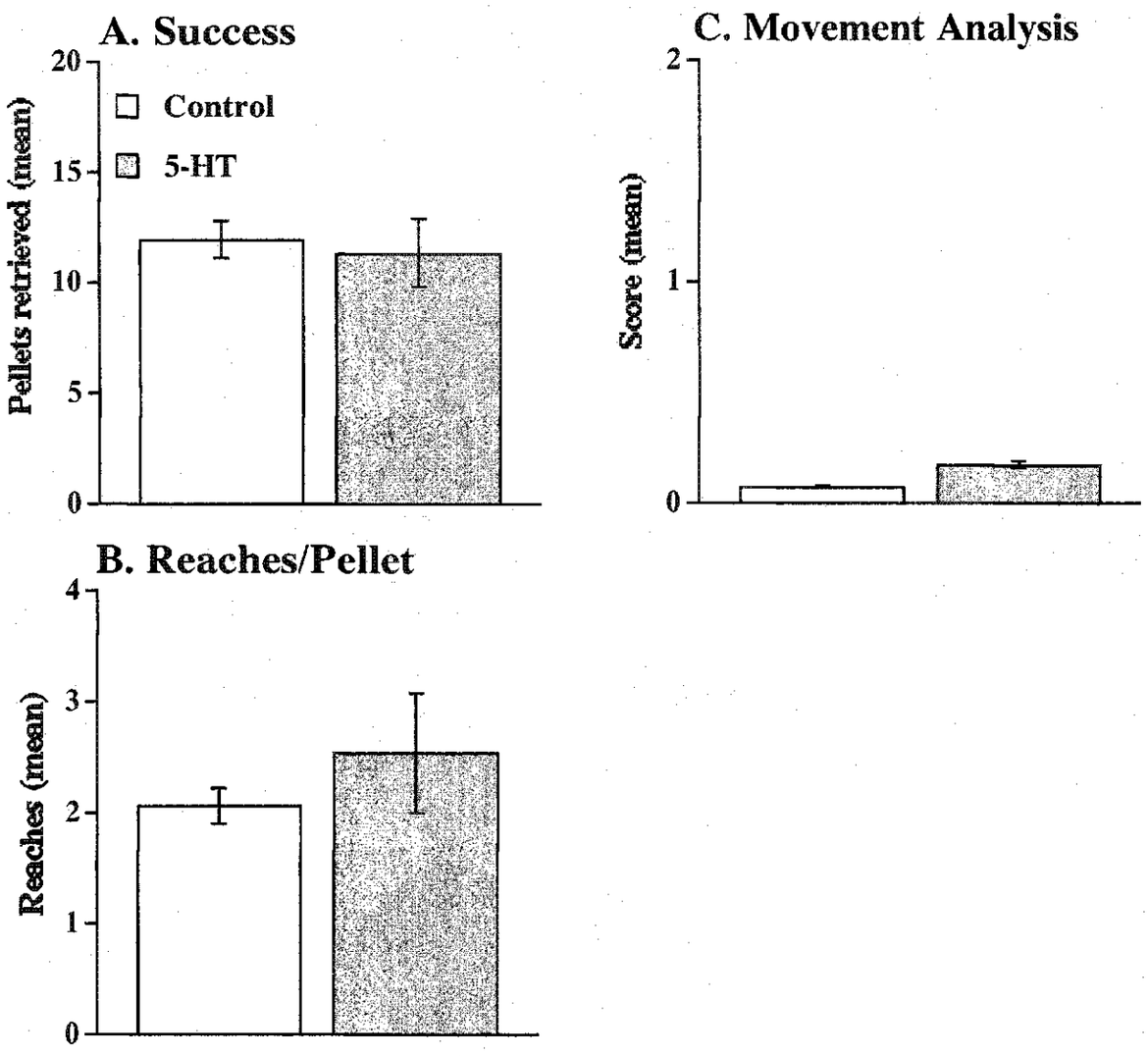
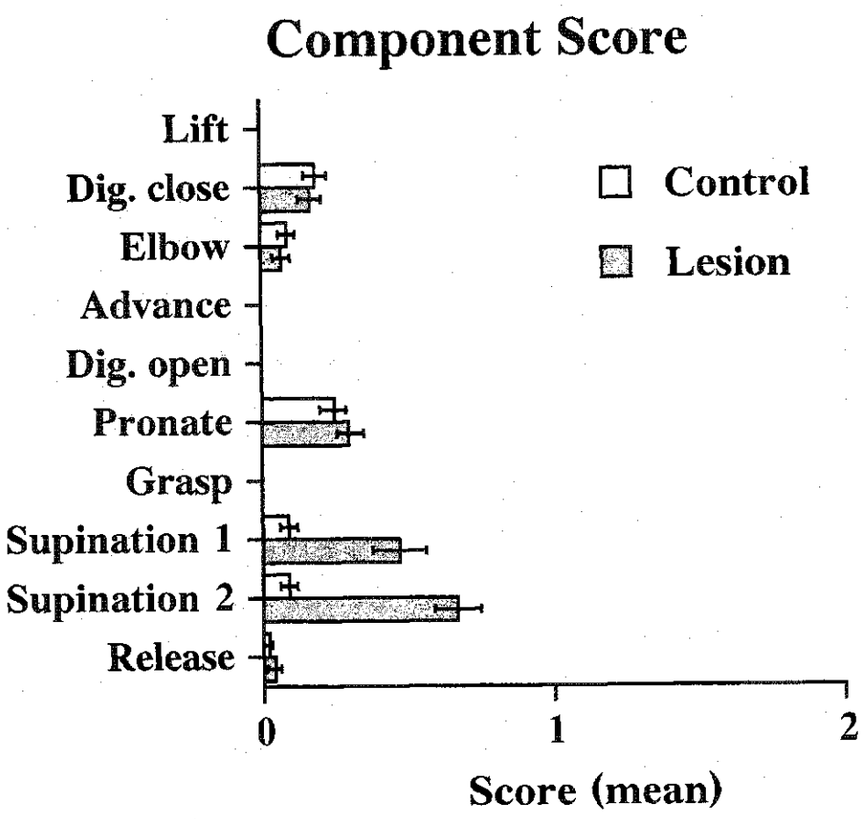

A. Success
Pellets retrieved (mean)
20
15
10
5
0
Control
5-HT
C. Movement Analysis
Score (mean)
2
1
0
B. Reaches/Pellet
Reaches (mean)
4
3
2
1
0

Figure 3.4: Reaching movement components (mean and standard error) on the 10 movement components of reaching for control and medial forebrain bundle lesion groups. Each of the movement components was rated on a 3-point scale, with 0=normal and 2=absent.


Component Score
Lift
Dig. close
Elbow
Advance
Dig. open
Pronate
Grasp
Supination 1
Supination 2
Release
Control
Lesion
0
1
2
Score (mean)

Skilled Reaching (methiothepin mesylate): All animals continued to use their contralateral paw to reach for food items located on a shelf following the administration of methiothepin mesylate, except at the highest dose (0.3 mg/kg) at which animals did not attempt to reach and appeared drowsy and immobile (Fig. 3.5). A simple ANOVA revealed no significant main effect of treatment on reaching success score ($F(4,1) = 4.339$; $p = 0.1057$). A repeated measures ANOVA revealed a significant difference in the success rate between the various drug doses and control ($F(4,4) = 4.376$; $p = 0.014$). A post hoc Fisher LSD ($p<0.5$) analysis showed that animals had a higher success rate at 0.15 mg/kg than control and 0.1 mg/kg, and the 0.3 mg/kg dose produced the lowest success rate.

Rung Walking: The ability of lesion and control groups to cross a horizontal ladder with randomly spaced bars was assessed by counting the number of foot faults. Animals from both groups walked across the horizontal ladder equally well. Both control and lesion groups committed a few foot faults of both forelimbs and both hindlimbs. The scores from the previously described five error categories were summed for each group and a total number of foot faults (contralateral+ipsilateral) including forelimbs and hindlimbs was calculated for each group. A simple ANOVA found no significant main effect of treatment on total number of foot faults ($F(1,11) = 0.561$; $p = 0.4694$). The ratio of foot faults committed by both forelimbs and hindlimbs was calculated for the contralateral and ipsilateral sides of the body, and a simple ANOVA found no significant main effect of treatment in foot fault asymmetry ($F(1,11) = 3.072$; $p = 0.1074$) (Fig. 3.6A).

Figure 3.5: Single pellet reaching success score (mean and standard error) in control and following the administration (i.p.) of one of five doses of methiothepin mesylate: 0.1, 0.15, 0.2, 0.25, 0.3 mg/kg.

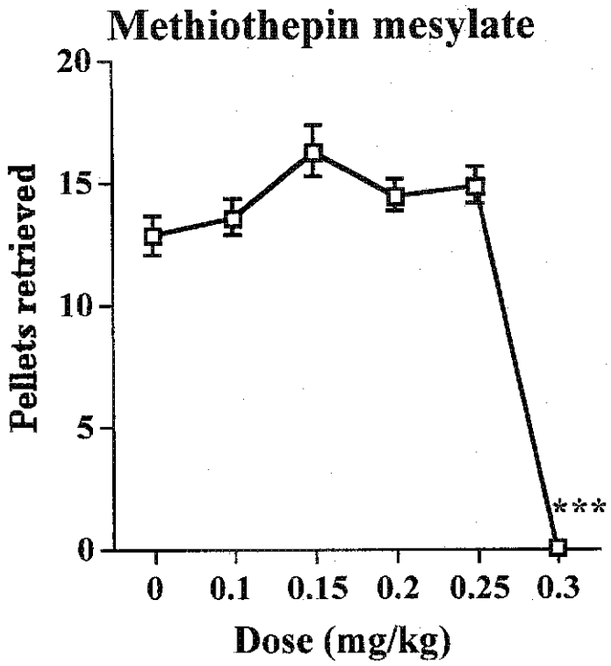
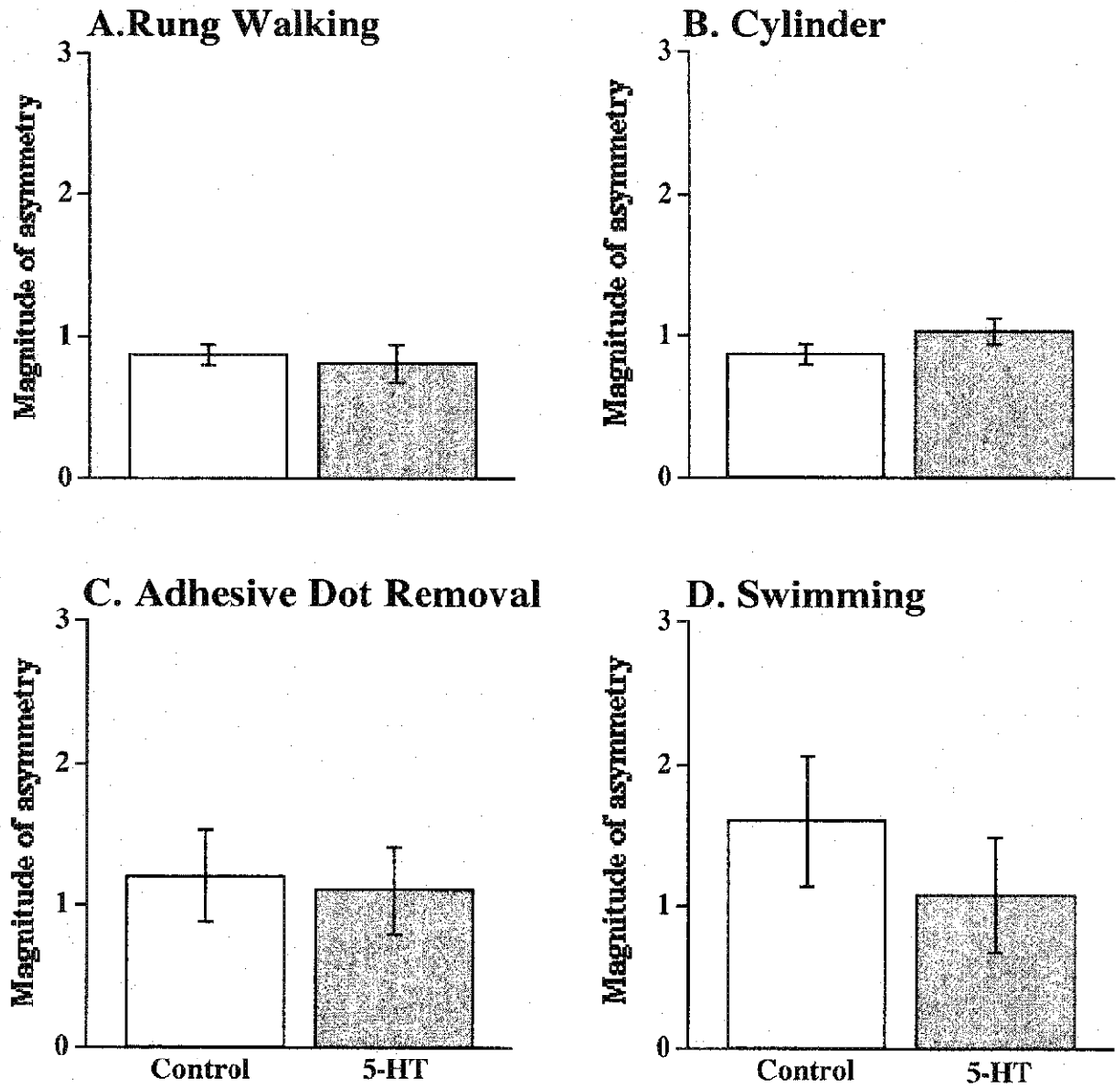

Methiothepin mesylate
Pellets retrieved
20
15
10
5
0
***
0
0.1
0.15
0.2
0.25
0.3
Dose (mg/kg)

Figure 3.6: Asymmetry score (mean and standard error) of contralateral/ipsilateral limbs on (A) rung walking, (B) cylinder test, (C) adhesive dot removal, (D) swimming for control and medial forebrain bundle lesion groups. Note, there are no group differences.


