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Medial prefrontal modulation of the nucleus basalis magnocellularis during attention-dependent learning

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MEDIAL PREFRONTAL MODULATION OF THE NUCLEUS BASALIS MAGNOCELLULARIS DURING ATTENTION-DEPENDENT LEARNING

A Thesis

Presented to the

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ABSTRACT

The nucleus basalis magnocellularis (NBM) sends cholinergic projections to the neocortex. While NBM activation of the neocortex regulates attention and contributes to memory formation, the mechanisms controlling NBM activity during attention-dependent learning are not well understood. The medial prefrontal cortex (mPFC) sends projections to the NBM, as evidenced by neuroanatomical studies. Additionally, pharmacological manipulation of mPFC influences cortical cholinergic efflux. However, it is not known if this mPFC-NBM circuit is involved in regulating NBM activity during natural learning. The current experiment tested the hypothesis that the mPFC is critically involved in modulating NBM activity during attention-dependent learning in rats. The mPFC was inactivated by infusing muscimol (2 μg/μl concentration at a volume of 0.5 μl) 40 min prior to behavioral testing in the final phase of the "incremental attention" paradigm. Training in this task involves first establishing an association between two serially presented CSs and a US. Next, animals are exposed to conditioning trials where the expected relationship between CSs is violated. As a result of this prediction error experience, attention to the relevant CS is enhanced. This enhancement

of attention results in an increase in associability of the affected cue. Compared to animals that have not experienced prediction error in the second phase of the task, rats in the prediction error condition show enhanced acquisition of new associations involving that cue. Previous research has shown that NBM activity is critical for the expression of attention-dependent enhancement of associative learning during the final phase of this task, but is not necessary for associative learning following consistent stimulus contingencies. Consequently, if the mPFC modulates NBM activation, where this activation is necessary for the enhancement of attention-dependent learning, then inactivation of the mPFC by muscimol should prevent the NBM from contributing to associative learning in the final phase of the incremental attention paradigm. In contrast, performance in animals that have not undergone prediction error trials was not expected to suffer as a result of muscimol treatment. Results supported these hypotheses; compared to saline-treated controls, muscimol-treated rats showed impaired attention-dependent learning following prediction error trials in the Predictive Shift condition of the incremental attention task. In contrast, muscimol-treated rats in the consistent stimulus contingency condition

performed as well as saline-treated controls. Results strongly suggest that the mPFC-NBM circuit serves a functional role in regulating NBM output during attention-dependent learning.

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CHAPTER ONE

THE ROLE OF THE NUCLEUS BASALIS MAGNOCELLULARIS IN ATTENTION-DEPENDENT LEARNING

Introduction

Research investigating rodent models of Alzheimer's disease has implicated the basal forebrain cholinergic system in several aspects of cognition, including learning, memory, and attention. Furthermore, the cortically-projecting cholinergic neurons of the nucleus basalis magnocellularis (NBM) within the basal forebrain, play a crucial role in these cognitive processes. Additionally, simple associative learning tasks (Butt & Bowman, 2002; Butt, Noble, Rogers, & Rea, 2002) and tasks that do not explicitly place demands on attention (Himmelheber, Sarter, & Bruno, 2000; Turchi & Sarter, 2001) remain unaffected following NBM damage. These findings suggest that the NBM cholinergic projections are critically involved in the attentional aspects of learning. According to Sarter, Gehring, and Kozak (2006), attentional systems are set off when difficulties such as extended time on task, altered stimulus appearance, and presentation of distracters occur during behavioral tasks.

The following studies examine the effects of NBM disruption on attention demanding tasks. These behavioral tasks, which reliably measure attentional function, include many of the task difficulties described above. NBM disruption can be achieved either through pharmacological manipulation or excitotoxic lesions, which result in either cholinergic deafferentation or NBM inactivation. The behavioral impairments seen following NBM disruption are consistent with the argument that the NBM cholinergic system is critically involved in attention.

Nucleus Basalis Magnocellularis Lesions and Attentional Impairments

Investigators Muir, Dunnett, Robbins, and Everitt (1992) assessed performance of the five choice serial reaction time task following basal forebrain cholinergic lesions. In the first experiment, the high affinity uptake blocker, hemicholinium, was used to pharmacologically damage the cholinergic system. The anticholinesterase inhibitor physostigmine was also administered either alone or along with hemicholinium to test its effects. In the second experiment, cholinergic and hippocampal grafts were implanted into the neocortex in an attempt to attenuate the detrimental effects of basal forebrain lesions

produced by quisqualic acid. These experiments are described in more detail below.

In experiment one, rats were trained to criterion perform levels in the five choice serial reaction time task. A cannula guide was implanted into the right lateral ventricle of each rat to allow subsequent drug infusion into the ventricles of the brain. Following surgical recovery, rats received one intracerebroventricular infusion of a vehicle solution containing no drug, and were then returned to the five choice serial reaction time task training regimen. Upon reaching stable performance, rats received an intracerebroventricular infusion of hemicholinium or vehicle, and an intraperitoneal injection of physostigmine or saline before being tested on the task. The combination of drug treatments were administered once per session, followed by two rest days, with baseline performances were measured between each drug session.

Results revealed that hemicholinium treatment produced significant impairments in performance of the five choice serial reaction time task. Furthermore, subsequent treatment with physostigmine reversed the behavioral impairments produced by hemicholinium.

Animals in experiment two received pre-training identical to that in experiment one. Rats were then given

bilateral infusions of quisqualic acid or vehicle into the basal forebrain. Behavioral testing recommenced after surgical recovery. Following the testing sessions, one group of rats received ventral forebrain graft transplants, rich in cholinergic cells, and another group received hippocampus graft transplants which have low levels of cholinergic cells. Animals who did not receive transplants served as controls. Following recovery, testing in the standard version of the task began. In addition to the five choice serial reaction time task, a set of additional behavioral challenges were administered. Results showed that lesioned rats displayed impairments in some aspects of performance during both baseline and behavioral challenge testing. Interestingly, cholinergic grafts had some attenuating effect on performance.

Based on the overall results, these researchers suggested that the basal forebrain plays an important role in attentional performance. They further concluded that damaging cholinergic function can have detrimental effects on performance which can be attenuated by administrations of the cholinesterase inhibitor, physostigmine or by cholinergic graft transplants.

Further evidence for NBM involvement in attention is provided by Pang, Williams, Egeth, and Olton (1993), who

investigated the role of the NBM in attention. In this experiment, the NBM was inactivated with the $GABA_A$ agonist muscimol and its effects were assessed using a two-choice reaction time task. Rats received training on the two-choice reaction time task, which assesses attentional processes. Upon reaching stable performance, rats were surgically implanted with bilateral cannulae targeting the NBM. Training began two days after surgery and continued until stable performance was regained. During testing sessions, rats were infused with muscimol (0.5, 1.0, or 2.5μg/hemisphere) and were placed in the chambers to perform the two-choice reaction time task. Performance measures consisted of error rate, discriminability, bias, and reaction time for correct responses to an auditory and visual stimulus. Throughout testing, rats were infused with muscimol two times a week, with non-infusion days serving as control data.

Results revealed that, when compared to baseline during non-infusion testing, muscimol infusions increased RT and discriminability for both the visual and auditory stimuli. Muscimol infusions also increased error rate when data from both stimuli were combined. However, bias and error rates for the individual stimuli were not affected. These data provide evidence that the NBM is involved in

those aspects of attention pertaining to discriminability, but not to response bias. It is suggested that changes in discriminability result in modifications in perceptual processing. Effects on bias were interpreted as reflecting changes in response approach. Overall, the findings implicate the NBM in attention, more specifically in the regulation of the perceptual components of attention.

It is important to note that since muscimol inactivates both cholinergic and non cholinergic cells found in the NBM, the attentional deficits seen in the above study could not be specifically attributed to cholinergic cell inhibition (Pang et al., 1993). To address this issue, excitotoxic lesions have been performed in several studies to target the cholinergic cells in the NBM to some degree of specificity. The immunotoxin 192 IgG-saporin has been shown to produce cholinergic cell loss specific to the NBM (Wenk, Stoehr, Quintana, Mobley, & Wiley, 1994). Such selective NBM lesions are accompanied by behavioral impairments in attentional performance, as is shown in the following studies.

For example, McGaughy, Dalley, Morrison, Everitt, and Robbins (2002) investigated the effects of cholinergic deafferentation using saporin infused into the NBM on

cortical acetylcholine (ACh) release and attention. In their first experiment, all animals underwent behavioral training of the five choice serial reaction time task prior to surgery. Once criteria had been reached, animals were randomly assigned to either the saporin or saline (sham) group. Saporin group rats were infused with a high dose of the immunotoxin. All animals also received cannula implantations into the prefrontal cortex for microdialysis testing. Following surgical recovery, animals were re-trained in the standard version of the task, followed by exposure to a series of behavioral obstacles designed to increase attentional demands.

