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The role of frontal cortical-basal ganglia circuits in simple and sequential visuomotor learning

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THE ROLE OF FRONTAL CORTICAL- BASAL GANGLIA CIRCUITS IN SIMPLE AND SEQUENTIAL VISUOMOTOR LEARNING

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DEDICATION

I would like to dedicate this endeavor to my brother, Clifton Bailey, whose creative talents and intellectual curiosity were silenced prematurely. I would also like to thank my children, Matthew, Donald and Shannon, for their love and inspiration. To my soul mate, Donna, this would not have been possible without your unending support. Thank you for encouraging the pursuit of my dreams...for our future.

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TABLE OF CONTENTS

DEDICATION iii

ACKNOWLEDGMENTS iv

LIST OF TABLE vi

LIST OF FIGURES vii

ABSTRACT ix

SECTION PAGE

INTRODUCTION 1

EXPERIMENT 1: FRONTAL CORTEX 23

Methods 23

Results 32

EXPERIMENT 2: BASAL GANGLIA 44

Methods 44

Results 46

Discussion 56

LIST OF REFERENCES 79

APPENDIX A 87

APPENDIX B 97

APPENDIX C 121

LIST OF TABLES

Table 1: Stereotaxic Coordinates of Cortical Infusion Sites 88

Table 2: Summary of Experimental Tasks and Performance Measures 89

Table 3: ANOVA Summary Table Examining Short-term Visuomotor Sequence Learning (Experiment 1) 90

Table 4: Stereotaxic Coordinates of Striatal Infusion Sites 91

Table 5: ANOVA Summary Table Examining Changes in RT Patterns for Random and Repeat Sequence Sessions (Experiment 2) 92

Table 6: ANOVA Summary Table for Long-term Visuomotor Sequence Learning (Experiment 2) 93

Table 7: ANOVA Summary Table Examining Short-term Visuomotor Sequence Learning (Experiment 2) 94

Table 8: ANOVA Summary Table Examining Short-term Visuomotor Sequence Learning for the Initial Repeat Session 95

Table 9: ANOVA Summary Table Comparing Initiation of a Single Motor Response vs. the Initial Response in a 5-Response Sequence 96

LIST OF TABLES

Table 1: Stereotaxic coordinates of cortical infusion sites 88

Table 2: Summary of Experimental Tasks and Performance Measures 89

Table 3: ANOVA Summary Table Examining Short-term Visuomotor Sequence Learning (Experiment 1) 90

Table 4: Stereotaxic coordinates of striatal infusion sites 91

Table 5: ANOVA Summary Table Examining Changes in RT Patterns for Random and Repeat Sequence Sessions (Experiment 2) 92

Table 6: ANOVA Summary Table for Long-term Visuomotor Sequence Learning (Experiment 2) 93

Table 7: ANOVA Summary Table Examining Short-term Visuomotor Sequence Learning (Experiment 2) 94

Table 8: ANOVA Summary Table Examining Short-term Visuomotor Sequence Learning for the Initial Repeat Session 95

Table 9: ANOVA Summary Table Comparing Initiation of a Single Motor Response vs. the Initial Response in a 5-Response Sequence 96

LIST OF FIGURES

Figure 1: Representative lesions of M1, M2, M1M2, and mPF cortex 98

Figure 2: Cortex visuospatial response task (VSRT) percent correct responding across stimulus durations 99

Figure 3: Cortex VSRT choice reaction time (RT) across stimulus durations 99

Figure 4: Cortex VSRT runway RT 100

Figure 5: Cortex short-term simple stimulus response (S-R) learning accuracy improvement 101

Figure 6: Cortex long-term simple S-R RT 102

Figure 7: Cortex serial reaction time (SRT) learning - Comparison of random and repeat session RT completing a five response sequence 103

Figure 8: Cortex SRT learning – Overall RT response patterns for random and repeat sequence sessions..... 104

Figure 9: Cortex short-term SRT learning – within session development of repeat sequence response patterns..... 105

Figure 10: Cortex short-term SRT learning – within session development of random sequence response patterns 106

Figure 11: Cortex visuomotor sequence motor initiation - comparison of single response RT vs. initial response of a five response sequence..... 107

Figure 12: Representative lesions of dorsal caudate putamen (C/Pu), dorsomedial C/Pu, dorsolateral C/Pu and ventral striatum..... 108

Figure 13: Striatal VSRT – response accuracy across stimulus durations..... 109

Figure 14: Striatal VSRT - choice RT across stimulus durations. 110

Figure 15: Striatal VSRT - runway RT..... 111

Figure 16: Striatal short-term simple S-R learning accuracy Improvement 112

Figure 17: Striatal short-term simple S-R learning- effect of improved accuracy on RT performance..... 113

Figure 18: Striatal long-term simple S-R learning 114

Figure 19: Striatal SRT learning - Comparison of random and repeat session RT completing a five response sequence (dorsolateral, ventral and control groups) 115

Figure 20: Striatal SRT learning – Comparison of random and repeat session RT for completing the entire five response sequence (dorsomedial, complete dorsal striatum and control groups) 116

Figure 21: Striatal SRT learning- Overall RT response pattern for random and repeat sequence sessions 117

Figure 22: Striatal short-term SRT learning – within session development of repeat sequence response patterns 118

Figure 23: Striatal short-term SRT learning – within session development of random sequence response patterns 119

Figure 24: Striatal visuomotor sequence initiation - comparison of single response vs. initial response of a five response sequence 120

ABSTRACT

THE ROLE OF FRONTAL CORTICAL-BASAL GANGLIA CIRCUITS IN SIMPLE AND SEQUENTIAL VISUOMOTOR LEARNING.

by

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University of New Hampshire, September, 2004

Imaging, recording and lesioning studies implicate the basal ganglia and anatomically related regions of frontal cortex in visuomotor learning. Two experiments were conducted to elucidate the role of frontal cortex and striatum in visuomotor learning. Several tasks were used to characterize motor function including: a visuomotor reaction time (VSRT) task, measuring response speed and accuracy to luminance cues; simple stimulus-response (S-R) learning, measuring VSRT improvements when cues occurred in consistent locations over several trials; and a serial reaction time (SRT) task measuring motor sequence learning. SRT learning was characterized by incremental changes in reaction time (RT) when trained with the same sequence across daily sessions and by abrupt RT changes when switched to random sequence sessions.

In experiment 1, rats with excitotoxic lesions in primary (M1) or secondary (M2) motor cortex, primary and secondary (M1M2) motor cortices, medial prefrontal cortex (mPF) or sham surgery were tested on these tasks. Cortical lesions slowed RT in the VSRT task but did not impair short- or long-term simple S-R learning. Cortical lesions increased RTs for the initial response of a 5-response sequence in the SRT task that was exacerbated when performing repeated (learned)

sequences. All groups demonstrated visuomotor sequence learning including incremental changes in RTs for later responses in learned sequences that reversed abruptly when switched to random sequences.

Rats in experiment 2 were given lesions in dorsolateral striatum, dorsomedial striatum, complete dorsal striatum, ventral striatum and sham surgery. Rats with ventral striatal lesions were unimpaired on any visuomotor task demonstrating shorter RTs than controls on most measures. Dorsomedial striatal lesions significantly impaired all VSRT performance measures. Striatal lesions had no effect on short or long-term simple S-R learning. Lesions involving dorsomedial striatum disrupted initiation of motor sequences in the SRT task. This impairment was exaggerated when performing well-learned sequences. Striatal lesions did not disrupt the incremental RT changes of later responses in the sequence indicative of motor learning. Results suggest that cortico-striatal circuits are involved in initiating learned motor sequences consistent with a role in motor planning. These circuits do not appear essential for acquisition or execution of learned visuomotor sequences.

INTRODUCTION

The acquisition of knowledge occurs through two distinct processes of learning and memory; declarative and procedural. Declarative learning and memory involves the encoding and conscious recall of facts and explicit events whereas procedural learning and memory is characterized by changes in behavior resulting from direct experience without reliance on conscious recall (Ackermann, Daurn, Schugens, & Grodd, 1996; Graybeil, 1998; Salmon & Butters, 1995). There is convergent evidence from research with clinical populations, healthy human subjects, non-human primates and other species, suggesting that these processes are supported by distinct circuits within the brain.

Research with specific clinical populations has demonstrated preserved learning and memory performance on tasks designed to target declarative processes while these same populations show impaired performance on procedural memory tasks. Alzheimer's and Korsakoff's patients suffer profound amnesia demonstrating severe impairments in tasks requiring explicit recall of previously learned information (e.g. word lists) yet maintain intact performance on the serial reaction time (SRT) task designed to assess implicit learning and memory performance (Nissen & Bullemer, 1987; Nissen, Willingham & Hartman, 1989). Classic SRT learning involves pressing buttons on a keypad that correspond spatially to stimuli presented on a monitor. Subjects are not informed that during some sessions the series of stimuli will occur in a repeating (10-12 element) sequence. Subjects suffering from Parkinson's (PD) or Huntington's (HD)

disease, neurodegenerative disorders characterized by compromised basal ganglia function, display severe performance deficits on the SRT task with relatively preserved function on tasks requiring explicit recall (Dominey & Jeannerod, 1997; Knowlton, 2002).

The declarative learning and memory deficits associated with global amnesia characteristic of Wernike-Korsakoff syndrome and Alzheimer's disease can result from damage to the hippocampal formation, including adjacent cortices and projections from the basal forebrain, the diencephalon including the mammillothalamic tract, mediodorsal nucleus and the intralaminar nuclei, or from frontal lobe degeneration (Amaral, 1987, Mair, 1994; Zola-Morgan & Squire, 1993). In contrast, procedural learning and memory seems dependent on the intact functioning of the basal ganglia and associated cortical inputs and outputs (Graybeil, 1998; Mishkin, Malamut & Bachevalier, 1984; Salmon & Butters, 1995, Packard & Knowlton, 2002).

The caudate and putamen serve as the input structures for the basal ganglia receiving their primary input, organized in a topographical manner, from the cerebral cortex, such that the integrity of the medial/lateral, dorsal/ventral organization is essentially maintained. Alexander, DeLong and Strick (1986) specified five, parallel circuits originating in specific areas of frontal cortex, projecting through distinct regions of the basal ganglia to the thalamus, with primary projections back to their cortical areas of origin. They suggest that connectivity between these brain regions provides closed and open pathways for communication in each circuit. The closed portion begins in a specific cortical area,

projects to a specific region within the basal ganglia, on through pallidum and substantia nigra which in turn send projections to specific thalamic nuclei and finally back to the area of cortical origin. Alexander et al. (1986) propose that these basal ganglia circuits account for functional segregation through projections that ultimately target the cortical region of origin as well as accommodating functional integration by receiving inputs from multiple cortical areas in addition to the targeted cortical area of the return loop. The anatomical organization of these circuits could facilitate the integration of sensory and motor information critical for visuomotor learning.

Analogous cortico-striatal-thalamocortical circuits have been identified in primates and rodents. The primate 'motor circuit' involves the putamen, which receives afferent input from several cortical areas including primary motor, somatosensory, premotor and supplementary motor areas (SMA). Projections from putamen target ventrolateral globus pallidus then on to the ventrolateral thalamic nuclei and then back to the supplementary motor area of cortex (Alexander et al., 1986). The motor circuit in the rat involves the dorsolateral caudate/putamen (dlC/Pu) complex which receives inputs from the primary (M1) and secondary (M2) motor cortices then projects primarily to the entopeduncular nucleus (analogous to primate globus pallidus internal, GPi) less substantially to substantia nigra pars reticulata (SNr) on through ventroanterior/ ventrolateral (VA/VL) and lateral ventromedial (lVM) thalamic nuclei terminating in areas M1, M2 and parietal somatosensory cortices. The primate 'limbic circuit' involves the ventromedial region of the caudate, receiving input from lateral orbitofrontal

cortex then projecting to dorsomedial GPi and SNr then to the medial ventroanterior and mediodorsal thalamic nuclei each sending efferents to the cortical area of origin. The rat 'limbic circuit' includes inputs from medial orbital (MO), infralimbic (IL), prelimbic (PrL), agranular insular (AI), and ventral anterior cingulate (AC) cortices to ventral striatum, in addition, this region of striatum receives projections from other limbic associated structures (e.g. hippocampus and amygdala). Projections from ventral striatum target ventral pallidum which sends efferents to mediodorsal thalamus. Thalamocortical projections from this region demarcate the rat prefrontal cortex. The primate 'association circuit' includes central regions of caudate and putamen, receiving inputs from prefrontal cortical regions, which project to GPi and SNr on to VA and MD thalamic nuclei terminating in broad projections to frontal cortex. In the rat this circuit includes the inputs from medial prefrontal cortex (mPFC) to dorsomedial C/Pu (dmC/Pu) then projections to entopeduncular nucleus and SNr, then on to lateral and medial segments of the MD, the VA/VL and IVM, termination sites in cortex include the dorsomedial prefrontal cortex (Joel & Wiener, 1994).

The distinction between hippocampal based mnemonic processing and learning and memory processes demonstrated through improved skill performance is well established (Graybeil, 1998; Gabrieli, 1998; Salmon & Butters, 1995). It remains unclear, however, what brain regions are involved in complex skill acquisition, refinement, maintenance and expression. Several lines of research have attempted to elucidate the contribution of specific brain regions within these circuits on tasks associated with procedural learning with conflicting results.

Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies with humans show an increase in regional cerebral blood flow (rCBF) in the putamen when complicated sequential finger movements have been learned (Seitz, Roland, Bohm, Greitz & Stone-Elander, 1990; Seitz & Roland, 1992; Ungerleider, Doyon, & Karni, 2002), while other research suggests that the putamen is equally active during acquisition of a novel finger sequence and when the sequence is well-learned (Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994). Scanning of subjects engaged in rotor pursuit, another measure of visuomotor learning, showed a significant increase in relative CBF in the basal ganglia during motor execution but not during motor learning (Grafton, Mazziotta, Prest, Friston, Frackowiak, & Phelps 1992). It is unclear based on these findings what role the basal ganglia play in motor learning.

Clinical populations with degenerative disorders (PD and HD) affecting basal ganglia structures provide conflicting results on the involvement of these sub-cortical structures in visuomotor learning as well. It was demonstrated in two studies examining groups of individuals with basal ganglia dysfunction that aspects of general motor performance were disrupted, but subjects with basal ganglia lesions demonstrated normal motor sequence learning (Exner, Weniger, & Irle, 2001, Exner, Koschack, & Irle, 2002). In contrast, Willingham & Koroshetz (1993) demonstrated impaired SRT task performance with HD subjects. Yet, these subjects were able to demonstrate normal RT improvement across sessions when performing a random series of key presses incompatibly mapped to the visual cue (one position to the right of the visual cue). This suggests that

loss of dopaminergic input in to the putamen, in PD subjects affects motor skill learning when it involves performing a repeating sequence of visually guided, finger presses. However, caudate degeneration in HD subjects does not disrupt the ability to improve motor skill performance when finger presses are randomized and spatial responses are rule directed instead of under strict visual guidance. Another study from the same lab evaluated the ability of HD and PD patients to utilize advance information (a cue that signaled the position of an upcoming stimulus) to improve RT performance. Results from that study demonstrate improvements in RT similar to controls (Willingham, Koroshetz, Treadell, & Bennett, 1995). The authors suggest that advanced information could improve response speed by facilitating motor preparation, orienting attention to the target location or a combination of the two processes.

Other research with subjects suffering HD associated basal ganglia dysfunction, demonstrated impaired acquisition of an SRT task learned through trial and error (Brown, Redondo-Verge, Chacon, Lucas, & Channon, 2001). In contrast, when learning involved the incidental acquisition of the motor sequence using the standard SRT design HD subjects were unimpaired. Trial and error learning encourages active exploration of each response and the use of working memory to maintain a representation of elements within the sequence relative to other response elements, eventually leading to an awareness of the sequence. HD subjects with caudate degeneration are impaired on this measure suggesting that the caudate region and its cortical connections may be more involved with the intentional learning of visuomotor sequences.

A factor that complicates assessing cognitive deficits, in HD and PD populations, with tasks that involve motor responding is the persistent motor difficulties that characterize the disorders. Dissociating bradyphrenia from bradykinesia is particularly valuable in gaining a more complete understanding of the role of the corticostriatal system in cognitive aspects of motor learning. Smith, Seibert, McDowall, & Abernathy (2001) tested PD patients on a variation of the SRT task designed to mitigate motor deficits associated with PD by assessing response choices verbally. In this version of the task PD subjects were unimpaired on SRT learning. Another study designed to evaluate cognitive slowing independent of motor slowing required PD subjects to mentally manipulate, in a serial fashion, a visually presented stimulus on a target grid. Once subjects had determined the new location of the stimulus on the visual grid they provided a verbal response. Investigators varied the presentation speed of the instructions used for manipulating the location of the stimulus. As speed of presentation increased performance accuracy dropped for both control and PD groups. When the groups were matched in performance at the slowest presentation speed, however, performance accuracy declined significantly more for the PD group than for controls as stimulus presentation speed increased. This indicates an additional impairment in cognitive processing that is dissociable from deficits in motor task performance (Sawamoto, Honda, Hanakawa, Fukuyama, & Shibasaki, 2002).

Often tasks measuring declarative and procedural performance utilize very different paradigms making direct comparison and interpretation of performance

complex. Recently, Willingham, Salidas, & Gabrielli (2002) mitigated this issue when they examined the behavioral performance and neural activity associated with declarative and procedural learning of the same motor response. Subjects performed identical repeating sequences of finger presses. In one condition the subjects were aware of the repeating sequence and in another condition the identical sequence was presented with an unexpected stimulus characteristic. The authors used two circles of different colors appearing in one of four locations on a monitor to instruct participants to expect a repeating or random sequence of locations. Red circles occurred in a specific sequential order while black circles indicated that the order was random. Prior to being scanned subjects responded to a repeating sequence of red circle locations (explicit-overt condition) and a series of black circle locations. The subjects were unaware that some of the black circles occurred in a second repeating sequence of locations (implicit condition) while others occurred at random locations (random condition). When scanning began subjects were also presented with the original red repeating sequence of locations however the circles now appeared black (explicit-covert condition) indicating to the subject that the order was random. The subjects were utilizing the same motor processes to perform the sequence but in one condition they had explicit knowledge of the sequence while in the other they did not. Mean RT scores for the subjects indicated that explicit-overt, explicit-covert and implicit conditions were all significantly faster than the random condition. In addition, performance in the explicit-overt condition was significantly faster than explicit-covert and implicit conditions which were not significantly different from each

other. Scanning results indicated that the neural networks activated when RTs improve through procedural learning are also active when the sequence has been learned explicitly. These areas include (contralateral to the performing hand) left Brodmann's area, left inferior parietal cortex and the right putamen. Additional areas are active in the explicit-overt condition including the prefrontal cortex. The high degree of overlap in neural activation suggested that performance of this visuomotor task incorporated the same neural circuitry involved in procedural learning whether the initial learning process was incidental or guided by explicit knowledge.

It has been argued that improvements in RT characteristic of motor skill learning involves two distinct stages: a fast learning phase typically occurring within one training session in a time course of minutes and a second consolidation stage that develops in a latent fashion over several hours without additional training. This gradual improvement then continues over additional training sessions (Karni, Meyer, Rey-Hipolito, Jezzard, Adams, Turner, & Ungerleider, 1998).

Keele (1968) described the process underlying this learning as the formation of a motor program; a collection of muscle commands, coordinated prior to the execution of the movement sequence and implemented without external feedback. The author went on to suggest that preprogramming a series of predictable movements would decrease attentional demands and facilitate response speed (as cited in Marsden, 1984). Marsden (1984) extended this basic premise suggesting that the ability to combine singular motor movements into a complex sequence of motor activity involves a series of steps including; preparing the motor program,

selecting independent motor elements, organizing these into subunits, appropriately sequencing the subunits and then initiating the series of motor plans. Once the response objective is achieved the motor program is terminated. Support for this view is demonstrated by RT improvements of normal subjects completing the SRT task. Each individual motor element predicts the subsequent movement necessary to progress the response sequence. The repeated linking of the same sequential motor elements encourages the formation of a motor plan. Behavioral evidence of this process, improvement in the RT for completing the sequence of motor responses, may indicate a reduction in the time required to complete some or all of the specific movements within the sequence.

One possible explanation for the performance improvements over time involves the anatomical organization of the corticostriatal system. Graybeil (1998) suggests that the massive convergence of cortical neurons on striatal projection neurons, on the order of 10,000 to 1, reflects a convergence of information involving sensorimotor associations. In addition each cortical neuron can synapse on multiple striatal projection neurons allowing for divergence of information as well. The majority of striatal neurons demonstrate context specific, response characteristics. They respond only to certain movement sequences or remembered stimuli. The activity of tonically active interneurons (TAN's) in the striatum may underlie the gradual learning and subsequent chunking of motor subunits into a learned S-R association.

In recording studies with monkeys Aosaki, Kimura, and Graybeil (1995) found that as a behavioral response was learned there was a gradual increase in the

number of interneurons firing. A specific response to a previously neutral stimulus initially incorporated a small number of interneurons representing a transient S-R association. As the specific S-R association was repeatedly experienced, additional interneurons were recruited. These newly recruited interneurons demonstrated the firing characteristics of the established interneurons associated with the gradually learned S-R behavior. TANs influence the striatal projection neurons converging in pallidum (principal outflow of the basal ganglia). Continued experience with the S-R association produced a shift in striatal neural activity. Specifically, some striatal neurons displayed a temporal shift in their firing pattern corresponding to an earlier point in the behavioral trial. This anticipatory firing was suggested to coincide with a well-learned task (as cited in Graybeil, 1998).

Recording studies with non-human primates provide support for this view. Changes in neuronal firing in the caudate and rostral putamen (association areas) correspond to the trial and error acquisition of early stage visuomotor sequence learning. While increases in neural firing in more caudal regions of the putamen (sensorimotor areas) coincide with later stages when the visuomotor sequence is well learned (Miyachi, Hikosaka, & Lu, 2002). Reversible inactivation of these same areas on an identical task provided further support for these conclusions. Miyachi et al. (1997) temporarily inactivated the anterior striatum or posterior putamen and produced impairment in the acquisition of a new motor sequence and execution of a well-learned sequence, respectively. To summarize, the authors suggested that as a sequence becomes well-learned each two-response motor unit was consolidated or chunked into a longer ten-response (hyperset) sequence.

Monkeys began to anticipate successive responses facilitating response speed for well-learned sequences. If the order of the two-response elements within the ten-response sequence was altered from the original well learned hyperset sequence, response speed resembled that for new hypersets. If this same reordering is done during the early learning stage of a new hyperset performance is unaffected (as cited in Hikosaka, Nakahara, Rand, Sakai, Lu, Nakamura, Miyachi & Doya, 1999).

Anatomically related areas of frontal cortex have also been implicated in processes of S-R visuomotor sequence learning. Monkeys trained to perform visuomotor sequence tasks then given muscimol injections (GABA agonist) into the presupplementary motor area (pre-SMA) demonstrated increased performance errors for new sequences while errors for well-learned sequence sets remained stable. Muscimol injections into the supplementary motor area (SMA) produced a milder level of impairment (Nakamura, Sakai, & Hikosaka, 1999). Single unit recording studies demonstrate increased activity in the pre-SMA cells of monkeys performing previously trained motor response sequences. These cells displayed preferential firing when a current motor sequence plan was discarded for a new motor sequence (Hikosaka, Nakamura, Sakai, & Nakahara, 2002). Other research has implicated the premotor area in planning motor responses. Monkeys trained to perform a visually guided ready-set-go task displayed increased activity in premotor neurons after the signal cue and prior to the intended movement suggesting involvement in motor preparation (Weinrich & Wise, 1982). Using another instructed-delay task, Crammond & Kalaska (2000) observed similar increases in activity of premotor neurons prior to responding.

In humans, trial and error learning of a button press sequence activated the pre-SMA only during the initial visuomotor association pre-SMA activity decreased with continued practice of the repeating sequence (Sakai, Hikosaka, Miyachi, Sasaki, Fujimaki, & Putz, 1999). Unilateral damage to the SMA in one patient resulted in impaired SRT performance and mirror reversed tracking contralateral to the lesion (Ackermann et al., 1996).

Functional magnetic resonance imaging (fMRI) studies implicate the dorsolateral prefrontal cortex (dlPFC) in addition to the pre-SMA in early stages of motor skill learning. When a sequence becomes well-trained neural activity shifts from the dlPFC and pre-SMA to parietal areas of cortex (Hikosaka, Miyashita, Miyachi, Sakai, & Lu, 1998). Changes in the extent of activation were also noted in primary motor cortex with extended practice of a finger opposition sequence (Kami et al., 1998). Transcranial magnetic stimulation studies also support a role for dlPFC in motor skill learning. Using three variations of the SRT task researchers demonstrated that transcranial magnetic stimulation of the dlPFC impaired acquisition of motor response learning when the response was associated with a location cue. If the response was cued by color alone, or a color / location combination cue, motor responding was unimpaired (Robertson, Tormos, Maeda, & Pascual-Leone, 2001).

