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Top-down modulation by medial prefrontal cortex of basal forebrain activation of auditory cortex during learning

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TOP-DOWN MODULATION BY MEDIAL PREFRONTAL CORTEX OF BASAL FOREBRAIN ACTIVATION OF AUDITORY CORTEX DURING LEARNING

A Thesis

Presented to the

Faculty of

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In Partial Fulfillment

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ABSTRACT

Acetylcholine (ACh) release in the rat auditory cortex is greater in rats undergoing auditory classical conditioning compared to rats in a truly random control paradigm where no associative learning takes place. The current experiment tests the hypothesis that this associatively dependent modulation is mediated by prefrontal afferent projections influencing the nucleus basalis magnocellularis (NBM), which in turn modulates ACh release in neocortex. Rats with bilateral ibotenic acid lesions of medial prefrontal and agranular insular cortices were tested in an auditory classical conditioning task while ACh was collected from the primary auditory cortex. It was hypothesized that lesions of these prefrontal areas would prevent learning-related increases of ACh release in the primary auditory cortex. The hypothesized results were supported. Rats with lesions of the prefrontal cortex had significantly less ACh release than Sham lesion controls. This result suggests that prefrontal afferents act on the NBM to modulate cholinergic activity in sensory neocortex as a function of the behavioral or predictive significance of sensory stimuli. Results from this experiment provide unique evidence that medial prefrontal cortex projections to the

NBM are important for mediating cortical ACh release during associative learning. A prefrontal-basal forebrain circuit operating differentially on behaviorally significant versus irrelevant stimuli might serve as a neurobiological substrate for selective attention and for ACh-dependent representational synaptic plasticity in primary sensory cortex.

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CHAPTER ONE

NEUROBIOLOGY OF PAVLOVIAN CONDITIONING

Introduction

Classical or Pavlovian conditioning is an associative form of learning where a previously neutral conditioned stimulus (CS) is paired with an unconditioned stimulus (US) that elicits an unconditioned response (UR). After sufficient pairings, the previously neutral stimulus begins to elicit a learned or conditioned response (CR) (Frieman 2002). As simple as the process of Pavlovian conditioning may seem, there are many behavioral and neurological components involved in conditioned responding, stimulus detection, and stimulus discrimination.

Appetitive conditioning is just one subtype of classical conditioning that is widely used in research where the US is a rewarding or preferred stimulus. The majority of the research on the neurobiology of appetitive Pavlovian conditioning is focused on the dopaminergic reward structures and pathways. While this intrinsically makes sense, there are other structures involved in appetitive conditioning whose functioning allows for such basic components of Pavlovian conditioning as acquisition

of the task, conditioned responding, and stimulus differentiation.

Motivation and Pavlovian Conditioning

One basic prerequisite for appetitive Pavlovian conditioning is motivation for rewarding stimuli used as USs. Researchers ensure the motivation for food reward through the use of food deprivation. Rats on a food deprivation schedule are readily accepting of food rewards. However, Ito, Everitt, and Robbins (2005) show that the hippocampus also plays a role in incentive properties of food. The hippocampus (HPC) participates in associative learning involving spatial and contextual information. However, selective lesions of the HPC also cause alterations in appetitive conditioning using food reward.

Ito, Everitt, and Robbins (2005) hypothesized that the HPC plays an inhibitory role in appetitive Pavlovian conditioning. In their experiment, rats were separated into 2 groups, the HPC lesion and sham lesion control groups. After recovery, rats were placed in activity cages and were presented with a 10 s white rectangle visual stimuli (CS+) displayed on one side of a video display unit paired with sugar pellets (US) on some trials, and a

10s white rectangle displayed on the other side of a video display unit (CS-) paired with no delivery of sugar pellets on other trials. After acquisition, rats received omission training in which they were presented with both the CS+ and CS- without sugar pellets. Conditioned responding was measured through conditioned approach or autoshaping behavior.

Results showed that both the sham and HPC lesioned rats acquired a conditioned approach response. However, the HPC lesioned rats consistently performed the CR significantly faster than the sham lesion group. The HPC lesioned rats did not show a generalized arousal, and did show normal habituation, and the increased conditioned approach was only observed in anticipation of food in the testing cage. HPC lesions have been shown to increase appetitive conditioned responding in previous research and is further supported in this experiment.