Neurochemical results revealed that there were significantly less choline acetyltransferase immunoreactive cholinergic neurons present in the NBM of saporin animals compared to sham animals. In addition, compared to sham lesion controls, ACh release in the prefrontal cortex was significantly lower in the saporin group both before and during attentional testing. Norepinephrine release did not differ between saporin and sham animals either before or during testing. Saporin animals displayed severe impairments in postoperative performance of the five choice serial reaction time task compared to sham animals. Saporin rats showed decreased

accuracy, and an increase in trials omitted, latency, and number of preservative and premature responding.

In their second experiment, a separate group of animals received NBM infusions of either a low dose of saporin or vehicle. These animals were also implanted with cannula targeting the prefrontal cortex for later microdialysis testing. After recovery, animals were tested again under varying condition of attentional demands until they achieved stable performance.

Neuroanatomical results showed that the number of cholinergic neurons were significantly lower in the NBM of saporin rats than sham rats. Additionally, there was a preservation of non-cholinergic neurons in the NBM. Behavioral results revealed impaired choice accuracy for saporin animals. There were no differences in norepinephrine release between saporin and sham rats. However, in the low dose saporin group (but not the high dose group), ACh release in the prefrontal cortex was significantly higher in saporin rats than in sham rats on the first day of testing.

It was suggested that the increase levels of ACh in the saporin low dose group may have been due to the basal forebrain cholinergic system compensating for the small amount of damage caused by the low dose of the toxin. NBM

lesions caused dose-dependent effects, with decreases in performance accuracy in the saporin high dose group reflecting decreases in the number of surviving cholinergic neurons in the NBM, and a corresponding decrease in ACh release in the prefrontal cortex. Because behavioral performance wasn't correlated with norepinephrine release, these authors conclude that ACh, but not norepinephrine, is critically involved in sustained attention.

Collectively, these NBM inactivation or lesion studies resulted in behavioral impairments reflecting deficits in several aspects of attention. It can be concluded that performance in tasks that tax attentional function critically depends on the cholinergic neurons of the NBM. As the link between NBM function and the processes of attention has become more firmly established, research has increasingly focused on the role of the NBM in regulating attention during learning. An emerging area of research interest in NBM function addresses the question not only of NBM control of attentional processes in the neocortex (especially prefrontal cortex), but also on the question of which neural systems are responsible for modulating NBM activity itself.

CHAPTER TWO

ATTENTION-DEPENDENT TASK PERFORMANCE AND ACETYLCHOLINE RELEASE IN PREFRONTAL CORTEX

The cholinergic cells originating in the basal forebrain project to the entire cortex, including the prefrontal cortex (Lucas-Meunier, Fossier, Baux, & Amar, 2002; Zaborszky, Pang, Somogyi, Nadasdy, & Kallo, 1999). Moreover, studies utilizing attention-demanding tasks have shown heightened acetylcholine activity in the prefrontal cortex, when compared to baseline levels. For example, an experiment conducted by Arnold, Burk, Hodgson, Sarter and Bruno (2002) compared acetylcholine (ACh) release in three groups of rats tested in tasks varying in terms of attentional demands. One group was assigned to a sustained attention task while the other two groups were assigned to operant control tasks that did not place significant demands on attention. Arnold et al. (2002) hypothesized that ACh release would be highest during performance of the sustained attention task, and would also be greater than basal levels during performance of the operant tasks with comparatively lower attentional demands.

Rats were randomly assigned to perform under either the sustained attention task or one of the two operant

control tasks. The operant tasks were designed to control for the possible effects of the sensory and motor aspects of the sustained attention task. The purpose of one task, labeled the FI-9 task, was to diminish possible processing demands produced by the presentation and levers. The other task, known as the retracting lever task, was intended to replicate the extension of a lever into the chamber, and control for the amount of reinforcement and motor movement in the sustained attention task. All rats were initially trained to perform their designated task until achieving stable performance. Rats then received unilateral cannula implantations above the frontoparietal cortex. After recovery, rats received additional training in their designated task. Rats then received one session of microdialysis testing where ACh was captured from frontal cortex during task performance.

Results demonstrate that ACh release was elevated during performance of the sustained attention task when compared to baseline levels prior to task onset. Similarly, rats in the operant tasks showed higher levels of ACh release compared to their respective baseline levels. However, rats in the sustained attention task showed higher levels of ACh release than rats performing in either operant control task.

Given these results, Arnold and colleagues (2002) suggest that there is a clear relationship between the amount of attentional processing required in a task and the level of increase in ACh efflux during task performance relative to basal ACh release levels. These researchers note that ACh efflux may reflect the level of performance in a task requiring attention, with greater levels of ACh release being associated with higher levels of task performance. Another possibility suggested was that elevated levels of ACh may be associated with elevated levels of attentional effort. Overall, these findings were in accordance with the hypothesis that performance requiring attention is characterized by increased cortical cholinergic modulation.

In a related study by Dalley and colleagues (2001), ACh and norepinephrine input to the prefrontal cortex was measured during performance of the five choice serial reaction time task. Two distinct manipulations were used in this task in order to investigate neurotransmitter release under different cognitive demands. In one group, ACh and norepinephrine levels were measured during performance of the five choice serial reaction time task. In the other group, the reward contingency used in the task was manipulated by yolking reinforcement in one

animal to the operant response performance of a second rat. Thus, rats undergoing microdialysis were rewarded the same amount of times as their paired-controls but theses rewards were not contingent upon their own operant responding but instead were contingent upon correct responding of the paired control. Dalley et al. (2001) predicted that rats in the group with the manipulated reward contingency would shift their focus from performing the operant visual discrimination, to simply waiting near the place where reinforcement was delivered. In addition, a decrease in ACh release during this response shift was expected. Lastly, norepinephrine release was also expected to reflect this shift in reinforcement contingencies.

All rats were exposed to the five choice serial reaction time task for training until reaching stable performance. Behavioral baseline performance was collected to ensure that all animals were at the same performance level prior to contingency shifts. Rats were randomly assigned to either the contingent or non-contingent group. During surgery, rats received probe implantations into the prelimbic area of the prefrontal cortex. On the first day of microdialysis testing, the contingent group remained on the previously trained task while rats in the non-contingent group were given the same visual cue and

reward as their contingent pair in a manner unrelated to their own performance. Another microdialysis session took place the next day.

In the non-contingent group, results revealed greater levels of norepinephrine release when the reward contingency changed from performance-dependent to performance-independent reinforcement. However, this increase was only seen during the first day of microdialysis testing. The contingent group displayed elevated levels of norepinephrine as well, but this increase was apparently not related to task performance per se. Increased levels of ACh release were seen in the contingent group throughout the task. Non-contingent animals displayed similar increases but to a lesser degree.

These researchers suggest that the norepinephrine and ACh systems are critically involved in different attentional processes in the prefrontal cortex. Cortical norepinephrine activity in the prefrontal cortex is suggested to signal differences between instrumental occurrences and rewards. The authors suggest that a large portion of ACh release seen in contingent rats is associated with the cognitive requirements of the task. Furthermore, they conclude that the cortical cholinergic

system is involved in performance where attentional demand has been established. Conversely, the cortical noradrenergic system is suggested to play a role in situations where the established association between reinforcement and instrumental actions eliminated.

Previous lesion and drug studies demonstrate a relationship between cortical cholinergic activity and performance involving sustained attention. However, these studies were not able to directly indicate this relationship due to the fact that the effects of lesion and/or pharmacological manipulations on attention and ACh efflux were conducted in separate experiments. To address this, Himmelheber and colleagues (2000) assessed performance on a sustained attention task while measuring ACh release from the frontoparietal cortex at the same time. This method was intended to monitor the changes in ACh release throughout performance. These researchers also measured ACh release during the presentation of a visual distracter, which was intended to interfere with task performance. ACh release in the frontoparietal cortex was expected to display task-related changes as a function of attentional demand.

Prior to surgery, all animals went through a series of shaping, training, and habituation procedures. Rats

received training in the sustained attention task, in which visual signal lengths, distracter, and house light were manipulated. Rats were also habituated to the microdialysis procedures. Upon completion of these series of procedures, rats received microdialysis cannula guide implantations above the frontoparietal cortex. After recovery, rats were re-exposed to training and habituation procedures in the microdialysis chambers until re-establishing stable performance. Testing consisted of four microdialysis sessions, three tested performance in the sustained attention task and one assed the presentation of the distracter. On the day of a microdialysis session, rats were first placed in a plastic test bowl where baseline levels were collected. Rats were then transferred to the operant chambers and additional dialysate samples were collected during pre-task and task onset. After completion of the task, rats remained in the chambers for additional collection of ACh efflux.