Assessment of sequential motor skill learning in rodents has focused on the contribution of the striatum in different maze tasks. DeCoteau & Kesner (2000) tested sequence learning in explicit and implicit versions of an eight-arm radial maze. Explicit training required rats to make a series of arm entries based on a

repeating fixed sequence that had been acquired through trial and error. Awareness of the sequence was demonstrated by increased accuracy of orienting responses at closed maze gates across trials. Implicit training involved arm entries guided by gates opening as rats approached the center hub indicating the next arm entry. The sequence repeated on each trial. Procedural learning was demonstrated by decreased latency to reach the reinforcement in each subsequent arm. Rats were trained on one version of the task pre- or post-surgically to evaluate acquisition and retention. Rats with medial C/Pu lesions were unable to acquire the implicit version when trained post-surgically but were able to perform at the level of controls when the task was trained pre-surgically. Lateral C/Pu lesions did not affect acquisition or retention of the procedural task. Both groups demonstrated spared performance of the explicit version of the task. Results suggest that for acquisition of a sequence of spatial responses in rodents intact functioning of the medial C/Pu is necessary.

In another procedural learning task Jog, Kubota, Connolly, Hillegart & Graybeil (1999) trained rats to perform in a T-maze and then recorded from ensembles of sensorimotor (dlC/Pu) striatal neurons throughout the acquisition and overtraining phases. They identified a distinct shift in the pattern of neural activity as the task became well-learned. The shift involved increased neural activity coinciding to start and goal related behavioral events while neural activity associated with intermediate behavioral events decreased across sessions. The authors proposed that sensorimotor striatum may form an action template of a well-learned sequence of sensorimotor associations. When the initial sensorimotor elements occur it

triggers the entire sequence of responses associated with achieving the goal as a unit.

To further elucidate the role of specific striatal subdivisions in visuospatial learning and memory Mair, Koch, Newman, Howard, & Burk (2002) compared the effects of excitotoxic lesions of striatum on a visuospatial reaction time (VSRT) task involving stimulus guided responding with performance on a delayed match to sample lever task that required memory for spatial information from the previous trial to guide current responding. A double dissociation for the effects of dorsolateral and ventral lesions was observed across the two tasks. Dorsolateral lesions increased response latency on stimulus guided responding without affecting accuracy or general motor performance while ventral lesions impaired performance guided by spatial information retained in working memory. Dorsomedial striatal lesions impaired accuracy on the delayed matching task and produced a non significant trend of slower choice RT and increased errors on VSRT performance. This pattern of impairments suggests that the limbic circuit including ventral striatum is important for responding based on information retained in working memory while lesions of dorsolateral striatum affected motor performance only when a choice of response locations was involved.

The evidence from clinical literature, scanning and lesion studies implicates the basal ganglia in the gradual improvements in RT performance associated with sequential visuomotor learning. Functional differences have been elucidated between ventral (limbic) and dorsal (sensorimotor and association) areas of striatum on tasks measuring RT in visuospatial responding and sequential

visuomotor learning and memory. Anatomical connections maintain some level of functional segregation for the frontal cortical projections to striatum and the return loops through thalamus. This suggests that the frontal cortex may be another region mediating sequential visuomotor learning.

Human studies involving SRT task performance have shown impairments in long-term visuomotor learning for patients with Parkinson's disease (Jackson, Jackson, Harrison, Henderson, & Kennard, 1995; Dominey, & Jeannerod, 1997; Westwater, McDowall, Siegert, Mossman, & Abernethy, 1998; Knowlton, 2002) and Huntington's disease (Knopman & Nissen, 1991; Willingham, & Koroshetz, 1993; Brown, et al., 2001) degenerative diseases of the basal ganglia but not for focal lesions of the basal ganglia unless cortical dysfunction was also evident (Exner, et al., 2002). SRT task performance has been found to be impaired by prefrontal cortex (Beldarrain, Gafman, Ruiz de Velasco, Pascual-Leone, Garcia-Monco, 2002) and motor cortex lesions (Ackermann, et al., 1996). Functional imaging studies have also implicated the cortex (Hikosaka et al., 1998; Karni et al., 1998; Robertson et al., 2001; Sakai et al., 1999) and striatum (Doyon, Owen, Petrides, Sziklas, & Evans 1996; Rauch, Whalen, Savage, Curran, Kendrick, et al., 1997) in the short- and long-term performance changes demonstrated in sequence learning. This evidence suggests a role for frontal cortex in sequential motor learning but the specific contribution of different cortical areas has not been established.

This study examined the effect of excitotoxic lesions of functionally discrete regions of frontal cortex and striatum on short- and long-term simple and

sequential visuomotor learning. Lesion studies with rats have established a functional dissociation between ventral and dorsal regions of striatum with additional evidence that segregation of function may distinguish dorsomedial and dorsolateral regions (Decoteau & Kesner, 2000; Mair et al, 2002). Similar functional specificity has been identified in the frontal cortex of the rat (Bailey & Mair, 2004; Pasetti, Chudasama, Robbins, 2002; Muir et al., 1996) with support for this view based on anatomical projections to functionally discrete areas of striatum.

Only one previous study has examined sequential visumotor learning in rodents with a task that is similar to the human SRT task. Christie & Dalymple-Alford (2004) assessed visuomotor sequence learning using a nose poke chamber with four response locations. Rats responded to sequences of lit ports for intracranial self stimulation reinforcement. Rats with dorsal C/Pu lesions showed learning on short (4) response sequences but failed to demonstrate learning on longer (8 and 12) response sequences.

Subjects performing the human SRT task respond to a series of individually presented cues on a computer monitor. The position of each cue corresponds to a spatially aligned key on the keyboard. Typical sessions involve a random sequence of 100 cues or a repeating sequence of 10 cues repeated 10 times in a session. Subjects are not informed about the repeating nature of some sessions. Each repeating session starts at a different point in the sequence making it difficult for subjects to detect a start or end point for the repeating 10 elements. A random session is followed by a block of repeating sessions (5) and a final random session. General task performance is assessed based on changes in the mean RT

of the entire 10 element sequence in the initial and final random sessions. Sequence learning is measured as the change in mean overall RT to complete the 10 response sequence on the final repeat session with the subsequent random session. In addition, the change in mean overall RT to complete the 10-response sequence is compared across the repeating sessions as another measure of sequence learning.

We have developed a rat analogue of the SRT task that examines the classic measures of motor sequence learning: general task performance (improvement from one random session to the next), and sequence specific learning (improved RTs across repeating sessions and increased RTs when switched from a repeating sequence session to a random sequence) using overall RT to complete the sequences of responses. Rats were trained to respond to one of five nose-poke ports indicated by a luminance cue. A sequence consisted of five serially presented luminance cues. Response at a cued port extinguished the light in that port and triggered illumination in the next port in the sequence. Response to the fifth port in the sequence resulted in the delivery of water (2 - 0.1ml pulses) reinforcement. Rats had previous S-R training responding to light cues presented at random ports for water reinforcement. Each session consisted of 60 five-response trials. Reaction time for each response was recorded. Rats completed five training sessions to become familiar with the FR-5 reinforcement schedule. Rats completed three blocks of sessions. A block consisted of a random session followed by 5 repeating sessions.

A unique feature of the task design was that performance of each sequence was marked by discrete start and endpoints. This provided a means for identifying the initial response of the sequence from later elements. One of the hallmark features of PD is akinesia, difficulty initiating voluntary movement. Packard & Knowlton (2002) suggested that the motor difficulties inherent in basal ganglia dysfunction may overwhelm any demonstration of motor sequence learning. The ability to dissociate RTs specific to the first response of a learned response from RTs reflecting execution of the remaining response elements may identify deficits in initiating a motor plan distinct from motor learning.

In addition to the behavioral measures listed above we also used measures that tracked changes across each response. The first measure compared median RT to complete all five responses in a random sequence to the median RT for the entire 5-response sequence in a repeat sequence session. This measure is similar to the assessment measures of human performance in the SRT task. In addition, changes in the median RT for each response across sessions was also examined to elucidate the impact of these changes on the changes observed in the RT for the overall sequence.

It was predicted that different patterns of RT responding would be revealed for random and repeating sessions based on the adaptive benefit of developing a sequence specific motor program. Specifically in repeat sequence sessions, to the extent that rats made use of a motor program responding would be facilitated. It is expected that initiating a motor program consisting of several response elements would increase the RT for the initial response element or elements compared to

initiating a single motor element. Chunking of subsequent motor elements into a cohesive response unit would result in a reduction in RT for later motor elements. Random sequence sessions would not encourage development of a specific motor plan because prior response locations could not predict future response locations. To the extent that initiating a series of five motor responses involves additional motor planning than initiating a single response then RTs for the initial response element or elements should increase for sessions in which 5 response sequences are trained (whether random or repeating). To the extent that a specific learned sequence increases demands on motor planning the RT for initial elements should be greater for repeated than random sequences.

Short-term learning, a rapid change in RT performance indicative of motor sequence learning was assessed by comparing median RT performance across blocks of trials in the initial random and repeat sequence sessions. If the targeted lesion areas of frontal cortex or striatum were critical for the short-term learning anticipated in visuomotor sequence learning then impairment of this measure was expected. The effect of these lesions on long-term learning was assessed by comparing changes in median RTs across repeating sequence sessions and subsequent increases in median RTs when switched to random sequence sessions.

Other research has demonstrated that visuomotor sequence learning is robust after a gap in training or after interposing a random sequence session (Nissen & Bullemer, 1987; Exner et al., 2002). The effect of targeted lesions of frontal cortex and basal ganglia on this characteristic of visuomotor sequence performance was

also examined by comparing two repeating sequence sessions separated by a random session. Another factor of interest was the extent to which the pattern of RT performance for a learned motor response sequence (e.g. increased RT for initial elements combined with improved RT for later elements) was sequence specific. A sequence specific motor program would predict that switching to a random sequence or a new repeating five-response sequence would disrupt performance. Comparing the final session of the original sequence with the subsequent random session (R3) and the new repeat sequence (B1) that followed addressed these measures.

In experiment 1, the effect of lesions in regions of frontal cortex (mPF, M1, M2 and combined M1M2) projecting to areas of interest in striatum on the acquisition of simple S-R learning and sequence learning was examined. Clinical and imaging studies suggest that RT improvement characteristic of motor skill learning, in human subjects, occurs within the initial training session. Rapid acquisition suggests involvement of executive processes (i.e. attention, working memory, and chunking of motor elements) typically associated with cortical function. We expected large motor cortex lesions to disrupt speed of responding in a general manner across all tasks without affecting the ability to learn the simple S-R motor task but possibly impairing the sequential ordering of responses. Premotor cortex has been implicated in the acquisition of sequential visuomotor responding and chunking of repeating sequential motor elements (Nakamura et al., 1999; Hikosaka et al. 2002). We expected M2 lesions to affect the early acquisition and more gradual improvement characteristic of simple and sequential visuomotor learning.

Lesions of mPF have been shown to affect tasks requiring working memory and VSRT performance (Porter, Burk, & Mair, 2000; Burk & Mair, 2001; Bailey & Mair, 2004). To the extent that these processes are important in simple S-R learning and sequence learning we expected performance on these tasks to be disrupted.

Experiment 2 tested the effect of striatal lesions on the acquisition and expression of simple S-R learning and sequential visuomotor learning. It was expected based on cortical connections and previous findings that ventral lesions would not affect simple S-R learning or sequential visuomotor learning. Lesions of dorsolateral caudate putamen were expected to disrupt response speed similar to lesions in anatomically connected regions of motor cortex. The effect of lesions to this area on the gradual improvement seen in RT during sequential learning was also tested. Dorsomedial caudate putamen lesions were expected to disrupt VSRT performance and motor sequence learning. Ventral lesions were not expected to disrupt VSRT motor performance or motor sequence learning.

EXPERIMENT 1: FRONTAL CORTEX LESIONS

Methods

Subjects

For this experiment subjects consisted of 40 male Long Evans rats (Charles River Laboratories) eight weeks of age at the onset of behavioral training. Subjects were caged singly in a temperature regulated and 12 hour light / dark cycle (lights on 7:00 a.m. to 7:00 p.m.) controlled vivarium. Behavioral training occurred during the light cycle. During training rats were allowed ad libitum food and 30 minutes of water per day at the conclusion of the light cycle. Rats not scheduled for training on a particular day were given one hour of water. All handling and maintenance procedures for the rats complied with guidelines established by the University of New Hampshire's Animal Care and Use Committee.

Equipment

All training and behavioral testing was carried out in operant chambers (Env 007, Med Assoc., Georgia, VT.) equipped with a five port nose poke response wall (Env 115A) at one end and a runway alley with a retractable lever (Env 215A) directly opposite the response wall. Each response port was equipped with an infrared nose poke detector, 6.4 mm diameter yellow stimulus light mounted flush on the back wall, and a milled basin in the base of the port for dispensing two 0.1 ml pulses of water (solenoid valve - The Lee Company, Essex, CT.) as reinforcement. Mounted in the center of the opposite wall was a

clear polycarbonate covered arm 43L x 17H x 8W cm with a photocell located where the arm opens into the chamber. Each chamber was equipped with a whisper fan (IMC Magnetics Corp., model no. 4715FS-12T-B20) which served the dual function of ventilation and white noise. The experimental chamber was contained in a sound insulated, plywood box connected via an interface to a remote computer that activated the training programs and recorded response data.

Presurgical Behavioral Training

Rats were trained on a standard 5 choice visuospatial reaction task (VSRT) in three stages. Rats were initially shaped to a lighted port for reinforcement by placing them in the chamber with lights on in all the ports. When they made a response (nose poke) into a lighted port, reinforcement (two 0.1 ml. pulses of water) was delivered in that port and the light extinguished indicating subsequent responses to that port would not receive reinforcement. After receiving water at all five ports the lever extended from the opposite wall. The rat pressed the lever to initiate the next trial. Each session consisted of 10 trials. After 10 sessions the lever was moved to the end of the arm for an additional 5 sessions requiring the rat traverse the length of the arm to initiate each trial.

Stage two of the training, consisted of ten sessions, beginning with a lever press illuminating all five port lights. As the rat crossed the photocell at the chamber end of the arm four of the lights extinguished while one, selected at random by the computer, remained on for the duration of the 3 second response window. If the rat responded to the illuminated port (S+) within three seconds it

received reinforcement, if it failed to respond the light extinguished and the trial was recorded as an omission. The final stage of training was identical to stage two except that the duration of the light cue (.05, .11, .26, .58, 1.33, 3.0 s) varied randomly on each trial. Each session consisted of 96 trials. Rats were required to achieve criterion performance levels across three consecutive sessions of 70% correct responses averaged across stimulus durations. Once reaching criterion they were matched for performance in 8 blocks (five per block) and randomly assigned to the four treatment conditions (M1/M2, complete M1, complete M2, mPF) or sham controls.

Surgical Procedure

Anesthesia was administered via an intramuscular injection of ketamine (85 mg / kg) and xylazine (8.5 mg / kg). Rats were positioned in a Kopf stereotaxic instrument (Tujunga, CA) with the incisor bar set 3.3mm below the interaural plane. Aseptic surgical procedures were followed for opening the skull.

Cortical lesions were produced by infusing 0.1 µl N-methyl-D-aspartate (NMDA; 100 mM in phosphate buffer, pH = 7.4) with a 26 gauge cannula using a Kopf 5000 microinjection unit at the desired locations (locations in mm, AP coordinates relative to bregma, DV coordinates relative to dura). The cannula was left at each site for 60s following each injection to allow for diffusion at the site. Stereotaxic coordinates for each lesion are listed in Table 1. Sham surgery on controls involved the same preliminary surgical procedures as the lesion groups without opening the skull.

Post Surgical Testing

Each group was retrained on stage 2 VSRT (long duration stimulus cue) until achieving 95% correct responding across three consecutive sessions or completion of 10 total sessions. Rats were then tested for 10 sessions on the standard VSRT task. Results provided a measure of the lesion effects relative to each group on response accuracy and response speed unique to each treatment group.

Upon completion of VSRT training rats began simple S-R learning. Animals responded to the same port for seven consecutive trials. Response opportunities were initiated with a lever press. Crossing the arm photocell caused one randomly selected light to illuminate for 0.05s then extinguish. A response to the port within 3.0s received reinforcement. Responses to non-illuminated ports were recorded as errors. Responses outside the 3.0s response window constituted omissions. The stimulus duration for responses 1, 3, 5 and 7 was .05s. For responses 2, 4 and 6 the stimulus light remained on for 3.0s. Simple S-R learning was measured as increased accuracy across the 0.05s stimulus presentations. Reaction time was analyzed to determine if behavioral performance on this measure changed as a consequence of changes in response accuracy.

Any group that had demonstrated impaired accuracy at the briefest VSRT stimulus duration would have had difficulty detecting the cue on the brief trials of the short-term simple S-R learning task. We analyzed the VSRT accuracy data and found no significant [One way ANOVA $F(4, 35) = 1.186$, $p = .3338$] difference in accuracy performance between the lesion groups and sham controls at the

briefest stimulus duration. Therefore there was no adjustment in stimulus duration necessary to equate baseline performance levels.

Following short-term simple S-R learning rats were trained on two sessions of the long-term simple S-R task. Each session consisted of 50 trials in which the cued port was randomly selected on each trial. Testing involved one random port session followed by five sessions of 50 trials in which the same port was cued on each trial. The final session was a 50 trial session of randomly cued ports. Each single-port response in this task involved all of the same demands on motor execution as the initial response in the rat-SRT task. A difference in median RT comparing random port responses to a port that repeated on every trial would indicate a benefit for the ability to predict future responses guided by previous experience.

Upon completion of the long-term simple S-R training rats were acclimated to the reinforcement schedule (fixed ratio-5) of the rat-SRT task. In the initial session rats were presented a random sequence of five ports, indicated by a luminance cue that remained on until the animal responded to the port causing it to extinguish and the light in the next port in the sequence to illuminate. Animals received reinforcement (2 - 0.1ml pulses of water) when the photocell recorded a break at the fifth port in the sequence. Sessions consisted of 60 (5-response) trials. Animals initiated each trial with a lever press. When performance stabilized (5 sessions) each animal completed one additional session used as a baseline measure of RT. Then animals began testing on the repeat sequence sessions. These were identical to the random sessions except that the five serial

responses repeated on every trial and across sessions (e.g. port 3-1-3-5-3). The spacing of the response ports in the repeat sequences was equalized to assure that faster RTs were not an artifact of shorter distances between successive response locations. The average distance between successive random port responses was determined to be ~ 1.8 ports this guided the spacing of the repeat response sequence. Rats completed five sessions of the repeating sequence. Two blocks of this pattern of sessions, one random sequence, five repeating sequences were run followed by a third block with a different five session repeating sequence to determine whether changes observed in performance were sequence specific or reflected a general pattern of responding to a learned sequence. We assessed both short- and long-term visuomotor sequence learning as well as general improvements in motor response performance. In addition we examined individual response elements of random and repeating sequence responding to evaluate anticipated differences in the pattern of response speed that developed when a response location is predictable versus when response location is random.

Behavioral Performance Measures

The effect of frontal cortical lesions on the speed and accuracy of responding to a briefly presented visual stimulus was tested in the visuospatial reaction time (VSRT) task. Responses were characterized as correct, errors of omission, or errors of comission. Accuracy was calculated as correct responses / correct + errors of comission. General motor speed performance was assessed as the time it took the animal to traverse the runway arm after pressing the lever.

Choice response speed was defined as the time elapsed between the photocell break at the chamber entrance triggering the cued port until the photocell at that port recorded a nose poke.

Short-term simple S-R learning measured the ability of an animal to benefit from the presentation of stimuli in a consistent location for several consecutive trials. Stimulus duration alternated between .05s and 3.0s over seven presentations at one port location beginning and ending with .05s durations. Previous research has demonstrated that response accuracy to the .05s duration when presented at random locations is at chance. If lesions of frontal cortex disrupt learning to orient to a stimulus at a repeating location or disrupt visual perception of the brief cue performance would be impaired. Tests were conducted on the accuracy (correct responses /corrects + errors of commission) and reaction time for the brief .05s stimulus duration presentations.

Long-term simple S-R learning measured the change in reaction time of an animal required to respond to a single (3.0s) cued port on each trial within a session when demands on attention were minimal. Animals completed one session in which the response port changed randomly on each trial followed by five sessions of the response port remaining in the same port location for the session and a final random session. The motor demands of this reaction time measure were identical to the initial response of the sequence learning task and provided a comparison of initiating a single response versus a sequence of motor response elements.

The rat-SRT task measured short- and long-term sequence learning. Visuomotor sequence learning was assessed through changes in RT on several measures. The first measure compared median RT for the entire random sequence of motor responses to the median RT for the entire repeating sequence of responses. Long-term visuomotor learning was measured as the change in RT across repeating sequence sessions. Research involving clinical populations consistently report these measures as characteristic of motor sequence learning.

In addition to demonstrating a measurable decrease in RT across repeating sequence sessions it was expected that different patterns of RT responding would be revealed for random and repeating sessions. Specifically in repeated sessions, continued practice with the sequence would encourage the development of a motor program linking (chunking) the individual response elements into an efficient response unit facilitating performance. Initiating the motor program of several response elements was expected to impede RTs for the initial response element or elements. Chunking of subsequent motor elements into a cohesive response sequence was expected to reduce the RT for later response elements. Performing a random series of five responses that changed on every trial would not generate a specific motor program as prior response locations would not be indicators for subsequent response locations. It was expected however that initiating a sequence of five random motor responses would involve motor planning. Therefore, slowing in the initial element was also expected for random sequences compared to initiating a single response. In addition, the inability to plan future response locations based on prior responses would prevent chunking. Slowing across later

elements of the random sequence compared to the repeating sequence was predicted.

To the extent that visuomotor sequence learning is robust it was predicted that RT improvements demonstrated in repeating sequence sessions would not be disrupted by interposing a testing session of random sequences. By contrast to the extent that RT improvements seen in the later response elements reflected a sequence specific motor plan it was predicted that switching to a novel response sequence would disrupt RT performance, in particular the efficient execution of later response elements. The slowing in RT performance for final elements of the novel sequence would indicate that the performance benefit of a sequence specific motor plan does not generalize to a novel sequence of motor responses.

Short-term motor sequence learning is argued to involve executive processes typically associated with frontal cortical function. To determine if short-term learning was demonstrated in visuomotor sequence learning RT data from the initial random and repeating sequence sessions was divided into blocks of trials and examined for signs of RT change consistent with short-term learning.

It was predicted that the complexity of the motor planning needed to initiate a series of five motor responses would be greater than the motor planning for a single motor response. Increased planning would be reflected in longer reaction time for initial responses of a five-response sequence than for initiating a single response. In addition, a learned sequence repeating on every trial required a specific complex motor plan whereas random five response sequences generated

a general motor plan to make five consecutive responses without a specific plan (Table 2).

Histological Procedure

At the completion of all behavioral testing rats were sacrificed. Subjects received anesthesia (100 mg / kg ketamine with 10 mg / kg xylazine) through an intramuscular injection followed by transcardiac perfusion of physiological saline then 5% (vol / vol) buffered formalin. The brain was removed and submerged in a (10% glycerin / 4% buffered formalin) solution followed by 72 hours in 20% glycerin / 4% neutral buffered formalin. Frozen tissue was sectioned in the coronal plane. Every fifth, 30 μm slice, was mounted and stained with thionin. Slides were examined under a light microscope to confirm the location and extent of lesion damage.

Results: Experiment 1

Histological Analyses

Infusions of NMDA in cortex produced characteristic neuron loss and glial cell influx in all target sites. NMDA infusions targeted, bilaterally, at M1 were accurately located in all cases and in most cases damaged all of M1 as delineated in Paxinos & Watson (1998). Two animals had lesions that extended anterior more than intended damaging M2. The largest of the M1 lesions extended from +4.25 mm to 0.5 mm relative to bregma. Typical M1 lesions extended from 3.5 mm to – 0.1 mm relative to bregma (Figure 1).

M2 lesions consistently damaged the region medial to M1 although a few cases were asymmetrical with some unintended sparing of M2 at the most

anterior infusion (+ 4.2 mm) site. The AP extent of the largest lesion was + 4.60 mm to + 0.7 mm anterior to bregma. Most lesions were limited to M2, some evidence of M2 sparing occurred at the most anterior coordinate. One animal experienced some unilateral damage to M1 as well. A representative lesion of M2 extended from + 4.5 mm to +1.05 mm anterior to bregma (Figure 1).