A.Rung Walking
B. Cylinder
C. Adhesive Dot Removal
D. Swimming
Magnitude of asymmetry
0
1
2
3
Control
5-HT

Cylinder Test: The ability of lesion and control groups to use their forelimbs to support their weight during spontaneous exploration was assessed by comparing the number of wall contacts in a cylinder using the ipsilateral and contralateral forelimbs. Animals from both groups actively explored the cylinder, and when they reared touched and supported their body against the walls with their forelimbs. The total number of wall contacts (contralateral+ipsilateral) was calculated for each group and a simple ANOVA showed no significant effect of treatment ($F(1,11) = 0.009$; $p = 0.9249$). The lesion group equally touched the walls of the cylinder as the control group. The ratio of contralateral forelimb use to ipsilateral forelimb use was calculated for both groups, and a simple ANOVA revealed no significant main effect of treatment in forelimb use asymmetry ($F(1,11) = 2.187$; $p = 0.1673$) (Fig. 3.6B).

Adhesive Dot Removal: The ability of control and lesion groups to attend to sensory stimuli was assessed by comparing the latency to remove adhesive paper from their forelimbs. All animals successfully removed the adhesive dots from forelimbs within the three-minute time limit. Animals often removed the stimulus from their contralateral forelimbs then proceeded to remove the stimulus from their ipsilateral forelimbs. A simple ANOVA showed that the overall latency (contralateral+ipsilateral) to remove both stimuli was significantly higher in the lesion group than the control group ($F(1,11) = 19.643$; $p = 0.001$). A simple ANOVA revealed no significant main effect of treatment in asymmetry of dot removal latency, however ($F(1,11) = 0.067$; $p = 0.8007$) (Fig. 3.6C).

Swimming Task: The ability of control and lesion groups to inhibit their forelimbs was assessed by counting the number of forelimb strokes in a straight swim to a visible platform. On testing days all animals swam directly to the platform and successfully climbed onto it. Both control and lesion groups showed no signs of swimming impairment by holding their forelimbs still under their chins and only using their hindlimbs to propel through the water. A simple ANOVA revealed no significant main effect of treatment on the overall number of strokes ($F(1,11) = 0.768$; $p=0.3994$). A simple ANOVA also revealed no significant main effect of treatment on asymmetry of forelimb inhibition ($F(1,11) = 0.579$; $p=0.4628$) (Fig. 3.6D).

DISCUSSION

This is the first detailed analysis of the effects of either selective unilateral serotonergic fiber depletion or serotonergic receptor blockade on skilled motor behavior. Rats were trained to reach through a slot for food pellets located on a shelf. The subjects continued to use their contralateral paw for reaching and with the same proficiency as controls following damage to the ascending serotonergic projection or blockade of the serotonergic receptors. In addition, the lesion group did not show any signs of impairments on the other behavioral measures.

The intact performance of the lesion group on the behavioral measures is consistent with previous work (Lawson & Bland, 1993; Vanderwolf, 1989; Vanderwolf & Baker, 1986; Vanderwolf, Leung, Baker, & Stewart, 1989). It has been shown that the low voltage fast activity (LVFA) of both the serotonergic and the cholinergic projections

are intensely active during Type 1 behaviors (Vanderwolf, 1987b). Vanderwolf and Baker (1986) demonstrated that rats treated with the serotonin synthesis inhibitor *p*-chlorophenylalanine (PCPA) displayed normal LVFA and RSA, but the administration of the muscarinic receptor blocker atropine sulphate to PCPA-treated animals, resulted in severe abnormalities in brain waves and behavior. Walking, changing posture, swimming and manipulating objects with the forelimbs are examples of Type 1 behaviors that may be maintained with either cholinergic or serotonergic input. Type 1 behaviors are central to intact performance on the behavioral tests in the present experiment. The lack of impairment following the depletion of the serotonergic neurons may, therefore, be due to a compensatory mechanism assumed by the cholinergic projections.

Another explanation for the intact behavior is that the serotonin depletion was not large enough to produce impairment. Vanderwolf et al. (1989) report that following the depletion of the B7 and B8 raphe cell groups, which send serotonergic projections to the cortex, normal atropine-resistant cortical activation (ARCA) was still recorded despite the severely reduced levels of serotonin in the cortex. They further report that a depletion of the cell group B9, which also sends serotonergic projections to the cortex, in addition to B7 and B8, is necessary to abolish ARCA. A 90% reduction in serotonin levels had to be achieved for functional deficits to unveil. Similar results have been reported; for example, an incomplete depletion of dopamine cells does not produce behavioral impairments (Zigmond, Acheson, Stachowiak, & Stricker, 1984; Zigmond & Stricker, 1984). It must be noted, however, that the projections from the cell groups B7, B8, B9 pass though the medial forebrain bundle, which is the lesion site in present study. In other words, all three cell groups were targeted using the current lesion method and thus

should have produced almost a complete loss of ascending serotonergic fibers in the ipsilateral hemisphere.

Decrease in spontaneous explorative behavior following the lesion is indicated by the reduced wall contact seen in the cylinder test. This result is consistent with other studies that have produced selective depletion of ascending serotonergic fibers (File & Deakin, 1980; Lorens, Guldberg, Hole, Kohler, & Srebro, 1976). Although increased locomotor activity has also been reported after serotonergic fiber depletion, non-specific damage due to the use of electrolytic lesion methods may be the reason for the different behavioral outcomes (Jacobs, Wise, & Taylor, 1974; Kam & Moberg, 1977; Lorens et al., 1976).

The only tests of sensory responsiveness conducted in rats with serotonin depletions are ones that involve startle response, such as responsiveness to a loud auditory stimulus (Connor and Stalk, 1970) or an air puff stimulus (Geyer et al., 1976; Geyer, Warbritton, Menkes, Zook, & Mandell, 1975). Such studies have consistently reported a decrease in startle response following intraventricular injection of serotonin and an increased response after the depletion of serotonin. Although there is no previous work on forelimb sensitivity with respect to manipulations of serotonin in the brain, a loss of inhibition, which means faster orientation to stimuli, was expected. The lesion group did not show signs of asymmetry, however, on the adhesive dot removal task.

The intact performance of the lesion group on the swimming task is not consistent with the results of Vanderwolf (1989). The author reports that following the central depletion of serotonin, animals were severely impaired on a task where they had to swim and climb onto a visible platform. Vanderwolf (1989) reported that following the lesion,

swimming was erratic and aimless, and animals experienced motor impairment that reduced their ability to swim and climb onto the platform. It must be noted, however, that the observations made by Vanderwolf (1989) were produced using PCPA, which globally abolished the ARCA. He also reported that animals that sustained a lesion but did not experience a significant reduction in ARCA, which is likely similar to the present lesion group, behaved like controls in the swimming tank (Vanderwolf, 1989).

The present findings indicate no impairment in the production of movements following damage to the ascending serotonergic projections to the cortex. Serotonin has been reported as critical for proper development and wiring of the central nervous system in a number of studies (Benes, Taylor, & Cunningham, 2000; Lauder, 1990; Mazer et al., 1997), but its role in neuronal plasticity and behavior modification in adults remains controversial. One conclusion that could be reached from this study is that serotonergic projections are not instrumental for the production of skilled movements.

CHAPTER FOUR

Combined neurotoxin lesions of the nucleus basalis and the medial forebrain bundle produce mild motor deficits in rats

ABSTRACT

The interaction between acetylcholine and serotonin in the rat's brain has been reported as instrumental for intelligent behavior. The conjoint depletion of both neurotransmitters results in dementia like symptoms in rodents. The focus of this research however has been directed towards understanding the interaction between the two systems with respect to cognitive tasks while others such as skilled motor movements have been neglected. The role of the interaction of cholinergic and serotonergic projections in the production of skilled motor behavior was examined in the present experiment. Lesions of the nucleus basalis (quisqualate, 0.5 μg/μl) and the medial forebrain bundle (5,7-dihydroxtryptamine, 5 μg/4 μl) were produced unilaterally and in the same hemisphere either with a recovery and behavioral testing period in between (two-stage lesion) or conjointly (one-stage lesion). The use of the contralateral limbs in skilled movements of rats with unilateral combined lesions was compared with the ipsilateral limbs and with the limbs of control rats on tests of: limb use in support (cylinder test), limb placing while walking a horizontal ladder, sensory responsiveness to contact (adhesive dot removal test), forelimb inhibition during swimming, and limb use in reaching for food. The two-stage lesion group did not show a decline in pellet retrieval success but did display signs of qualitative impairments in certain aspects of the reaching movement. The one-stage

lesion group showed impairments in the quantitative and qualitative aspects of skilled reaching. In general, the combined lesion did not have a severe effect on motor behavior.

INTRODUCTION

The idea of a functional interaction between the cholinergic and serotonergic systems is not a novel one. Several lines of evidence suggest that these two systems interact in various structures of the brain such as the neocortex and the hippocampus, to modulate learning and memory (Nilsson, Strecker, Daszuta, & Bjorklund, 1988; Riekkinen, Sirvio, & Riekkinen, 1990a; Vanderwolf, 1988; Vanderwolf & Baker, 1986).

The interaction between the two neurotransmitters did not receive much attention, however, until the Bartus, Dean, Pontecorvo, and Flicker (1985) "cholinergic hypothesis of learning" was deemed too reductionistic. Studies done on aged rodents were among the first to redirect the field; aged animals exhibited more severe deficits in spatial navigation than animals sustaining a lesion to the nucleus basalis or blockade of the muscarinic receptors (Fischer, Chen, Gage, & Bjorklund, 1992; Gage & Bjorklund, 1986; Riekkinen, Sirvio, & Riekkinen, 1990b; Whishaw, 1985). Although a decline in the cholinergic projections is still considered the primary cause for the cognitive impairments in aged animals, it is not the only cause. The degeneration of serotonergic projections to the neocortex and the basal forebrain has been suggested as a contributing factor (Nilsson et al., 1988; Vanderwolf, 1987; Vanderwolf, 1988; Vanderwolf & Baker, 1986).

Although the mechanism of the interaction between the two systems remains unclear, a few lines of evidence support it. First, neuroanatomical approaches have demonstrated that afferents of serotonergic fibers innervate dense cholinergic regions such as the basal forebrain (Samanin, Quattrone, Peri, Ladinsky, & Consolo, 1978; Vertes, 1988) and a dense distribution of 5-HT_1 receptors in areas that are largely innervated by cholinergic neurons (Quirion & Richard, 1987). This is an avenue for

direct interactive processes. In addition, there is evidence of indirect interactions at the level of convergent projection areas of the two systems such as in the hippocampus, the neocortex, and the amygdala (Steckler & Sahgal, 1995). Second, electrophysiological studies have demonstrated that the cerebral activation, low voltage fast activity (LVFA) in the neocortex and rhythmical slow activity (RSA) in the hippocampus are controlled by the cholinergic and serotonergic projections (Vanderwolf, 1987; Vanderwolf, 1988; Vanderwolf & Stewart, 1986). These two neurotransmitters may interact by altering the activity of a common target such as the hippocampus or the cortex. Third, administering a serotonergic agonist increases the levels of acetylcholine in the hippocampus and the striatum (Samanin et al., 1978). Fourth, studies involving the depletion and or blockade of both systems have shown that the disruption of both systems results in a generalized behavioral disorganization similar to behavioral disorganization in decorticated animals, but interference with either alone has little effect on behavior (Beiko, Candusso, & Cain, 1997; Dringenberg & Zalan, 1999; Vanderwolf, 1987; Vanderwolf, Baker, & Dickson, 1990). Potential interactions between the cholinergic and serotonergic systems raise the possibility of one neurotransmitter modulating the efficacy of the other. Steckler and Sahgal (1995) use the term *additive synergism* to refer to behavioral deficits that are at least as large as the effect of the manipulation of either system individually. If the combined efficacy is larger than the additive effect, it is referred to as *potentiation*.