Results showed a significant increase in cortical ACh efflux when animals were transferred from the test bowl to the operant chamber. Further elevated levels of ACh efflux were seen during task onset, surpassing pre-task ACh levels. Upon completion of the task, ACh efflux decreased below pre-task levels. Task performance correlated with

stimulus length and was affected by the presentation of the distracter. The increase in ACh release when rats were transferred from the test bowl to the operant chamber reflected environmental changes, anticipation of reinforcement, and physical contact. However, because the increase in ACh efflux was so robust, these researchers further suggest that an increase in attentional processing in expectation of approaching task requirements may be partially responsible for the increase in ACh release.

Task performance-related ACh release was suggested by the finding that ACh efflux during task performance was elevated above pre-task levels, and declined after task completion. Himmerheber et al. (2000) suggest that attentional processes were responsible for in the elevated levels of ACh release seen during task performance. The behavioral and biochemical effects seen during the presentation of a distracter were interpreted as reflecting the disruption of active attentional processes. Together, these results provide supporting evidence for the relationship between high attentional demand and high cortical cholinergic activity.

To further investigate how manipulations on attentional load affects cholinergic activity, Himmelheber and colleagues (2001) conducted a similar study measuring

frontoparietal ACh efflux during both a standard and a low attention-demanding version of the sustained attention task. This study consisted of three experiments. Experiment one assessed ACh release during the standard version of the sustained attention task, and during unexpected changes in the attentional load during performance of the task. ACh efflux was predicted to be dependent upon the attentional demands of the task. In experiment two, ACh release was measured during performance of the low attention demanding task. Here, ACh release was predicted not to increase above pre-task levels. Lastly, in order to assess the involvement of cholinergic transmission during performance of the low demand task, experiment three investigated performance following saporin lesions to the NBM Lesions were hypothesized to have no effect on performance due to the argument that the low demand task requires little processing resources.

In experiment one, rats were first trained to perform in the sustained attention task followed by further training in the microdialysis chambers. Upon reaching stable performance, rats received two 'shift low' sessions and one 'shift high' session. The shift low sessions consisted of performance in the standard sustained

attention task during the first task block followed by a shift to the low demand task for the remainder of the blocks. The low demand task differentiated from the standard task in that only the correct lever was presented, signal length and the intertrial interval remained constant during performance. In the high shift session, rats performed the low task for the first three blocks and then shifted to the standard sustained attention task. Once stable performance was established, rats received microdialysis cannula implantations targeting the frontoparietal cortex. After recovery, rats were retrained in the sustained attention task and shift sessions until performance was once again stabilized. Rats were then tested over the course of five microdialysis sessions. Two of the five session tested performance of the sustained attention task, another two sessions tested one shift low session and one shift high session, and the fifth session consisted of contextual extinction sessions. During contextual extinction, rats were placed in operant chambers without a task or reinforcement. After contextual extinction, rats were exposed to the fifth and final microdialysis session.

Results from experiment were consistent with the Himmelheber et al. (2000) study in terms of ACh

enhancement during context transfer and task onset. In addition, during the extinction session, there were no changes seen in ACh efflux and ACh release did not fall below pre task levels.

Prior to surgery, rats in experiment two were trained in the low demand task only. Following recovery and re-established performance, rats received two or three microdialysis sessions. These sessions were similar to those conducted in experiment one including the contextual extinction session. The finding that ACh levels increased during performance in this experiment was similar to the finding of elevated levels of ACh efflux seen during performance of the sustained attention task in experiment one. ACh efflux during transfer and extinction were similar to those in experiment 1 as well.

In experiment three, following over training in the low demand task, rats received bilateral saporin lesions into the NBM. After recovery, performance on the low demand task was assessed. Results revealed that performance in the low demand task was unaffected by saporin induced NBM lesions.

The findings from experiment one provided further support for the relationship between task related ACh increases and task performance. Researchers suggest that

the results from experiment two indicate that cortical cholinergic activity is not exclusive activated by attentional demands. Furthermore, although ACh activity was seen during performance in the low demand task, there were no indications of performance being affected by cholinergic deafferentation.

Based on previous studies investigating the basal cholinergic system and medial prefrontal cortex (mPFC) ACh release during an attentional task, Passetti, Chudasama, and Robbins (2000) suggest that cortical ACh efflux may be regulated by behaviors occurring during attention demanding tasks. Based on this hypothesis, they conducted a study where they assessed ACh release during performance of the five choice serial reaction time task. In this task, the degree to which attention is required can be manipulated by the length of the visual stimuli. A stimulus duration of 0.5s is used on the standard version of the task. However, to investigate the effect of cortical ACh release during task performance, these researchers varied the length of the stimulus during each session (0.5s, 0.25s, and 5s). Longer stimuli durations were expected to tax less on attention during performance.

Rats were divided into two groups. One group consisted of rats trained to perform the five choice

serial reaction time task. The second group of rats served as controls and was habituated to the same testing environment but received no stimulus presentations or food delivery. Behavioral and microdialysis sessions consisted of several phases. During phase 1, trained animals received five choice serial reaction time task training while control rats were habituated to the testing chambers. On phase 2, all rats had plastic rods surgically attached to their heads for tethering purposes during microdialysis testing. Phase 3 consisted of tethering habituating for all rats. Microdialysis probe implantations into the mPFC were performed during phase 4. Finally, during phase 5, rats received microdialysis testing for three consecutive days. Each session varied in stimulus duration (session 1: 0.5s; session 2: 0.25s; and session 3: 5s).

Results revealed an increase of mPFC ACh release during task performance in trained rats when compared to baseline. This increase was seen across all three microdialysis sessions. ACh release then began to decrease along with performance during the end of the session. Importantly, ACh efflux of trained and control rats were similar prior to task performance. When comparing session days 1 and 2, the shorter stimulus duration (.25s)

resulted in lower choice accuracy. However, there were no differences in ACh release between the two stimulus lengths.

According to these researchers, the overall increases in ACh were task-related. This argument was based on the comparison of ACh levels between trained rats and control rats. The comparable levels of ACh efflux between sessions 1 and 2 were not in agreement with previous studies. Explanations to this finding were not evident but the notion of decreased ACh levels was ruled out. Support for this was suggested by the fact that animals displayed no decline in ACh efflux across days. Additionally, there was a positive correlation between ACh release and some measure of performance. Based on these findings, it was suggested that ACh release may have been affected by the presence of the visual stimuli, the motor factor of performance, or the food reward. Support for this was explained in the finding that the most significant changes in ACh efflux were seen during session 3, where there were more stimulus presentations, locomotor activity, and food rewards. It was also indicated that the lower number of omissions seen during session 3 when compared to sessions 1 and 2 may reflect the higher levels of ACh release found in session 3 when compared to 1 and 2.

In conclusion, the biochemical, histological, and behavioral findings provided in the studies investigating NBM disruption and ACh efflux during attention demanding tasks provide substantial evidence supporting the argument that intact NBM cholinergic projections to the prefrontal cortex are critical for the function of attentional processes during learning.

CHAPTER THREE

TOP-DOWN MODULATION OF THE NUCLEUS BASALIS MAGNOCELLULARIS BY THE PREFRONTAL CORTEX

NBM activation of the neocortex is known to regulate attention and contribute to memory formation in neocortex. However, the mechanisms controlling NBM activity during attention-dependent learning are not well understood. The medial prefrontal cortex (mPFC) has been identified as having descending afferent control over the nucleus basalis magnocellularis (NBM), as evidenced by neuroanatomical and pharmacological studies. However, it is not known if this putative mPFC-NBM circuit is involved in regulating NBM activity, and thereby regulating attentional processes during natural learning.

According to Sarter, Givens, and Bruno (2001), in situations where attention is required, top-down processes are recruited in order to augment cognitive processing facilitate attentional performance. With increased attentional demand, basal forebrain corticopetal neurons become activated, signaling task relevant information via cholinergic projections to the mPFC. Consequently, the mPFC is believed initiate the activity of top-down processes leading to the regulation of NBM activity

(Sarter, Gehring, & Kozak, 2006; Sarter et al., 2001; Sarter, Hasselmo, Bruno, & Givens, 2005a). Sarter and collegues (2001) suggest that this process depends on the prefrontal-basal forebrain circuit, where the mPFC controls NBM modulation of attention.