Ito, Everitt, and Robbins (2005) explain the observed increase in responding to the CS as a possible increase in the incentive for the reward or for the CS associated with reward. This idea is supported by the fact that the HPC works through its inhibitory influence on the nucleus accumbens, and lesions to this site reduce this inhibitory affect.

Conditioned Stimulus Detection

Another basic component of appetitive Pavlovian conditioning is the ability to detect and process the CS. Several bodies of research have demonstrated that different components of the brain may be responsible for the processing of components of conditioned stimuli. Mingote, Bruin, and Feenstra (2004) found that during appetitive classical conditioning, rats had an increase in noradrenaline (NA) and dopamine (DA) in the prefrontal cortex (PFC). However, rats showed an increase in NA only during extinction trials where the CS was presented alone. This shows that NA may be important to responding to CSs that predict appetitive stimuli.

Motivational processes, as well as cognitive functions of the PFC such as working memory, depend on the mesocorticolimbic DA system. With the use of in vivo microdialysis techniques, DA has been shown to increase in response to appetitive USs as well as CSs predicting appetitive USs. NA also modulates PFC functioning such as processing reward related information, and NA has been found to play a role in the motivational effects of drugs.

Previous research has found that both NA and DA increase in the PFC in response to aversive conditioning, indicating that both neurotransmitters may be important

for conditioning. Mingote, Bruin, and Feenstra (2004) hypothesized that the release of both NA and DA would increase during an appetitive classical conditioning task. In their experiment, male Wistar rats were anesthetized and two microdialysis probes were implanted bilaterally into the medial PFC. After recovery, rats began an appetitive Pavlovian conditioning task in a Skinner box. The Skinner box was equipped with a motion detector to record locomotor activity, a food dispenser with infrared beams to detect nose pokes into the food dispenser, and a wall mounted speaker used to deliver white noise. Rats were separated into three groups: paired, unpaired, and control. The paired group received six presentations of the white noise CS followed by sugar pellet US delivery into the food dispenser on three consecutive days. The unpaired group received six presentations of the CS and six presentations of the US unpaired on three consecutive days, and the control group received CS only presentations for three consecutive days. On the third day, two hours after training, rats received extinction sessions. NA and DA were measured throughout the third day.

Mingote, Bruin, and Feenstra (2004) found that rats in the paired group showed more conditioned responding, defined as an increase in nose pokes during the CS,

increased motor activity during the CS, and shorter latency to nose poke during the CS. They also found an increase in NA and DA in the paired group as well as the unpaired group. However, the increase was not observed in the control group during conditioning sessions. Interestingly, an increase in NA was observed during the extinction phase in rats in the paired group only. These results show that both NA and DA respond to rewarding stimuli. However the DA may respond solely to stimuli that resemble rewards, whereas NA is implicated in selective attention to explicit CSs that are not necessarily similar to rewarding stimuli.

Bonardi (2001) found that the dorsal hippocampus is also important in processing conditioned cues. Specifically, Bonardi (2001) found that lesions of the dorsal hippocampus caused impairments in appetitive conditioning with localized cues. Previous research suggested that Pavlovian conditioning was left intact by lesions of the hippocampus, while spatial learning was impaired by these lesions. However, lesions of the hippocampus have been shown to disrupt Pavlovian trace conditioning, taste aversion, and sensory preconditioning. Rats were tested in three experiments.

In Bonardi's (2001) experiments, rats were separated into a dorsal hippocampus lesion group and a sham lesion control group. Lesions were performed and, after recovery, rats were placed in an operant chamber for Pavlovian conditioning training. The operant chamber was equipped with a food dispenser with a transparent plastic door such that snout entries would cause the door to be pushed in and recorded electronically. Snout entries during the CS were used as a measure of conditioned responding. The first experiment used a light inside the food dispenser as a CS and presented rats with eight conditioning sessions in which they received eight CS-US (food pellet) pairings. Some of these rats progressed to experiment two (in which white noise served as the CS) and where they recieved four training sessions. In the final experiment, naive rats were separated into two groups. Both groups received fourteen sessions of eight CS-US pairings, but the first group had an overhead light as the CS and the second group had a light inside the food dispenser as a CS. After the initial fourteen sessions, rats recieved another fourteen sessions of CS-US pairings that reversed the CSs for each group so that group one received the light inside the food dispenser as a CS and the second group received the overhead light as CS.