Studies examining the interaction between the two neurotransmitters have used models of global depletion or blockade of the neurotransmitters (Dringenberg & Zalan, 1999; Lehmann et al., 2000; Vanderwolf, 1987), which means that neuroanatomical specificity was neglected. It remains unclear whether the interaction between the two

systems is effective in specific structures such as the neocortex, for example. Furthermore, the focus of these studies has been to understand the interaction with respect to cognitive behaviors, and it is not clear whether the interaction is instrumental for the production of other behaviors such as skilled movement.

The present study examined whether the interaction between cholinergic and serotonergic systems is central to the production of skilled movements controlled by the motor cortex. Nucleus basalis lesions (quisqualic acid) and medial forebrain bundle lesions (5,7-dihydroxytryptamine) were produced unilaterally and in the same hemispheres of rats. The animals were allowed two weeks to recover and were then given a series of sensorimotor tests, all of which have been demonstrated to be sensitive to sensorimotor lesions. The assessment included tests of forelimb support (cylinder test), limb placing while traversing a horizontal ladder with variably spaced rungs, sensory responsiveness to sensory contact (adhesive dot removal test), forelimb inhibition during swimming, and limb use in reaching for food. The performance of the rats was videotaped and the tapes were scored in order to assess the quantitative and qualitative performance of the animals. In addition to being compared to a control group, performance related to the limbs ipsilateral to the lesion was compared to performance of the limbs contralateral to the lesion with the expectation that the contralateral limbs should be more affected as typically occurs following frank cortical injury.

METHODS

The present experiment was conducted using two different lesion methods. (1) Two-stage lesion: subjects received either a unilateral nucleus basalis lesion (quisqualic acid, 0.5 μg/μl) or a unilateral medial forebrain bundle lesion (5,7-dihydroxytryptamine, 5 μg/4 μl). After eight weeks, they received an additional unilateral lesion to produce a combined nucleus basalis and medial forebrain bundle lesion in the same hemisphere. (2) One-stage lesion: a separate group of rats received both nucleus basalis and medial forebrain bundle lesions in one operation. The two experiments were conducted in separate buildings at the University of Lethbridge, and therefore, the results from the two control groups were not pooled for consistency.

Subjects

Subjects were 33 Long-Evans hooded female-adult rats, 120 days old and weighing 250-300g raised in the University of Lethbridge Vivarium. In the first experiment, the animals were assigned to two different groups, control (n=7), two-stage lesion (n=9). The animals were housed in groups of three or four individuals in hanging wire mesh cages. In the second experiment the animals were assigned to one of two groups, control (n=10), one-stage lesion (n=7). The animals were housed in groups of 2 or 3 in clear Plexiglass transport tubs. Both colony rooms were maintained on a 12/12h light/dark cycle (08:00-20:00 h).

Feeding

For the experiments, rats were food deprived but with *ad lib* water access. One week prior to surgery, rats were food deprived to 85% of their original body weight by providing 15g of solid chow per rat per day to maintain body weight.

Surgery

One-stage lesion: Each animal received an injection of desipramine HCl (25 mg/kg i.p.) (Sigma-Aldrich, St. Loius, MO), a norepinephrine reuptake inhibitor 30 min prior to neurotoxin infusions. Animals then received an injection of atropine nitrate (0.1 mg/kg i.p.) (Sigma-Aldrich, St. Loius, MO) to facilitate respiration throughout surgery. Under sodium pentobarbital anaesthesia (0.5 ml/kg, i.p.) (Sigma-Aldrich, St. Loius, MO), each rat received stereotaxic infusions of 0.12 M Quisqualic Acid (Sigma-Aldrich, St. Loius, MO) via a 30-gauge cannula connected to a micro drive pump by a polythene tube. Two 0.5 µl infusions were made unilaterally in the nucleus basalis. Each infusion was delivered over three min, and an additional five min allowed for diffusion before the cannula was retracted. Stereotaxic coordinates anterior (A), lateral (L) and ventral (V) for the two infusions were A=0.2 mm, L=3.4 mm, V=7.0 mm (below dura) and; A=1.0 mm, L= 2.6 mm, V=7.3 mm (below dura), with the incisor bar set 5.0 mm above the interaural line (see Fig. 2.1). The skull was then flattened, and each rat received stereotaxic infusions of 5,7-dihydroxytrptamine (5 µg/4 µl, Sigma-Aldrich, St. Loius, MO). Two 2.0 µl infusions were made unilaterally in the medial forebrain bundle in the same hemisphere as the nucleus basalis lesion. Each infusion was delivered over five min, and an additional five min allowed for diffusion before the cannula was retracted.

Stereotaxic coordinates anterior (A), lateral (L) and ventral (V) for the two injections were A=-2.0 mm, L=1.5 mm, V=9.5 mm (below skull) and; A=-1.5 mm, L=1.5 mm, V=9.5 mm (below skull). The lesion group was allowed two weeks to recover before behavioral testing.

Two-stage lesion: The nucleus basalis and the medial forebrain bundle lesions were produced in separate surgeries but using the same methods described in the one-stage lesion.

Atropine Sulphate

Three doses of atropine sulphate (Sigma-Aldrich, St. Loius, MO): 5,10, and 25 mg/kg were prepared in 0.9% sterile saline solution. A single dose was administered (i.p.) to both control and lesion groups 20-30 min. prior to testing on the single pellet reaching task, each dose was only used once. The drug doses were administered starting with the lowest dose to minimize tolerance effects. After each rat was injected with the drug, it was returned to its home cage until it was due for testing on the reaching task.

Behavioral Training and Test Analysis

Tray reaching boxes and training: This task was only used in the one-stage lesion experiment without any training prior to surgery. The training phase involved placing each animal individually in a reaching box 26 cm high, 28 cm deep, and 19 cm wide, for 30-40 min each day for 14 days (Whishaw, O'Connor, & Dunnett, 1986). The front of the boxes were constructed of 2mm bars separated from each other by a 9 mm gap. Clear Plexiglass tops allowed access to the inside of the box. A 4 cm wide and 0.5

cm deep tray was mounted in front of the bars. The tray contained food fragments weighing approximately 30 mg each. Animals had to reach between the bars, grasp the food and retract it where they were able to freely eat. The animals were free to use either of their forelimbs. Following this training period, animals were tested for 5 min on three consecutive days. Each time the rat places its forelimb towards the tray, a "reach" was scored. If the animal was successful in obtaining food, a "hit" was scored. The percentage of reaches and hits was calculated and regarded as an indication of reaching accuracy. Following this testing phase, the use of the preferred paw for reaching was restricted by wrapping a bracelet made of Elastoplast fabric adhesive tape (Smith & Nephew Inc., Lachine, Quebec) as previously described (Whishaw et al., 1986). The bracelet prevented the animal from inserting its paw through the gap to reach the food, thus forcing it to reach with its other paw. The bracelets did not impede the subject's movement and could be easily slipped off by the experimenter without damaging the animal's forelimb or ripping hair off. Once habituated to the bracelet, the animals ignored it and did not attempt to remove it, the rats learned to use the other limb and could do so even without the bracelet.

Single pellet reaching boxes and training: All animals were pre-trained to reach through a slot for single pieces of food for two weeks prior to surgery or drug administration (Whishaw, 2000). Reaching boxes were made of clear Plexiglas. Each box was 45x14x35 cm high. In the center of each front wall was a 1 cm-wide slit which extended from 2 cm above the floor to a height of 15 cm. On the outside of the wall, in front of the slit, mounted 3 cm above the floor, was a 2 cm-wide shelf. Two indentations

on the floor of the shelf were located 2 cm from the inside of the wall and were centered on the edges of the slit were rats could reach. Food pellets (45 mg Rodent Chow food pellets, Bioserve Inc.) were placed in the indentation contralateral to the limb with which the rat reached (Whishaw & Pellis, 1990). Following each reach, a short pause preceded the presentation of the next pellet and an additional pellet could be dropped in the back of the box. This encouraged animals to return to the back of the box after each reach and so forced them to reposition themselves and prepare for the next reach. The animals were trained for ten minutes each day for the first week and were presented with 20 pellets each day for the second week. Reaching performance was assessed on two measures: "reaching success" = number of pellets retrieved and "reaches/pellet retrieved" = number of reaching attempts/successful retrieval. After the recovery period following surgery, the animals were tested every day for two weeks. They were presented with 20 pellets in each testing session.

For a qualitative analysis of reaching, a reach was subdivided into ten components (Whishaw, Pellis, Gorny, Kolb, & Tetzlaff, 1993). (1) Limb lift: the limb is lifted from the floor with the upper arm and the digits adducted to the midline of the body. (2) Digits close: as the limb is lifted, the digits are semiflexed and the paw is supinated so that the palm faces the midline of the body. (3) Aim: using the upper arm, the elbow is adducted so that the forearm is aligned along the midline of the body, with the paw located just under the mouth. This movement involves fixation of the distal portion of the limb, so that digits remain aligned with the midline of the body. This is likely produced by a movement around the elbow that reverses the direction of movement of the paw to compensate for the adduction of the elbow. (4) Advance: the head is lifted and the limb

is advanced directly forward above and beyond the food pellet. (5) Digits open: as the limb is advanced the digits are extended and opened. (6) Pronate: using a movement of the upper arm, the elbow is abducted, pronating the paw over the food. Full pronation of the paw onto the food is aided by a movement of the paw around the wrist. (7) Grasp: as the pads of the palm or the digits touch the food, the food is grasped by closure of the digits. This can occur as an independent movement or the grasp can occur as the paw is withdrawn. (8) Supination I: as the limb is withdrawn, the paw is dorsiflexed and is supinated 90^0 by a movement around the wrist and by adduction of the elbow. These movements can occur as soon as the food is grasped or can occur as the limb is withdrawn. (9) Supination II: as the rat sits back with food held in the paw, the paw is further supinated by 90^0 and ventroflexted to present the food to the mouth. (10) Release: the digits are opened and the food transferred to the mouth.

Five successful reaches were analyzed frame-by-frame on the video tapes. Each movement was rated on a three-point scale. If the movement appeared normal, it was given a score of "0", if it appeared slightly abnormal but recognizable it was given a score of "1", and a score of "2" was assigned if the movement was absent or completely unrecognizable.

Rung Walking: The runway consisted of a straight section 1m in length with walls 19 cm high and a square goal box at one end in which food was located (Metz & Whishaw, 2002). The width of the alley was adjusted to the size of the animal allowing 1 cm on either side of the animal to prevent it from turning around. The floor of the runway was made of a readily changeable arrangement of horizontal steel rods 3 mm in

diameter. An irregular but unchanged rung pattern was maintained throughout all trials, gap sizes varied from 1 cm to 5 cm. A high-8mm camera was positioned at a slight ventral angle, so that the positions of all four limbs can be filmed simultaneously from a ventral view.

The novel foot-fault scoring system (Metz & Whishaw, 2002) was modified and used to assess forelimb and hindlimb qualitative placement. Each step was rated on a five-point scale: if the foot placement appeared normal where the midportion of the palm was placed on the rung, it was given a score of "0"; if placement on the rung was done using the wrist or digits of the forelimb or the heel or toes of the hindlimb, it was given a score of "1"; if a limb was placed on a rung and slipped off during weight shifting without disturbing balance, it was given a score of "2"; if a limb was placed on a rung and slipped off during weight shifting causing a fall, it was given a score of "3"; and if a limb missed the targeted rung completely and fell through the gap compromising body posture and balance, it was given a score of "4". Animals received three trials during each testing day. The asymmetry score, which is a ratio of foot faults committed by both sides of the body, was calculated for each group; this is: contralateral limb faults/ipsilateral limb faults.