Lesions involving both primary (M1) and secondary motor cortex (M2) typically reflected extensive tissue loss and glial cell congregation at the perimeter of the lesion. The AP range of the largest M1M2 lesion extended from + 4.75 mm to + 0.7 mm relative to bregma. Typical M1M2 lesions extended from + 4.0 mm to + 0.4 mm anterior to bregma and were confined to the M1 and M2 regions (Figure 1).

mPF lesions targeted prelimbic (PrL) and cingulate cortices areas 1 and 2 (Cg1 and Cg2, respectively). Damage included neuron loss, tissue loss and gliosis. Lesions in a few animals extended into anterior regions of M2 sparing posterior sections of Cg1 and Cg2. The most extensive mPF lesion ranged from + 4.3 mm to + 0.1 mm relative to bregma, with more typical AP ranges of +3.7 mm to + 0.35 mm measured from bregma (Figure 1).

Visuospatial Reaction Time (VSRT)

Two measures of VSRT performance were compared across the treatment levels. Response accuracy scores improved for all groups as stimulus duration increased (Figure 2) This finding was confirmed in a split plot factorial [SPF5.6 treatment (M1, M2, M1&M2, mPF, control) x stimulus duration (0.05. 0.11, 0.26, 0.58, 1.33, 3.0s)] ANOVA (Kirk, 1995 p.515). Results indicated no main effect of

treatment $p > .1$, a significant main effect of stimulus duration [Greenhouse-Geisser adjusted $F(3, 82) = 1296.01$, $p < .0001$] and no interaction [$F(20,175) = .94$, $p = .531$].

Preliminary examination of the reaction time data for all behavioral tasks indicated heterogeneity of variance. Median RTs were calculated for each session or block within a session to minimize variability. Conservative post hoc testing and Greenhouse-Geisser adjusted degrees of freedom for sphericity violations were used where convention dictated.

Groups with lesions involving M2 (M1M2 and M2) had slower choice reaction time at all stimulus durations. Characteristic of all groups was an improvement in reaction time as stimulus duration increased (Figure 3). A SPF5.6 (treatment x stimulus duration) ANOVA (Kirk, 1995 p. 515) using correct responses only supported these findings. Results showed a main effect of treatment $F(4, 35) = 3.269$, $p = .022$, significant effect of stimulus duration [Greenhouse-Geisser adjusted $F(2, 67) = 139.24$, $p < .0001$ and a significant interaction [Greenhouse-Geisser adjusted $F(8, 67) = 2.682$, $p = .014$. One-way ANOVAs performed at each stimulus duration with corresponding post hoc testing (Games-Howell $\alpha = .05$) identified the M2 group as significantly slower responding to cued ports compared to controls except at the briefest (0.05s) duration (Kirk, 1995 p 147). At the brief duration the M1M2 group was significantly slower than controls.

The groups did not differ on runway reaction time (Figure 4). A one-way ANOVA comparing median RT indicated no significant differences ($F(4, 35) = .912$, $p = .468$) among the treatment levels. These results indicate that lesions of

mPF and motor cortex lesions do not impair VSRT accuracy or runway RT. All lesions slowed responding when a choice was required between alternate spatial locations to guide correct responding.

Short-Term Simple S-R learning

Animals were tested on their ability to improve accuracy performance to a brief stimulus cue presented in a consistent location. All groups were highly accurate and showed little change in accuracy performance responding to the 3.0s stimulus duration and these findings will not be included in further discussion. All groups demonstrated improved accuracy scores across the four (0.05s) brief stimulus cue presentations (Figure 5). The analysis [SPF5.4 treatment x stimulus presentation (4 levels: responses 1, 3, 5, 7)] yielded no effect of treatment [$F(4, 35) = 1.889$, $p = .134$] and no interaction with stimulus presentation [$F(12,105) = .840$, $p = .609$] (Kirk, 1995 p. 515). The main effect of stimulus presentation was significant [Greenhouse-Geisser adjusted $F(2, 48) = 70.635$, $p < .0001$].

The reaction time for all groups was unaffected by improved accuracy performance. A SPF5.4 (treatment x stimulus presentation) ANOVA confirmed this interpretation (Kirk, 1995 p. 515). Results indicated no significant main effects [$F(4, 35) = 1.870$, $p = .138$ and $F(3, 105) = 1.208$, $p = .310$, respectively] or interaction ($F(12, 105) = .709$, $p = .739$). These results indicate that lesions of motor cortex and medial prefrontal cortex do not disrupt the ability to improve response accuracy to a brief visual cue occurring in a consistent location.

Long-Term Simple S-R learning

Comparisons of group reaction time when responding to a single randomly selected port or a single port repeated on every trial indicated that all groups responded faster to a single repeated port location, than to an unpredictable, randomly occurring port location (Figure 6). Data were analyzed in a SPF5.2 [treatment x session type (random or repeat)] ANOVA (Kirk, 1995 p. 515). Results indicated no main effect of treatment $F(4, 35) = 2{,}621$, $p = .0761$ and no interaction ($F < 1.0$). The main effect of session was significant $F(1, 35) = 14.945$, $p = .0005$.

Sequential Visuomotor Learning

The effect of cortical lesions on sequence learning was assessed using the behavioral measures previously described. Seven animals (two in each of the following groups control, M2, and mPF and one M1M2 animal) missed running the final repeating sequence session in block two of the original A sequence. Data from the session immediately prior to the missed session were used to replace the missing data points. RT performance on the entire five-response repeat session (A10) was compared with RT performance for the subsequent random (R3) session. Reaction time to complete five responses of the learned repeat sequence was significantly faster than the time to complete five responses of the random sequence (Figures 7 & 8, graph 1). Analysis [SPF5.25 (treatment x sequence type x response) ANOVA (Kirk, 1995 p. 553)] of the median RTs for the five-response repeat (A10) and random sequence (R3) sessions confirmed no significant effect of treatment [$F(4, 35) = 2.093$, $p = .1017$]. Significant main effects of sequence type [$F(1, 35) = 21.411$, $p < .0001$] and response [F (4, 140)

= 22.567, p < .0001]. Two-way interactions, treatment x response and response x sequence type were also significant [F (16, 140) = 1.917, p = .0234 and F (4, 140) = 31.426, p < .0001, respectively]. The lack of a significant three-way interaction suggested that the differences in response RTs depending on which sequence was performed was similar for all groups.

The treatment x response interaction reflected increased RTs for lesion groups on the first response in a sequence of responses compared to controls. This increase was exacerbated for learned sequences. One-way ANOVAs at each response, collapsed on the sequence type (random / repeat), indicated that the treatment effect approached significance for response 1 only [F (4, 35) = 2.403, p = .0683]. The response x sequence type interaction reflected different RT patterns of responding for the two sequence types. Specifically for later responses (3-5) of the repeating sequence RTs were faster than for later responses of the random session. All lesion groups demonstrated slowing in the initial responses of the repeating sequence sessions compared to the random sequence sessions (Figure 8). The two way interaction response x sequence type was examined in one-way ANOVAs for response across the two sequence types showed significantly faster RTs on response 1 & 2 for the random session [F (1, 39) = 6.913, p = .0122; F (1, 39) = 4.627, p = .0377, respectively] while responses 3-5 were significantly faster on the repeat sequence (A) session [F (1, 39) = 58.168, p < .0001; F(1, 39) = 43.365, p < .0001; and F (1, 39) = 184. 958, p < .0001, correspondingly). The analyses of individual responses in the

sequences provides convincing evidence for learning; faster RTs for later elements of learned sequences.

Changes in RTs within the first repeating session were identified as short-term learning improvements. Trials from the first random and repeat sequence session were divided into five blocks of 12 trials. Plotting the median RTs for each block indicated that across the blocks of the first random sequence session, all groups showed slowing in the first response across blocks of the session and slowing in the final response as well. For repeating sessions all groups demonstrated slowing across blocks for responses 1 and 2 while improving RTs for response 5.

A SPF5.255 (treatment x sequence type x response x block) ANOVA (Kirk 1995 p. 562) supported these findings. All main effects were significant as were the two-way interactions sequence type x response and response x block and the three-way interaction sequence type x response x block (Table 3). Separate SPF5.55 (treatment x response x block) ANOVAs (Kirk, 1995, p. 553) were run to gain insight into the source of the three-way interaction. For the repeated sequence session all main effects were significant [treatment $F(4, 35) = 3.20$, $p = .024$; response Greenhouse-Geisser adjusted $F(2, 70) = 19.435$, $p < .0001$; and block Greenhouse-Geisser adjusted $F(3, 77) = 6.045$, $p = .003$] but there were no treatment x within factor interactions. Post hoc testing (Games-Howell $\alpha = .05$; Kirk, 1995 p. 147) confirmed that the M1M2 and M2 groups were slow responding to the cued series of ports compared to controls (Figure 9).

The two-way interaction response x block was also significant [F (5, 143) = 15.246, p < .001]. One-way ANOVAs for response across the levels of block identified the source of the interaction as significant changes across blocks for responses 1 [F (4, 156) = 15.035, p < .0001, 2 [F (4, 156) = 9.487, p < .0001] and 5 [F (4, 156) = 29.983, p < .0001. Response 3 and 4 showed no significant change [Fs < 1.0].

The SPF5.55 (treatment x response x block) ANOVA (Kirk, 1995 p. 553) for the random sequence session showed no treatment main effect [F (4, 35) = 1.782, p = .155]. Within subject main effects of response [Greenhouse-Geisser adjusted F (2, 51) = 52.529, p < .0001] and block [F (4, 140) = 3.082, p = .018] and the two-way interaction response x block [Greenhouse-Geisser adjusted F (9, 309) = 3.002, p = .002] were all significant. One-way ANOVAs for response across the levels of block indicated that the interaction resulted from significant changes across the blocks of the session for response 1 [F (4, 156) = 5.883, p = .0002] and 5 [F (4, 156) = 2.882, p = .0245] only. Both responses slowed considerably across the blocks of the session (Figure 10). Responses 2, 3 and 4 did not change distinctly across the blocks with (ps > .05).

Graphs plotting the change in RT for each response across the blocks of the initial random and repeating sequence session highlight the unique pattern of RTs characteristic of increasingly predictable motor responses linked to repeating response locations and RTs that reflect random motor elements generated by random unpredictable response locations (Compare Figures 9 & 10). Short-term learning (within session) results support and extend the findings

illustrated by the long-term learning (between sessions) results. In both short- and long-term learning RTs for the initial response in a series of responses (random or repeat) increased with practice while RTs for the later responses in particular the final response slowed in random sequences and improved in repeat sequences.

Long-term learning was measured by improvement in RTs across the A1-10 repeating sequence sessions. Examination of RTs for each response indicated that all groups experienced an increase in RT for responses 1 and 2 in the initial sessions before stabilizing or improving in later sessions. In contrast later response elements, particularly response five, showed consistent improvement in RT across sessions demonstrating the incremental improvement associated with long-term visuomotor learning. These findings were supported in a SPF5.105 (treatment x session x response) ANOVA (Kirk, 1995 p. 562). All main effects of treatment [$F(4, 35) = 2.648$, $p = .0496$], session [Greenhouse-Geisser adjusted $F(6, 206) = 39.894$, $p < .0001$] and response [Greenhouse-Geisser adjusted $F(2, 55) = 38.748$, $p < .0001$] were significant as was the two-way interaction session x response [Greenhouse-Geisser adjusted $F(11, 373) = 5.726$, $p < .0001$]. Post hoc testing (Games-Howell $\alpha = .05$) identified M1M2 and M2 as significantly slower responding to a cued port compared to controls. One-way ANOVAs examining each response confirmed that there was a significant effect of session on all responses ($ps < .0001$) and no treatment x session interaction ($ps > .36$). In addition, for the first response there was a marginally significant [$F(4, 35) =$

2.634, p = .0505] effect of treatment. All lesion groups were markedly slower on the first response element than the control group (Figure 8).

Comparing performance in sessions A5 and A6 showed little effect of interposing training with a random sequence session. Retention of the learned sequence is most noticeably reflected in the unchanging RTs for responses 3, 4 and 5 across the two sessions (Figure 8, graph 1). This demonstrates that effects of learning are maintained over 48 hours even when rats are trained to perform different sequences in the interim.

To the extent that memory for sequence specific information improved reaction time it was predicted that the introduction of a novel sequence would disrupt visuomotor sequence learning. Rats displayed a disruption in sequence learning when switched from the original sequence to a new five response sequence location (Figure 8). A SPF5.25 [treatment x session (sequence A-session 10 vs. sequence B-session 1) x response ANOVA (Kirk, 1995 p. 553) supported this finding. Results indicated significant main effects of treatment [F (4, 35) = 3.665, p = .014], session [F (1, 35) = 54.761, p = .0001] and response [Greenhouse-Geisser adjusted F (2, 62) = 30.758]. Post hoc testing (Games-Howell α = .05; Kirk, 1995 p. 147) indicated that RT for the M1M2 and M2 were significantly slower than controls. The two-way interactions response x treatment [Greenhouse-Geisser adjusted F (8, 62) = 2.515, p = .024] and session x response [Greenhouse-Geisser adjusted F (2, 69) = 24.221, p < .0001] were also significant. One-way ANOVAs at each response collapsed on the treatment factor indicated that there was no effect on response one switching from a well

learned 5 response sequence to a novel sequence [F (1, 39) < 1] but significant effect for responses 2-5. Response two was faster for all groups in the novel sequence than the well learned sequence but the most dramatic change was demonstrated in the final three elements of the novel sequence. All groups were significantly [all ps < .0001] slower on the final three elements of the new five response sequence than the well learned sequence. This provides convincing evidence that the benefit in RT performance associated with learning a sequence of motor responses is demonstrated by improvements in later responses in the motor sequence.

Response initiation, characterized as the reaction time for the initial response in a sequence was predicted to be affected by demands on motor planning; with longer RTs for sequences than for single responses and for repeated sequences than random sequences. The results were consistent with these predictions (Figure 11). A SPF 5.22 [treatment x response type (single response vs. initial response of a 5 response sequence) x session type (random or repeat)] ANOVA showed a main effect of treatment [F (4, 35) = 3.277, p = .022], response type [F (1, 35) = 35.131, p < .0001], and session type [F (1, 35) = 15.879, p < .0001]. Post hoc testing (Games-Howell α =.05; Kirk, 1995 p. 147) indicated that M1M2 and M2 were significantly slower than controls in speed of responding. The two-way interaction response type x session type was also significant [F (1, 35) = 33.935, p < .0001]. One-way ANOVAs examining response type across the levels of session type indicated that RTs to initiate a repeated series of responses were significantly slower for all groups [F (1, 35) = 25.721, p < .0001]

than RTs to initiate a random sequence of responses. In addition, RTs to initiate random series of responses were significantly longer [$F(1, 39) = 14.929$, $p = .0004$] than RTs to make a single response to a randomly cued port. These results are consistent with the idea that the complexity of the information contained in a motor plan directing a series of movements affects the time to begin the first response.

EXPERIMENT 2: STRIATAL LESIONS

Methods

Subjects

Subjects consisted of 43, male Long Evans rats (North Carolina) ~ seven weeks of age at the onset of behavioral training. Housing and handling protocol were identical to experiment 1.

Equipment

All equipment was identical to the equipment used in experiment 1.

Pre-surgical Behavioral Training

Training followed the same schedule and tasks as described previously. As in the first experiment once animals reached criterion on the standard VSRT task (accuracy levels > than 70% averaged across all stimulus durations) they were randomly assigned to one of five treatment conditions (dorsomedial C/Pu, dorsolateral C/Pu, ventral C/Pu, large dorsal C/Pu, and sham surgery controls) using a block randomization process.

Surgical procedure

General surgical protocol was identical to that described in Experiment 1 infusing the same neurotoxin concentration and volume at the following stereotaxic coordinates to produce lesions in striatum. Anterior-posterior (AP) locations for all lesions were relative to bregma, dorsal-ventral (DV) coordinates were relative to the interaural line (IA) and medial-lateral sites relative to midline. Each group consisted of 8 randomly assigned animals. Three additional animals

were added to the large dorsal C/Pu lesion group after losing one animal during post surgical recovery. See Table 4 for the stereotaxic coordinates for each lesion site.

Sham surgery on the controls involved the same preliminary surgical procedures as the lesion subjects without drilling the skull. Rats were monitored (post-surgical healing, weight, hydration, and species typical behavior) during the two week recovery period and then put on water restriction to begin post surgical testing.

Post-Surgical Behavioral Testing

Behavioral testing followed the same schedule as experiment 1 except for the following: some rats in the large dorsal C/Pu lesion group revisited stage-one VSRT training after demonstrating a high percentage of omissions during initial stage-two training. After completion of simple S-R learning animals completed the sequential learning task before completing the single response reaction task. The behavioral measures, used to test response accuracy, reaction time, simple S-R learning, and visuomotor sequence learning were identical to experiment 1.

Histological Procedures

At the culmination of the study rats were killed using identical procedures as experiment 1. Brains were fixed in the same manner and sectioned coronally, stained with thionin, and examined under a light microscope for verification of lesion damage.

Results: Experiment 2

Histological Analyses

NMDA lesions were characterized by glial cell proliferation neuronal loss and collapse of tissue resulting in ventricular enlargement. Bilateral lesions of the dorsal striatum affected the target areas in all rats. The data for three animals that were unable to perform any of the behavioral tasks was not included in any analyses, although their pathology was not unusual. Pathology of the remaining eight animals involved extensive gliosis and significant tissue collapse in dorsal striatum. In the largest lesions the AP extent was from + 2.45 mm to b 1.30 mm relative to bregma. Typical AP lesion extent was + 2.0 mm to -1.0 mm from bregma (Figure 12).

Bilateral dorsomedial lesions affected the target region in all cases. One animal's data excluded from analyses as an outlier did not exhibit unusual pathology. Larger lesions in this group extended from + 1.85 mm to – 0.7 mm relative to bregma. Representative lesions with more restricted AP ranges extended from + 1.40 mm to -1.0 mm from bregma. Gliosis and tissue loss was primarily confined to medial C/Pu leaving dorsolateral and ventral regions intact (Figure 12).

Dorsolateral lesions were made bilaterally in all animals. NMDA infusion in lateral C/Pu target sites spared dorsomedial and ventral regions producing neuron loss and gliosis within the immediate dorsolateral area. The maximum AP extent of the largest dorsolateral lesion was + 1.25 mm to – 1.45 mm with more typical AP ranges of + 1.70 mm to – 0.55 mm (Figure 12).

Lesions of ventral striatum consistently damaged the shell and core regions of nucleus accumbens. Infrequently some unintended damage occurred in ventral pallidum and dorsomedial C/Pu near the cannula tracks. The greatest AP extent of the ventral lesion was + 3.10 mm to – 0.65 mm with a more typical lesion range of + 2.55 mm to + 0.10 mm. Lesion damage involved neuron loss and increased glial cell concentration in the accumbens (Figure 12).

Visuospatial Reaction Time (VSRT)

Rats were tested post surgically on 10 sessions of the five-choice VSRT task. Rats with large dorsal C/Pu lesions were unable to respond consistently to the short stimulus durations and within the 3.0s response window. They were therefore eliminated from this analysis. The remaining groups were compared on accuracy and reaction time performance (Table 2). Preliminary examination of the reaction time data indicated heterogeneity of variance between the groups across the multiple tasks to be analyzed. Median RTs were calculated and used in statistical tests to reduce the variability of the reaction time data.

Response accuracy for each group improved as stimulus duration increased. This result was confirmed in a SPF4.6 treatment (medial, lateral, ventral, and control) x stimulus duration (.05s, .11s, .26s, .58s, 1.33s, and 3.0s) ANOVA (Kirk, 1995 p. 515). The main effect for treatment [$F(3, 26) = 9.301$, $p = .0002$], stimulus duration [Greenhouse-Geisser adjusted $F(3, 68) = 857.77$, $p < .0001$] and the interaction [$F(8, 68) = 3.589$, $p = .002$] were all statistically significant. One-way ANOVAs at each stimulus duration indicated that there were no differences in response accuracy between lesion groups and controls at the brief

(.05s and .11s) or long (3.0s) stimulus durations ps > .05. However at all remaining durations, (Games - Howell α =.05) post hoc analyses identified the medial group as significantly less accurate than the control group (Figure 13).

Examining choice reaction time indicated that as stimulus duration increased reaction time improved for all treatment levels. In addition, the dorsomedial lesion group was slower to respond to cued ports than controls (Figure 14). These results were supported in a SPF4.6 (treatment x stimulus duration) ANOVA (Kirk, 1995 p. 515). The main effects of treatment [$F(3, 26) = 5.560$, $p = .004$] and stimulus duration [Greenhouse-Geisser adjusted $F(2, 52) = 79.422$, $p < .0001$] were both significant. The interaction was not $F < 1.0$. Post hoc testing (Games - Howell α =.05) did not identify any group as significantly different in response time from controls.

The dorsomedial group was also slower than controls traversing the runway (Figure 15). A one-way ANOVA yielded a significant treatment effect $F(3, 26) = 6.548$, $p = .002$. Post hoc (Games - Howell α =.05) testing confirmed that the dorsomedial lesion group was considerably slower than controls traveling the length of the runway arm. In summary, this measure of visuospatial responding indicates that lesions involving dorsomedial striatum impair response accuracy to illuminated ports. At brief durations this deficit is mitigated by a floor effect. In addition, these lesions also impair general motor RT and RT when a choice among alternating response locations is required.

Short-Term Simple S-R Learning

All groups were highly accurate and showed little change across trials responding to the 3.0s stimuli, thus these data were not further analyzed. Accuracy performance for the brief stimulus cue was examined using a SPF4.4 (treatment x stimulus presentation) ANOVA (Kirk, 1995 p. 515). There were significant treatment [$F(3, 26) = 3.253$, $p = .038$], and stimulus presentation main effects [Greenhouse-Geisser adjusted $F(2, 38) = 34.533$, $p < .0001$] with no interaction $F < 1.0$. Post hoc testing (Games - Howell $\alpha = .05$) indicated that accuracy scores of the dorsomedial C/Pu group were significantly poorer than all other groups. The lack of an interaction is consistent with the similar improvement in accuracy performance observed for all groups (Figure 16).

The dorsomedial group was consistently slower to respond to the cued port across brief stimulus presentations. A SPF4.4 (treatment x stimulus presentation) ANOVA (Kirk, 1995 p. 515) supported this finding (Figure 17). There was a significant main effect of treatment [$F(3, 26) = 3.188$, $p = .040$], and no effect of stimulus presentation or the interaction [p values >.2]. The dorsomedial group demonstrated the slowest RT although post hoc testing (Games-Howell $\alpha = .05$) did not indicate significant group differences. On this measure of simple S-R learning all groups demonstrated similar improvements in response accuracy across the presentations of a brief stimulus cue.

Long-Term Simple S-R Learning

Rats with large dorsal striatal lesions were able to complete this task and thus were included in the analysis. Rats in the medial and M & L lesion groups

tended to respond more slowly (Figure 18). A 5.2 treatment (M & L, medial, lateral, ventral and control) x session (random or repeat port) ANOVA (Kirk, 1995 p. 515) demonstrated a main effect of treatment [$F(4, 32) = 14.247$, $p < .0001$]. Post hoc testing (Games - Howell $\alpha = .05$) showed that the M & L group responded significantly slower to the cued port than the control, lateral and ventral groups. The main effect of session was significant [$F(1, 32) = 4.892$, $p = .034$] as was the session x treatment interaction [$F(4, 32)$ 2.730, $p = .046$].

Sequential Visuomotor Learning

All groups took more time to complete repeat than random sequence trials. This trend was exacerbated by dorsomedial C/Pu lesions (Figure 19). These trends were confirmed in a SPF5.25 (treatment x sequence type x response) ANOVA (Kirk, 1995 p. 553) of the median RTs for the five-response repeat (A10) and random sequence (R3) sessions (Table 5). The main effect of treatment and response were significant [$F(4, 33) = 9.754$, and $F(4, 132) = 28.683$, $ps < .0001$] as were all two-way interactions response x treatment, sequence type x treatment, and response x sequence type [$F(16, 132) = 3.151$, $p = .0001$; $F(4, 33) = 3.352$, $p = .0208$; and $F(4, 132) = 11.431$, $p < .0001$, respectively]. The three-way interaction (response x sequence type x treatment) was also significant [$F(16, 132) = 2.929$, $p = .0004$]. Separate two-way ANOVAs (SPF 5.2 treatment x sequence type) at each response indicated interaction effects for responses 1 and 2 [$F(4, 33) = 3.364$, $p = .0205$ and $F(4, 33) = 3.129$, $p = .0275$]. One-way ANOVA's indicated that the control group [$F(1, 7) = 16.748$, $p = .0046$] showed significantly faster RTs for the initial response of the random sequence.

Rats with large dorsal striatal lesions were also markedly faster performing the first response of a random sequence [F (1, 7) = 5.285, p = .0551 compared to the first response of a repeat sequence. For response 2, One-way ANOVA's did not indicate significant effects of treatment although the large dorsal striatal group demonstrated a slowing trend for repeat sessions that approached significance (p = .0978). Responses 3 - 5 showed significant main effects of treatment and for responses 3 & 5 sequence type but no treatment interactions. Examination of the means indicates that, for all groups, response 3 and 5 are faster in repeat sessions than random sessions. In summary, all groups showed an increase in RT for the initial response of a learned repeating sequence and a decrease in RT for the fifth response in repeating sequences. Rats with lesions involving dorsomedial striatum demonstrated greater absolute RT deficits when initiating the first elements of a repeating sequence compared to all other groups and to random sequence sessions (Figures 20 & 21).