Results showed that rats with dorsal hippocampus lesions had impaired conditioning to cues within the food dispenser, normal conditioning to the overhead light as a CS, and enhanced conditioning to the auditory cue. Bonardi (2001) suggested that the differences in the ability for the CS to elicit a CR in the rats with the dorsal hippocampus lesions could be due to the inability of these rats to learn about a localized cue despite their ability to learn about more diffuse cues.

Ascending projections from the amygdala central nucleus (CeA) are important for conditioned orienting responses when a CS is paired with a food US (Lee et al., 2005). Lee et al. (2005) examined the role of the amygdalo-nigral circuitry in an appetitive conditioning task where a visual stimulus was paired with food. Previous research had shown that lesions to either the CeA or lesions that disconnected the CeA from the dorsolateral striatum (DLS) attenuated conditioned orienting responding, although rats maintained normal unconditioned orienting responses and conditioned food cup behavior (2005).

This evidence implicated the ascending projections from the CeA to the substantia nigra pars compacta (SNc), because since it is the only pathway between the CeA and

the DLS. Lee et al. (2005) examined the function of the ascending pathway between the CeA and the DLS using a retrograde axonal tracer in conjunction with a neuronal activation tracing method to visualize whether the CeA neurons that project to the SNc were in fact activated by exposure to a CS. Activation was defined as double labeling in neurons with both the axonal tracer Fluoro-Gold and the neuronal activation c-fos.

Male rats were anesthetized and received injections of the retrograde axonal tracer Fluoro-Gold into SNc and, after recovery, were individually placed in a chamber containing a food cup equipped with phototransistors to record head entries as a CR as well as a video camera to record orienting responses as a CR. Rats were separated into three groups consisting of unpaired, paired, and paired II groups. Rats in the paired group received 16 CS-US (light-food) pairings, rats in the unpaired group received 16 CSs and 16 USs explicitly unpaired, and rats in the paired II group received 48 CS-US pairings to assess the consequences of extended training. At the end of behavioral training, rats received a 16 min test session in which they were presented with CS alone and were then sacrificed 90 min after the beginning of the

test session in order to detect c-fos protein expression, a marker for activity in the CeA in response to the CS.

Behavioral results showed that rats in the paired II group showed greater conditioned responding than the paired group, and the paired group showed greater conditioned responding than the unpaired group. Lee et al. (2005) also found that rats in the paired groups had greater amounts of Fos expression indicating more activity in the medial CeA and the majority of the Fos positive neurons projected to the SNc and were double labeled with Fluoro-Gold. The Fos expression along with conditioned responding provide evidence that the amygdalo-nigral circuitry is important in an appetitive associative learning task and is important for responding to the CS.

Differential Conditioning

Another component that is important for appetitive Pavlovian conditioning to occur is the differential conditioning or discrimination of a CS predicting a rewarding US event (CS+) from another CS predicting the absence of the US(CS-). Cardinal et al. (2003) investigated the role of the anterior cingulate cortex (ACC) in appetitive classical conditioning. Lesions of the ACC have been shown to impair autoshaping in a task where

a CS+ and CS- are presented. Rats with lesions of the ACC fail to discriminate between these cues and instead approach in response to both the CS+ and CS-. Male rats were separated into two groups (ACC lesion vs. sham lesion), lesioned, and allowed to recover. Rats were then placed in a testing chamber with a display on one wall and a pellet dispenser located in the center of the display. Pressure sensitive floors were located in the center, right, and left of the display screen to electronically measure conditioned approach responses. Rats were trained for two days with 50 trials per day of CS+ (white vertical rectangles presented on one side of the display) - US (food pellets) pairings, and CS- (white vertical rectangles presented on the other side of the display) and no US. Finally, after training, CS+ and CS- were presented simultaneously without the presentation of food and conditioned approach was measured. Rats with lesions of the ACC showed significantly less conditioned responding than sham lesion rats. Cardinal et al. (2003) states that the ACC may be important for conditioned responding when the reward is not located in the same area as the CS, or it is necessary for discriminating between stimuli that are differentially associated with reward.