Cylinder test: Forelimb use for weight support during explorative activity was examined by placing rats in a transparent cylinder 20 cm in diameter and 30 cm high for four minutes (Schallert, Kozlowski, Humm, & Cocke, 1997). A mirror was placed underneath the cylinder at an angle to allow the experimenter to videotape the animal's activity from a ventral view. The cylindrical shape encouraged vertical exploration of the

walls with the forelimbs. The cylinder was high enough so that animals could not reach the top and was wide enough to allow 2 cm between either end of the animal and the walls. Forelimb use was measured during vertical exploration following rearing. Independent use of each forelimb during wall contact was scored during weight shift initiation or to regain center of gravity while moving laterally in a vertical posture. The asymmetry score of forelimb use in wall exploration was calculated for each group, this is: contralateral forelimb wall contact/ipsilateral forelimb wall contact.

Adhesive Dot Removal: Procedures for this task have been described previously by Schallert et al. (1982). Animals were removed from their home cages and their forelimbs were washed with 50% ethanol solution, then wiped with cotton gauze and allowed to dry. Two parallel creases were formed in adhesive paper stimuli (113 mm^2, manufactured by Avery International) to facilitate wrapping them around the forelimb. The stimuli were attached to the distal-radial aspect of both forelimbs. Immediately after, the experimenter firmly touched both forelimbs simultaneously and placed the animal in a clear Plexiglas tub (45x26x20 cm) without bedding for ease of recording. A stainless steel lid was used to cover the tub and contain the rat. The fine forelimb hair was not pulled out in the process, however, the stimuli were sticky enough that they rarely fell off when the animal moved around, groomed or shook its forelimb. Trials in which either stimulus fell off spontaneously were disregarded and repeated. The order of stimulus attachment to the contralateral and ipsilateral forelimbs was counterbalanced for all animals. Subjects contacted and attempted to remove the adhesive paper. The order and latency of removal was recorded for each forelimb for four trials. Each trial was ended

after both labels were removed or after three minutes. The asymmetry score of latency of dot removal was calculated for each group contralateral forelimb latency/ipsilateral forelimb latency.

Swimming Test: Video recordings were made in a large rectangular aquarium (120x43x50 cm) as described by Whishaw, Nonneman, and Kolb (Whishaw, Nonneman, & Kolb, 1981). Water was high enough to prevent animals from touching the bottom of the aquarium but at the same time low enough to prevent them from escaping to the edge of the pool, temperature was maintained at 21^0C. At one end of the pool was an escape wire mesh platform onto which the animals could climb. The platform was visible to the animals at all times. During the training phase, animals were released close to the platform, after they learned to swim and climb onto the platform, they were released at progressively longer distances until they swam directly from the opposite end of the tank. Initially, most animals used all four limbs to stroke, rapidly changed direction, and sometimes swam aimlessly. Once animals learned to swim directly to the platform and were more familiar with the task, they held their forelimbs immobile under their chins and only used their hindlimbs to propel through the water. Each animal performed four trials during which they had to swim directly to the platform. Animals were dried and returned to their home cages after completing four trials. Disruption to the normal swim pattern was quantified by counting the number of strokes by each forelimb. The asymmetry score of forelimb inhibition was calculated for each group, this is: contralateral forelimb strokes/ipsilateral forelimb wall strokes.

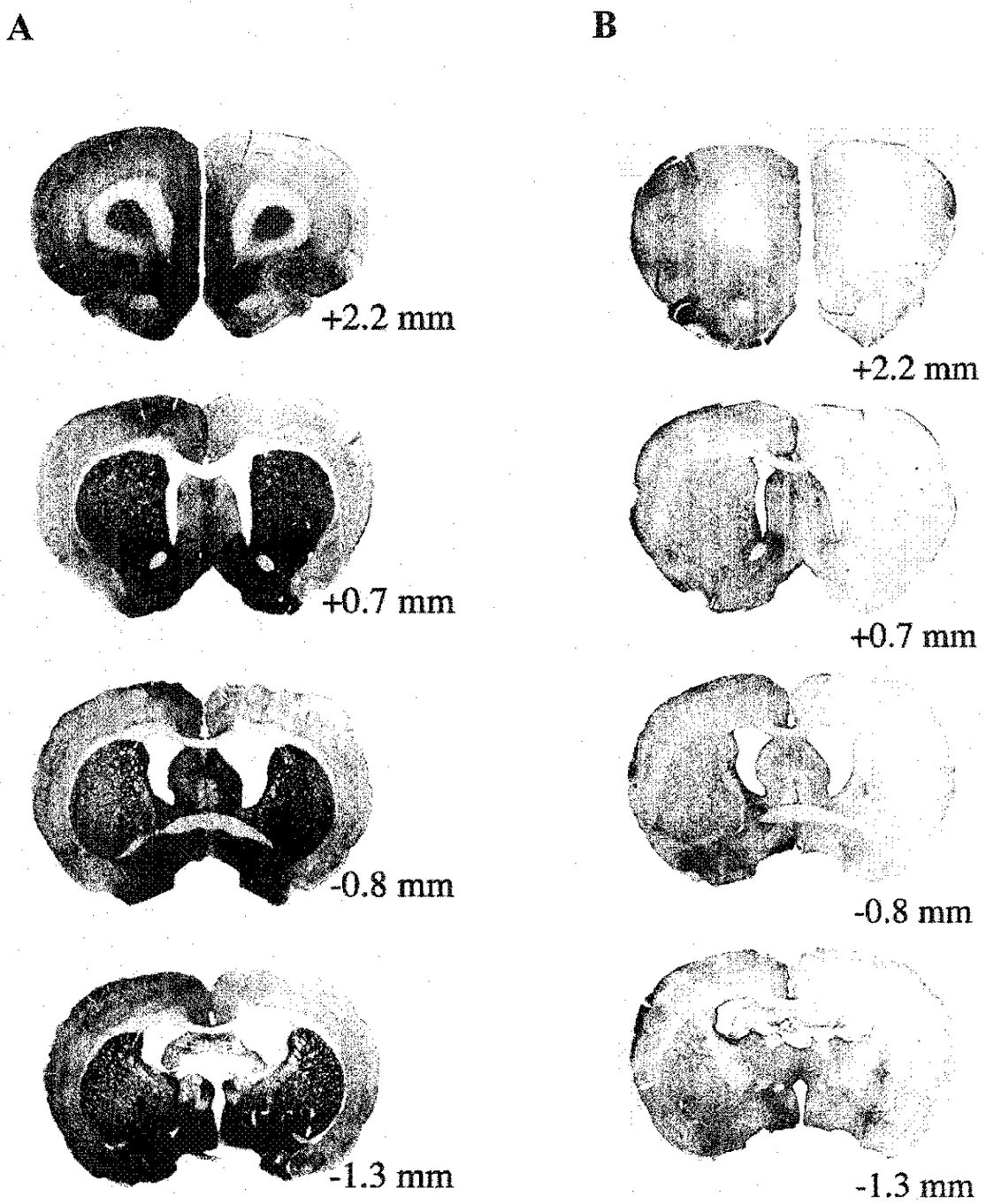
All subjects were tested on the rung walking, cylinder, swimming, and adhesive dot removal tests once a week for four weeks after surgery.

Histological procedures

After six weeks of behavioral testing, all animals were sacrificed using a lethal dose of sodium pentobarbital. They were intracardially perfused, first with saline in 0.1 M PBS followed by 4% paraformaldehyde in 0.1 M PBS. The brains were removed, post-fixed and cryoprotected in a solution of 30% sucrose in 4% paraformaldehyde solution for three days in 4°C. All brains were then sectioned into 40 μm using a cryostat (2800 Frigocut, Reichert-Jung). Sections from the nucleus basalis lesion and combined lesion groups were mounted onto glass slides and stained for acetylcholinesterase using a procedure modified from Karnovsky and Roots (1964) (Fig. 4.1A) . Sections from the medial forebrain bundle lesion and combined lesion groups were stored in 0.1 M PBS solution. The following day, free-floating sections were incubated for 15 min in a quench solution, 20 ml of 3% H_20_2 in 180 ml 0.1 M PBS, to reduce background staining. The sections were then washed three times with 0.1 M PBS. The tissue was then incubated in a primary antibody solution, 15 ml 0.1 M PBS, 3 drops goat serum, 100 μl of 3% Triton-X, 20 μl of serotonin antibody (donated by Dr. Richard Dyck); bovine serum albumin 150mg was added to reduce background staining. Six sections per centrifuge tube were rotated at 40 rpm while refrigerated at 4°C for 20 hrs. All sections were washed three times with 0.1 M PBS and incubated in a secondary antibody solution, 10 ml 0.1 M PBS, 3 drops goat serum and 1 drop anti-rabbit IgG (Vector Laboratories, Burlingame, CA). The sections were rotated at 40 rpm at 4°C for 1 hr. The tissue was washed three times

with 0.1 M PBS and then incubated in an AB complex solution (Vector Laboratories, Burlingame, CA), 5 ml 0.1 M PBS, 2 drops solution A, 2 drops solution B and centrifuged at 40 rpm at 4°C for 30 min. The tissue was washed three times with 0.1 M PBS and then dipped into a solution containing: 5 ml distilled H_2O, 2 drops 7.5 M PBS, 4 drops D amino benzodine (DAB), 2 drops H_2O_2, and 2 drops Ni^{2+} (Vector Laboratories, Burlingame, CA). All sections were finally rinsed three times with 0.1 M PBS, mounted onto slides, and had cover slips placed on top (Fig. 4.1B).

Figure 4.1: Representative photomicrographs of coronal sections of the motor cortex stained for: (A) acetylcholinesterase; and (B) immunohistochemical staining for serotonin. The left hemisphere is contralateral to the lesion and appears darker than the ipsilateral hemisphere indicating the presence of more acetylcholinesterase and serotonin.


A
B
+2.2 mm
+0.7 mm
-0.8 mm
-1.3 mm
+2.2 mm
+0.7 mm
-0.8 mm
-1.3 mm

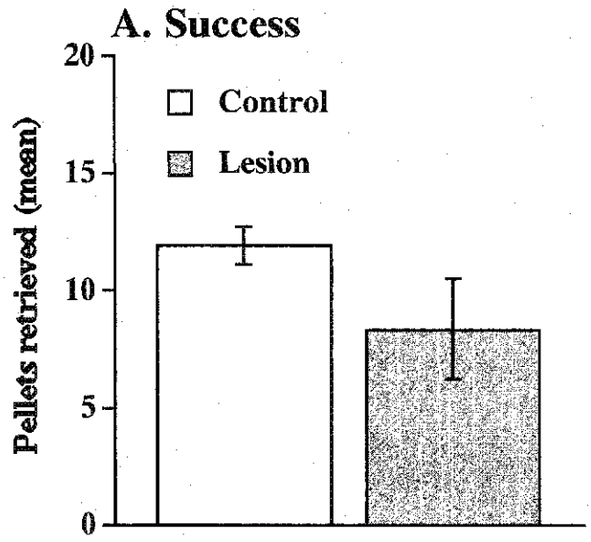
RESULTS

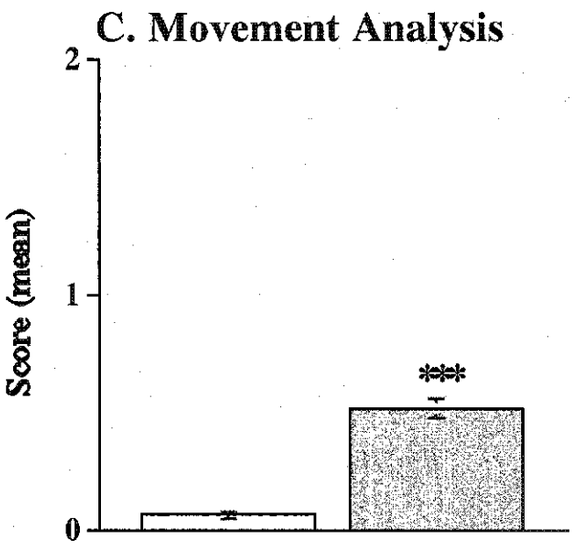
Behavioral Observations (two-stage lesion)

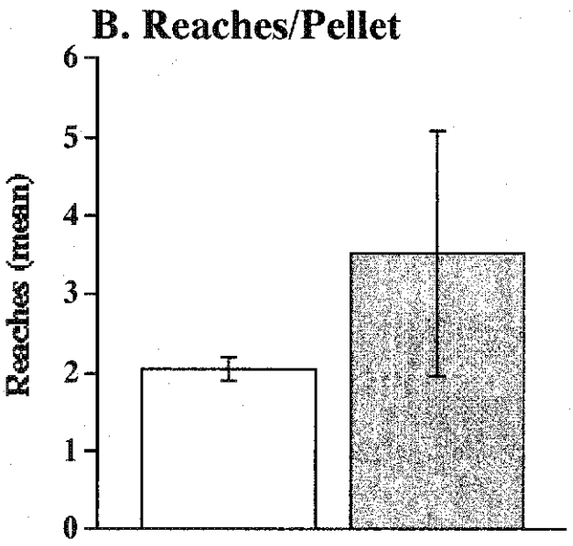
Single pellet reaching: The ability of control and two-stage lesion groups to use their forelimbs was assessed in a skilled reaching task in which animals reached through a narrow slot onto an elevated shelf to retrieve a food reward. Both the two-stage lesion and the control groups continued to use their contralateral forelimb for reaching as they did before surgery. A mean success score was calculated for all animals and compared across all groups. A simple ANOVA revealed no significant main effect of treatment on success score ($F(1,14) = 1.755$; $p=0.2065$) (Fig. 4.2A). A mean reaches/pellet retrieved rate was calculated for all animals as well and compared across all groups. A simple ANOVA revealed no significant main effect of treatment on the number of reaches/pellet ($F(1,14) = 0.042$; $p=0.9395$) (Fig. 4.2B). The two-stage lesion did not quantitatively affect skilled reaching.