The presumed existence of a prefrontal-basal forebrain circuit is largely based on anatomical and physiological studies identifying direct connections between these two regions. The basal forebrain is comprised of four main structures, which include the medial septum, vertical and horizontal limbs of the diagonal band of Broca and the magnocellular nucleus or NBM. Together, these structures comprise the basal forebrain cholinergic system (basal forebrain cholinergic system). The neocortex receives significant cholinergic input from the NBM. Physiological studies reveal that the prefrontal cortex has influence over the basal forebrain (Lucas-Meunier et al., 2003). According to Golomayo, Nuñez, and Zaborszky (2003) and Gyengesi, Zaborszky, and Detari (2008), basal forebrain neurons become active following electrical stimulation of the prefrontal cortex. The argument that these two regions are functionally related is also based on the fact that both basal forebrain and prefrontal cortex share a similar

topographical organization. Substantial evidence suggests that basal forebrain projections innervate the cortical mantel in a consistent and orderly fashion (Gaykema, Van Weeghel, Hersh, & Luiten, 1991; Golomayo et al., 2003; Zaborszky, Gaykema, Swanson, & Cullinan, 1997). Likewise, regions within the prefrontal cortex display differential projections onto specific areas in the basal forebrain. These topographical similarities indicate a possible reciprocal relationship between both regions. Further support for this notion is provided in findings from neuronal tracing studies. Gaykema and colleagues (1991), as well as Zaborszky and colleagues (1997) conducted similar experiments in which the anterograde tracer, Phaseolus vulgaris (PHA-L), was injected into the prefrontal cortex in order to track prefrontal cortex projections. In addition, AChE and ChAT staining was performed to identify the presence of cholinergic cells in the basal forebrain. Results revealed PHA-L labeled prefrontal cortex projections innervating all regions of the basal forebrain cholinergic system. In addition, primary PHA-L labeled prefrontal cortex efferents were shown to stem away from the major corticofugal pathways and exclusively innervate the basal forebrain cholinergic system. Various prefrontal cortex projections were also

shown to terminate next to cholinergic components. Based on these findings, Gaykema et al. (1991) suggest that prefrontal projections may directly synapse onto cholinergic neurons in the BF. Zaborszky et al. (1997), however, explains that although prefrontal cortex projections may terminate near cholinergic neurons, they do not make direct synaptic connections. Furthermore, prefrontal cortex terminal buttons directly synapse onto non-cholinergic cells. Gyengesi et al. (2008) similarly show that prefrontal projections selectively terminate on non-cholinergic neurons such as GABA and glutamate. In light of these findings, it can be reasonably concluded that prefrontal cortex afferents to basal forebrain influence the activity of the cholinergic cells of the NBM.

According to Lucas-Meunier et al. (2003), cholinergic neurons in the NBM are controlled by GABAergic interneurons and glutamatergic neurons. GABA released in the neocortex can spread extrasynaptically in significant amounts and stimulate $GABA_A$ receptors on the terminal endings of cholinergic NBM cells, thereby inhibiting the release of ACh in neocortex. Activation of these $GABA_A$ receptors can also stimulate NBM neurons and remove the tonic inhibition of ACh release. Overall, the findings

from the present anatomical and electrophysiological studies are all in agreement that prefrontal projections provide input to the cholinergic cells in the basal forebrain. Although prefrontal cortex afferents may not have direct contact with cholinergic cells, they have been shown to provide a strong influence over ACh release through its direct connections with non-cholinergic cells.

Prefrontal influence over ACh release can be seen in studies assessing ACh activity following pharmacological manipulations prefrontal cortex. Researchers Nelson, Sarter, and Bruno (2005) investigated the potential mechanisms behind glutamatergic and cholinergic transmission during prefrontal cortex regulation of ACh release in the posterior parietal cortex cortex. In this study, rats with microdialysis cannula guides implanted in the posterior parietal cortex and prefrontal cortex were treated with one of thirteen different drug infusion combinations. The conditions were as follows: prefrontal cortex infusions of 1) artificial cerebral spinal fluid; 2) NMDA; 3) AMPA; 4) AMPA + atropine; 5) AMPA + mecamylamine; 6) AMPA + DNQX 7) carbachol; 8) nicotine; 9) carbachol + atropine; 10) carbachol + mecamylamine; 11) carbachol + DNQX and posterior parietal cortex infusions of 12) carbachol or 13) nicotine. On all four

days of microdialysis testing, rats received infusions of either their designated drug or artificial cerebral spinal fluid. Following drug infusion, all rats were switched back to artificial cerebral spinal fluid infusions and post-drug microdialysis samples were collected.

Results showed that prefrontal cortex administration of AMPA, but not NMDA, increased ACh efflux in the posterior parietal cortex. This suggested that glutamatergic transmission within the prefrontal cortex plays a role in the regulation of posterior parietal cortex cholinergic transmission. Both AMPA and NMDA are selective agonists mimicking the effects of glutamate. NMDA receptors, however, are located mostly on projections between cortices and show relatively low levels of basal activity. It was also revealed that prefrontal cortex infusion of carbachol, but not nicotine, elevated ACh efflux in the posterior parietal cortex. This supports the argument that prefrontal cortex cholinergic regulation also plays a role in cholinergic transmission to the posterior parietal cortex. Interestingly, neither carbachol nor nicotine perfusions into the posterior parietal cortex resulted in prefrontal cortex ACh efflux, suggesting that the relationship between these two regions is not bidirectional. Overall, these results provide

evidence supporting that the prefrontal cortex regulates ACh release in the posterior parietal cortex, and perhaps throughout the neocortex.

Rasmusson, Smith, and Semba (2007) further investigated the role of the prefrontal cortex in regulating ACh in other cortical areas. It was hypothesized that the pathway by which sensory information travels in order to reach basal forebrain cholinergic neurons passes through the prefrontal cortex. This was assessed by measuring induced ACh release in the visual, auditory, and somatosensory cortices prior to and following prefrontal cortex inactivation by muscimol. Results supporting their assumption that prefrontal cortex inactivation would result in diminished ACh release, would confirm the prefrontal cortex's regulatory role in the proposed prefrontal-basal forebrain circuit.

Rats had microdialysis probes implanted into the mPFC for muscimol delivery and into the somatosensory, visual, or auditory cortex for ACh collection. Rats with a somatosensory implantations received peripheral stimulation of the contralateral forepaw or electrical stimulation of the ipsilateral specific thalamic nucleus, an area that innervates the cortical collection area. Visual cortex stimulation was evoked through thalamic

stimulation of the dorsal lateral geniculate nucleus. Rats with an auditory probe implantation received electrical stimulation of the ventral medial geniculate nucleus. Additionally, dialysate samples were collected during baseline and following muscimol infusion, allowing ACh release to be compared before and after mPFC inactivation. Control animals received artificial cerebral spinal fluid infusions instead of muscimol in order to compare the pattern of ACh efflux evoked by cortical stimulation. As predicted, electrical stimulation produced increased levels of ACh release in all cortical regions. This elevation in ACh release was significantly reduced following muscimol infusion. Additionally, evoked ACh levels prior to muscimol delivery were similar to ACh levels observed in artificial cerebral spinal fluid infused control animals.

Overall, these findings strongly implicate the prefrontal cortex as an essential component of the circuitry mediating cortical projections to BF cholinergic neurons. Data from these neuroanatomical and pharmacological studies have identified descending mPFC afferent control over the NBM. This arrangement is consistent with the argument that a mPFC-NBM circuit is

involved in regulating NBM activity and attentional processes.

CHAPTER FOUR

THESIS EXPERIMENT

Introduction

The cholinergic NBM has been implicated in several aspects of cognition including learning, attention, and memory. Cholinergic neurons in the NBM release the neurotransmitter ACh throughout a vast area of the neocortex, including the mPFC. While it is well-established that NBM cholinergic activation of the neocortex is involved in regulating attentional processes in learning and memory, little is known about the underlying mechanisms controlling NBM activity itself. However, a growing body of literature suggests that descending projections from the mPFC synapse on basal forebrain neurons are capable of modulating NBM activity, and in turn modulating cholinergic activation of the neocortex.

Neuroanatomical studies have identified mPFC afferent projections as possessing top-down control over the NBM (Gaykema et al., 1991; Sarter et al., 2001, 2005, 2006; Zaborszky et al., 1997). Additionally, pharmacological inactivation of the mPFC has been shown to diminish electrically stimulated ACh release in the neocortex

(Rasmusson et al., 2007; see also Nelson et al., 2005). Overall, current research suggests the existence of a mPFC-NBM circuit. However, whether this circuit plays a role in mediating NBM-dependent attentional processes during learning has yet to be investigated.

Attention-dependent learning can be assessed through the use of a complex Pavlovian conditioning task known as the incremental attention paradigm. This paradigm allows both attention-dependent and attention-*in*dependent learning to be evaluated under comparable conditions. Importantly, the attention-dependent enhancement of conditioning seen in the predictive shift condition of this paradigm depends on the release of ACh in the neocortex from the cells of the NBM (Chiba, Bucci, Holland, & Gallagher, 1995; Holland et al., 2006). In contrast, attention-*in*dependent learning in the consistent prediction condition of this task does not depend on cholinergic NBM activity (Chiba, Bucci, Holland, & Gallagher, 1995; Holland et al., 2006).