Cassaday and Norman (2005) observed that lesions to the nucleus accumbens shell and core have differential effects on conditioning to discrete and contextual cues in appetitive procedures. They found that lesions of the nucleus accumbens shell resulted in an increase in contextual conditioning but had no effect on discrete cues. Previous studies have shown the nucleus accumbens is divided into different regions and these divisions may represent functional divisions. Previous research using lesions of the nucleus accumbens in aversive conditioning have shown conflicting data. Cassaday and Norman (2005) examined the role of the nucleus accumbens shell and core in several appetitive trace conditioning tasks. Rats were separated into nucleus accumbens shell lesion or core lesion groups, lesioned, and allowed to recover. After recovery, rats were placed in an appetitive classical conditioning task. Conditioning took place in conditioning chambers equipped with a food magazine illuminated in the presence of food, and a photobeam was used to record nose pokes into the magazine. Rats were further separated into two conditioning groups, one receiving a 10 s trace conditioning task, the other receiving a 0 s trace conditioning task. Conditioning consisted of eight CS-US presentations (noise-food). Finally, two days of

extinction were used to determine the extent of contextual conditioning (day 1 background light contextual CS) and discrete cue (sound CS) conditioning.

Conditioned Responding

Another important component of appetitive Pavlovian conditioning is the acquisition and maintenance of a CR. Previous research has shown that conditioned orienting responses depended on the amygdala central nucleus (CN) (Grosheck et al., 2005). Increased activity in CN and its projections to the substantia nigra pars compacta, as well as the dorsolateral striatum, have been shown to increase responsiveness to sensory stimuli.

Grosheck et al. (2005) examined the role of the CN in the acquisition of a CR and its maintenance during an appetitive Pavlovian conditioning task. Rats were separated into two groups. One group received an injection of the antagonist NBQX into the CN, thus inactivating CN function unilaterally, and the other group received vehicle injections. All rats subsequently received unilateral lesions of the CN contralateral to the site of injections. Rats received three conditioning sessions in which 16 noise-food pellet (CS-US) pairings were presented. Rats then underwent two test sessions in which

they received presentations of a 78 dB white noise CS for 10 s with no food delivery. During the first test session, half of the rats received a vehicle injection, while the other half received a NBQX injection. In the second session these conditions were reversed.

Groshek et al. (2005) found that rats that received vehicle injections quickly acquired conditioned orienting responses to the conditioned stimuli, while rats that received injections of the antagonist NBQX during training phases showed lower conditioned orienting responding. During the testing session, rats that who received injections of NBQX during the training phase showed lower conditioned orienting responding than those that received vehicle injections during the training period. Conditioned food cup behavior was not different between groups. These results show that CN integrity is important for acquiring a CR but are not necessary for maintaining a previously learned CR.

Parkinson et al. (2000) also found impairments with conditioned responding following lesions of the ACC or to the nucleus accumbens (NAcc). Previous research has shown that lesions of the NAcc disrupt spatial learning, Pavlovian conditioning, instrumental learning, and declarative memory. Lesions of both the NAcc and the

interconnected ACC impair conditioned approach, where those brain structures have been interpreted as having an important influence on appetitive classical conditioning.

Parkinson et al. (2000) examined the affects of the connected NAcc and ACC structures in an appetitive classical conditioning task. Rats were separated into 4 groups: ACC lesioned, NAcc core lesioned, NAcc shell lesion, and sham lesion. After recovery, rats were placed in a Skinner box equipped with a video display unit, a magazine hopper, and a pressure sensitive floor that recorded conditioned approach electronically. During conditioning CS+ (10 s stimuli presented on one side of the video display unit) was paired with US (sugar pellet) and CS- (10 s stimuli presented on the other side of the video display unit) was not paired with US. 50 trials per day for two days and conditioned approach were recorded. After training was complete, subjects received an extra session of omission training in which they received 50 trials of CS+ and CS- without the delivery of a sugar pellet.

Analysis show that rats with lesions of the ACC had normal CS+ conditioned approaches however, they also showed and increase in CS- approaches which may indicate a form of impulsivity produced by the ACC lesion, because

the ACC is implicated in attention, memory, and emotion. These rats also showed an increase in CS- responding during omission training. Rats that received NAcc core lesions showed less conditioned responding than sham lesioned rats during conditioning and omission training which may be due to a disruption in the ability to discriminate, produce, or express a CR. Rats that received NAcc shell lesions did not differ from the sham lesion control group in their acquisition of a CR or during omission training.