To assess the extent to which reaching elements were changed in the two-stage lesion group relative to the control group, the ten-element reaching data were subjected to a repeated measures ANOVA. The analysis revealed a significant main effect of treatment on movement component score ($F(1,14)= 158.709$; $p=0.0001$). The lesion group had a significantly higher score, meaning more movement abnormalities as compared to the control group (Fig. 4.2C). An interaction of movement x treatment showed that the high impairment score for the two-stage lesion group may be attributed to deficits in some movement components but not others ($F(1,9) = 32.298$; $p=0.0001$). A follow up LSD post hoc ($p<0.05$) analysis showed that the lesion group was impaired on

Figure 4.2: Single pellet reaching scores (mean and standard error) in control and two-stage lesion groups. (A) success, number of pellets retrieved out of 20; (B) reaches/pellet, number of reaches performed for each successfully retrieved pellet; (C) the qualitative movement error scores of five representative reaches in control and lesioned animals, ***p=0.001.


A. Success
Control
Lesion
Pellets retrieved (mean)
20
15
10
5
0


C. Movement Analysis
Score (mean)
2
1
0
***


B. Reaches/Pellet
Reaches (mean)
6
5
4
3
2
1
0

the advance, pronation, supination I, and supination II components of the reaching movement (Fig. 4.3). This showed qualitatively that the lesion affected skilled reaching.

Rung Walking: The ability of control and two-stage lesion groups to cross a horizontal ladder with randomly spaced bars was assessed by counting the number of foot faults. Animals from both groups traversed the length of the ladder on each trial. The scores from the previously described five error categories were summed for each group, and a total number of foot faults (contralateral+ipsilateral) including forelimbs and hindlimbs was calculated for each group. A simple ANOVA found a significant main effect of treatment on total foot faults ($F(1,14) = 7.953$; $p = 0.0136$). The lesion group committed more overall foot faults than the control group. The ratio of foot faults committed by both forelimbs and hindlimbs was calculated for the contralateral and ipsilateral sides of the body. A simple ANOVA found a significant effect of treatment on foot fault asymmetry ($F(1,14) = 5.275$; $p=0.0376$). The lesion group committed more foot faults with their contralateral side of the body while the control group did not show asymmetry (Fig. 4.4A).

Cylinder Test: The ability of control and two-stage lesion groups to use their forelimbs during spontaneous vertical exploration was assessed by comparing the number of wall contacts in a cylinder using the ipsilateral and the contralateral forelimbs. Animals from both groups actively explored the cylinder and when they reared touched

Figure 4.3: Reaching movement components (mean and standard error) on the 10 movement components of reaching for control and two-stage lesion groups. Each of the movement component was rated on a 3-point scale, with 0=normal and 2=absent.

*$p<0.05$

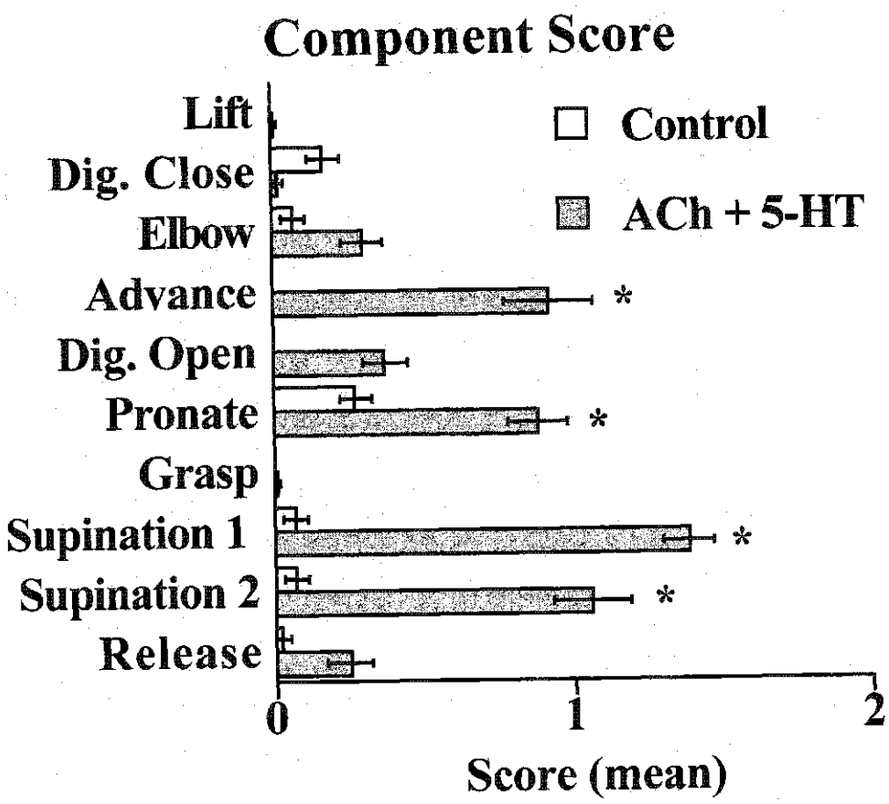
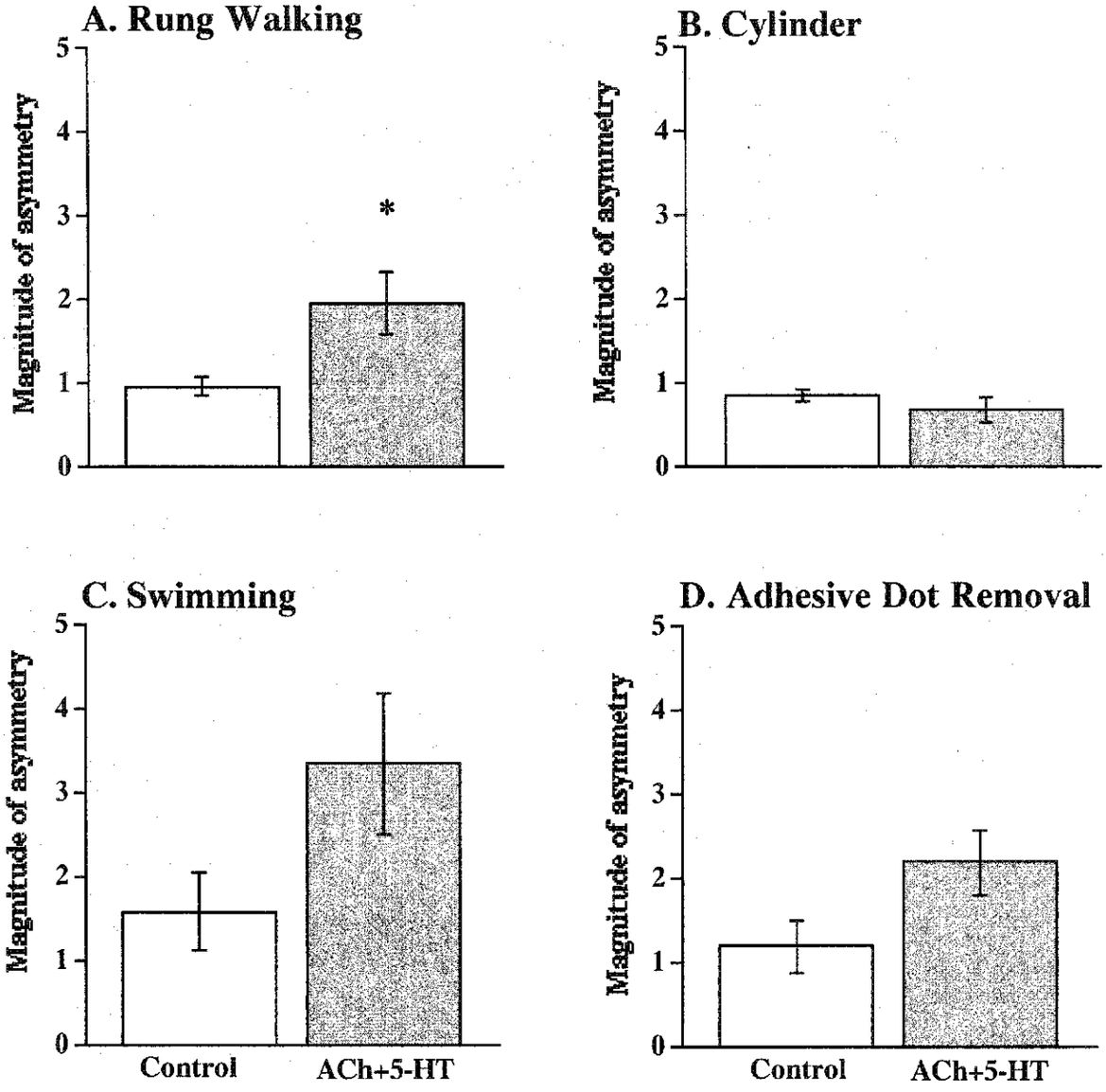

Component Score
Lift
Dig. Close
Elbow
Advance
Dig. Open
Pronate
Grasp
Supination 1
Supination 2
Release
Control
ACh + 5-HT
*
*
*
*
0
1
2
Score (mean)

Figure 4.4: Asymmetry score (mean and standard error) for control and two-stage lesion groups of contralateral/ipsilateral limbs on (A) rung walking, (B) cylinder test, (C) adhesive dot removal, (D) swimming. *$p<0.05$


A. Rung Walking
B. Cylinder
C. Swimming
D. Adhesive Dot Removal
Magnitude of asymmetry
0
1
2
3
4
5
*
Control
ACh+5-HT

and supported their body against the walls with their forelimbs. The total number of wall contacts (contralateral+ipsilateral) was calculated for each group and a simple ANOVA showed no significant effect of treatment ($F(1,14) = 4.037$; $p = 0.0642$). The ratio of contralateral forelimb use to ipsilateral forelimb use was calculated for both groups and a simple ANOVA revealed no significant main effect of treatment in forelimb use asymmetry ($F(1,14) = 0.834$; $p = 0.3765$) (Fig. 4.4B).

Swimming Task: The ability of control and two-stage lesion groups to inhibit their forelimbs was assessed by counting the number of forelimb strokes in a straight swim to a visible platform. On testing days all animals swam directly to the platform and successfully climbed onto it. Animals from both groups showed no signs of swimming impairment by holding their forelimbs still under their chins and only using their hindlimbs to propel through the water. A simple ANOVA revealed no significant main effect of treatment on the overall number of strokes ($F(1,14) = 1.814$; $p = 0.9712$). A simple ANOVA revealed no significant main effect of treatment on asymmetry of forelimb inhibition ($F(1,14) = 2.394$; $p = 0.1458$) (Fig. 4.4C).

Adhesive dot removal: The ability of control and two-stage lesion groups to attend to sensory stimuli was assessed by comparing the latency to remove adhesive paper from their forelimbs. All animals successfully removed the adhesive dots from both paws within the three-minute time limit. Animals often removed the stimulus from their contralateral forelimbs then proceeded to remove the stimulus from their ipsilateral forelimbs. A simple ANOVA revealed a significant main effect of treatment on the

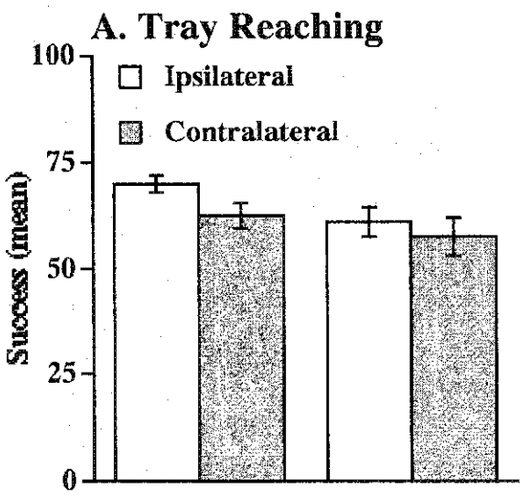
overall latency (contralateral+ipsilateral) to remove both stimuli ($F(1,14) = 55.654$; $p = 0.0001$). The lesion group took more time than the control group to remove both stimuli. A simple ANOVA revealed no significant main effect of treatment in asymmetry of dot removal latency, however ($F(1,14) = 3.757$; $p = 0.0730$) (Fig. 4.4D).