Short-term visuomotor sequence learning was examined across blocks of trials in the initial random and repeat sequence sessions. The slow motor responding of the large dorsal striatal lesion group resulted in completion of approximately 15-25 trials per session, therefore their data were not included in the analysis. Examination of RTs across blocks indicated that within the initial repeating session RTs for responses 1 and 2 increased across successive blocks (Figure 22). In contrast, for responses 3-5 RTs decreased across the blocks. A different response pattern was generated across the blocks of a random sequence. RTs for response one increased steadily across the blocks.

All other responses showed no major change in RT across the blocks of the random session (Figure 23). A SPF4.255 (treatment x session x response x block) ANOVA (Kirk, 1995 p. 562) indicated this difference in response RTs for the separate sequence types (Table 7). The four way interaction was significant [Greenhouse-Geisser adjusted $F(20, 166) = 2.125$, $p = .006$] as was the three-way interaction session x response x block [Greenhouse-Geisser adjusted $F(7, 166) = 4.418$, $p < .0001$].

These effects were further analyzed in separate SPF4.55 ANOVAs (Kirk, 1995 p. 553). Results for repeating sessions indicated no main effect of group [$F(3, 26) = 2.633$, $p = .071$] but the two-way interaction of response x block [$F(5, 129) = 11.562$, $p < .0001$] was statistically significant (Table 8). The significant three-way interaction response x block x group [$F(15, 129) = 3.032$, $p < .0001$] reflected a significant group x block interaction for response one only [$F(12, 104) = 4.547$, $p < .0001$]. The medial group demonstrated a dramatic increase in RT for the final block of response 1. For all other responses group x block did not interact indicating that the main difference between the dorsomedial group and other groups was the increased cost to initiate the repeating sequence. This effect was most striking at the end of the session when the sequence was most familiar (Figure 22). The increasing RT trend in the first response was uniform across all groups including controls supporting a performance cost when a learned sequence of motor responses was generated.

An analysis of the RT block data for the random sequence session confirmed the development across blocks of the response pattern characteristic of random

sequence sessions (Figure 23). The results of a SPF4.55 (treatment x response x block) ANOVA (Kirk, 1995 p. 553) indicated a significant main effect of treatment [$F(3, 26) = 4.424$, $p = .012$] response [Greenhouse-Geisser adjusted $F(2, 35) = 37.106$), $p < .0001$] and a response x block interaction [Greenhouse-Geisser adjusted $F(6, 152) = 3.487$, $p = .003$]. Post hoc testing (Games - Howell $\alpha = .05$) did not identify any significant differences among the groups. The response x block interaction was explained in separate SPF5.5 ANOVAs at each response (Kirk, 1995 p. 515). The effect of block was only significant for the first response. Examination of the mean table indicated an increasing trend across the blocks of response one. There is no significant effect of block on the remaining responses in the random sequence comparing graphs of each sequence type across blocks highlights the gradual shift from very similar response patterns in the initial block of the two sessions to a distinct response pattern indicative of the random vs. repeat sequence. Consistent across the two response patterns is an increase in RT for the first response across the blocks of the session.

Long-term visuomotor sequence learning was demonstrated by all groups in the gradual improvement in RT for the final element of the repeat sequence and by comparison with random sequences. An analysis [SPF5.105 (treatment x session x response) ANOVA (Kirk, 1995 p. 553)] of all repeating sessions of the A sequence showed significant main effects of treatment [$F(4, 33) = 11.209$, $p < .0001$], and response [$F(6, 48) = 41.195$, $p < .0001$]. The effect of session was not significant [Greenhouse-Geisser adjusted $F(4, 130) = 2.272$, $p = .066$]. All

two-way interactions were significant with Greenhouse-Geisser adjusted Fs (ps < .035). The three-way interaction was not significant [Greenhouse-Geisser adjusted F (25, 207) = 1.494, p = .069] (Table 6). This nearly significant interaction may reflect the greater impairment of the dorsomedial lesion groups initiating a repeating sequence. This impairment increased across the sessions in the first repeating block before peaking in the second block and showing some reduction in RT. All other groups showed a smaller increase in RT across the sessions in the first block (Figures 20 & 21).

Retention of a learned sequential motor response should result in stable or improved RTs when the learned sequence is again tested. Comparison of the RT of each response from session A5 to A6, between which a random testing session occurred, indicated that RTs for most responses remained relatively stable across the two sessions for all groups consistent with retention of visuomotor sequence learning.

Memory for sequence specific information has been demonstrated to improve reaction time especially for the later elements of the motor program. To the extent that RT improvement was sequence specific and not general mastery of the response task, then switching to a novel motor sequence was expected to impair RTs for later response elements compared to the original sequence. Comparison of response RTs for the final learned sequence session (A10) and the initial session of a novel sequence (B1) confirmed this result (Figures 20 & 21). Responses 3-5 show increased RTs after switching to the novel motor sequence. A SPF5.25 (treatment x session x response) ANOVA (Kirk, 1995 p.

553) examining the shift to the novel sequence indicated significant main effects of treatment and response [F (4, 33) = 7.40, and F (3, 68) = 22.080, ps < .0001, respectively]. The two-way interactions (treatment x response and response x session) were also statistically significant [Greenhouse-Geisser adjusted Fs (9, 68) = 2.748, p = .011 and F (3, 75) = 7.299 p = .001, respectively]. Post hoc testing (Games - Howell α .05) confirmed that complete dorsal striatal lesions slowed RT responding to a series of cued ports.

Examining the mean of each response across sessions indicated that the response x session interaction reflected an obvious improvement in mean RT for responses 1 and 2 of the new sequence and a marked slowing in RT for the remaining elements of the new sequence (Figures 20 & 21). Failure of the three-way interaction to reach significance confirmed that this pattern was similar among all groups. These findings indicate that the savings in RT demonstrated in the final elements of a well-learned sequence of visuomotor responses does not transfer to a novel visuomotor response sequence. In addition the cost (slower RTs) to initiate a well-learned motor plan is greater than the cost to initiate a novel motor plan. These findings supported previously reported effects comparing the final learned sequence session (A10) with the random session that followed (R3); faster RTs for the initial elements when switched to the random sequence and slower RTs for later elements.

Motor initiation was examined by comparing single port response RTs in the long-term simple S-R task to the initial RTs of five response sequences. To the extent that more complex motor planning is necessary to respond to a series of

ports than to a single port we expected the initial RT of the series of responses to be impaired. All groups demonstrated slower RTs beginning a repeat sequence than for initiating a random sequence. In addition, all groups demonstrated slower RTs for response 1 of the random sequence sessions than for the single response repeat session (Figure 24). The SPF5.22 [treatment x response type (single response / sequence learning) x session (random / repeat)] ANOVA conducted on the RT data supported these conclusions (Kirk, 1995 p. 553). All main effects and interactions were significant (Table 9). Previous analysis on the single response dataset demonstrated that, although all groups had marginally slower RTs on single repeating port trials than on single random port trials this difference was not statistically significant. One-way ANOVAs for each group across random and repeating sequence sessions indicated that all groups, excluding the dorsolateral lesion group, were significantly slower on the first response of a repeating sequence [significant ps < .0393]. In addition, all groups were significantly slower [all ps <.05] for response 1 of the random sequence session than for the single response repeat session.

Discussion

Effects of Frontal Cortical Lesions

Cortical lesions had significant effects on the RT but not the accuracy of choice responses in the VSRT task. In general, when cues were brief, all groups demonstrated poorer performance. Although cortical lesion groups tended to be less accurate than controls at shorter stimulus durations these differences were not statistically significant. Runway RT was unaffected by cortical lesions. This

suggests that the increase observed for choice RT was not the result of a general motor impairment. Runway RT began when the rat depressed the lever and concluded when the photocell at the chamber end of the arm was crossed. Choice RT began from the time the arm photocell was crossed until the photocell at the illuminated port was broken. Both responses were initiated by the rat's behavior and involved similar travel distances. Choice responding required rats to modify responses on every trial based upon the location of the luminance cue. Thus the effects of the cortical lesions are consistent with a specific impairment in sensory-guided responding. Although all groups exhibited a similar pattern of impairment, post hoc testing showed that only the M2 group was significantly slower than controls.

Previous findings in our lab have shown that lesions of mPF or M2 cortex affect response accuracy for shorter duration stimuli and increase choice RT (Burk & Mair, 2001; Bailey & Mair, 2004). In the present study there were non-significant trends toward impairment in accuracy and significant effects on choice RT consistent with these findings. The more limited effects of lesions may reflect the smaller sizes of the lesions in the present study.

Short-term simple S-R learning tested the ability of rats to learn the location of a reinforcer held constant for seven consecutive trials. Lesions of frontal cortex did not affect the rate at which accuracy improved for the brief luminance cues presented on trials 1, 3, 5, and 7. There was no effect on RT for any group. Thus, none of the cortical lesions affected the ability of rats to take advantage of short-term consistencies in stimulus location. There were non-significant trends for all

lesion groups to respond more slowly than controls consistent with results for the VSRT task. The analyses of short-term S-R learning showed improvement in accuracy in relatively few trials.

Long-term S-R learning was measured by comparing RTs for responses to 3.0s luminance cues when the location of the reinforced port varied randomly to when it remained constant at one location for 5 sessions. All groups demonstrated significantly faster RTs when the location of the port remained constant. Although rats with lesions of frontal cortex tended to have longer RTs for both types of session, they resembled controls in the extent to which they benefited when the location of the reinforced port was held constant. Thus frontal lesions did not appear to affect either of these measures of short- or long-term simple S-R learning.

Stimulus-guided motor responding presupposes a series of neuronal events linking the stimulus cue and an appropriate response needed to achieve the objective. These events have been termed motor planning or motor programming. Marsden (1984) describes motor planning as a process of selecting and ordering smaller motor sub-plans; that precedes the initiation and then the execution of the action occasioned by the plan. Convergent evidence from single unit recording and pharmacological manipulation studies with monkeys; lesion experiments with rodents; and human brain imaging studies have implicated the mPF and pre-motor regions in the preparation of motor responses (Sakai et al., 1999; Nakamura et al. 1999; Crammond & Kalaska,

2000; Weinrich & Wise, 1982; Averbeck, Chaffee, Crowe, & Georgopoulos, 2002; Jenkins et al., 1994; Delatour & Gisquet-Verrier, 2001)

To the extent that visually-guided responding depends upon motor planning, the findings of Henry & Rogers (1960) suggest that response latency for movement initiation increases as the number of movements performed increased (as cited in Sternberg, Monsell, Knoll, & Wright, 1978). Similarly, Sternberg et al. (1978) found that the latency initiating the first of a series of typing keystrokes increased linearly as the number of letters in the sequence increased. They termed this the sequence length effect. Sternberg argued that this effect reflected an increase in motor planning necessary to efficiently perform the series of responses.

In keeping with this premise, a sequence of nose pokes would generate a more complex motor plan than a single nose-poke. Similarly, as more information is encoded into a motor plan (e.g. a series of fixed response locations in a specific order) the plan should become increasingly complex. Long-term simple S-R learning showed that lesions of frontal cortex had no effect on the latency to initiate single port response. All groups were responded significantly faster on repeating than random port trials. Responding faster for repeating trials is consistent with the idea that predictability of response location improves response speed. It is inconsistent with the notion that creating a more complex motor plan, including information about response location, impedes response initiation. An alternative interpretation is that the random port task requires scanning ports for a luminance cue, increasing latency even of the simple motor

plan while more efficient orienting of attention in trials that cue the same port could reduce response latency of a more complex motor plan.

The results of the sequence learning tasks are consistent with Sternberg's hypothesis. All lesion groups took longer moving from the arm photocell to the port indicated by a signal light, when well practiced in responding to that light, as the first of a sequence of five rather than as a single port response (as in VSRT or in long-term simple S-R learning). The increase in initial responding was exacerbated in repeated sequence learning when rats performed the same sequence on every trial (Fig. 11). This increase seems paradoxical when considered in terms of motor learning. Practice responding to the same port at the start of every sequence should serve to decrease RT. However, learning the specific sequence that will be executed should increase the amount of information that must be processed at the motor-planning stage. Thus the increase in completing the first element of a repeating sequence seems in keeping with increased demands on motor planning. By this argument, the relatively constant RT of controls initiating single or multi-port responses can be taken as evidence of a relatively intact capacity for motor planning that is not taxed by the demands of the 5-port response sequence.

Differences in delay to reinforcement provide an alternative explanation for the increase in RT initiating the 5-port response sequence. For single port response tasks (VSRT, simple S-R learning) rats received reinforcement immediately after making a single port response. Although the initial response in sequence learning tasks was comparable in other aspects, reinforcement was

not delivered until after the last (fifth) response in the sequence was completed. Spence (1956) argued that delay of reinforcement adversely affects learning response reinforcer associations by allowing intervening behavior to become associated with the reinforcer. Renner (1963) and Sgro et al. (1967) found that rats traversed an alley more slowly if the food reward was delayed 3 – 30s. Logan & Spanier (1970) found a smaller effect on performance when water was the primary reinforcer (c.f. MacKintosh, 1974).

The present sequence learning tasks differed in important ways from the tasks in which increased delay to reinforcement was associated with increased RT. The initial nose pokes were followed immediately by consistent stimulus events (the port light turning off and the light in the next port turning on) that may have served as a secondary or conditioned reinforcer predicting the primary reinforcer. Further, the interval between the initial nose poke and the primary reinforcer was bridged by a series of nose pokes, each of which were followed by the same potential secondary reinforcer. Thus the present task involved a consistent chain of responses linked to the primary reinforcer that would have provided little opportunity for other intervening behaviors to become associated with the primary reinforcer.

Lesions of mPF and motor cortex slowed initiation of a series of responses, yet on the final four responses of each sequence, performance of the lesion groups was comparable to controls. This is important for several reasons. First, normal RT performance for later responses in the sequence suggests that, latency to initiate the first response is not indicative of a general deficit in

voluntary motor function, motivation for reinforcement, or a perceptual impairment. Second, all groups exhibited consistent improvement in RT for later responses in repeated sequences both within (Figure 9) and between (Figure 8) sessions. Improved RT for later sequence elements was a unique characteristic of learned sequences. Responses 3 – 5 of random sequence sessions slow consistently with each successive response whereas responses 3 - 5 of repeat sequence sessions are faster than the RTs of the initial two responses (Fig 8). Improvement in meeting the general demands of the task was evidenced by faster RTs for all responses in both sequence types across sessions; however this effect had greater impact on later responses of repeat sequences. This suggests that sequence specific learning, consistent with executing a learned motor sequence was unaffected by cortical lesions.

Comparison With Previous Experimental Research

There is a paucity of comparative literature examining the effect of frontal cortical lesions on motor sequence learning in rats. However, several studies have explored the effects of cortical lesions on accuracy of responding and motor preparation in reaction time tasks. Convergent evidence from our lab and others indicate that large, excitotoxic mPF lesions impair response accuracy on VSRT type tasks, especially when luminance cue durations are brief (Burk & Mair, 2001; Bailey & Mair, 2004; Passetti et al., 2002; Muir, Everitt & Robbins, 1996). Muir et al. (1996) characterized the accuracy deficit associated with mPF damage as a disruption of attentional processes and the response latency deficit associated with motor cortex lesions in their study as an impairment of decisional

processes. Trends towards accuracy and RT deficits in the VSRT and short-term simple S-R tasks of the current study are consistent with a role for mPF in attentional processing.

In a study examining motor planning, rats with complete mPF lesions and lesions limited to ventral mPF (prelimbic-infralimbic) exhibited disrupted motor readiness in a reaction time task (Risterucci, Terramorsi, Nieoullon, & Amalric, 2003). Another study found that unilateral lesions of AGm (M2) increased SRT response latency bilaterally (Brown, Bowman & Robbins, 1991). Findings from the present study support a role for mPF and motor cortex in increased response latency particularly for lesions involving M2.

The role of the prefrontal and motor cortices on motor sequence acquisition and retention has been extensively studied in monkeys and humans using versions of a (2 x 5) trial and error sequential button pressing task described earlier. Recording and reversible inactivation studies with monkeys, and scanning studies on humans, have consistently identified the presupplementary motor area (pre-SMA) and its projection areas in striatum as critical for performing new motor sequences (Nakamura et al. 1999; Sakai et al. 1999; Hikosaka et al. 1999, 2002 for reviews). Activity in the supplementary motor area (SMA) and its projection areas in striatum have been associated with performance of new and well-learned button press sequences (Ackermann et al., 1996; and see Hikosaka et al. 1999, 2002 for reviews). In the rat, M2 (AGm) has been identified as containing areas, analogous to the primate SMA, premotor cortex, and frontal eye fields, although these are not well differentiated within M2

(McGeorge & Faull, 1989). In the current sequence learning task lesions of M2 did not disrupt acquisition or execution of a new motor response sequence, however, it did increase RT for the initial motor response of new (random) and learned sequences suggesting a disruption in planning or activating the learned response sequence.

Examining the cognitive demands presumed to maintain performance on trial and error sequence learning and incidental SRT motor sequence learning tasks may explain the inconsistent results. Trial and error (2 x 5 hyperset task) learning involves working memory processes and sustained attention to maintain each of the correct pairs of responses on line while working out subsequent response pairs. This process should encourage forming motor sub-programs that would be subject to ongoing modification as movement errors are corrected and appropriate responses remembered. Early response pairs would be combined with later pairs until the final motor plan was determined (Sakai, Kitaguchi, & Hikosaka, 2003). To the extent that monitoring prior response information and incorporating new response information depends upon pre-SMA functioning then learning new sequences would be expected to be disrupted. Some support for this view comes from another study of human motor sequence learning. Subjects performing long continuous sequences of finger presses (12-element sequence) were found to subdivide the sequence into 'chunks" or smaller sub-sequences. Disrupting pre-SMA activity using repetitive transcranial magnetic stimulation (rTMS) produced RT slowing when each sub-sequence was initiated, but not when performing the 1st response of the overall sequence (Kennerley, Sakai, &

Rushworth, 2003). The (2 x 5) trial and error learning task naturally divides the ten-element sequence into 5 sub-sequences. Pre-SMA activity appears to correlate with initiating and monitoring the order of response pairs. In contrast, in the present SRT task rats complete a short sequence of visually guided responses. Training the sequence as a relatively short five-response series may not be conducive to subdividing the sequence into sub-sequences. This would suggest that, in rats, intact mPF and motor cortical functioning is important for initiating a sequence of visually-guided motor responses especially when the sequence is well-learned. The ability to form a motor plan and execute the sequence once initiated seems independent of these cortical regions.

The Effect of Ventral, Dorsomedial, Dorsolateral or Complete Dorsal Striatal Lesions on Visuomotor Performance

The present study confirmed previous research suggesting a role for dorsal striatum in stimulus-guided motor responding (Brasted et al., 1998; Mair et al. 2002). Large dorsal, dorsomedial and to a lesser extent dorsolateral C/Pu lesions increased RTs for all tasks (VSRT, short-term simple S-R, long-term simple S-R, and sequence tasks). Paradoxically, the ventral lesion group exhibited shorter RTs than controls for most of these tasks. Dorsomedial lesions also produced deficits in stimulus-guided response accuracy (VSRT and short-term simple S-R tasks). Interestingly, reduced accuracy for rats with dorsomedial lesions did not translate into an impairment learning the new S-R association indicated by improved accuracy in simple short-term S-R learning.

Two factors are important to point out about the present S-R learning tasks. First, rats were well trained on the primary S-R strategy; respond to the lighted port, prior to surgery. Therefore, unlike other S-R learning tasks which require learning a new S-R strategy; for instance, respond only to lit maze arms for reinforcement (McDonald & White, 1993) this task required rats to apply a well-established strategy while learning to respond to a specific location reinforced on immediately preceding trials. Learning-set paradigms share a common feature with this S-R learning task specifically; animals maintain a basic expectation about task events (associability of a class of stimuli with reinforcement) and map them onto novel stimuli. In this task the basic expectation is that the luminance cue signifies the S+ port; this is mapped onto a port location that is modified based on response history. Acquisition of the new contingency may reflect contributions of mPF cortex in orienting attention towards the response location more consistently, or an ability to anticipate the alternating nature of the stimulus duration which could enhance detection of brief (.05) luminance cues. Although dorsal striatum may be essential for learning certain S-R strategies, like associating a previously neutral class of stimuli with a specific response, spared performance of all lesion groups on this simple S-R task demonstrates an ability to modify responding based on learned associations (location of recently reinforced responses).

The basal ganglia have been implicated in motor sequence learning although it remains unclear what specific aspects of the process are striatal dependent. We tested the effect of basal ganglia lesions on motor sequence

learning in the rat-SRT task. In accordance with experiment 1 results, all groups failed to demonstrate motor sequence learning when overall RT for random and repeating sequences was compared. Groups failed to demonstrate the characteristic increase in RTs when switched from a learned sequence to a random sequence. In fact, rats with lesions involving dorsomedial striatum demonstrated faster RTs completing random sequences (Fig. 20). These preliminary findings appear to confirm previous reports that in rats dorsal C/Pu is critical for motor sequence learning (Christie & Dalrymple- Alford, 2004; DeCoteau & Kesner, 2000).

Further examination of separate response RTs, for both sequence types, revealed the same unique RT pattern development of random and repeat sequences found in experiment 1 (compare figures 8 and 22). A sequence of five responses produced increased RTs for the first response of the sequence (compared to initiating a single response) and an even larger increase when the response initiated a learned sequence. This pattern was evident across blocks in the initial session (figures 23 and 24) and across sessions (figures 20 and 21). Rats from all groups demonstrated faster RTs for the final response of repeat sequence sessions compared to the final response of random sequence sessions.

Particularly surprising was the performance of rats with lesions compromising the entire dorsal caudoputamen. Although dramatically slowed in their RT to the ports; rats with combined dorsal medial and lateral lesions exhibited response patterns similar to controls for the final four responses of random and learned

sequences. Dorsomedial lesions produced an intermediate level of slowing but again the response pattern for the two sequence types was comparable to control rats. These results provide evidence that rats with lesions of the caudoputamen are able to demonstrate reaction time improvements, across sessions of repeating sequences of motor responses, consistent with motor sequence learning.

Examination of individual response RTs also elucidated the impact of generating the first response of a learned sequence on the increase seen in the time to perform the overall sequence. This is particularly evident in the groups with dorsomedial C/Pu damage, where the increase in RT performing the entire learned sequence was almost entirely explained by the latency for the first response (figure 21). This suggests that rats with dorsomedial C/Pu damage, display impaired motor speed evidenced by consistently slower RT performance for all responses; however the greatest effect appears to involve a disruption in initiating a learned sequence of motor responses. In contrast, when initiating single-port responses, (long-term simple S-R learning) although rats with lesions involving dorsomedial C/Pu were slower than controls initiating the response this did not increase if the response location was predictable (same port on every trial). This suggest that it is not the repetitious nature of the response location that increases initiation time but rather the complexity of the motor events to follow that slows the first response of the series.

Accuracy of responding to a luminance cue is a factor that could affect response speed. In the current study cortical lesions had no effect on response

accuracy; but rats with dorsomedial C/Pu lesions demonstrated accuracy deficits in the VSRT and short-term simple S-R tasks. These were significant even at long (3.0s) durations. It is reasonable to expect that this would also affect RT to a cued port in the sequence task. It would not be expected to differentially slow the first response of a 5-poke series. It would also not be expected to slow the initial response to a predictable port (learned sequence) more than the initial response to an unpredictable port (random sequence). In fact, when the duration of the luminance cue was fixed (3.0s), as it was in long-term simple S-R (random) sessions, rats with lesions involving dorsomedial C/Pu responded at 90 % accuracy. When location was also fixed as in the long-term simple S-R (repeat) sessions accuracy was greater than 99 %. This would suggest that the accuracy deficits demonstrated by rats with dorsomedial lesions in VSRT may reflect a deficit involving divided attention (efficient monitoring of all five ports) and cue detection. Learned sequence sessions presented a fixed series of response locations in conjunction with a stable cue duration which should have minimized response accuracy deficits mitigating effects on response latency.

Comparison With Previous Experimental Research

Several studies have confirmed a role for the basal ganglia in reaction time performance. Brown & Robbins (1989) found that medial C/Pu lesions in rats increased latency to initiate nose poke responses in a visual reaction time task without affecting motor execution, while lateral lesions created an ipsilateral response bias without affecting response initiation or execution. Brasted et al. (1998) demonstrated that complete unilateral striatal lesions produced deficits

initiating contralateral responses that did not significantly slow response execution in a nine-hole SRT chamber. Prior research in our lab indicated that rats with dorsolateral C/Pu lesions and, to a lesser extent, dorsomedial lesions are impaired making visually guided responses in a 7-hole nose poke chamber (Mair et al., 2002). Findings from the present study are consistent with these results. Lesions involving dorsomedial C/Pu impaired VSRT response accuracy and increased choice RT. Dorsolateral lesions produce an intermediate level of accuracy and choice RT deficits.