Using the $GABA_A$ agonist drug muscimol, it is possible to pharmacologically inactivate the mPFC such that cholinergic transmission from the NBM to neocortex is diminished (see Rasmusson et al., 2007). Muscimol inactivates mPFC neurons by acting on inhibitory $GABA_A$

receptors, ultimately preventing the release of ACh in the neocortex (Nelson et al., 2005; Rasmusson et al., 2007). If the mPFC-NBM circuit is critical for attention-dependent learning as argued here, then muscimol-induced inactivation of the mPFC should impair attention-dependent learning, but should spare attention-*in*dependent learning in the current experiment.

Attention and the Prediction Error Model of Conditioning

The proposed research experiment investigates the role of the mPFC in modulating NBM activity during attention-dependent learning in rats. This experiment behaviorally assessed attention-dependent performance using the incremental attention paradigm. This paradigm involves the manipulation of attention to cues as a function of variation in "prediction error" on a given conditioning trial. Prediction error refers to the difference between a predicted outcome and the actual outcome that occurs on each trial of an associative learning task.

In the standard classical conditioning procedure, prediction error is highest during early conditioning trials where the unconditioned stimulus (US) is not yet well-predicted by the conditioned stimulus (CS) in the

naive animal. This prediction error is believed to enhance attention to the CS, which in turn increases the rate that the animal learns the CS-US association. According to Pearce and Hall (1980), the magnitude of the prediction error influences the associability of the CS on a given trial. When there is a large prediction error, attention levels are increased and the CS is therefore more likely to enter into an association with the US than when attention to the CS is low. This argument suggests that with continued training, the CS comes to predict the US with more and more accuracy. Note that according to this model, prediction error is highest in cases of predictive uncertainty (e.g., early in training), and lowest when the predictive relationship between the CS and US is most firmly established in the animal's mind. This leads to the somewhat counter-intuitive effect of animals initially paying a great deal of attention to CSs whose predictive meaning is uncertain (as evidenced by minimal conditioned responding early in training), to animals paying minimal attention to CSs that have a well-established predictive relationship with the US (as evidenced by maximal conditioned responding late in training).

Violations of Conditioned Expectations and Attention

The unexpected violation of previously established predictive relationships among CSs and USs similarly influences attention to otherwise familiar CSs (Pearce & Hall, 1980). The incremental attention paradigm is a Pavlovian conditioning task involving the violation of conditioned expectations (Wilson, Boumphrey, & Pearce, 1992). In this task, a surprising prediction error is introduced to previously trained animals when an expected outcome fails to occur following the previously accurate CS signal. In response to this prediction error, attention is returned to the CS such that its degree of associability is increased. With this attention-dependent increase in associability, the CS involved can enter more quickly into new associations than it otherwise would (Holland & Gallagher, 2006; Wilson et al., 1992).

The incremental attention task takes advantage of the fact that violating existing conditioned expectations results in an increase in the amount of attention paid to a particular CS. Through the use of this task, we can isolate and manipulate the level of attention paid to conditioned stimuli. In this way, we can use the incremental attention task to discover the relative

importance of particular brain systems in supporting the attentional processes involved in associative learning.

Training in the incremental attention task involves three conditioning phases imposed on two separate groups of animals. One group, the Consistent Prediction group, is exposed to a consistent relationship among several cues that leads to a progressive decrease in the associability of those cues as their predictive inter-relationships are learned. A second group, the Predictive Shift group, is originally trained in the same way as the Consistent Prediction group but then is exposed to a surprising shift in the previously established predictive relationship among cues. This violation of conditioned expectations leads to an increase in the associability of that cue that had its predictive meaning violated. This increased associability is evidenced during subsequent rapid conditioning involving the affected cue.

In Phase I of training, animals are presented with serial conditioning trials where a visual CS (CS Light) is followed by an auditory CS (CS Noise). On 50% of the trials, the light-noise sequence is followed by the delivery of a sucrose pellet US (CS Light-CS Noise-US), and on the other 50% of the trials, the CS Light-CS Noise sequence is not followed by the US (CS Light-CS Noise).

The CS Noise acquires substantial associative strength due to its close temporal proximity to the US, whereas the CS Light acquires less associative strength because it is more temporally remote from the US and therefore is not as obvious a predictor of sucrose pellet delivery. With continued training, the relationship between the CS Light and the CS Noise becomes better established. Consequently, in agreement with Pearce and Hall (1980), animals begin to pay progressively less attention to the CS Light because its predictive relationship with the CS Noise (and with the US) becomes well-learned. A similar decrement in attention to the CS Noise also takes place as its predictive relationship to the US is more firmly established across the training experience (Holland & Gallagher, 2006; Wilson et al., 1992).

In Phase II, the Predictive Shift group is exposed to "surprising" trials where attention to the CS Light is enhanced as a result of changing its relationship to the CS Noise. During phase II trials, rats in this group continue to receive CS Light-CS Noise-US trials on 50% of the trials as before, but instead of the usual CS Light-CS Noise trials occurring on the other 50% of trials, animals are presented with CS Light alone trials. The resulting violation in the predictive relationship between the CS

Light and CS Noise results in an increase in the attentional processing of the CS Light by the animal. During Phase II in the Consistent Prediction group, animals receive the CS Light-CS Noise-US and CS Light-CS Noise trials just as they did during Phase I. For these animals, attention to both CSs continue to diminish.

Changes in associability resulting from the prediction error occurring in the Predictive Shift group during Phase II are subsequently assessed in the final phase of the task. In Phase III, the CS Light is paired directly with the US (CS Light-US) for both the Consistent Prediction and Predictive Shift groups. During initial training in this phase of the task, animals in the Predictive Shift group acquire the CS Light-US association and begin to show a conditioned response (CR) faster than animals in the Consistent Prediction group. This is because animals in the Predictive Shift group experienced prediction error associated with CS Light during Phase II, resulting in a subsequent increase in attention to that cue. Animals in the Consistent Prediction group, on the other hand, do not experience prediction error and therefore continue to pay minimal attention to CS Light. Consequently, these animals learn the CS Light-US

association more slowly than rats in the Predictive Shift group during Phase III.

Hypotheses

The current research examines the role the mPFC-NBM circuit plays in attention-dependent learning. The first hypothesis concerns the validity of the incremental attention task effect, where it was expected that saline-treated control animals trained in the Predictive Shift condition of the task would show superior performance in CR acquisition compared to saline-treated animals trained in the Consistent Prediction condition of the task. This expectation is based on previous research (e.g., Holland & Gallagher, 2006) showing that control animals that experience prediction error (i.e., predictive shift condition) show superior performance in CR acquisition compared to control animals that do not undergo prediction error (i.e., consistent prediction condition) in the incremental attention paradigm.

The second hypothesis is that muscimol-induced inactivation of the mPFC should selectively disrupt attention-dependent but not attention-*in*dependent learning in the incremental attention paradigm. It was therefore expected that an interaction effect between task condition

and drug condition would occur. Specifically, in rats tested in the Predictive Shift condition, where attention-dependent learning is involved, the muscimol-infused group was expected to show poorer performance in CR acquisition than the saline-infused group. In contrast, in rats tested in the Consistent Prediction condition, which does not involve attention-dependent learning, the muscimol-infused group was not expected to differ in their performance compared to the saline-infused group.

Because a significant interaction effect between the task condition and the drug condition could nullify the potential main effects of these two variables, it was expected that, when all rats (including the muscimol-treated and the saline-treated rats) were considered, no significant difference in CR acquisition between the Consistent Prediction condition and the Predictive Shift condition should be observed. It was also expected that when all rats (including those tested in the Consistent Prediction condition and those in the Predictive Shift condition) were considered, no significant difference in CR acquisition between the muscimol-treated condition and the saline-treated condition should be observed.

CHAPTER FIVE

METHODS

Experimental Design

A univariate, 2 x 2 x 3, mixed factorial design was adopted. The first independent variable, "task condition," was a between-subjects variable with two levels (the Predictive Shift condition and the Consistent Prediction condition). The second independent variable, "drug condition," was also a between-subjects variable with two levels (the muscimol-treated condition and the saline-treated condition). The third independent variable "test block" was a within-subjects variable with three levels (one for each of three blocks) in Phase III of the incremental attention task. Each testing block consisted of five trials. The dependent variable was "performance in CR acquisition," operationally defined as a difference score reflecting the duration of the CR relative to baseline responding in the absence of the CS (for details, see Data Analysis below).

Subjects

A total of 80 male Long-Evans rats (weight 275-299 g; 71-79 days old upon arrival) were purchased from a commercial research animal vendor (Harlan, Indianapolis,

IN). Rats were individually housed under a reversed 12 hr light/dark cycle with ad libitum water and standard rat chow prior to surgical and behavioral manipulations. After surgery, rats were reduced to and maintained at approximately 85% of their free-feeding body weight in order to motivate appetitive conditioned approach behavior. An animal technician and veterinarian attended to all research animals in the CSUSB Social & Behavioral Sciences vivarium.