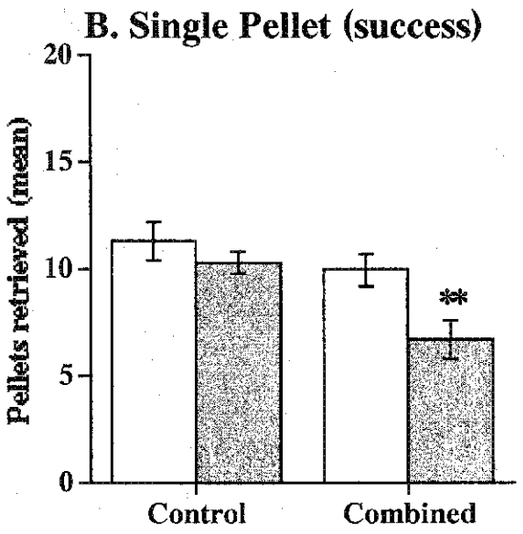
Behavioral observations (one-stage lesion)

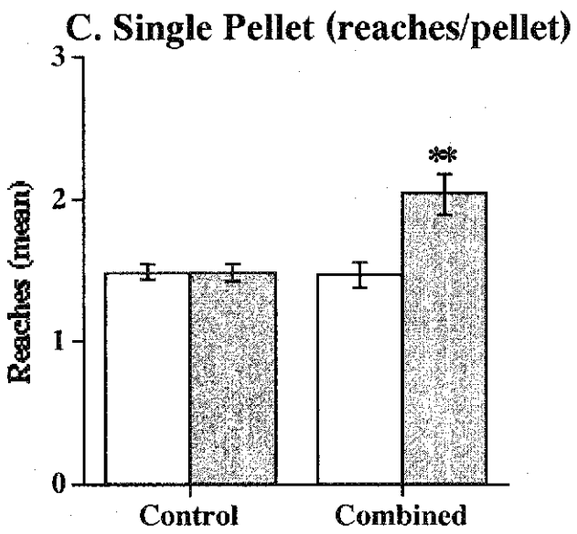
Tray reaching: The ability of control and one-stage lesion groups to use their forelimbs was assessed in a skilled reaching task in which subjects reached between adjacent bars to retrieve food pellets located in a tray. The animals were free to choose either forelimb for reaching for the first two weeks. The lesion group reached with their ipsilateral forelimbs. The control group selected one forelimb for reaching as well, which will be referred to as the contralateral forelimb. Performance was assessed after two weeks of training. Elastoplast bracelets were then used to force both lesion and control groups to use their contralateral and ipsilateral forelimbs, respectively. A repeated measures ANOVA revealed no significant effect of treatment on the overall hit score ($F(1,15) = 2.207$; $p = 0.1581$). In addition, there was no significant difference between forelimbs used on success rate ($F(1,1) = 2.247$; $p = 0.1546$), or a treatment x paw interaction ($F(1,1) = 0.324$; $p = 0.5775$). Both lesion and control groups performed equally as well with both their contralateral and ipsilateral forelimbs (Fig. 4.5A).

Single pellet reaching: Subjects were free to reach with either forelimb. Both the control and lesion groups reached with their ipsilateral forelimbs. A mean success score was calculated for all animals and compared across all groups. A simple ANOVA

Figure 4.5: Skilled reaching scores (mean and standard error) in control and one-stage lesion groups in (A) tray reaching, hit percentage; (B) single pellet, success, number of pellets retrieved out of 20; (C) single pellet, reaches/pellet, number of reaches performed for each successfully retrieved pellet, $^{**}p<0.01$


A. Tray Reaching
Ipsilateral
Contralateral
Success (mean)
100
75
50
25
0


B. Single Pellet (success)
Pellets retrieved (mean)
20
15
10
5
0
**
Control
Combined


C. Single Pellet (reaches/pellet)
Reaches (mean)
3
2
1
0
**
Control
Combined

revealed no significant main effect of treatment on success rate ($F(1,15) = 0.013$; $p=0.9102$). Elastoplast bracelets were then used to force both control and lesion groups to use their contralateral forelimbs. A simple ANOVA revealed a significant main effect of treatment on success score ($F(1,13) = 12.869$; $p=0.0033$). The control group was more successful in retrieving food pellets than the lesion group. One rat from each group failed to reach when the bracelet was on its ipsilateral paw. The asymmetry of success rate was also compared between groups. A simple ANOVA showed that the lesion group had a significantly larger asymmetry than the control group ($F(1,13) = 7.475$; $p=0.0171$) (Fig 4.5B). This means that the lesion group performed significantly better with their ipsilateral paw than their contralateral paw, but such asymmetry was not present in the control group.

A mean reaches/pellet retrieved score was calculated for all animals as well and compared across all groups. A simple ANOVA revealed a significant main effect of treatment on the number of reaches/pellet ($F(1,13) = 16.382$; $p=0.0014$). The lesion group used more reaches/pellet retrieved than the control group. A simple ANOVA revealed an asymmetry in the lesion group but not in the control group in the number of reaches/pellet retrieved ($F(1,13) = 11.346$; $p=0.005$) (Fig. 4.5C). The lesion group used more reaches/pellet retrieved with their contralateral forelimbs than their ipsilateral forelimbs, but the control group did not show such asymmetry.

To assess the extent to which reaching elements were changed in the one-stage lesion group relative to the control group, the ten-element reaching data were subjected to a repeated measures ANOVA. The analysis revealed a significant main effect of treatment on movement component score ($F(1,13)= 5.538$; $p=0.0350$). The lesion group

had a significantly higher score, meaning more movement abnormalities as compared to animals in the control group (Fig. 4.6A). An interaction of movement x treatment showed that the high impairment score for the two-stage lesion group may be due to deficits in pronation and supination of the forelimb (Fig. 4.6B).

Single pellet reaching (atropine sulphate): Both control and lesion groups continued to use their contralateral forelimbs for reaching while drugged with atropine sulphate. A repeated measures ANOVA was used to analyze the success score and showed an overall significant effect of treatment ($F(1,13)= 51.272$; $p=0.001$). The control group had an overall higher success score than the control group. The low dose of atropine reduced reaching success in the lesion group but had no effect in the control group. The medium dose abolished reaching in the lesion group but only reduced reaching slightly in the control group. Neither control nor lesion groups reached for pellets when administered the high dose (Fig. 4.7).

Rung Walking: The ability of lesion and control groups to cross a horizontal ladder with randomly spaced bars was assessed by counting the number of foot faults of each limb. Both control and lesion groups committed a number of foot faults of both forelimbs and both hindlimbs. The scores from the previously described five error categories were summed for each group and a total number of foot faults (contralateral+ipsilateral) including forelimbs and hindlimbs was calculated for each group. A simple ANOVA found a significant main effect of treatment on overall foot faults ($F(1,17) = 27.213$; $p= 0.001$). The lesion group committed more overall foot faults than the control group. The ratio of foot faults committed by both forelimbs and

Figure 4.6: Single pellet reaching scores (mean and standard error) in control and one-stage lesion groups. (A) The qualitative movement error scores of five representative reaches in control and lesioned animals, (B) reaching movement components on the 10 movement components of reaching. Each of the movement components was rated on a 3-point scale, with 0=normal and 2=absent. *$p<0.05$

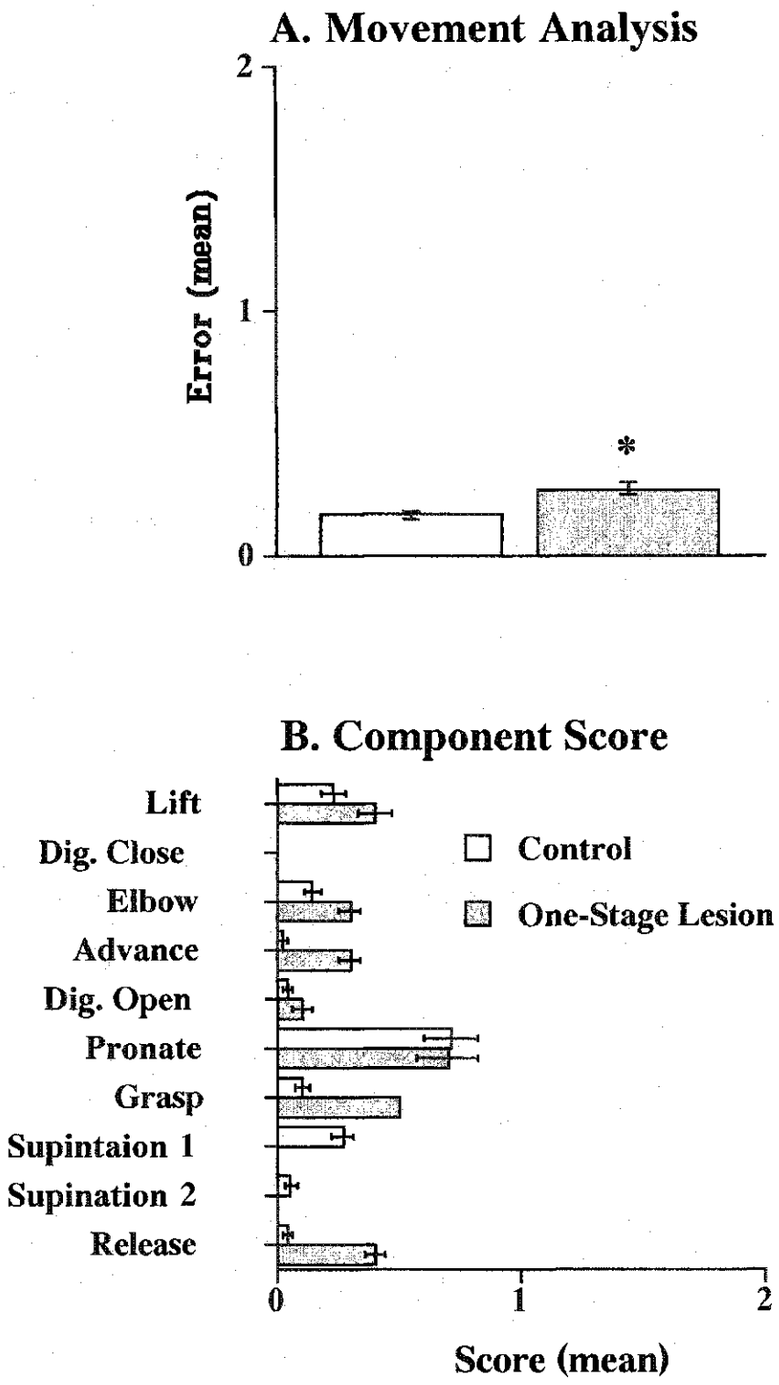
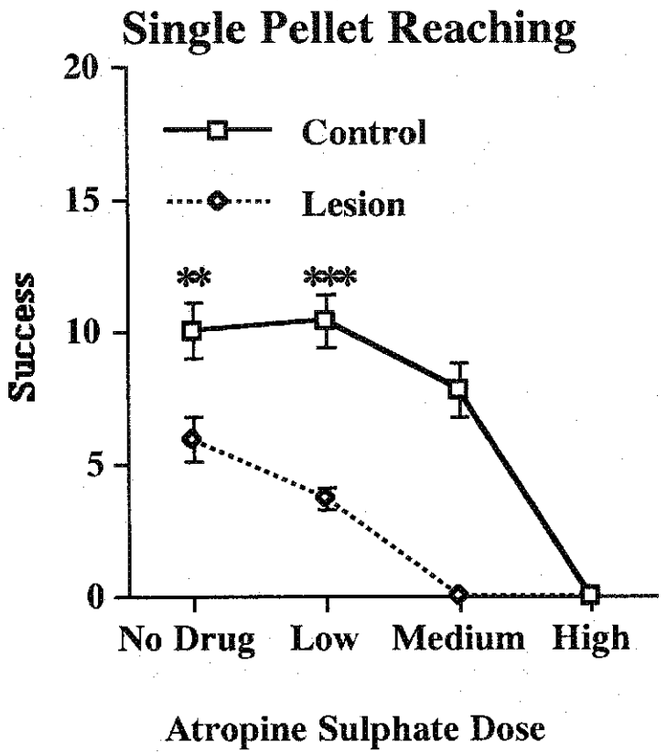

A. Movement Analysis
Error (mean)
2
1
0
*
B. Component Score
Lift
Dig. Close
Elbow
Advance
Dig. Open
Pronate
Grasp
Supintaion 1
Supination 2
Release
Control
One-Stage Lesion
0
1
2
Score (mean)

Figure 4.7: Dose response curve of atropine sulphate on single pellet reaching success scores (mean + standard error) in both control and one-stage lesion groups.