It is unclear whether DA originating from the NAcc enables learning, mediates expression of the CR, or mediates the selection of an already learned response (Parkinson et al., 2002). DA in the NAcc has been argued to be critical for reward, and correlations have been observed with predictive stimuli and DA release within the NAcc. Parkinson et al. (2002) examined whether DA in the NAcc was necessary for Pavlovian learning or for the performance of an already acquired Pavlovian response. Rats were anesthetized and microdialysis probes were implanted into the NAcc to measure DA levels. Upon recovery, rats were randomly assigned to 3 groups. The first group received intra-NAcc injections of 6-OHDA (a DA depleting lesion) or sham injections, and was conditioned

10 days later. The second group received injections of 6-OHDA or sham lesions and was conditioned 2 months later. The third group received injections of 6-OHDA or sham lesion after demonstrating discriminated approach to the CS. Conditioning was conducted inside a Skinner box with a video display unit and a food magazine. Conditioning consisted of a 10 s stimulus presented on one side of the video display unit (CS+) followed by a sugar pellet (US) delivered into the food magazine, and another stimulus presented on the other side of the video display unit (CS-) that was not followed by delivery of a sugar pellet. Rats received 50 trials per day across two days and conditioned approach was measured via a pressure sensitive floor in front of the stimulus. After conditioning, one day of extinction commenced in which both the CS+ and CS- were presented without the US and conditioned approach was measured. All lesions were confirmed to cause a decrease in DA levels in the NAcc as well as in the prefrontal cortex and NA were also reduced in the NAcc and prefrontal cortex for all groups except those tested two months after injections.

Animals receiving lesions 10 days prior to testing failed to show an increase in conditioned responding across trials, and in fact showed an overall reduction in

responding across trials. Animals receiving a lesion 2 months prior to testing showed a general increase in responding across trials. However, there were significant fluctuations in their approach behavior. Animals that had been trained for conditioned responding prior to NAcc lesions showed significantly fewer approaches to the CS+ during the extinction phase. All rats, regardless of when the NAcc lesion was introduced, showed significant impairments in conditioned responding whereas spontaneous locomotor was similar to sham lesion rats.

These findings support the idea that the NAcc is important for performing appropriate conditioned responses and the differential effects of lesions induced during the learning phase and lesions produced after the learning phase indicate that the NAcc may play a different role in each phase.

Previous studies have found conflicting reports on the role of the nucleus basalis of Meynert (or nucleus basalis magnocellularis/NBM in the rat) in conditioned responding (Olmstead, Robbins, & Everitt, 1998). Lesions of the ventral pallidum cells in the NBM have disrupted cocaine self-administration in rats. However, more specific lesions of the NBM have increased cocaine self-administration behaviors. One hypothesis on the role of

the NBM is that cortical ACh levels increase in response to conditioned and unconditioned stimuli such that memory consolidation is enhanced.

Olmstead, Robbins, and Everitt (1998) examined the role of cholinergic NBM and non-cholinergic ventral pallidal neurons in reward learning. Rats received one of three lesion conditions where either the cholinergic cells or ventral pallidal cells of the NBM were lesioned or rats received a sham lesion. Rats were then habituated to an activity chamber for 120 min each day until stable locomotor activity was reached. Then contextual conditioning began in which rats received access to food in the activity chamber for 30 min after their initial introduction into the chamber and 90 min before they were removed from the chamber. Conditioned responses were measured as a number of beam breakages during the 30 min food presentation interval. Rats then received extinction trials where feeding began in home cages and food was removed from activity chambers. After the extinction period, rats were allowed 1 h access of free feeding and then placed back in the activity chamber to examine drug induced locomotion. During the drug induced locomotion test, one group of rats were given systemic injections of d-amphetamine (0, 0.5, 1.5, and 5.0 mg/kg) and a separate

group was tested after recovery given intra-NAcc infusions of d-amphetamine.

Lesions of the NBM and ventral pallidum resulted in increased locomotor activity in response to a novel environment during the habituation phase. Activity scores during conditioning increased across all subjects and both NBM and ventral Pallidum lesions produced a greater amount of hyperactivity. Locomotor decreased for all rats during the extinction phase, but less significantly for the ventral pallidum lesioned rats. Injections of d-amphetamine increased locomotor activity for all groups, but significantly more activity was observed in the sham lesion group compared to the other groups. These effects are consistent with reports finding that in lesions of the NBM increased conditioned responding. This increase in responding can be due to a disregulation of cortical ACh which then could have caused a compensatory release in cortical DA levels such that behavioral responses were experienced as being more rewarding.