DeCoteau & Kesner (2000) assessed the effect of caudoputamen lesions on motor sequence learning through a succession of maze arm entries acquired through "procedural" or "declarative" training. Rats with medial C/Pu lesion failed to exhibit learning (measured as a reduction in arm-entry response latency) the procedural version of this task but were able to retain and perform the task if trained prior to surgical lesions. In addition, medial C/Pu lesions had no effect on acquisition or retention of the declarative version of the task. Lateral C/Pu lesions had no effect on acquisition or retention of either task version.

The maze SRT task used by DeCoteau & Kesner (2000) shares some features with the rat-SRT task in the current study but also differs in fundamental ways. Both tasks use sequences that contain distinctive start and end points. The maze task reinforces (food reward) each arm of the 6 consecutive arm entries in a trial, whereas this rat-SRT task reinforces only the final (5th) response of the sequence, promoting a continuous sequence of associated motor responses uninterrupted by reinforcement factors. Decoteau & Kesner's (2000) rats

completed two trials per day whereas the rats in the current study completed 60 - 5 response trials per day. Extensive practice is one feature consistently associated with the incremental improvements demonstrated in motor sequence learning (Mishkin & Petrie, 1984; Marsden, 1984). Thus the training process utilized by DeCoteau & Kesner (2000) may not have been effective in encouraging a motor learning process.

The failure of rats with medial C/Pu lesions to demonstrate sequence learning in the maze task does not contradict the present findings. Similar to DeCoteau and Kesner's (2000) results, rats with lesions involving medial C/Pu in the current study were unable to demonstrate motor sequence learning when measured by the overall RT for the entire sequence, the measure used in the rat maze task and in human SRT performance. Results from the present study also indicated that rats failed to show increased overall RTs when switched from a repeat session to a random session. Thus it is possible that the response speed measure used in the maze task (sum of all responses in the sequence) was insensitive to the learning that might have occurred.

A very recent study incorporating visually-guided nose poke sequences in an SRT type task has also demonstrated impaired motor sequence learning in rats with dorsal C/Pu lesions (Christie & Dalrymple-Alford, 2004). The authors utilized a 4-hole, nose-poke response chamber and trained rats to perform a range of nose-pokes (4, 8 or 12 responses) in random or repeating sequences. They used RT (summed across all responses in the sequence) and error rate as the behavioral measures. Sequence learning was assessed by comparing RT for the

final 10 blocks of repeating trials with the RT for the first ten blocks of random trials. Although these tasks share some obvious characteristics (nose-poke response to a light cue, prior S-R training, sequential performance of responses) there are important differences between them.

Rats in the Christie & Dalrymple-Alford (2004) study were tested for short-term, but not long-term learning. Rats were trained in three massed sessions (one for each sequence length) equated for overall number of nose pokes. This resulted in 645 4-response (322 8-response and 215 12-response) fixed sequence trials immediately followed by 60 4-response (30 8-response and 20 12-response) random sequence trials. Therefore each session involved 2820 continuous responses. In the current study each 5-response trial had a discrete start and end point. Rats performed 60 5-response sequence trials per session, for multiple daily sessions. Prior research with humans suggests that when completing long continuous sequences of finger presses there is a tendency to break the sequence into smaller sub-sequences (Sakai, Kitaguchi, & Hikosaka, 2003; Kennerley et al, 2003). Rats with dorsomedial C/Pu lesions in the Christie & Dalrymple-Alford (2004) study were able to demonstrate sequence specific learning on the 4-response sequence (similar to our findings on the 5 response sequence) but not the 8- or 12- response sequences. If rats spontaneously chunk long sequences of responses into two or more sub-sequence, latency to initiate each learned sub-sequence could account for the failure to show motor learning (faster RTs on repeating compared to random sequences). Thus rats in the Christie & Dalrymple-Alford (2004) study were only tested for short-term learning,

and the RT measure did not separate time to initiate and execute the sequence of responses.

Another important factor to consider is the measure used to define sequence learning; interference effects or the increase in RT when switching to a random sequence from a well trained repeating sequence. It has been well established in clinical and experimental literature that one characteristic of basal ganglia dysfunction is bradykinesia. When using reaction time as the primary indicator of motor learning a failure to improve RT may indicate impairment in motor learning, however it may also reflect an inability, because of underlying motor speed deficits, to demonstrate sufficient improvement in RT to be consistent with learning. Findings from the present experiments suggest that systematic changes (increased RTs for initial elements and improved RTs for later elements) may obscure evidence of motor learning when RTs are combined for the entire sequence. Finally, Christie & Dalrymple-Alford's (2004) results exclude the initial 60 trials of each session (warm-up trials). Therefore their RT analysis leaves out the RT improvements in the initial blocks of trials. This may have had more impact on the results for the dorsal striatal group given their general motor slowing compared to intact rats.

The role of the striatum in sequence learning has also been examined with non-human primates performing a (2 x 5) trial and error sequence task. Single unit recording in the striatum identified neurons in the associative striatum (dorsomedial C/Pu in rats) that fired selectively when performing new sequences, neurons in the sensorimotor striatum (dorsolateral C/Pu in rats) that fire

preferentially for learned sequences and a third type of neuron, more prevalent in sensorimotor striatum that was non-selective, increasing activity when new or learned sequences were performed (Miyachi et al., 2002). Although rats with compromised dorsomedial C/Pu function were severely delayed in beginning a learned sequence especially in the initial sessions they were able to demonstrate improved RTs for later responses in the sequence consistent with motor learning. Impaired performance in the (2 x 5) task may require initiating several sub-sequence motor plans (Sakai et al., 2003). Findings from the present study suggest that dorsomedial striatum would play a critical role in initiating sub-sequences and thus might account for the observed deficits.

The Role of Frontal Cortical - Basal Ganglia Circuits in Motor Sequence Learning

Motor learning is characterized by incremental improvements in reaction time and response accuracy. The SRT task assesses motor sequence learning in several ways; incremental changes in RT within and between sessions and as abrupt changes in RT when switched to a random sequence or a different repeating sequence of responses. Neuropsychological studies have identified motor sequences learning deficits in clinical populations including those with basal ganglia dysfunction (Helmuth, Mayr, & Daum, 2000; Jackson, Jackson, Harrison, Henderson, & Kennard, 1994), cerebellar lesions (Shin & Ivry, 2003) focal thalamic lesions (Exner et al., 2001), pre-SMA damage (Ackermann et al., 1996; Exner et al., 2002) and prefrontal dysfunction (Beldarrain et al., 2002). Other research has specifically implicated the contribution of anatomically linked

cortico-striatal circuits in motor sequence learning (see Hikosaka et al, 1999 for a review).

Marsden (1984) contends that performing a sequence of motor responses involves several stages. A motor plan is first developed by selecting and ordering smaller sub plans. This is then followed by the initiation and then execution of the motor plan to achieve an objective. Several researchers have experimentally dissociated movement initiation from execution (Brasted et al., 1998; Brown & Robbins, 1989). The present rat-SRT paradigm was designed to isolate individual response RTs in an attempt to understand how the improvements in RT performance, indicative of motor skill learning evolve. Designing a task with distinct start and endpoints provided an opportunity to measure changes in initiating the first response of a sequence from executing subsequent elements in the series.

Response initiation measured from the time a rat entered the chamber from the arm (onset of the luminance cue) until the photo beam was broken at the first illuminated port on the far side of the chamber. Motor learning was inferred from improvements in response time. Execution was reflected in the RTs for each of the remaining nose pokes in the sequence. Motor learning specific to a learned sequence was demonstrated through improved RTs on later responses in repeated sequences. Motor learning associated with practice performing 5-poke sequences unrelated to sequence-specific information was demonstrated by patterns of responding during training with random sequences. Consistent with Sternberg's (1978) premise that sequence length increases latency, the time to

initiate responses increased when rats performed a series of 5 nose pokes and increased even more when the response began a well-learned sequence.

There are factors that may have contributed to the ability of rats with cortical and striatal lesions in the present study to demonstrate visuomotor learning. We utilized a short sequence length to test visuomotor sequence learning. Rats with dorsal striatal lesions demonstrated spared motor learning in a similar SRT task, when sequences of 4 responses were tested, but failed to show learning at longer (8 & 12 response) sequences (Christie & Dalrymple-Alford, 2004). It could be that testing rats on longer sequences would have resulted in impaired motor learning. Another factor that could have mitigated visuomotor learning deficits was the duration of training. Rats in this study were trained extensively in the sequence learning task. Motor sequence learning is exhibited as incremental improvements in RT occurring after practice with a particular task. To the extent that lesions of dorsal striatum and cortex slow the acquisition of motor learning, long-term training may serve to mitigate learning deficits. This interpretation seems inconsistent however with the incremental changes in RT performance demonstrated within the first session and across initial sessions of the repeating sequence in our study.

Nissen & Bullemer's (1987) original SRT presents a 10 or 12 element repeating sequence in a continuous flow of 100-120 responses. Normal subjects demonstrate overall RT improvements for the sequence with repeated practice and demonstrate disrupted performance when switched to a random sequence. These changes have been considered classic indicators of motor sequence

learning. Consistent with these RT changes, detailed examination of individual responses in our study indicated that improvements in RTs for later responses in the sequence are consistent with existing evidence that motor learning improves reaction time (Nissen & Bullemer, 1987; Sakai et al., 2002; Robertson et al 2001; Helmuth et al., 2000; Honda et al., 1998; Jackson et al., 1994; Christie & Dalrympl-Alford, 2004). However, this fine-grained analysis also demonstrates increased RTs for the initial response. Thus there appears to be both a cost and a benefit for RTs associated with repeated performance of a motor sequence. These results suggest that the effects of basal ganglia disease on RT measures of motor sequence learning may reflect deficits in motor planning (or response initiation) rather than in learning processes.

Results form experiments 1 and 2 suggest several characteristics of motor sequence learning. Performing a sequence of motor responses produced distinct patterns of responding depending on whether the responses occured in a random or repeated order. Repeated sequences were characterized by slower RTs for initial elements and faster RTs for final elements (compared to random sequences). These consistent changes developed incrementally both within the initial session (short-term learning) and between sessions (long-term learning). Once a sequence of motor responses was well-learned the RT improvements indicative of motor learning (improved RT for later elements) were reversed abruptly when switched to a random sequence or to a new repeated sequence.

Results from the current study indicate that lesions involving M2 and mPF regions of cortex and anatomically related areas of dorsomedial C/Pu affect

initiation but not acquisition or execution of learned sequences. These findings are consistent with clinical and experimental evidence that point to a critical role for striatum and frontal cortex in motor planning (Graybeil, 1998; Hikosaka, et al. 1999; Crammond & Kalaska, 2000; Doyon et al, 1996). Results from this study are inconsistent with clinical and experimental literatures that suggest a significant role for these cortico-striatal circuits in visuomotor sequence learning (see Hikosaka et al. 1999, Packard & Knowlton 2002 for review). The present findings support the idea that cortico-striatal circuits participate in initiation of learned sequences of visuomotor responses, however, they suggest that other neural circuits mediate the acquisition and the central representation of movements reflected in the execution of the sequence once initiated. This last point is consistent with evidence implicating parietal cortex and cortico-cerebellar circuits in these processes (Nixon & Passingham, 2000; Seidler, Purushotham, Kim, Ugurbil, Willingham, & Ashe, 2002; Lu, Hikosaka, & Miyachi, 1998; Doyon, Song, Karni, Lalonde, Adams, & Ungerleider, 2002).

SRT tasks that present a continuous repeating response cycle have no defined start or endpoint and thus prevent distinguishing response initiation and execution. They thus confound the cost to initiate the sequential response with the RT benefits of later elements apparent in the present study. Utilizing an SRT design that isolates component processes of motor sequence learning (e.g. motor planning, initiation and execution) may provide a more useful measure for distinguishing the specific contribution of cortico-striatal circuits to visuomotor sequence learning.

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APPENDIX A

Table 1.

Cortical injection sites (0.1 µl of 100mM NMDA)

Lesion	AP	ML	DV
M1	+3.2	2.6, 3.5, 4.4	1.3
	+2.2	2.4, 3.4	1.3
	+1.2	2.4	1.3
	+0.2	1.8	1.3
M2	+4.2	1.4, 2.5, 3.6	1.3
	+3.2	1.2	1.3
	+2.2	1.0	1.3
	+1.2	1.0	1.3
M1M2	+3.7	1.8, 2.8, 3.8	1.3
	+2.7	1.8, 2.8, 3.8	1.3
	+1.7	1.8, 2.8, 3.8	1.3
	+0.7	1.2, 2.2	1.3
mPF	+3.7	0.8	2.5, 3.5
	+2.7	0.8	2.3, 3.3
	+1.7	0.8	2.5
	+0.7	0.8	2.3

Coordinates were measured in millimeters with AP relative to bregma, ML sites bilateral of midline and DV measurements located below the surface of cortex.

Table 2.

Experiments 1 & 2: Summary Table of Experimental Tasks and Performance Measures

Behavioral Task	Performance Measures	
VSRT- measure of visuospatial responding	Accuracy	% correct responses / % correct + errors of commission compared across six luminance cue durations
	Choice RT	RT from crossing the arm photocell beam to the photocell break at the cued response port
	Runway RT	RT from depressing the lever to crossing the arm photocell beam.
Simple S-R Learning- assesses the ability to utilize consistency in response location to improve accuracy	Accuracy	% correct (calculated as above) compared across four 0.05 luminance cue presentations
	RT	RT from crossing the arm photocell beam to the photocell break at the (0.05s) cued response port.
Single response RT- a measure of RT to initiate a single motor response	RT	RT from crossing the arm photocell beam to the photocell break at the (3.0s) cued response port.
Visuomotor Sequence Learning – a measure of motor skill learning demonstrated through improved RT completing a repeated series of visuomotor responses	RT for entire sequence	RT from crossing the arm photocell beam to the completion of the fifth response at the cued port
	RT pattern	Comparing RT of responses 1-5 for random and repeating sequences sessions
	Long-term	Comparing RT for responses 1-5 across all sessions of the same repeating sequence
	Short term	Comparing RT for response 1-5 across blocks of trials in the initial random and repeat sequence sessions
	Sequence specific learning	Comparing changes in RT for responses 1-5 between original sequence session (A10) and a subsequent random session (R3) and when switched to a novel sequence session (B1)
	Motor initiation	RT comparison of initiating a single response and the initial response in a five response sequence

Table 3.

Experiment 1: SPF5.255 (treatment x session x response x block) ANOVA Summary Table Examining Short-term Visuomotor Sequence Learning

Source	Mean square	Df	F	p
Treatment	519.263	4	2.798*	.041
Error (treatment)	185.560	35		
Session	246.753	1	4.997*	.032
Session x treatment	104.528	4	2.117	.099
Error (session)	49.377	35		
Response	8693.16	• 2	38.258****	<.0001
Response x treatment	426.778	• 7	1.878	.097
Error (response)	227.227	• 56		
Block	129.064	• 3	7.683****	<.0001
Block x treatment	23.683	10	.827	.598
Error (block)	28.648	• 83		
Session x response	338.536	• 3	6.620**	.001
Session x response x treatme	358.071	11	.692	.731
Error (session x response)	51.135	• 89		
Session x block	11.565	4	1.799	.132
Session x block x treatment	11.565	16	.772	.715
Error (session x block)	14.978	• 113		
Response x block	331.028	• 6	12.052****	<.0001
Response x block x treatment	7.667	64	.836	.812
Error (response x block)	27.466	• 187		
Session x response x block	141.163	8	8.421****	< .0001
Session x response x block x treatment	7.992	64	1.047	.384
Error (session x response x block)	16.763	256		

Note. Bullet • indicates Greenhouse-Geisser adjusted df
*p < .05. **p < .01. ***p < .001. ****p < .0001.

Table 4.

Experiment 2: Striatal injection sites (0.1 µl of 100 mM NMDA)

Lesion	AP	ML	DV
dorsolateral	+1.7	3.0	4.0, 4.8, 5.6
	+1.0	3.4	4.0, 4.8, 5.6
	+0.3	3.8	3.4, 4.5, 5.6
	-0.4	4.2	3.4, 4.2, 5.0
dorsomedial	+1.7	2.0	4.0, 5.0, 6.0
	+1.0	2.2	4.0, 5.0, 6.0
	+0.3	2.4	3.0, 4.5, 6.0
	-0.4	2.6	4.0, 5.0, 6.0
dorsal striatum	+1.7	2.0	4.0, 5.0, 6.0
		3.0	4.0, 4.8, 5.6
	+1.0	2.2	4.0, 5.0, 6.0
		3.4	4.0, 4.8, 5.6
	+0.3	2.4	3.0, 4.5, 6.0
		3.8	3.4, 4.5, 5.6
	-0.4	2.6	4.0, 5.0, 6.0
		4.2	3.4, 4.2, 5.0
ventral	+2.7	1.0, 2.0	3.0
	+1.7	1.0, 2.0	2.0, 3.0
	+0.7	1.0, 2.0	1.8, 2.8

Coordinates were measured in millimeters with AP relative to bregma, ML sites bilateral of midline and DV located relative to the interaural line.

Table 5.

Experiment 2: SPF5.25 (treatment x sequence type x response) ANOVA Summary Table Examining Changes in RT Patterns for Random (R3) and Repeat (A10) Sequence Sessions

Source	Mean square	df	F	p
Treatment	92.983	4	9.754****	< .0001
Error (treatment)	9.533	33		
Sequence Type	5.304	1	2.900	= .098
Sequence type x treatment	6.131	4	3.352*	= .021
Error (sequence type)	1.829	33		
Response	107.353	•1	28.683****	< .0001
Response x treatment	11.793	•7	3.151**	= .005
Error (response)	239.361	•64		
Sequence Type x Response	29.007	•2	11.431****	< .0001
Sequence Type x Response x Treatment	7.432	•8	2.929**	= .007
Error (sequence type x response)	2,538	•66		

Note. Bullet • indicates Greenhouse-Geisser adjusted df
*p < .05. **p < .01. ***p < .001. ****p < .0001

Table 6.

Experiment 2: SPF5.105 (treatment x session x response) ANOVA Summary Table for Long-term Visuomotor Sequence Learning

Source	Mean square	Df	F	p
Treatment	74307.297	4	11.209****	< .0001
Error (treatment)	6629.180	33		
Session	1139.000	•4	2.272	= .066
Session x treatment	1220.678	•16	2.435**	= .003
Error (session)	501.332	•131		
Response	150646.980	•2	41.195****	< .0001
Response x treatment	14458.698	•6	3.954**	= .003
Error (response)	3656.902	•48		
Session x Response	2093.949	•7	2.289*	= .035
Session x Response x Treatment	1366.570	•25	1.494	.069
Error (session x response)	914.893	•207		

Note. Bullet • indicates Greenhouse-Geisser adjusted df
*p < .05. **p < .01. ***p < .001. ****p < .0001

Table 7.

Experiment 2: SPF5.255 (treatment x session x response x block) ANOVA Summary Table Examining Short-term Visuomotor Sequence Learning

Source	Mean square	df	F	p
Treatment	2716.746	3	3.800*	= .022
Error (treatment)	714.927	26		
Session	549.251	1	3.088	= .091
Session x treatment	155.617	3	.875	= .467
Error (session)	177.853	26		
Response	20006.056	• 2	35.847****	< .0001
Response x treatment	277.840	12	.498	= .912
Error (response)	558.097	• 44		
Block	246.550	• 3	4.127*	= .013
Block x treatment	124.006	•8	2.076	= .052
Error (block)	59.742	• 68		
Session x response	1059.052	• 3	6.404**	= .001
Session x response x treatme	106.688	12	.938	.512
Error (session x response)	165.371	• 72		
Session x block	182.829	•4	3.991**	= .009
Session x block x treatment	61.646	12	1.687	= .080
Error (session x block)	45.808	• 83		
Response x block	912.921	• 7	12.032****	< .0001
Response x block x treatment	166.840	•19	2.199**	=.005
Error (response x block)	75.873	• 158		
Session x response x block	295.840	•7	4.418****	< .0001
Session x response x block x treatment	142.292	•20	2.125**	= .006
Error (session x response x block)	66.966	•166		

Note. Bullet • indicates Greenhouse-Geisser adjusted df
*p < .05. **p < .01. ***p < .001. ****p < .0001.

Table 8.

Experiment 2
SPF5.55 (treatment x response x block) ANOVA Summary Table Examining Short-term Visuomotor Sequence Learning for the Initial Repeat Session

Source	Mean square	df	F	p
Treatment	1584.382	3	2.633	= .071
Error (treatment)	601.645	26		
Response	9513.279	• 3	21.643****	< .0001
Response x treatment	352.784	12	.803	.647
Error (response)	439.563	• 58		
Block	480.578	• 3	6.814**	= .001
Block x treatment	196.699	•8	2.789*	= .011
Error (block)	70.525	• 67		
Response x block	1255.689	• 5	11.562****	< .0001
Response x block x treatment	329.256	•15	3.032****	< .0001
Error (response x block)	108.607	• 129		

Note. Bullet • indicates Greenhouse-Geisser adjusted df
*p < .05. **p < .01. ***p < .001. ****p < .0001.

Table 9. Experiment 2

SPF5.22 (treatment x session type x response type) ANOVA Summary Table for Initiation of a Single Motor Response vs. the Initial Response in a 5-Response Sequence.

Source	Mean square	df	F	P
Treatment	5215.79	4	9.687****	< .0001
Error (treatment)	538.43	33		
Session type (single vs. seq.)	1581.71	1	18.935****	< .0001
Session type x treatment	246.13	4	2.946*	= .035
Error (session)	83.53	33		
Response type (ran. vs. rep.)	1824.69	1	20.632****	< .0001
Response type x treatment	331.89	4	3.753*	= .013
Error (response)	88.44	33		
Session type x response type	16937.63	1	50.718****	< .0001
Session x response x treatme	1911.68	4	5.724**	= .001
Error (session x response)	333.96	33		

Note. Bullet • indicates Greenhouse-Geisser adjusted df
*p < .05. **p < .01. ***p < .001. ****p < .0001

APPENDIX B

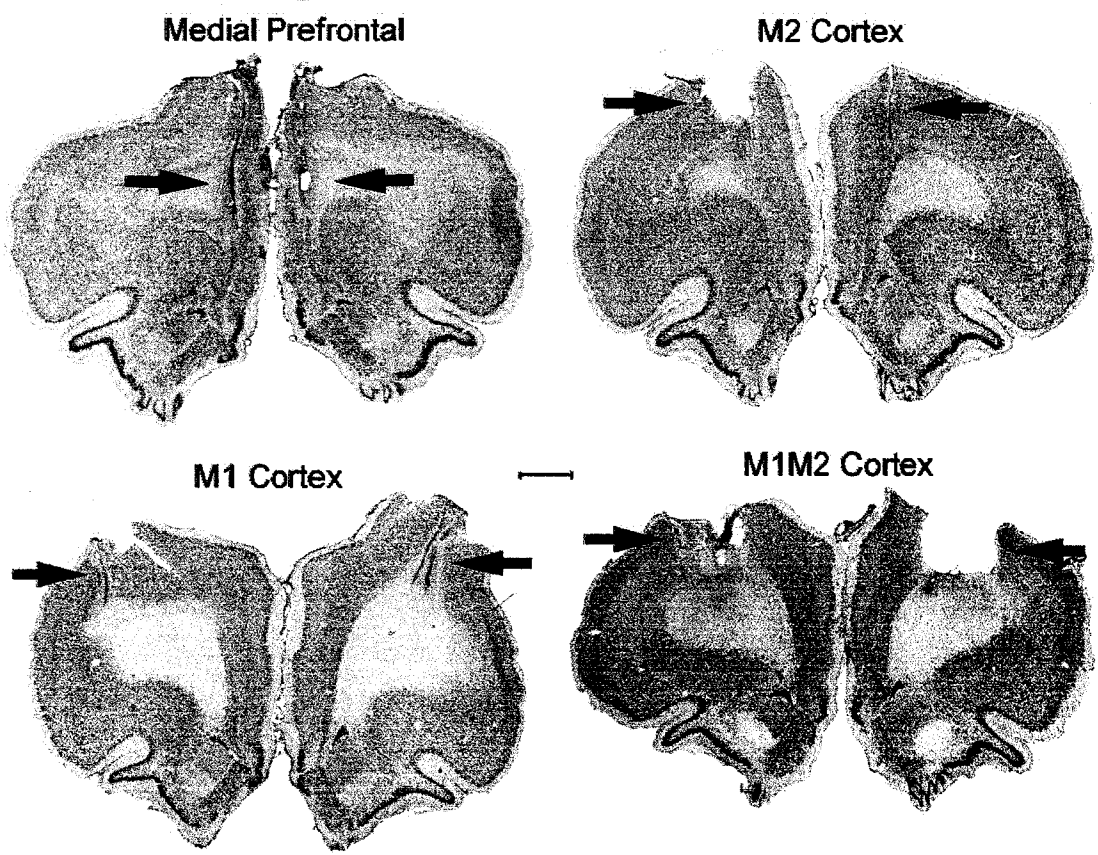


Figure 1. Photomicrographs of representative medial prefrontal (mPF) cortex, primary motor (M1) cortex, secondary motor (M2) cortex and combined primary and secondary motor cortices (M1M2) lesions with arrows indicating cortical damage in the region of interest.