Single Pellet Reaching
20
15
10
5
0
Success
Control
Lesion
**
***
No Drug
Low
Medium
High
Atropine Sulphate Dose

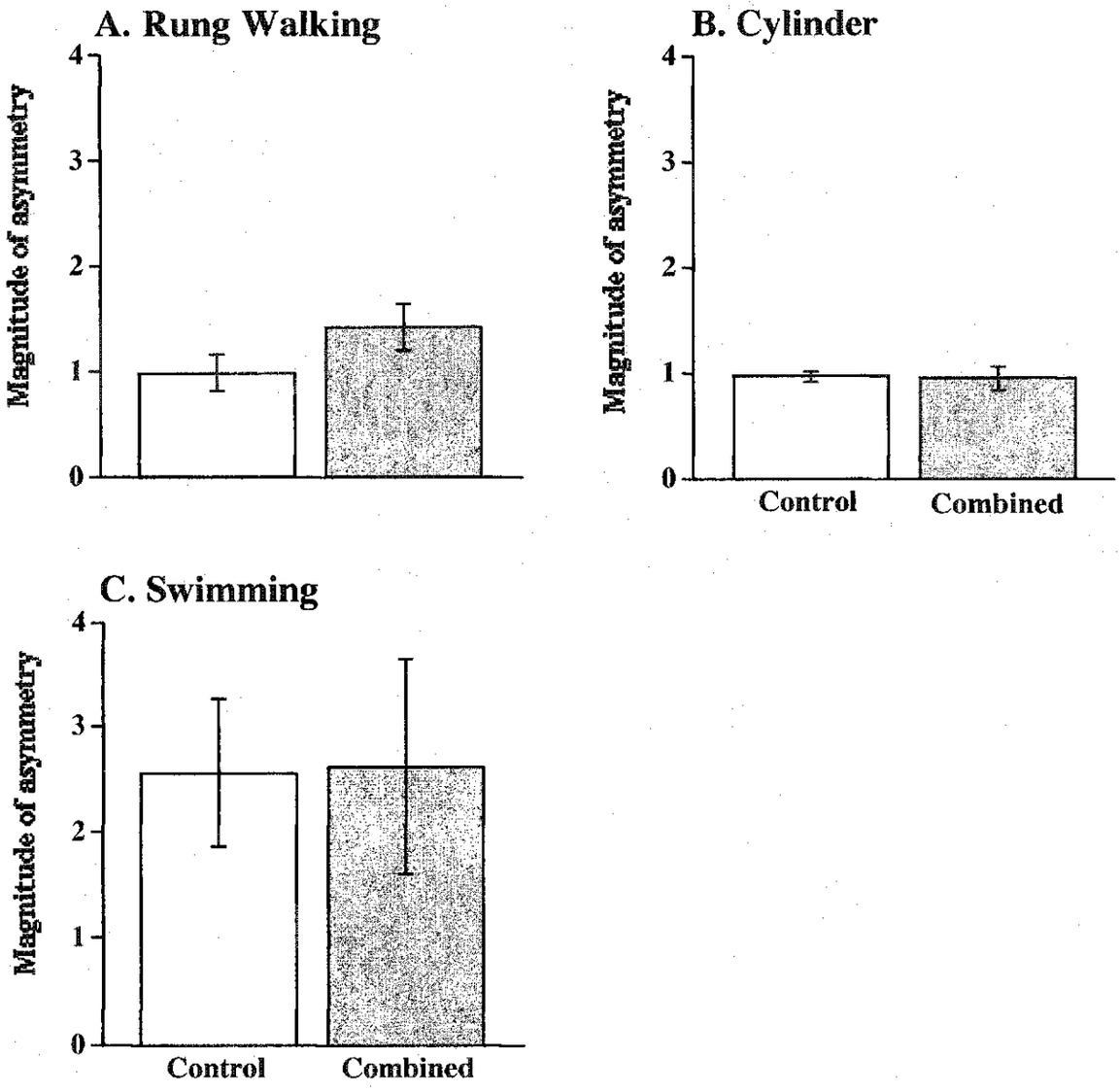
hindlimbs was calculated for the contralateral and ipsilateral sides of the body. A simple ANOVA found no significant main effect of treatment in foot fault asymmetry ($F(1,17) = 2.617$; $p=0.1241$) (Fig. 4.8A).

Cylinder Test: The ability of lesion and control groups to use their forelimbs during spontaneous vertical exploration was assessed by comparing the number of wall contacts in a cylinder using both the ipsilateral and the contralateral forelimbs. Animals from both groups actively explored the cylinder and when they reared touched and supported their body against the walls with their forelimbs. The total number of wall contacts (contralateral+ipsilateral) was calculated for each group. A simple ANOVA showed no significant effect of treatment on overall wall contact frequency ($F(1,17) = 0.106$; $p= 0.7489$). The ratio of contralateral forelimb use to ipsilateral forelimb use was calculated for both groups, and a simple ANOVA revealed no significant main effect of treatment on forelimb use asymmetry ($F(1,17) = 0.038$; $p= 0.8473$) (Fig. 4.8B).

Swimming Task: The ability of control and lesion groups to inhibit their forelimbs was assessed by counting the number of forelimb strokes in a straight swim to a visible platform. On testing days all animals swam directly to the platform and successfully climbed onto it. Animals from both groups rarely stroked and showed no signs of swimming impairment by holding their forelimbs still under their chins and only using the hindlimbs to propel through the water. A simple ANOVA revealed no significant main effect of treatment on the overall number of strokes ($F(1,17) = 2.297$;

p=0.148). A simple ANOVA revealed no significant main effect of treatment on asymmetry of forelimb inhibition (F(1,17) =0.001; p=0.9712) (Fig. 4.8C).

Figure 4.8: Asymmetry score (mean and standard error) for control and one-stage lesion groups of contralateral /ipsilateral limbs on (A) rung walking, (B) cylinder test, (C) swimming.


A. Rung Walking
Magnitude of asymmetry
0
1
2
3
4
B. Cylinder
Magnitude of asymmetry
0
1
2
3
4
Control
Combined
C. Swimming
Magnitude of asymmetry
0
1
2
3
4
Control
Combined

DISCUSSION

The interaction between acetylcholine and serotonin has been reported in numerous studies as central to normal behavior (Lehmann et al., 2000; Nilsson et al., 1988; Riekkinen et al., 1990a; Vanderwolf, 1987). These studies propose that the disruption of either system alone has a mild effect on behavior, but the conjoint depletion or blockade of both neurotransmitters causes severe behavioral disorganization. The results obtained in the present experiment demonstrate that the two-stage lesion caused mild deficits on the rung walking task, and the one-stage lesion produced mild deficits in the single pellet reaching. In general, however, the combined lesion did not abolish the production of movement.

The present results indicating mild impairments following the combined damage to the cholinergic and the serotonergic projections are not consistent with the suggestion of Vanderwolf (1987). He proposes that interrupting the neurotransmission of both systems abolishes the neocortical low voltage fast activation (LVFA) and causes severe behavioral disorganization similar to that seen in decorticated animals (Vanderwolf, Kolb, & Cooley, 1978; Whishaw, Schallert, & Kolb, 1981). There are a number of potential explanations for the differences between the present results and previous work.

(1) The present lesions may not be complete. The nucleus basalis lesion depletes 70-75% of the choline acetyltransferase in the neocortex (Dunnett, Whishaw, Jones, & Bunch, 1987), and the medial forebrain bundle lesion depletes 90% of the neocortical serotonin (Frankfurt, Renner, Azmitia, & Luine, 1985). The remaining acetylcholine and serotonin may have been sufficient to maintain LVFA and sustain normal behavior. (2) The present lesions were produced unilaterally, yet colossal connections from the intact

hemisphere may have compensated for the cholinergic and serotonergic deafferentation. (3) The sensorimotor cortex receives afferents from elsewhere in the cortex. Such connections may have maintained activation of the motor cortex. (4) Thalamic nuclei send projections to the neocortex, which may have maintained activation of the motor cortex as well. (5) The motor cortex may be intrinsically different than the rest of the neocortex and thus dependent on ascending projections other than the cholinergic and serotonergic afferents. (6) The current lesions were restricted to the ascending cholinergic and serotonergic projections. Most studies examining the interaction between the two systems however, produced either depletions or blockade of both systems throughout the brain (Cassel & Jeltsch, 1995; Steckler & Sahgal, 1995).

Despite the improved specificity and sparing of the descending serotonergic projections, the serotonergic projections to the basal forebrain and the hippocampus were not spared. Restricting the lesion to the serotonergic afferents of the neocortex is difficult to achieve due to the dense organization of the various raphe nuclei in the brain stem. Given that the cholinergic depletion was achieved by means of damaging the nucleus basalis, which mainly sends projections to the neocortex, it is possible to assume that the present results are due to the reduced levels of both neurotransmitters in the neocortex and the basal forebrain.

The mild deficits produced by the combined lesion were comparable to ones reported earlier on nucleus basalis damage alone. The present effects of atropine sulphate on skilled reaching further support the hypothesis that the ascending cholinergic projections are involved in the production of movement. Both the low and medium doses of atropine sulphate reduced reaching success in the one stage lesion group but did not

affect performance of the control group. This is indicative that the drug blocked the muscarinic receptors that were already experiencing a reduced neurotransmission of acetylcholine in the lesion group.

Previous work has shown that the depletion or blockade of both systems interrupts learning processes (Cassel & Jeltsch, 1995; Steckler & Sahgal, 1995 for review). The effects of combined damage of both systems on task retention have not been explored. The two experiments in this study were designed to examine the effects of the depletion on learning novel motor tasks and to assess performance and to assess retention of the same tasks following the depletion of both systems. The combined lesion generally did not affect the acquisition of novel tasks or performance of movements learned prior to surgery.

The combined depletion of acetylcholine and serotonin in the neocortex and nucleus basalis only produced mild motor deficits. The results are not consistent with Vanderwolf's (1987) suggestion that the combined depletion of acetylcholine and serotonin produces severe behavioral disorganization. The lesion methods adopted in the present experiment are useful in restricting damage to the ascending cholinergic and serotonergic projections and should be used to further explore the topic.

CHAPTER FIVE

GENERAL DISCUSSION

This thesis examined the hypothesis that the conjoint action of acetylcholine and serotonin that maintains the neocortical electroencephalogram (EEG) is essential for normal behavior. Groups of rats received unilateral cholinergic, serotonergic or combined neurotoxic lesions and were tested on a battery of motor tests sensitive to unilateral damage. Whereas slight impairments were obtained on skilled reaching and rung walking in some test conditions, in general, neither single nor combined lesions disrupted performance.

Vanderwolf (1987) and others (Dringenberg & Zalan, 1999) have demonstrated that combined but not single lesions of cholinergic and serotonergic cortical projections abolish the active low voltage fast activity (LVFA) EEG of the neocortex. Accompanying the loss of LVFA, animals, although still able to walk, are unable to acquire maze tasks or perform simple motor behaviors. In summarizing these findings, Vanderwolf (1987) proposed that the LVFA pattern is essential for intelligent behavior. Thus, Vanderwolf proposes that the loss of appropriate neuronal excitability produced by the cholinergic and the serotonergic projections disables the normal functions of the neocortex.

Since the afferent and efferent projections of the rat brain are organized within hemispheres, it might be expected that unilateral inactivation of the neocortex should disrupt behaviors dependent upon that hemisphere. For example, unilateral decortication disrupts a wide range of behaviors dependent on the cortex (Rose, Whishaw, & van Hof, 1992). In addition focal lesions to the neocortex (Whishaw, Gorny, & Sarna, 1998),

unilateral depletion of dopamine (Miklyaeva, Castaneda, & Whishaw, 1994), or damage to cortical efferents including the pyramidal tract (Whishaw et al., 1998) also disrupts behavior dependent on that cortex. Given that the integrity of the cortical hemisphere is central for the production of normal behavior especially on the contralateral side of the body, it follows that the loss of its LVFA produced by depletions of acetylcholine and serotonin should result in contralateral deficits similar to those produced using bilateral lesions.