Guidelines for Animal Use

The following procedures involving research animals meet the requirements in the Guidelines for Ethical Conduct in the Care and Use of Animals (American Psychological Association, 2005) and the California State University, San Bernardino Animal Care and Use committee.

Apparatus

Training and testing was conducted in individual computer-controlled, sound-attenuating operant chambers (Coulbourn Instruments, Allentown, PA) equipped with a speaker capable of producing a white noise conditioned stimulus (CS) and a white light located on the front panel of the chamber was used as the visual CS. A sucrose pellet (45 mg; MedAssociates, Lancaster, NH) served as the

unconditioned stimulus (US). Pellets were delivered into a magazine located at floor level. Snout entries into the food magazine were assessed using photobeam response detectors (MedAssociates, Lancaster, NH) located inside the food magazine. A 5 W white light located at the top of the chamber provided ambient illumination. The presentation of CS and US, and response detection were controlled via computer interface (WINLINC, Coulbourn Instruments, Allentown, PA).

Surgery

Animals were anesthetized prior to surgery with sodium pentobarbital (40 mg/kg, i.p.; Sigma, St. Louis, MO). Following the shaving, cleaning (90% ethyl alcohol), and application of a topical antibacterial solution (Betadine) to the scalp, rats were placed in a stereotaxic frame (David Kopf Instruments, Tajunga, CA). A commercially available ophthalmic lubricant was used to lubricate the animals' eyes. A 1.5 cm incision was made in the scalp along the midline, the periosteum above the skull was deflected, and the surrounding skin and musculature was deflected laterally. Using a stereotaxic drill (David Kopf Instruments, Tanjunga, CA) with a sterile bit, craniotomies were made in the skull as

follows. The craniotomies for the medial prefrontal cortex infusions were located 3 mm anterior to bregma and +/- 1.5 mm lateral to midline. Stainless steel double guide cannula (26 gauge; Plastics One, Roanoke, VA, USA) was then stereotaxically lowered to a level 1.5 mm below dura. The cannulae guides were secured to the surface of the skull by embedding the lower portion of each guide in a mound of dental acrylic anchored to the skull via sterile, stainless-steel screws. Stainless steel stylets were used to seal the cannulae guides until time of muscimol infusion. The incision site was cleaned and sutured around the dental acrylic mound allowing the experimenter to have access to the ends of the cannulae guides. Topical lidocaine (0.1%) was applied to injection site. Immediately following surgery, rats received a single injection of analgesic (Ketaprofen; 2 mg/kg, s.c.), and an injection of antibiotic (Baytril; 5 mg/kg, s.c.) prior to being returned to their home cages.

Procedures

Rats were randomly assigned to one of four groups as follows. For each task condition (i.e., Predictive Shift and Consistent Prediction), separate groups of rats received mPFC infusions of either muscimol or saline,

where saline served as a control for potential confounds associated with the infusion procedure. These groups included saline-treated (SHIFT-SAL; n = 20) or muscimol-treated rats (SHIFT-MUSC; n = 20) tested in the Predictive Shift condition, and saline-treated (CONSIST-SAL; n = 20) or muscimol-treated rats (CONSIST-MUSC; n = 20) tested in the Consistent Prediction condition.

Behavioral Training and Testing

Upon surgical recovery, rats were placed in the testing chambers with 10 sucrose pellets in the magazine tray in order to habituate to the testing environment. On the following day, rats began training in the incremental attention task. In Phase I of this task, rats were exposed to 60 serial conditioning trials per day for 10 days. In these trials, a visual conditioned stimulus (CS Light) and an auditory conditioned stimulus (CS Noise) were presented sequentially. In half of these trials, the CS Light-CS Noise sequence was followed by the US (CS Light-CS Noise-US), and in the other half of trials no US occurred (CS Light-CS Noise). In Phase II, rats were exposed to 60 serial conditioning trials for one day. Half of the rats trained in Phase I continued to receive the CS Light-CS

Noise-US sequence on half of their trials, but the CS Light-CS Noise trials without US occurrence was replaced by CS Light alone trials in this "Predictive Shift" condition. The remaining rats, in the Consistent Prediction condition, trained in Phase I simply continued to receive CS Light-CS Noise-US and CS Light-CS Noise trials during Phase II. Behavioral testing in Phase III, which consisted of 15 serial conditioning trials for one day, began 24 hours after the previous Phase II testing session. Muscimol or saline infusion into the mPFC occurred immediately before placing animals in the testing chambers. In this phase, CS Light was paired directly with the US (CS Light-US). For all three phases, the presentation of each stimulus lasted 10 seconds. The intertrial interval for phases I and II averaged 40 seconds and the intertrial interval for phase III averaged 110 seconds.

Drug Infusion

Immediately prior to testing in Phase III, rats received intra-cerebral infusions of either muscimol (2 μg/μl concentration at a volume of 0.5 μl) or physiologic saline depending on their group assignment. The muscimol concentration used was based on previous

studies showing impaired performance following muscimol administration into the mPFC (Pang et al., 1993; Rasmusson et al., 2007; Izaki et al., 2001).

Temporary stylets and caps were removed and replaced with an infusion cannulae (33 gauge; Plastics One), extending guide cannulae 0.5 mm into mPFC. The cannulae were connected to microsyringes (10 μl) attached to a dual infusion pump (World Precision Instruments, Sarasota, FL, USA). Muscimmol or saline was continuously infused over a period of 60 seconds. Cannulae remained in place for another 60 seconds prior to removal of the cannulae and return of stylets. Rats were then placed in the testing chambers and the final phase of the incremental attention task began.

Histology

Upon completion of behavioral testing, rats were administered a lethal dose of sodium pentobarbital (80 mg/kg, i.p.; Sigma, St. Louis, MO), followed by cardiac perfusion with 0.9% saline solution and with 10.0% formalin solution (preparatory procedures for brain tissue histology). Brains were extracted and placed in a 10.0% formalin and 30.0% sucrose solution for 48 hrs prior to freezing and sectioning on a sledge microtome (Model 860,

American Optical Co., Buffalo, NY). Sections (60 μm) were stained with cresyl violet and examined to verify placement of cannulae.

Data Analysis

A 2-way ANOVA for a mixed design was used to test the hypothesized task effect. A 3-way ANOVA for a mixed design was used to test the hypothesized interaction effect. Two additional analytical comparisons (i.e., independent-samples *t*-tests) were used to further explore hypothesis 2. A significance level of $p = .05$ was adopted to conclude statistical significance.

The dependent variable was CR acquisition performance, operationally defined as a difference score reflecting the duration of conditioned approach to the food-cup during CS presentation relative to the duration of baseline responding in the absence of the CS. To calculate this CR difference score, the total duration of food-cup approach during the last 6 s of each 10 s CS-Light presentation for each of the 5 trials per block was subtracted from the mean duration of food-cup approach during a comparable interval during the ITI when the CS was absent.

The decision to include only the last 6 s of each 10 s CS presentation in calculating the CR dependent measure in this experiment was based on the fact that visual cues predicting food evoke a CR characterized by initial rearing and orienting towards the visual cue, followed by approach to the food cup during the latter part of the CS interval (Bucci et al., 1998). Because of this initial rearing behavior, snout entry into the food cup is delayed until late in the CS presentation. Therefore, only the last 6 s of the 10 s CS presentation was considered.

To determine the mean duration of responding in the absence of the CS (i.e., mean baseline responding) for use in calculating the CR difference score, the average duration of responding per 30 s of inter-trial interval (ITI) was obtained. During Phase III testing, the mean ITI was 110 s, with a total of 550 s of ITI per 5-trial block. However, the interval of responding used to calculate the duration of food-cup approach during CS presentations was 6 s per trial (with a maximum possible response of 30 s per 5-trial block). Therefore, in order to derive a mean baseline ITI response duration equivalent to the 30 s possible CR duration, the total duration of food-cup approach during each ITI (550 s total per block) was

calculated as follows: 550 ÷ 18.3 ≈ 30 s. Thus, the CR was calculated by subtracting CS accumulation duration of responding from this mean ITI duration of responding. This calculation of baseline responding resulted in equivalent intervals used to compare responding during the CS to responding in the absence of the CS (i.e., baseline responding).

CHAPTER SIX

RESULTS

Due to surgical complications (8 animals) or isolated equipment malfunctions (9 animals), behavioral data for only 63 of the 80 animals tested in this experiment were analyzed. The total number of subjects in each condition included in the statistical analysis was as follows: SHIFT-SAL, n = 16; SHIFT-MUSC, n = 15; CONSIST-SAL, n = 17; CONSIST-MUSC, n = 15. Table 1 provides a summary of the results.