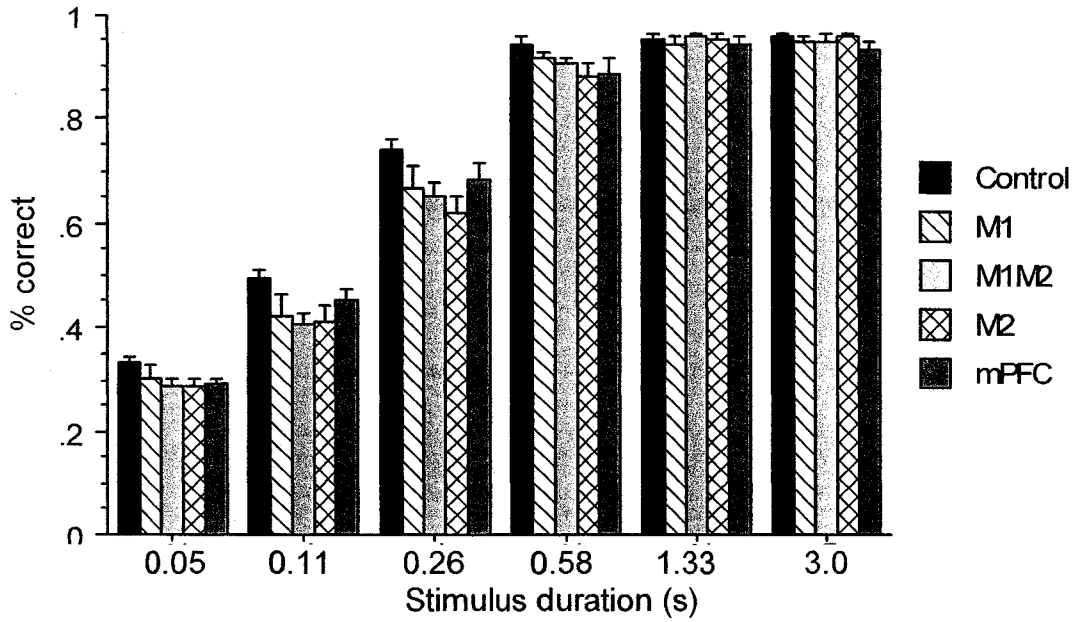


Figure 2. Effects of cortical lesions on mean percent correct (with SEM) in the visuospatial reaction time task. Cortical lesions had no significant effect on accuracy performance.

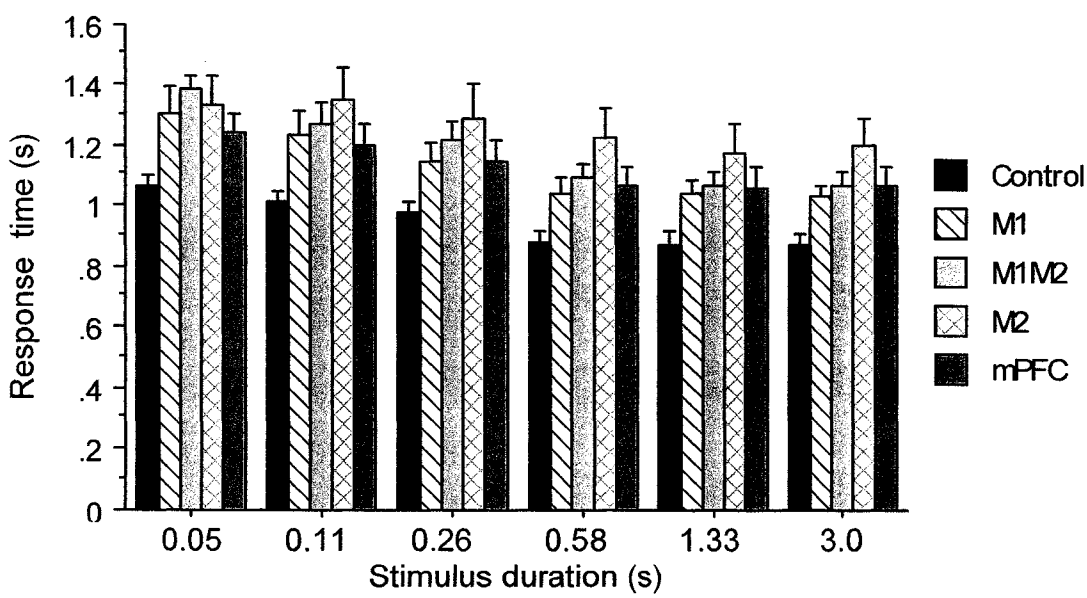


Figure 3. Effects of cortical lesions on choice response time (median ± SEM) for luminance cues of varying durations in the visuospatial reaction time task. All groups demonstrated slowing across durations compared to controls. For the M2 group this was statistically significant.

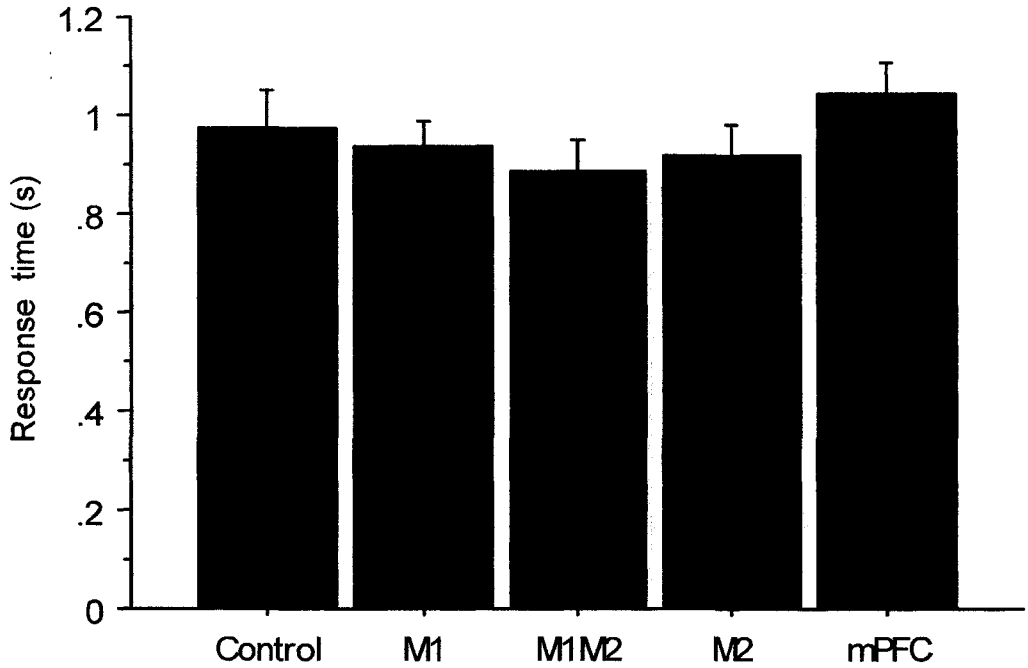


Figure 4: Effects of cortical lesions on response time (median ± SEM) traversing the runway arm in the visuospatial reaction time (VSRT) task. Cortical lesions did not significantly affect runway response time.

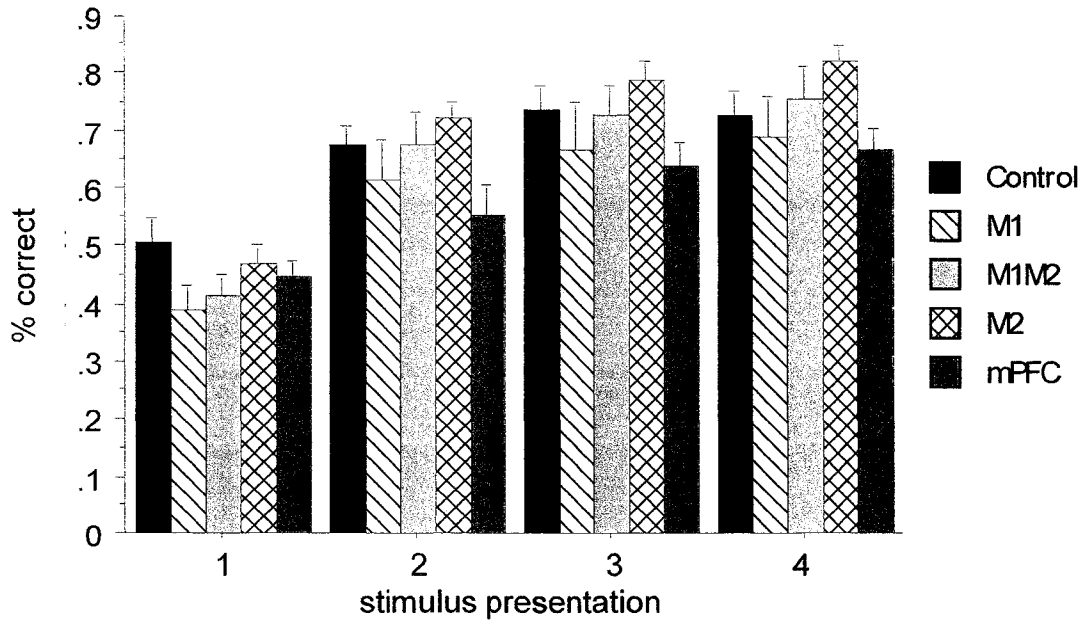


Figure 5. Effects of cortical lesions on mean percent correct (with SEM) performance across brief (0.05) luminance cue trials in the short – term simple stimulus-response (S-R) task. Cortical lesions had no significant effect on accuracy improvement compared to performance of controls.

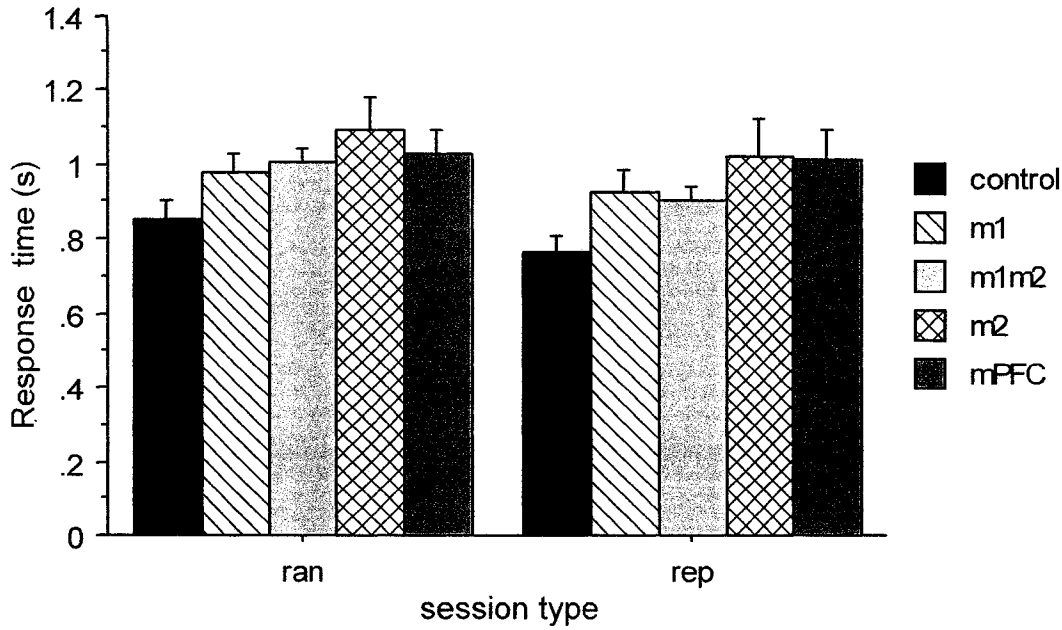
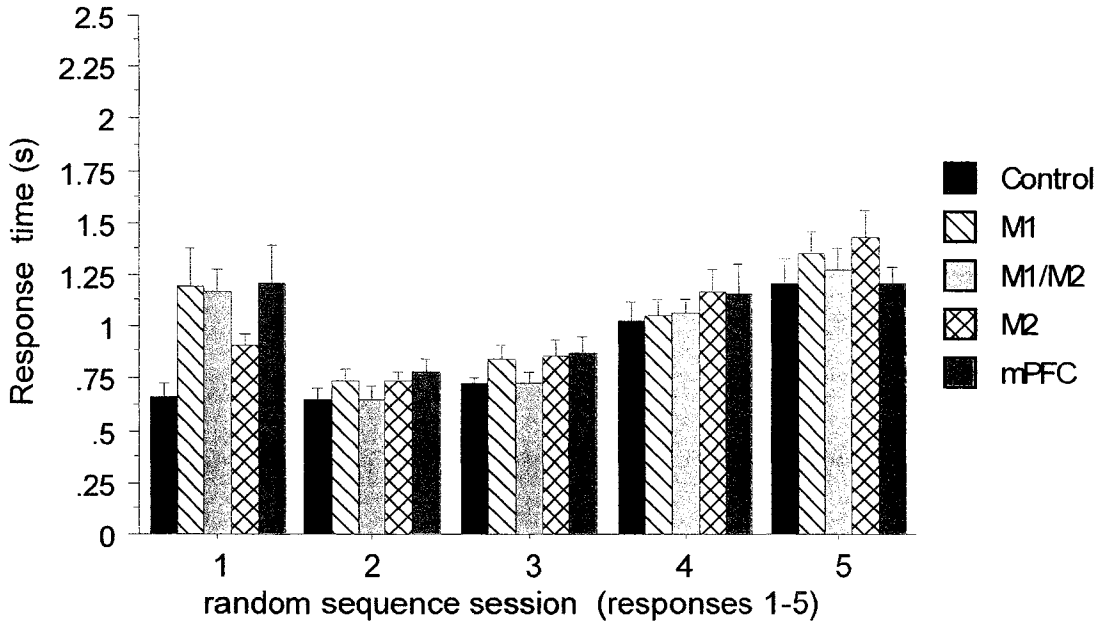


Figure 6. Effects of cortical lesions on response time (median ± SEM) in the long – term simple stimulus – response task (responding to a randomly cued port on every trial versus responding to the same cued port on every trial). Cortical lesions had no significant effect on performance.



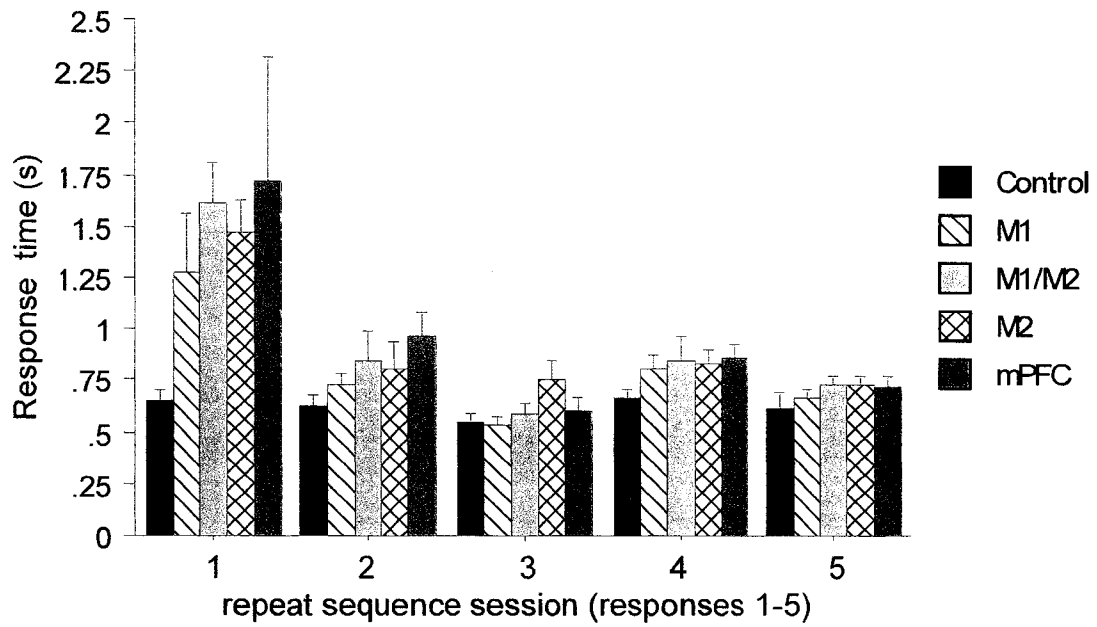


Figure 7. Effects of cortical lesions on response time (median ± SEM) performing a 5 response sequence in a random or repeating order. Cortical lesions significantly slowed the time to complete the first response of a sequence. This effect was exacerbated when performing a repeating (learned) sequence. Cortical lesions did not affect improvement in the later elements of repeating sequences consistent with motor learning.

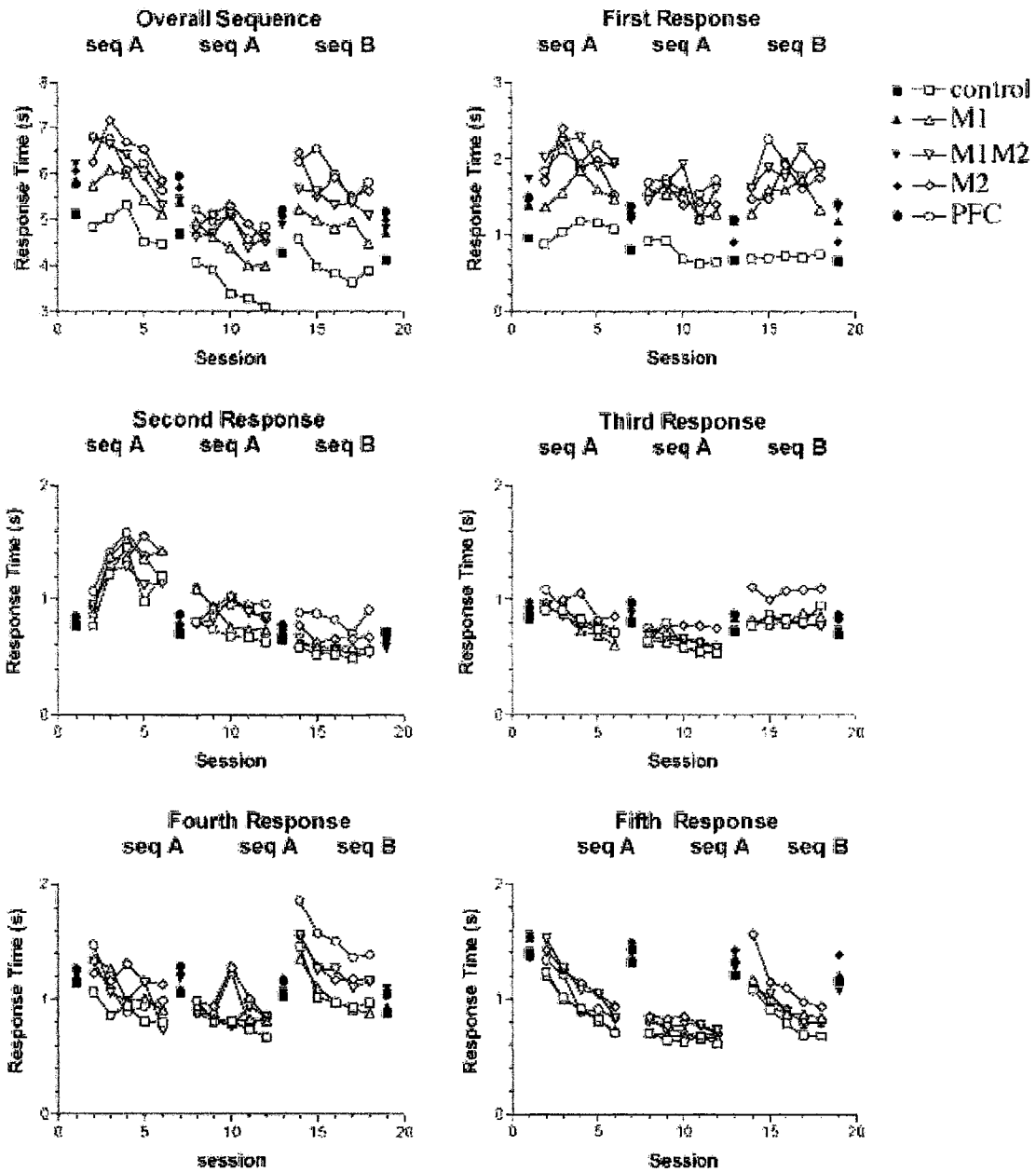


Figure 8. Effects of cortical lesions on response (median ± SEM) in the serial reaction time (SRT) task across sessions. Cortical lesions significantly impaired the time to initiate a sequence of responses but had no effect on motor learning demonstrated through faster response times on later response in the sequence. Graph 1 illustrates overall median time to complete random and repeat sequences. Graphs 2 – 6 reflect the response times completing each response within the random and repeat sequences.