In order to evaluate the unilateral contribution of the cholinergic and serotonergic projections, six behavioral tests sensitive to unilateral damage of the neocortex were used. (1) In the single pellet reaching task, the subject reached for single pieces of food rewards located on a shelf (Whishaw & Pellis, 1990). Success rate was recorded during each testing session, that is the number of pellets successfully retrieved and eaten by the subject out of 20 possible pellets. The reaching movement is made up of ten components according to Whishaw and Pellis (1990); a video recording was used for frame-by-frame analysis to detect any postural or forelimb deficits. (2) In the tray reaching task, the rat reaches in between bars for food pellets located in a tray (Whishaw, O'Connor, & Dunnett, 1986). Successful reaches involve the animal retrieving food and transferring to its mouth. (3) In the rung walking task, the animal traversed randomly spaced rungs to reach a goal box (Metz & Whishaw, 2002). Foot faults of the forelimbs and hindlimbs were recorded. (4) In the cylinder test, the rat reared and used its forelimbs to support its weight on the walls (Schallert, Fleming, Leasure, Tillerson, & Bland, 2000). Contact with the walls was recorded for each forelimb. (5) In the swimming task, forelimb inhibition was recorded in a straight swim towards a platform (Whishaw, Nonneman, &

Kolb, 1981). (6) In the somatosensory detection task, small adhesive stimuli were attached to the radial surface of each forelimb simultaneously, and the latencies to remove each stimulus were recorded (Schallert & Whishaw, 1984). All of the tests described involve both learning and performance components.

Cholinergic depletion or blockade

For the experiments, cholinergic depletions were produced by unilaterally infusing quisqualic acid into the nucleus basalis. Based on previous work comparing various neurotoxin lesions of the nucleus basalis, quisqualic acid produces the most selective lesion with the fewest side effects (Dunnett, Whishaw, Jones, & Bunch, 1987). Damage in the present experiment was restricted to the ipsilateral cortical cholinergic pathway as assessed by acetylcholinesterase reactivity in the neocortex. Previous work suggests that such a lesion produces a 70-75% depletion of neocortical choline acetyltransferase (Dunnett et al., 1987). The lesion did not affect performance in the rung walking, cylinder, swimming, adhesive dot removal, or success rate in single pellet reaching. Despite the unimpaired performance on these tests, qualitative deficits in skilled reaching were observed in forelimb advancement and supination of the paw. Similar qualitative impairments are observed after unilateral motor cortex lesions, but the movement impairment is usually accompanied by reduced success (Whishaw, Pellis, Gorny, & Pellis, 1991). Thus, the qualitative deficit without a quantitative deficit suggests that the cortical cholinergic innervation has only a small, although interesting, effect on skilled reaching.

Atropine sulphate was administered to block the central muscarinic receptors, which reduces the neurotransmission of acetylcholine. A dose response curve demonstrated that performance was unaffected by the low dose, the medium dose reduced reaching success slightly and caused mild movement deficits similar to the ones produced by the nucleus basalis lesion, and most subjects appeared drowsy and immobile under the high dose. The mild movement deficits observed in skilled reaching following the nucleus basalis lesion or central muscarinic receptor blockade suggest that acetylcholine does play a role in movement control. This is consistent with evidence showing that the iontophoretic administration of acetylcholine or stimulation of the nucleus basalis enhances the excitability of single-cells in the somatosensory cortex (Metherate, Tremblay, & Dykes, 1988; Tremblay, Warren, & Dykes, 1990). Acetylcholine is also central for the reorganization of cortical maps in the somatosensory and auditory cortex (Kilgard & Merzenich, 1998; Webster, Hanisch, Dykes, & Biesold, 1991). Acetylcholine may be involved in motor cortex function as revealed by qualitative analysis of movement and warrants further investigation.

The present findings indicating little, if any, permanent movement deficits following nucleus basalis lesions are consistent with previous studies. Dunnett et al. (1987) assessed animals with bilateral nucleus basalis lesions produced by various neurotoxins on a battery of sensorimotor tasks. The authors reported initial impairments that disappeared with recovery time. Other studies confirmed that lesions restricted to the nucleus basalis do not affect the organization of behavior, such as learning and memory in maze tasks (Baxter et al., 1996; Baxter & Gallagher, 1996; Berger-Sweeney et al., 1994). Abdulla, Calaminici, Stephenson, and Sinden (1994), on the other hand, described

sensorimotor deficits following unilateral AMPA neurotoxic lesions of the nucleus basalis. Furthermore, Dubois, Mayo, Agid, Le Moal, and Simon (1985) used radiofrequency current and ibotenic acid to bilaterally damage the nucleus basalis and reported profound disturbances in spontaneous behaviors. Both findings of Abdulla et al. (1994) and Dubois et al. (1985) should be interpreted with caution because the lesions produced were not selective. Thus, with the exception of the qualitative impairments observed in reaching, the main result that motor behavior is spared by cholinergic depletion was confirmed.

Serotonergic depletion or blockade

To produce serotonin depletions in the neocortex, the neurotoxin 5,7-dihydroxytryptamine was unilaterally infused into the medial forebrain bundle. Based on Giambalvo and Snodgrass (1978), this method produces chronic damage restricted to the ipsilateral ascending serotonergic projections. The lesion was assessed using immunohistochemical staining for serotonin and revealed decreased levels of serotonergic innervation in the ipsilateral hemisphere several weeks after the surgery. The lesion did not affect performance on any of the behavioral measures.

Methiothepin mesylate was administered to control rats to block the central serotonergic receptors (Jacoby, Shabshelowitz, Fernstrom, & Wurtman, 1975). Performance was unaffected by the drug, except the high dose under which subjects appeared drowsy, immobile and did not attempt to reach. The results suggest that serotonin does play a role in the production of movement but not by inactivating the neocortical serotonin.

The present findings indicating no movement deficits following serotonergic depletion or receptor blockade are consistent with the findings of Dringenberg and Vanderwolf (1995). They administered the serotonin synthesis inhibitor para-chlorophenylalanine (PCPA, 1000 mg/kg, i.p.), which depleted 90% of the serotonin in the rat's brain as detected using biochemical assays, and found no deficits on a battery sensorimotor tests. This lesion does not affect maze learning either (Altman, Ogren, Berman, & Normile, 1989). Beiko, Candusso, and Cain (1997), on the other hand, used the same lesion and demonstrated increased foot faults and slips on the beam walking task. Lehmann et al. (2000) infused 5,7-dihydroxtryptamine into the lateral ventricles of rats, which caused a permanent impairment in beam walking as well. Both studies by Beiko et al. (1997) and Lehmann et al. (2000) attributed the deficits to decreased serotonin in the frontoparietal cortex. This interpretation is questionable, however, because serotonin levels were reduced throughout the brain. Furthermore, the lesion likely damaged the descending serotonergic projections that synchronize pattern generators in the spinal cord (Baumgarten & Grozdanovic, 1995). In addition, the PCPA dose (1000 mg/kg) used by Beiko et al. (1997) has been shown to cause a reduction in locomotor activity (Dringenberg & Vanderwolf, 1995), which may be a factor in the poor performance on the beam walking task. The depletion of serotonin from the brain has been reported to increase (Blokland, Lieben, & Deutz, 2002) and decrease (Stein, Wise, & Belluzzi, 1975) anxiety. This debate is beyond the scope of this thesis but should not be neglected when comparing the present findings to Beiko et al. (1997) and Lehmann et al. (2000). Thus, the main finding was that damage to the ascending serotonergic

neurons alone does not affect skilled movements, and this result is generally consistent with other investigations.

The conjoint cholinergic and serotonergic depletions

Depleting the neocortex of both acetylcholine and serotonin was achieved using two different methods. (1) Two-stage lesion: subjects received either a nucleus basalis lesion or a medial forebrain bundle lesion. After eight weeks, they received the second lesion in the same hemisphere. (2) One-stage lesion: a separate group of rats received both nucleus basalis and medial forebrain bundle lesions in one operation. Acetylcholine and serotonin levels were reduced in the ipsilateral hemispheres of both the one-stage and the two-stage lesion groups as assessed by acetylcholinesterase reactivity and immunohistochemical staining for serotonin.

The lesions in both experiments had no effect on the cylinder, swimming, or adhesive dot removal tasks. The two-stage lesion group exhibited more foot faults in the rung walking task than controls. The impairment was mild, however, when compared to the severe deficits exhibited following unilateral focal motor cortex stroke or unilateral dopamine depletion (Metz & Whishaw, 2002). In addition, this group demonstrated qualitative movement impairments in the single pellet reaching task similar to those observed in the nucleus basalis lesion group but had normal success scores.

The one-stage lesion group performed equally as well as controls in the rung walking task. The one-stage lesion group was tested in both the tray and the single pellet reaching tasks with both ipsilateral and contralateral forelimbs. The control group was equally successful with both ipsilateral and contralateral forelimbs on both the tray and

the single pellet reaching tasks. The lesion group was equally successful with both paws on the tray reaching task but had less success and more movement abnormalities with the contralateral paw on the single pellet reaching task. Despite the deficits revealed, the impairments of the one-stage lesion group were still milder than the ones reported following focal motor cortex stroke (Whishaw, 2000). The results from both experiments indicate mild impairments in the rung walking task and the single pellet reaching task but otherwise intact sensorimotor abilities following the conjoint depletion of neocortical acetylcholine and serotonin.

Atropine sulphate given in a low dose to the one-stage lesion and control groups reduced reaching success in the lesion group but had no effect on the control group. The medium dose produced mild deficits in the control group, but prevented the lesion group from reaching. Both control and lesion groups did not attempt to reach at the high dose.

In general the combined lesion did not abolish skilled movements on any tasks, which confirms the results of Lehmann et al. (2000). They demonstrated that the depletion of acetylcholine using 192 IgG-saporin increased foot faults in the beam walking task but that the deficits were unchanged following intraventricular administration of 5,7-dihydroxytryptamine. The results are not consistent, however, with Beiko et al. (1997). They argue that scopolamine, a cholinergic blocker, potentiates the effects of PCPA treatment causing more foot faults in the beam walking than the administration of either agent alone. Thus, with the exception of mild impairments in the rung walking and single pellet reaching tasks, the results suggest that motor behavior is spared following the conjoint depletion of acetylcholine and serotonin.

Conclusions

The conjoint depletion of acetylcholine and serotonin only caused some mild deficits in single pellet reaching and rung walking but generally behavior was intact, which is not consistent with Vanderwolf's (1987) suggestion for all intelligent behavior to be abolished by combined lesions. There are a number of potential explanations for the different results. First, the present lesions may not have been complete. The nucleus basalis lesion only depletes 70-75% of the acetylcholine in the neocortex (Dunnett et al., 1987), and the medial forebrain bundle lesion depletes 90% of the serotonin in the neocortex (Frankfurt, Renner, Azmitia, & Luine, 1985). The remaining acetylcholine and serotonin in the neocortex may have been sufficient to produce normal behavior. Second, most input to the motor cortex arises from other cortical areas (Kolb & Tees, 1990, chap. 10) allowing for the possibility that the motor cortex may be activated by afferents from elsewhere in the cortex. Third, the use of a unilateral model restricted damage to the ascending ipsilateral projections; colossal connections, however, arise in one hemisphere and terminate in contralateral cortical areas and may have compensated for the dennervation. Fourth, the thalamus sends afferents to all cortical areas, which is generally sensory, topographically ordered and specific to certain cortical areas (Shepherd, 1998, p.466). Cortical activation may have been achieved via the thalamic nuclei afferents. Fifth, Vanderwolf's (1987) suggestion was based on observations in animals drugged with cholinergic and serotonergic blockers, and the results from the present experiments are mainly based on unilateral lesions. Furthermore, Vanderwolf (1987) used high doses of atropine sulphate; however, in the present experiments, high doses of either atropine sulphate or methiothepin mesylate alone were sufficient to

produce an immobile state. It is, therefore, not surprising that subjects appeared impaired on behavioral tests under the combined drug treatment (Dringenberg & Zalan, 1999; Vanderwolf, 1987). This impairment is likely due to the side effects of the drugs and not the cortical inactivation. Sixth, it is possible that the cholinergic and serotonergic projections may not be instrumental in the functioning of the motor cortex. Vanderwolf (1987) generalized his conclusion about the role of acetylcholine and serotonin for the activation of the neocortex, but his hypothesis does not account for potential intrinsic difference in the motor cortex. Other non-specific projections from subcortical structures may be more central to the integrity of the motor cortex. It is well known, for example, that the dopaminergic projections of the substantia nigra are instrumental for intact motor behavior (Ungerstedt, 1968).

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