Task Effect

To validate the existence of the task effect (i.e., the effect the incremental attention task has on performance in CR acquisition- more specifically, the existence of the task advantage of the Predictive Shift condition over the Consistent Prediction condition, without the interference of the drug), a two-way [2 (Task condition) x 3 (Test Block)] ANOVA for a mixed design was performed on the behavioral data from all the saline-treated control rats (n = 33). As shown in figure 1, results confirmed superior learning in saline controls in the Predictive Shift condition compared to saline controls in the Consistent Prediction condition

($F(1, 31) = 5.997$, $p = .020$; SHIFT-SAL: M = 655.26, SD = 697.10; CONSIST-SAL: M = 144.31, SD = 489.48).

Task Condition by Drug Condition Interaction

To test that an intact mPFC-NBM circuit is necessary for attention dependent learning, a three-way [2 (Task condition) x 2 (Drug condition) x 3 (Test Block)] ANOVA for a mixed design was performed on data from all rats in the experiment (n = 63). Results confirmed the existence of a significant interaction effect between task condition and drug condition, $F(1, 59) = 8.099$, $p = .006$. As expected, muscimol treatment disrupted performance in the Predictive Shift condition but not in the Consistent Prediction condition (see Figure 2).

An independent samples *t*-test was performed on data from the muscimol-treated rats (n = 15) and the saline-treated rats (n = 16) tested in the Predictive Shift condition to further examine the effect of muscimol treatment. As expected, when compared to saline-treated rats tested in the Predictive Shift condition, muscimol-treated rats tested in the same task condition showed significantly poorer performance ($t(29) = 2.975$, $p = .006$; SHIFT-SAL: M = 655.26, SD = 697.10; SHIFT-MUSC: M = -7.32, SD = 547.07).

A second independent samples *t*-test was performed on data from the muscimol-treated rats (n = 15) and the saline-treated rats (n = 17) tested in the Consistent Prediction condition to test the effect of muscimol treatment. As expected, the results revealed no significant differences in performance between the muscimol-treated rats and the saline-treated rats tested in the Consistent Prediction condition ($t(30) = -.880$, $p = .386$; CONSIST-SAL: M = 144.31, SD = 489.48; CONSIST-MUSC: M = 312.62, SD = 592.49).

Main Effects of Task Condition and Drug Condition

As predicted, results revealed no significant main effects of task condition or drug condition. When all rats (including the muscimol-treated and the saline-treated rats) were considered, no significant difference in CR acquisition between the Consistent Prediction condition and the Predictive Shift condition was observed (CONSIST: M = 223.21, SD = 538.03; SHIFT: M = 329.82, SD = 706.67). When all rats (including those tested in the Consistent Prediction condition and those in the Predictive Shift condition) were considered, no significant difference in CR acquisition between the muscimol-treated condition and the saline-treated

condition was observed (MUSC: M = 147.65, SD = 584.90; SAL: M = 392.04, SD = 644.07).

Histology

Brains were sectioned and stained to verify placement of cannulae. For each of the 63 rats included in the data analyses, the placement of cannulae was found within the range of the mPFC (as shown in Figure 3).

Table 1. Mean (SD) Conditioned Response Difference Scores (Expressed in 20 ms Units): Task x Drug x Blocks

	Block			
	Block 1	Block 2	Block 3	Total
Predictive Shift				
Saline (n = 16)	67.49 (209.75)	248.72 (314.48)	339.05 (307.52)	655.26 (697.10)
Muscimol (n = 15)	-122.55 (188.78)	32.10 (246.44)	73.12 (242.58)	-17.32 (547.07)
Total (Saline + Muscimol) (n = 31)	-24.46 (218.97)	143.90 (299.84)	210.38 (304.95)	329.82 (706.67)
Consistent Prediction				
Saline (n = 17)	-99.35 (202.20)	57.06 (145.08)	186.60 (225.18)	144.31 (489.48)
Muscimol (n = 15)	17.26 (171.21)	145.74 (218.31)	149.62 (334.48)	312.62 (592.49)
Total (Saline + Muscimol) (n = 32)	-44.69 (194.51)	98.63 (185.50)	169.27 (277.57)	223.21 (538.03)
Saline Infusion				
Total (Shift + Consistent) (n = 33)	-18.46 (219.63)	149.98 (257.57)	260.52 (275.08)	392.04 (644.07)
Muscimol Infusion				
Total (Shift + Consistent) (n = 30)	-52.64 (190.81)	88.92 (235.94)	111.37 (289.71)	147.65 (584.90)
Total (n = 63)	-34.74 (205.48)	120.91 (247.44)	189.49 (289.74)	275.67 (623.91)

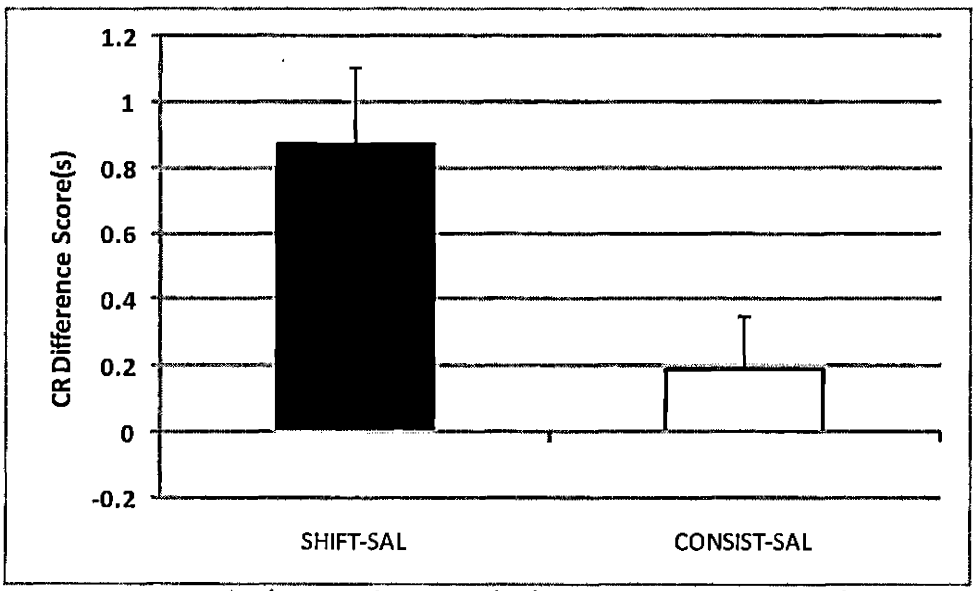

Figure 1. Mean (+/- SEM) Conditioned Response Difference Scores Collapsed Across Testing Blocks in Saline Control Rats Trained in the Predictive Shift Condition (SHIFT-SAL) and in Saline Control Rats Trained in the Consistent Prediction Condition (CONSIST-SAL)

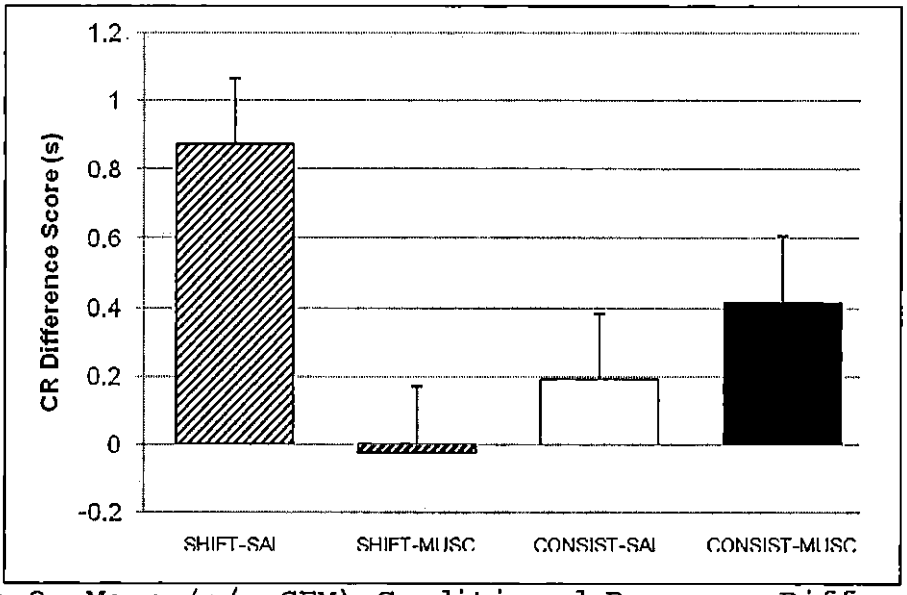

Figure 2. Mean (+/- SEM) Conditioned Response Difference Scores Collapsed Across Testing Blocks in Rats Trained in the Predictive Shift Condition Following Saline (SHIFT-SAL) or Muscimol Treatment (SHIFT-MUSC), and in Rats Trained in the Consistent Prediction Condition Following Saline (CONSIST-SAL) or Muscimol Treatment (CONSIST-MUSC)