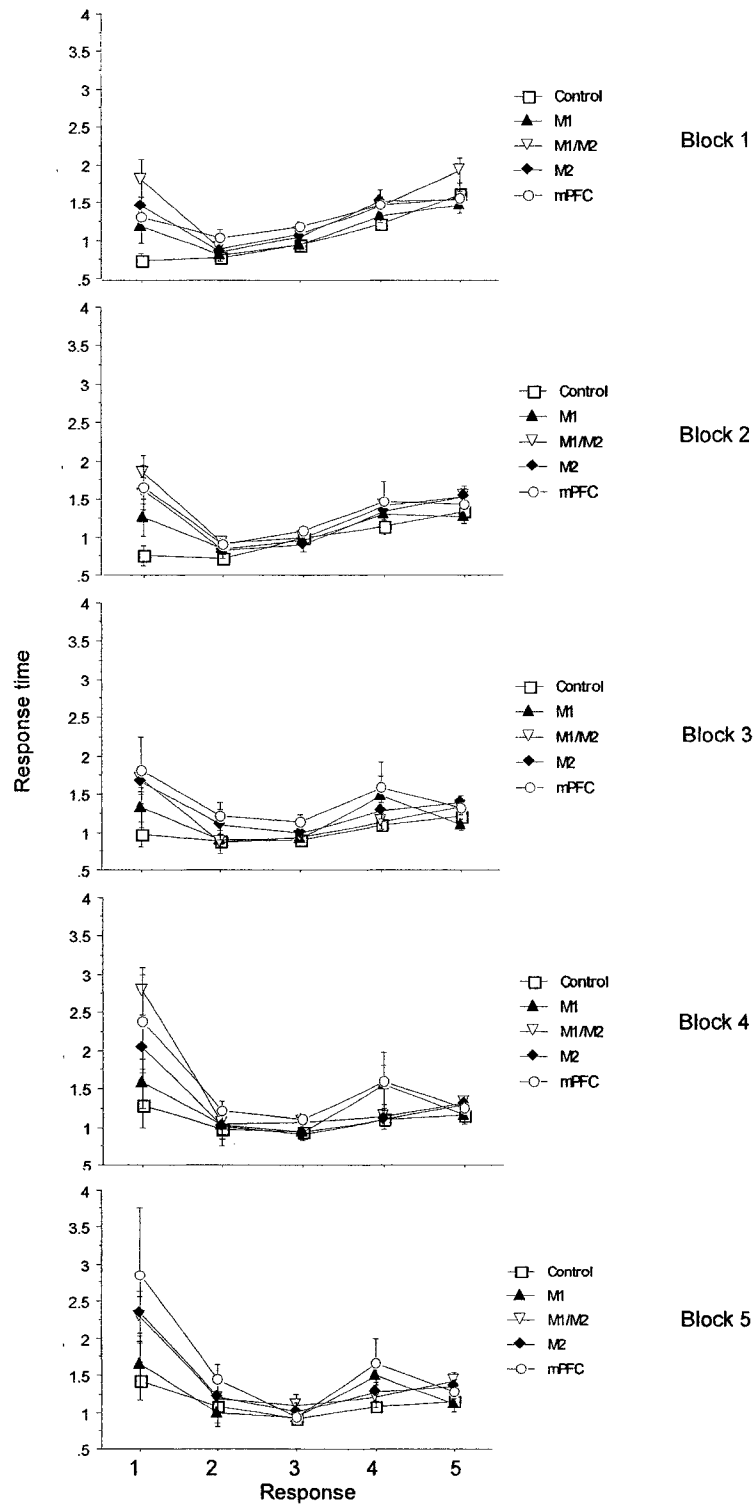


Figure 9. Effects of cortical lesions on response time (median ± SEM) for each response across 5 blocks (12 trials) within the initial repeat sequence session. All lesions significantly slowed response time for the first response but did not affect later responses

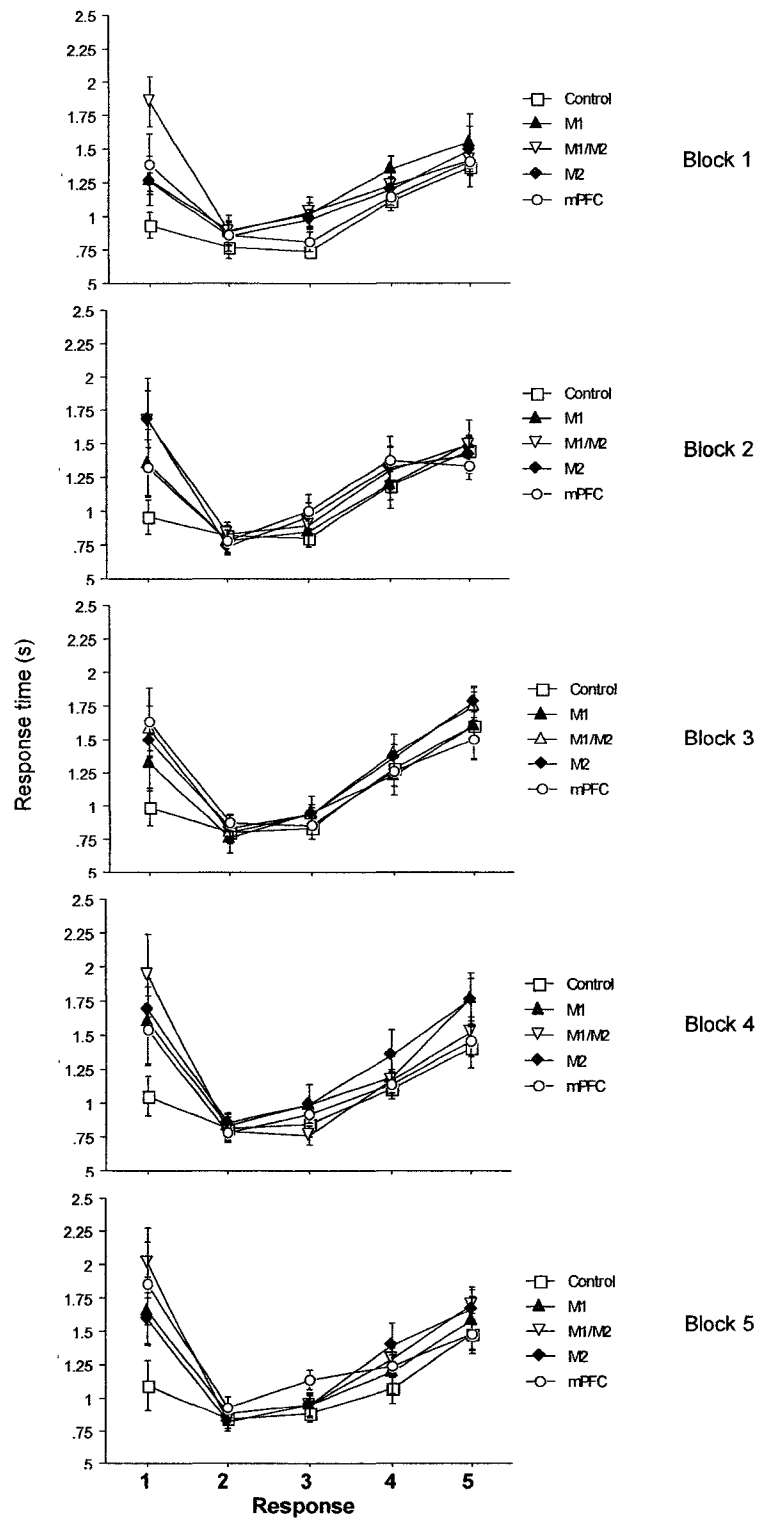


Figure 10. Effects of cortical lesions on response time (median ± SEM) for each response across 5 blocks (12 trials) within the initial random sequence session. All lesions increased response time to complete the first response but had no significant effect on later responses in the sequence

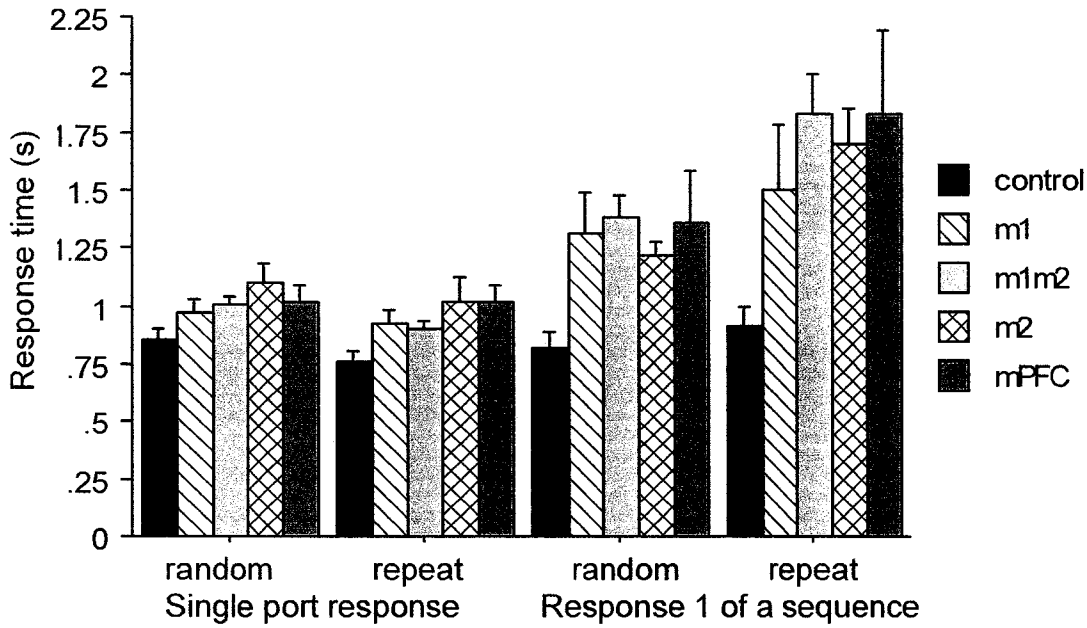


Figure 11. Effects of cortical lesions on response time (median ± SEM) initiating a single random or repeating port response (long – term simple stimulus response (S-R) task) compared to the identical motor response when it was the first response in a 5-response (random or repeating) sequence. All cortical lesions significantly slowed response time for the first response in a sequence. This effect was exaggerated for repeating (learned) sequences.

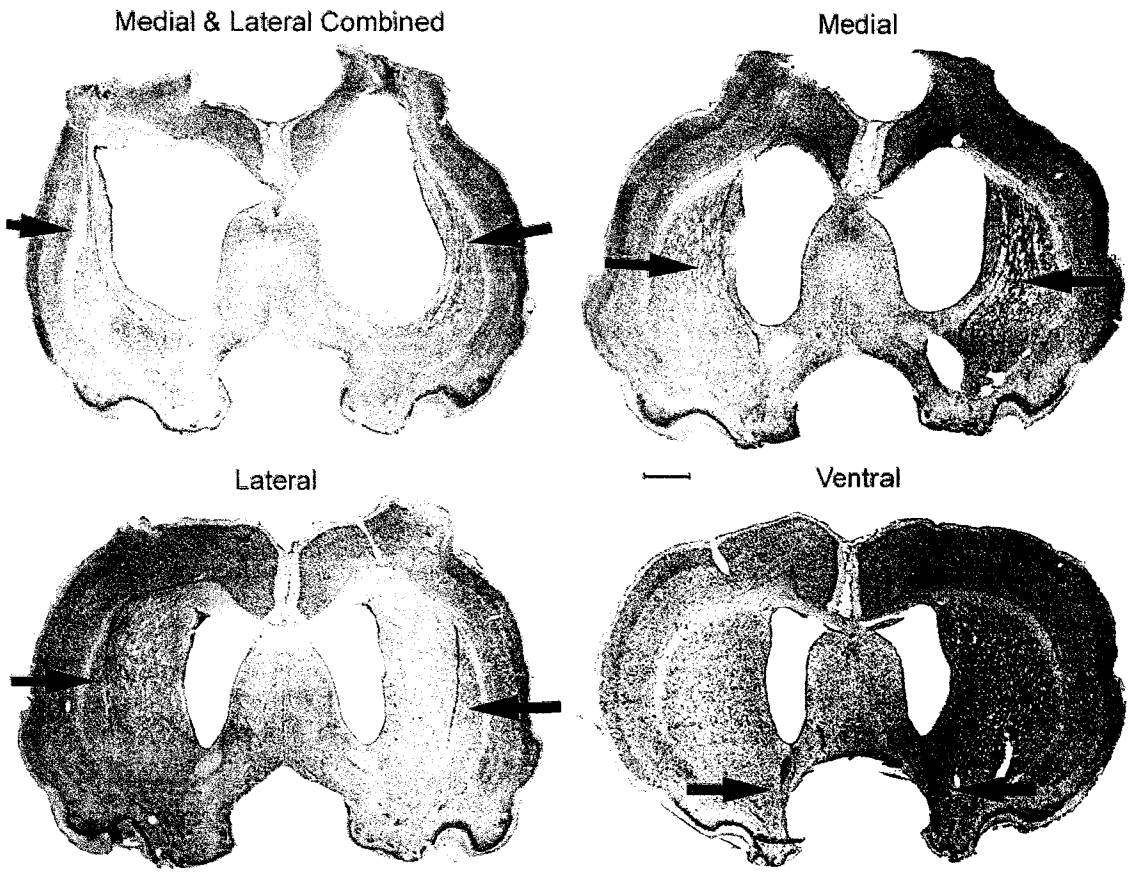


Figure 12. Photomicrographs of representative striatal lesions including: a combined dorsomedial and dorsolateral (M &L) lesion, lesions limited to dorsomedial, dorsolateral, and ventral striatum. Arrows indicate damage to the striatal regions of interest.

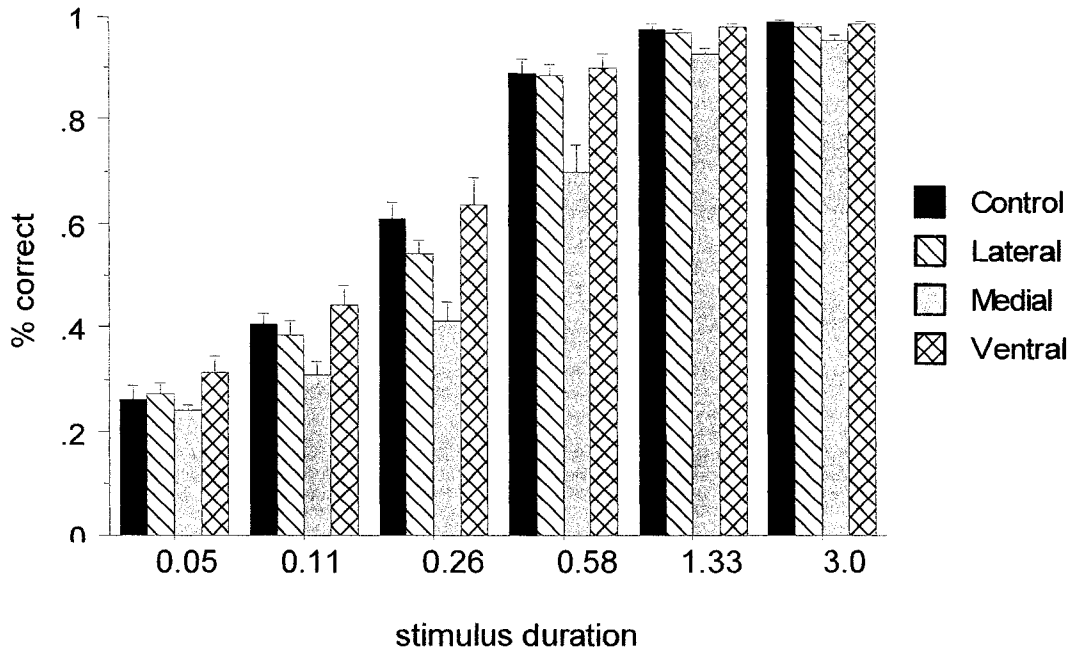


Figure 13. Effects of striatal lesions on mean percent correct (with SEM) in the visuospatial reaction time (VSRT) task. Dorsomedial lesions significantly impaired response accuracy at mid-range (0.26, 0.58, and 1.33s) stimulus durations.

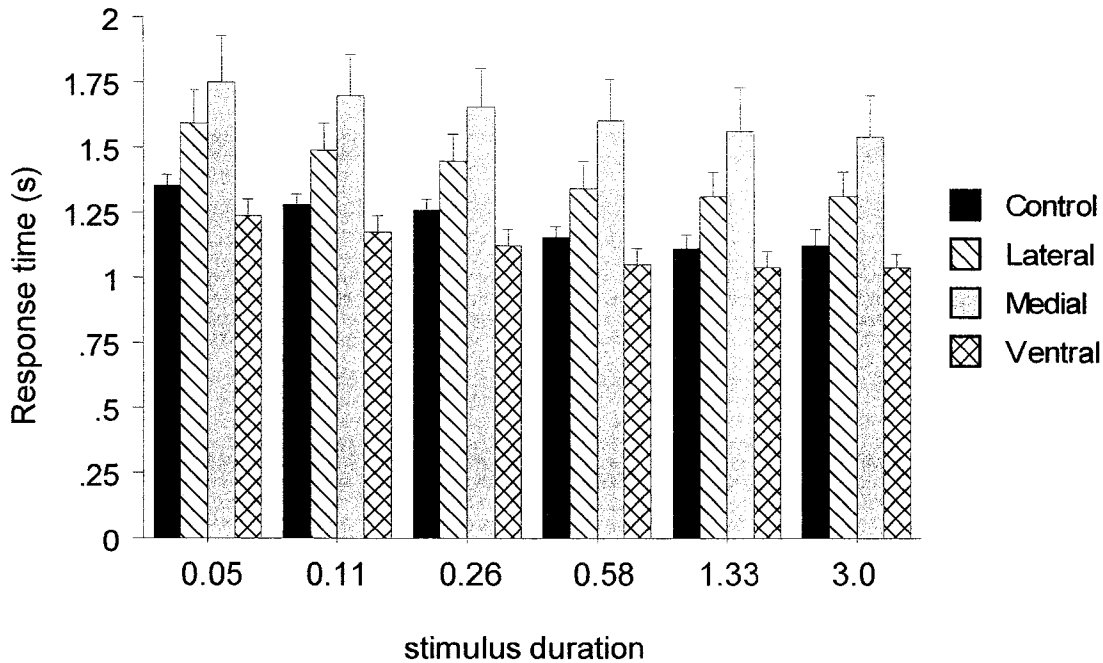


Figure 14. Effects of striatal lesions on choice response time (median ± SEM) for luminance cues of varying durations in the visuospatial reaction time (VSRT) task. Rats with dorsomedial lesions were slower responding to cued ports than controls. Dorsolateral lesions produced an intermediate level of impairment.

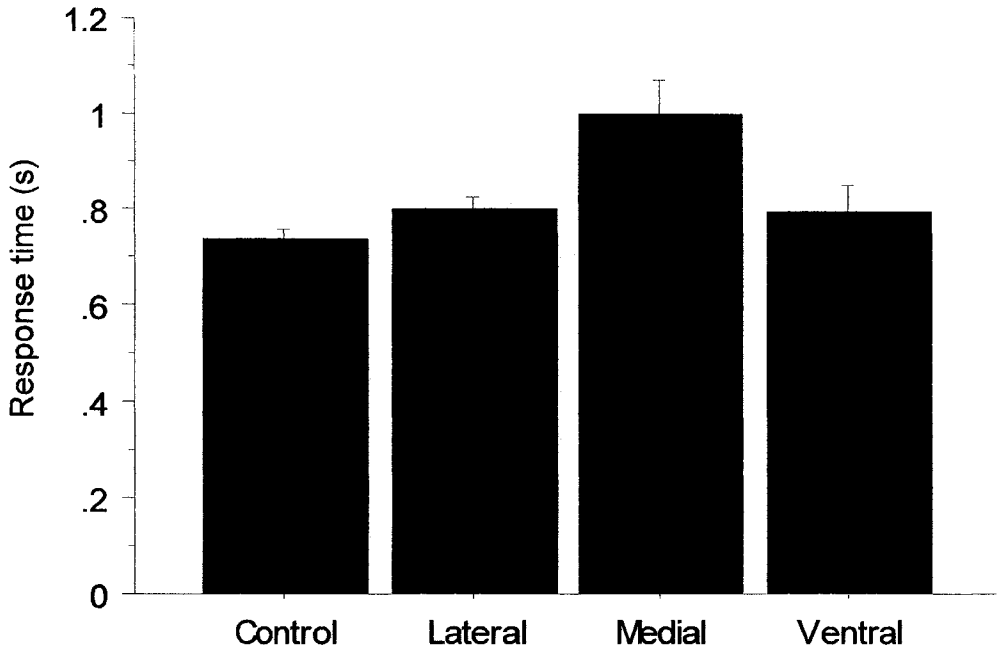


Figure 15. Effects of striatal lesions on response time (median ± SEM) traversing the runway arm in the visuospatial reaction (VSRT) time task. Dorsomedial lesions significantly slowed runway response time.

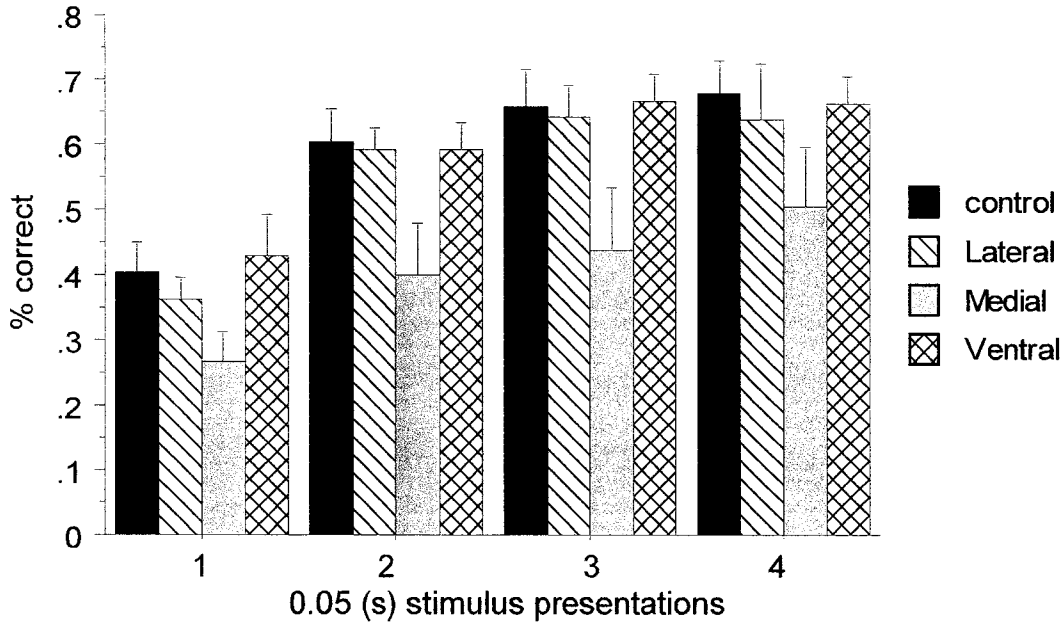


Figure 16. Effects of striatal lesions on mean percent correct performance (with SEM) across brief (0.05) luminance cue trials in the short – term simple stimulus-response (S-R) task. Striatal lesions had no significant effect on accuracy improvement compared to control group performance.

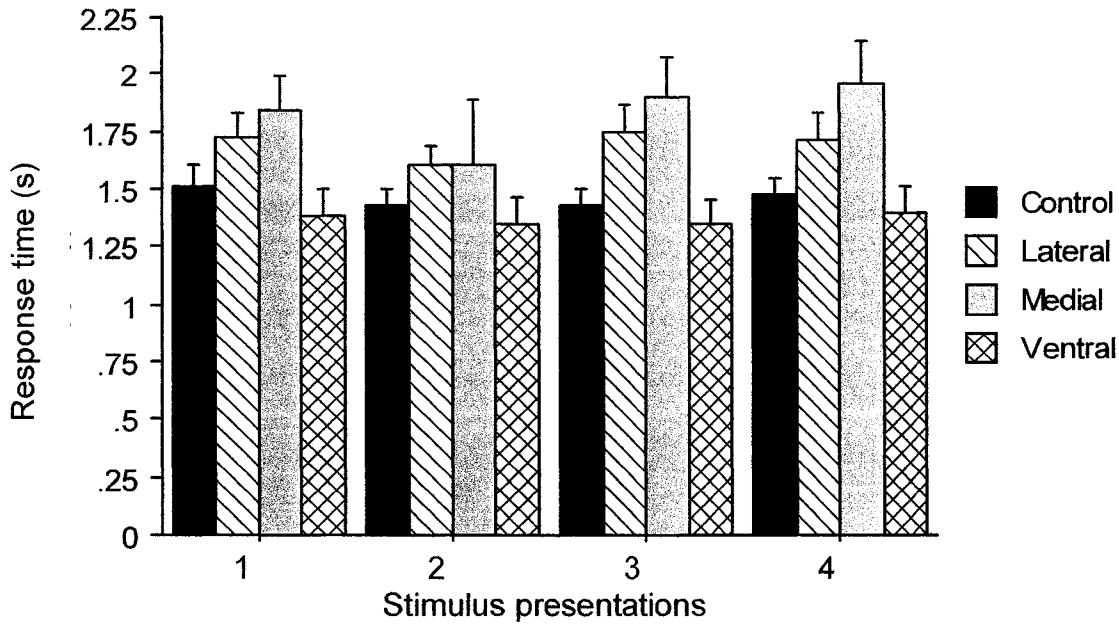


Figure 17. Effects of striatal lesion on short-term simple stimulus – response (S-R) response time performance (median ± SEM). Dorsomedial lesions significantly slowed response time. Dorsolateral lesions produced an intermediate level of slowing that was not significant.

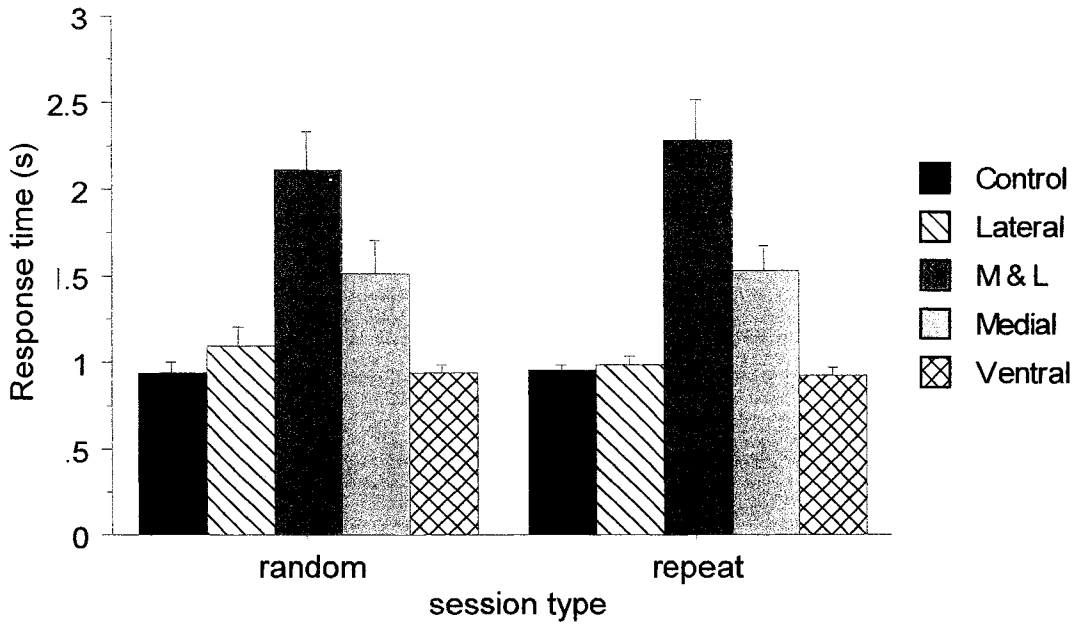
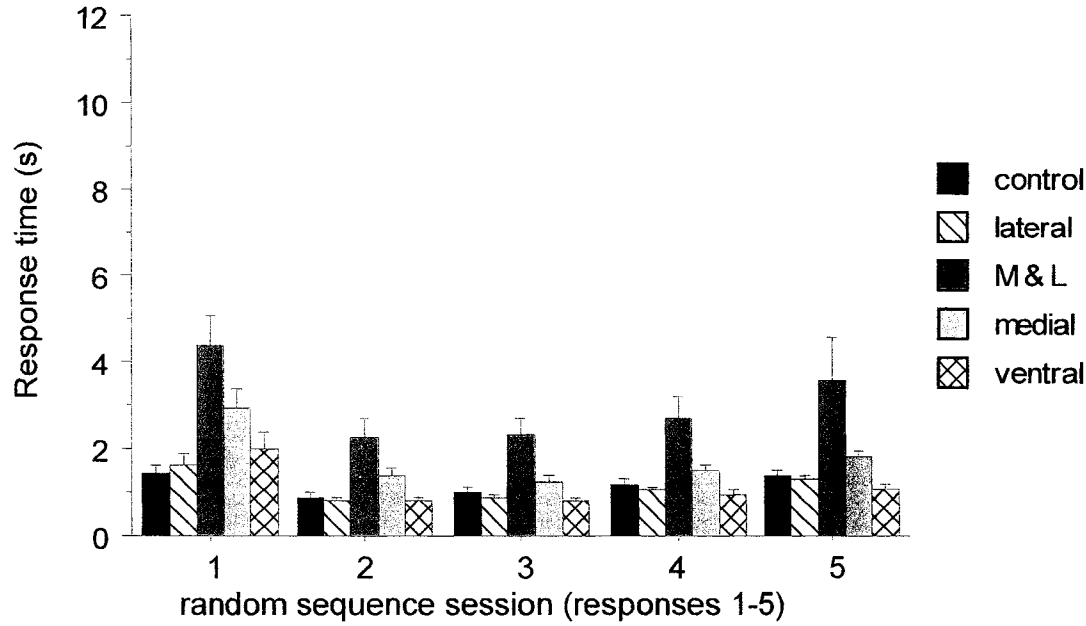


Figure 18. Effects of striatal lesions on response time (median ± SEM) in the long – term simple stimulus – response (S-R) task (responding to a randomly cued port on every trial versus responding to the same cued port on every trial). Complete dorsal striatal (M & L) lesions significantly slowed response time to the cued port across conditions. Dorsomedial lesions produced an intermediate level of slowing that was not significant.



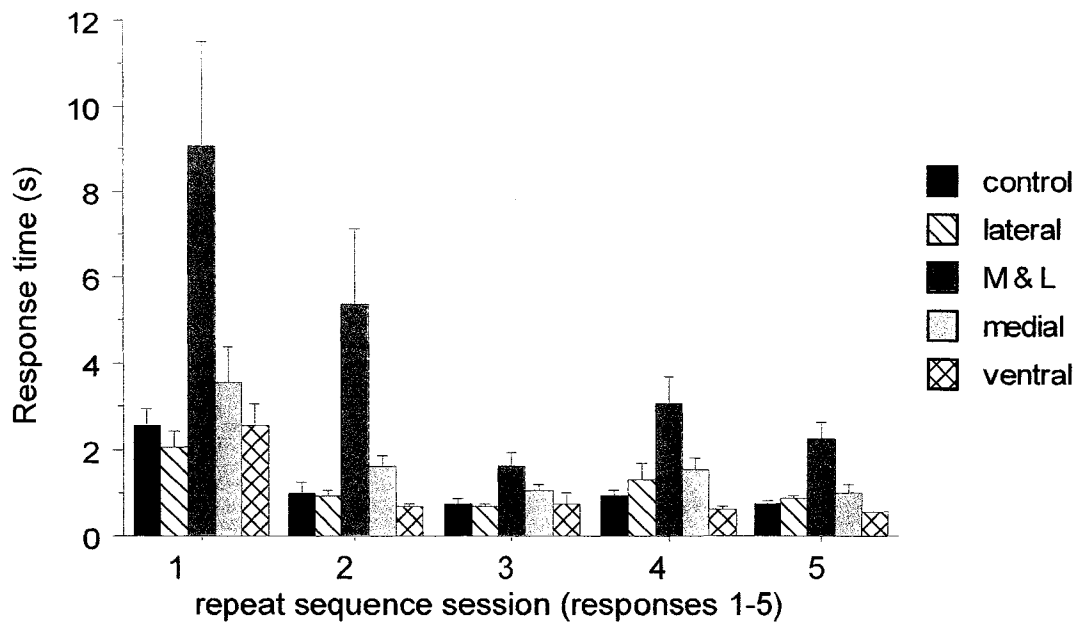


Figure 19. Effects of striatal lesions on response time (median ± SEM) performing a 5 response sequence in a random or repeating order. Complete dorsal striatal (M & L) lesions significantly slowed the time to complete the first response of a sequence. This effect was exacerbated when performing a repeating (learned) sequence. Striatal lesions did not affect the response time improvement of later elements in repeating sequences consistent with motor learning.

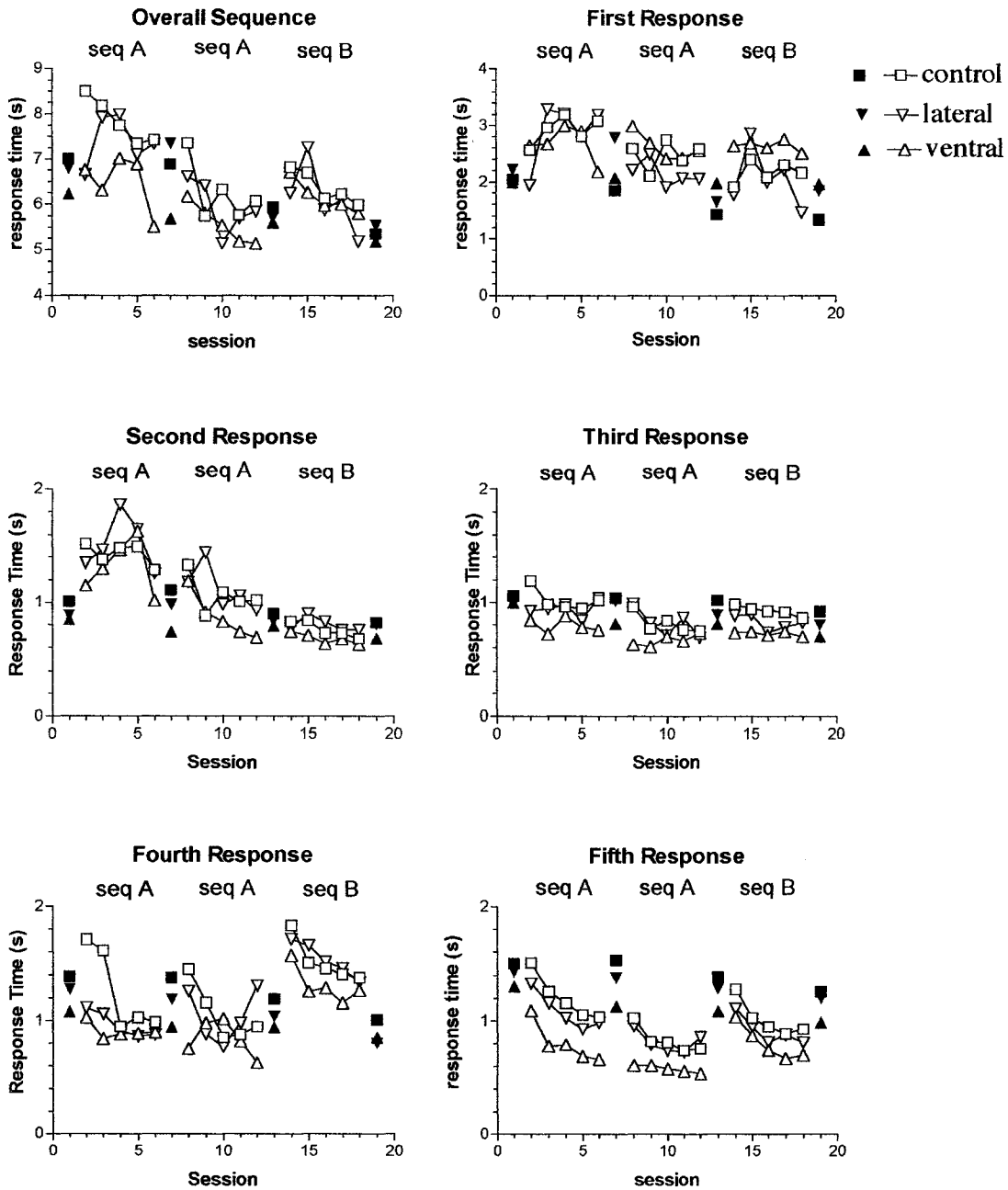


Figure 20. Effects of dorsolateral and ventral striatal lesions, across sessions, on response time (median ± SEM)) in the serial reaction time (SRT) task. Dorsolateral and ventral striatal lesions had no significant effects on SRT performance. Graph 1 illustrates overall median time to complete random and repeat sequences. Graphs 2 – 6 reflect the time to complete each response within random and repeat sequences.

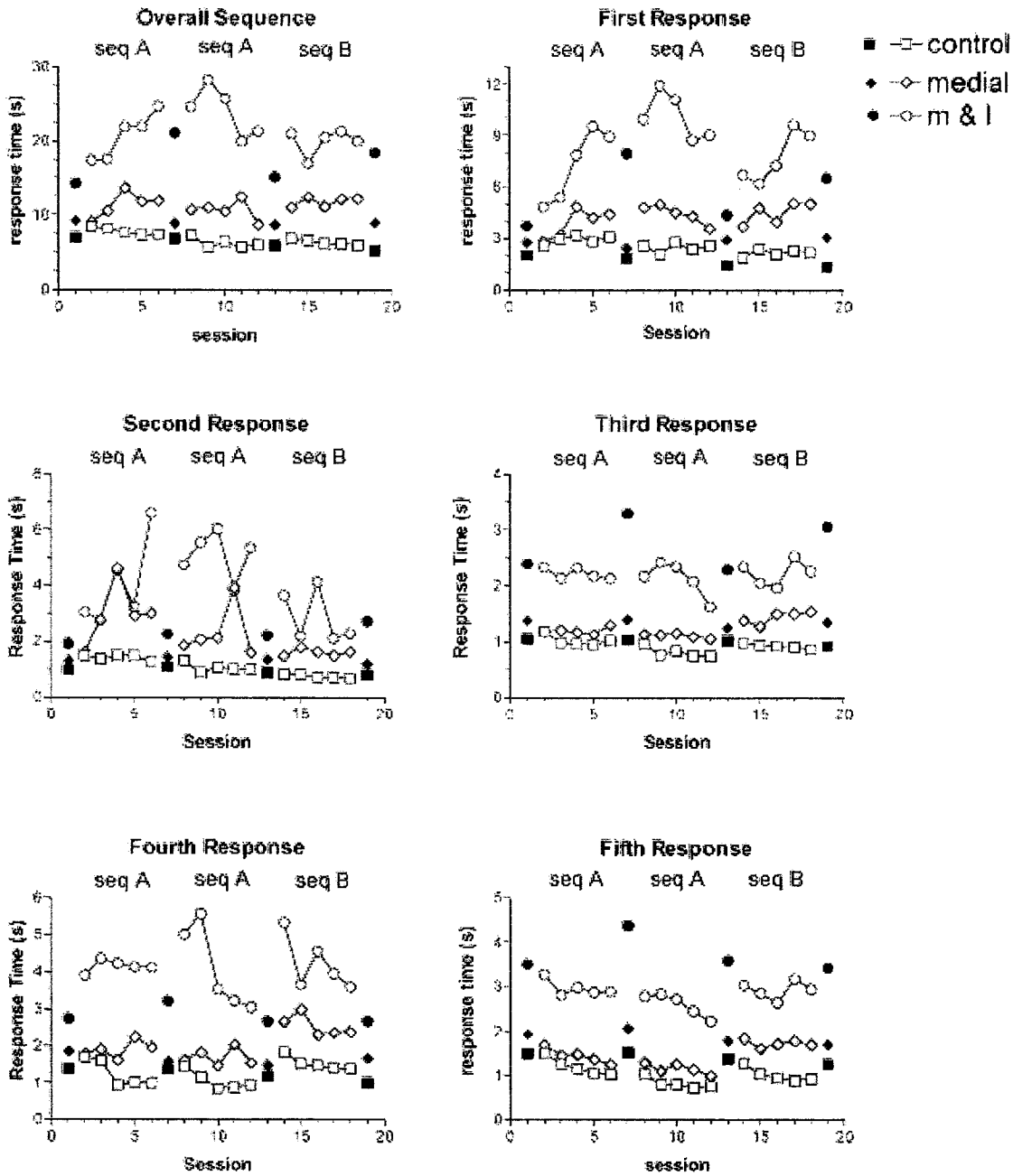


Figure 21. Effects of complete dorsal (M & L) and dorsomedial striatal lesions, across sessions, on response time (median ± SEM) in the serial reaction time (SRT) task. M & L lesions significantly slowed response time to initiate a sequence of 5 responses that was exaggerated when performing a repeating (learned) sequence. Dorsomedial lesions produced an intermediate level of impairment that was not significant. Lesions had no effect on motor learning. Graph 1 illustrates overall median time to complete random and repeat sequences. Graphs 2 – 6 reflect the time to complete each response within random and repeat sequences.

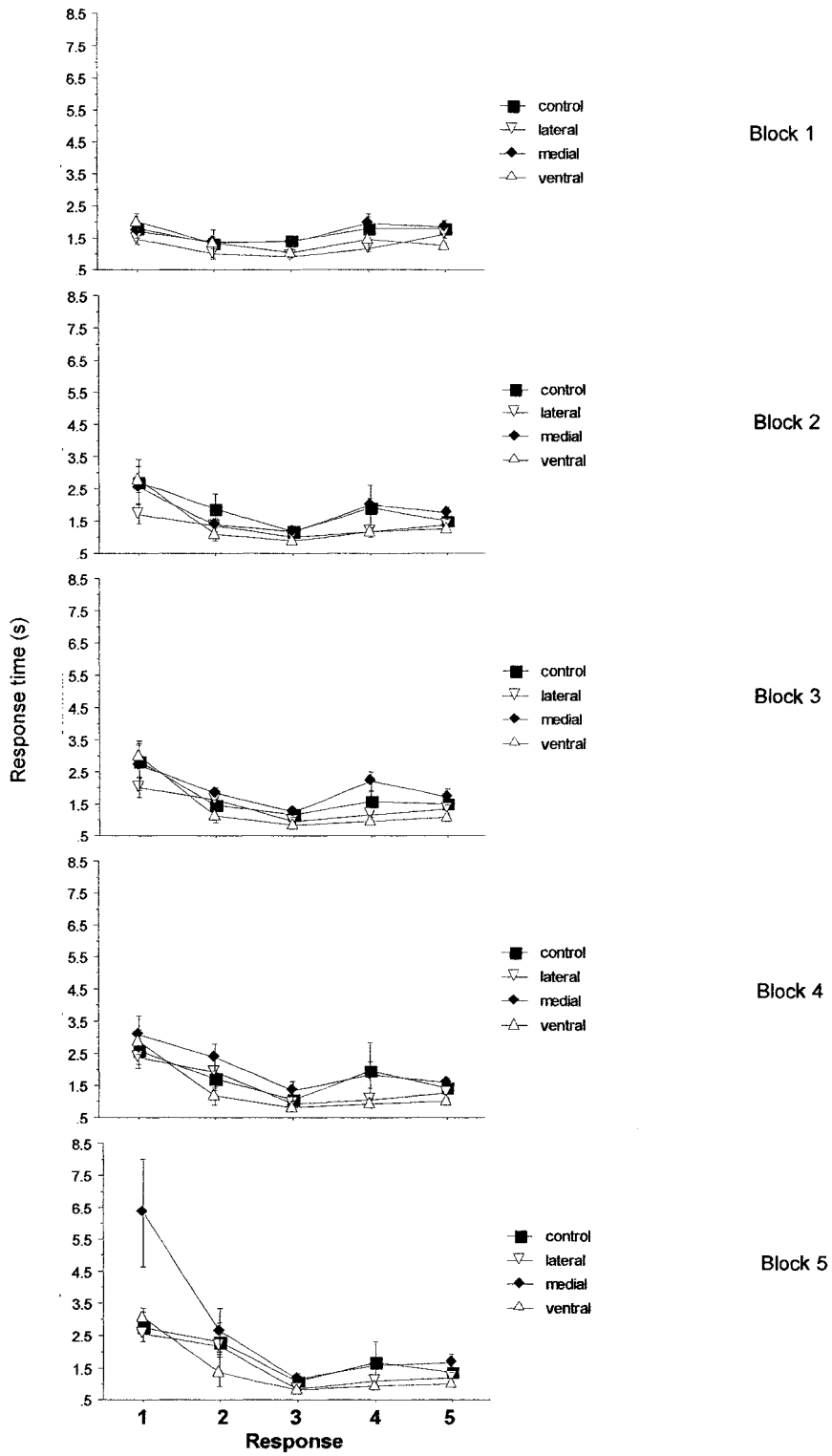


Figure 22. Effects of striatal lesions on response time (median ± SEM) for each response across 5 blocks (12 trials) within the initial repeat sequence session. Dorsomedial lesions significantly increased response time for the first response across the blocks of the session compared to controls

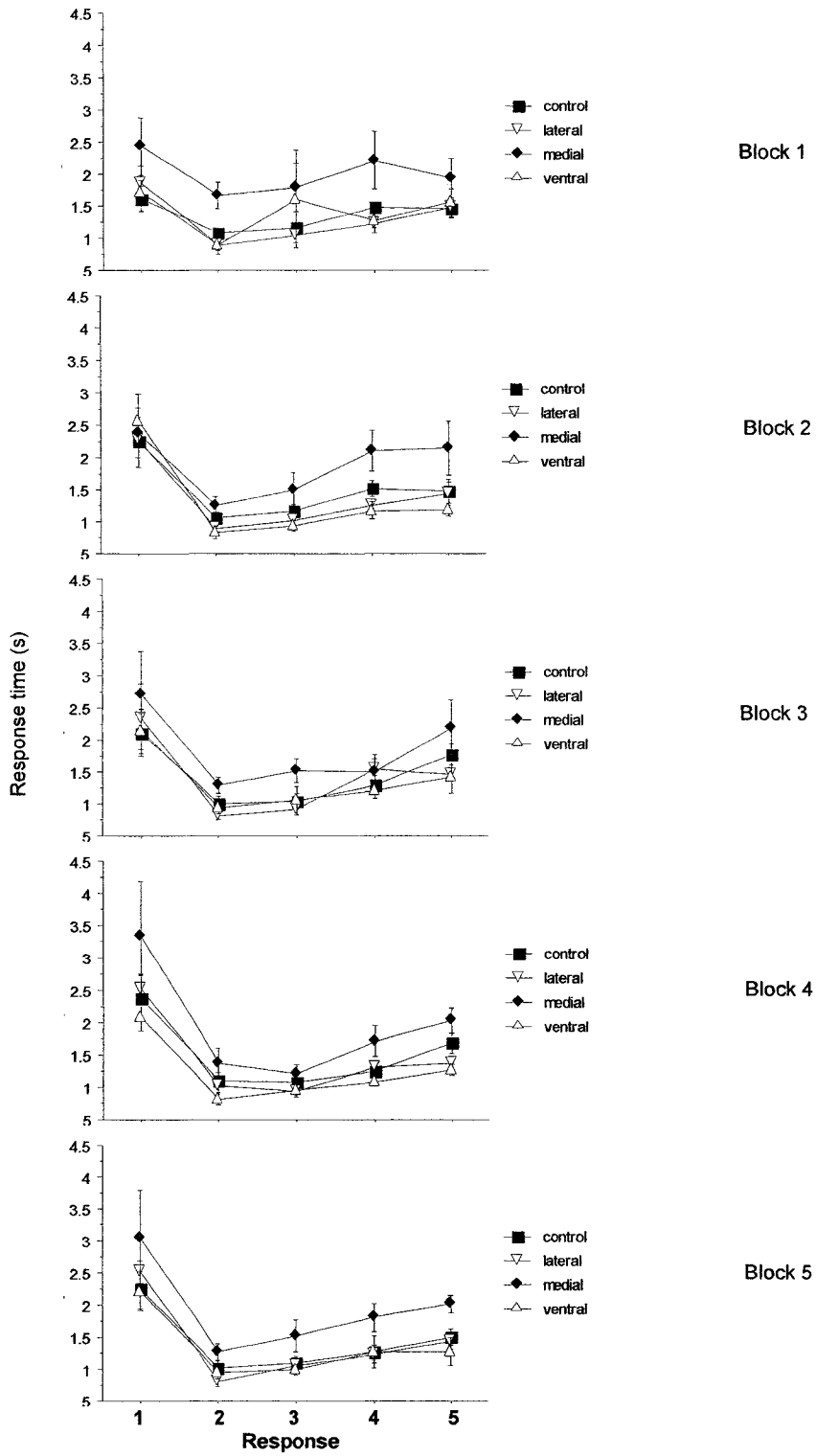


Figure 23. Effects of striatal lesions on response time (median ± SEM) for each response across 5 blocks (12 trials) within the initial random sequence session. All groups demonstrated a significant increasing in response times across the blocks for response 1 only.

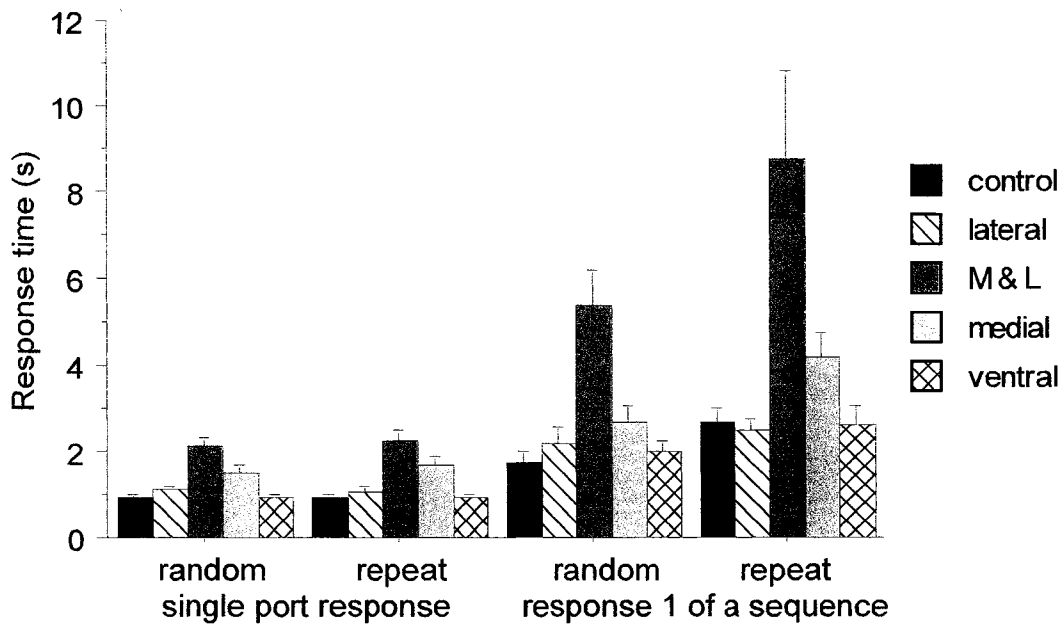


Figure 24. Effects of striatal lesions on response time (median ± SEM) initiating a single random or repeating port response (long – term simple stimulus response (S-R) task) compared to the identical motor response when it was the first response in a 5-response (random or repeating) sequence. Complete dorsal lesions significantly slowed response time for the first response in a sequence. This effect was exaggerated for repeating (learned) sequences.

APPENDIX C

UNIVERSITY OF NEW HAMPSHIRE

Office of Sponsored Research
Service Building
51 College Road
Durham, New Hampshire 03824-3585
(603) 862-3564 FAX

LAST NAME	Mair	FIRST NAME	Robert G.
DEPT	Psychology, Conant Hall	APP'L DATE	1/27/2004
		IACUC #	040103
OFF-CAMPUS ADDRESS (if applicable)	Psychology Conant Hall	REVIEW LEVEL	D
		TODAY'S DATE	2/2/2004
PROJECT TITLE	Neurobiology of Diencephalic Amnesia		

All cage, pen or other animal identification records must include your IACUC Protocol # as listed above.

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the protocol submitted for this study under Category D on Page 4 of the "Application for Review of Animal Use in Research or Instruction" - the study involves chronic maintenance of animals with a disease/functional deficit and/or procedures potentially inducing moderate pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics. The IACUC made the following comments on this protocol -- *comments* are usually minor editorial changes or clarifications that do not affect approval status (unlike contingencies, which require investigator action for initial or continuing approval):

1. On the Surgical Procedures form, #9 (post-operative medication), the IACUC restated Frequency of administration of butorphenol as "x1 for cannula surgeries."

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please note: Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. *Participation is mandatory* for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanys this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services. Thank you.

If you have any questions, please contact either Van Gould at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jessica Bolker

Jessica A. Bolker, Ph.D.
Chair

cc: File