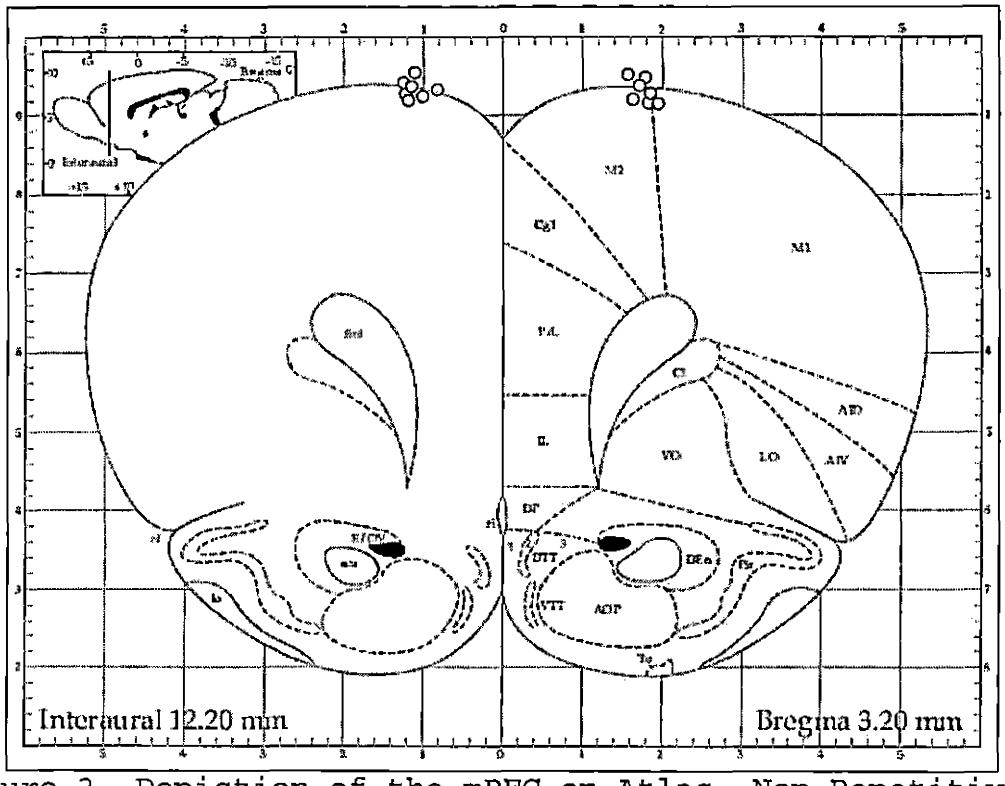

Figure 3. Depiction of the mPFC on Atlas. Non-Repetitive Sites of Cannulae Termini (Marked by Grey Circles) in the mPFC from a Representative Sample of Animals. Cannulae were Found within the Area of the Secondary Motor Cortex

CHAPTER SEVEN

DISCUSSION

The purpose of the present experiment was to provide behavioral evidence supporting the existence of an mPFC-NBM circuit, with the mPFC purportedly modulating NBM cholinergic activity during attention-dependent learning. Attention-dependent learning was assessed using the incremental attention task, where superior Phase III CR acquisition in the Predictive Shift condition, compared to the Consistent Prediction condition of the task demonstrates attention-dependent learning. The experiment was designed to disrupt the mPFC-NBM circuit by inactivating the mPFC with muscimol. Overall, results revealed that mPFC inactivation resulted in impaired attention-dependent performance but not attention-*in*dependent performance, suggesting that the mPFC plays a critical role in regulating NBM activity during attention-dependent learning.

Inactivation of the mPFC prevented the attention-dependent enhancement in associative learning normally seen in the Predictive Shift condition of the incremental conditioning paradigm (see Holland & Gallagher, 2006). As hypothesized, muscimol-treated rats

in the Predictive Shift condition showed impaired CR acquisition during Phase III of the task when compared to saline-treated rats. In contrast, muscimol treatment-induced inactivation of the mPFC had no effect on performance in the Consistent Prediction condition of the incremental attention task.

These findings support the hypothesis that muscimol infusion into mPFC would result in a dissociation of impairment in attention-dependent and *in*dependent learning; performance was disrupted in the muscimol-treated rats tested in the attention-dependent Predictive Shift condition but not in the attention-*in*dependent Consistent Prediction condition. Because enhanced performance in the Predictive Shift condition of the task depends on increased attention to the predictive cue (see Holland & Gallagher, 2006; Wilson et al., 1992), the impairment observed in the muscimol-treated rats is argued to result from a disruption in attention processes. In contrast, attention-*in*dependent performance in muscimol-treated rats trained in the Consistent Prediction condition was not affected by muscimol infusion into mPFC. These data strongly suggest that mPFC inactivation causes a selective impairment in attention-dependent learning, which itself

is known to depend on cholinergic activation of the neocortex by the NBM within the basal forebrain (Bucci et al., 1998; Chiba et al., 1995; Holland & Gallagher, 2006).

The argument for the specificity of mPFC inactivation effects on attention-dependent learning, but not attention-*in*dependent learning, is supported by the statistically significant finding of superior CR acquisition in the saline-treated rats trained in the Predictive Shift condition as compared to saline-treated rats trained in the Consistent Prediction condition of the task. This facilitation of learning in normal animals is consistent with earlier prediction error studies demonstrating improved learning performance in animals that previously experienced a violation of the conditioned expectations associated with at particular cues (e.g., Bucci et al., 1998; Chiba et al., 1995; Holland & Gallagher, 2006; Wilson et al., 1992).

Although the role of the mPFC in the cognitive process of attention is well-established (see Sarter et al., 2001, 2005, 2006), this study is the first to demonstrate the necessity of the mPFC in attention-dependent learning in the incremental attention task. Similarly, while muscimol inactivation of the mPFC is known to impair performance in working memory in the

delayed alternation task (Izaki et al., 2000) and to disrupt memory consolidation in inhibitory avoidance learning (Souza et al., 2000), the current findings are the first to demonstrate mPFC muscimol infusion-induced impairments in the cognitive process of attention.

Beyond the immediate interpretation of these findings, which demonstrate mPFC involvement in attention-dependent learning, the current results also inform our understanding of how the mPFC might interact with the cholinergic NBM. In particular, findings from this study are consistent with the hypothesis that the mPFC exerts modulatory control over the NBM through a descending projection system (see Gaykema et al., 1991; Zaborszky et al., 1997) that is engaged during attention-dependent learning (see Sarter et al., 2001, 2005, 2006). Impaired performance in the Predictive Shift condition due to inactivation of the mPFC, suggests that the mPFC may play a critical role in modulating basal forebrain activity, which itself is necessary for attention-dependent learning (Bucci et al., 1998; Chiba et al., 1995; Holland & Gallagher, 2006). While the role of the NBM in activating neocortex and thereby contributing to cognition, learning, and memory has long been established (Arnold et al., 2002; Himmelheber et al.,

2000; Lucas-Meunier et al., 2003; Wenk et al., 1994), little has been published on the question of how the NBM itself is controlled in attention, learning, and memory. The observation of a selective impairment in attention-dependent learning in mPFC-inactivated animals in the current experiment, therefore, provides valuable indirect evidence suggesting that the mPFC-NBM circuit plays a critical role in mediating attentional processes during normal learning.

Findings from the current research bring us one step closer to understanding the brain's ability to control which features of the environment are attended to, where selective attention, in turn, influences what aspects of experience are best remembered. We found that rats with an inactivated mPFC failed to increase their attention to sensory cues with uncertain predictive value, and consequently did not benefit from the enhanced attention normally resulting from prediction errors (see Holland & Gallagher, 2006; Wilson et al., 1992). These data demonstrate the critical importance of the mPFC in regulating attention and thereby influencing the ability to learn new associations.

While neuroanatomical and pharmacological studies have implicated the mPFC in the top-down control over NBM

(Gaykema et al., 1991; Nelson et al., 2005; Rasmusson et al., 20007; Zaborszky et al., 1997), the findings presented here are unique in suggesting that such a circuit is involved in regulating attentional processes during natural learning. By considering how the mPFC and NBM interact as part of a larger system, we can achieve a more complete understanding of the neurobiological mechanisms controlling attention during learning.

Future research might include electrophysiological studies designed to directly measure activity in the mPFC, the NBM, and its cortical targets during performance in the incremental attention paradigm. Similar mPFC inactivation studies could also be done using other behavioral tasks involving manipulations of attention during learning. Information coming from such experiments will contribute to a broader understanding of the dynamic interactions among brain regions during complex, cognitively demanding forms of learning.

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