A study conducted by Pirch (1993) found that this neuronal response in the basal forebrain to a CS+ during a visual discrimination task was different than the neuronal response to a CS-. Importantly, this study provided evidence that basal forebrain response due to associative

learning is different than its response to a US alone. In this study, a light (CS+) was presented to one eye of a rat and then paired with stimulation of the medial forebrain bundle (MFB); the same light was then presented to the other eye (CS-) in the same manner but with no stimulation of the MFB. Neuronal activity was recorded from both the frontal cortex and the basal forebrain. Neuronal representation of associative learning consisted of negative slow-potential (SP) responses that result from tone, light, or brain stimulation that precede food, foot shock, or rewarding MFB stimulation. These SP responses are a result of associative learning.

Pirch (1993) found that light paired with MFB stimulation resulted in significantly larger SP responses in both the cortex and basal forebrain than light that was not paired with MFB stimulation. Also, lights of different intensities that were paired with MFB stimulation had similar SP responses providing evidence that predictive value rather than the cue saliency was affecting the neuronal response. This study also tested the modality of the CS by pairing MFB stimulation with tone instead of light. Pirch (1993) found that tone elicited the same SP response as the light when paired with MFB stimulation, demonstrating that the SP responding in the basal

forebrain was not modality specific and was general to associative learning. This study also found that basal forebrain neuronal responses to the CS differed from the neuronal responses to the US.

ACh released by the cholinergic neurons of the basal forebrain modulate and influence information processing and attention (Baxter & Chiba 1999). Baxter and Chiba (1999) note that damage to the cholinergic neurons in the basal forebrain result in cognitive impairments. Particularly, cholinergic projections from NBM to the neocortex seem to be responsible for particular types of attention. Lesions to the NBM have been shown to eliminate learning by expectancy violation and have been shown to cause disruptions in visual discrimination tasks in rats. Also, stimulation of the NBM paired with auditory cues has shown reorganization of the primary auditory cortex suggesting that cholinergic projections from the NBM are involved in learning. The report by Baxter and Chiba (1999) supports the idea that cholinergic neurons in the NBM are associated with attention and learning processes but also suggests that selective damage to these neurons also correspond to selective cognitive impairment in the cortex.

Although appetitive Pavlovian conditioning may seem like a simple task, impairments of this task can result from many different things. The basal forebrain, nucleus accumbens, anterior cingulate cortex, and other brain structures have their own influence on many aspects of appetitive Pavlovian conditioning. Many complex experiments have been devised in order to pinpoint the exact effects of lesions to each of these structures. Some lesions have been shown to increase conditioned responding while most lesions cause deficits in conditioned learning. This review has provided evidence that Pavlovian conditioning is in fact a complex paradigm capable of identifying several brain structures of importance.

CHAPTER TWO

MICRODIALYSIS STUDIES OF CORTICAL ACETYLCHOLINE RELEASE

Introduction

Attentional processes as well as learning seem to be involved with activation of the basal forebrain, particularly the nucleus basalis magnocellularis (NBM) (Baxter & Chiba, 1999). Several bodies of research have found that activation of the basal forebrain result in acquisition of conditioned responding to cues even without pairing the cue with a US (Bakin & Weinberger, 1996; Dimyan & Weinberger, 1999; Weinberger & Bakin, 1998). ACh released by the NBM in the cortex causes receptive field plasticity or physical changes in the cortex due to learning (Weinberger, 1998). Also, basal forebrain activation or stimulation results in the initiation of receptive field plasticity (Weinberger, 2002).

Acetylcholine and Sensory Cortical Plasticity

Auditory cortex receptive fields refer to sound frequency and how it is coded within the auditory cortex. For example, a hair cell at the base of the cochlea would be activated by a high-frequency sound and those at the other end of the cochlea would be activated by a low-

frequency sound (Gazzaniga, 1998). These receptive fields work on a continuum where the output from the cochlea enters the cochlear nucleus and the inferior colliculus. This information would than be sent to the medial geniculated nucleus, where it is finally sent to the auditory cortex. The result is a "tonotopic map", where the particular frequency tuning of an auditory cortical neuron corresponds to a given frequency of sound, which in turn corresponds to the place along the basilar membrane of the cochlea giving rise to the auditory stimulation of the cortex. So neurons in one region of the auditory area would be activated by a low frequency, and cells in an adjacent area would respond to middle frequencies, and adjacent cells would respond to high frequencies. An auditory neuron's best frequency refers to the maximum responsiveness in frequency tuning. When a cell responds the most to a particular frequency, that frequency is its best frequency.

According to a study by Weinberger and Bakin (1998), learning based receptive field plasticity is based on classical conditioning. In their study, adult guinea pigs were implanted with microelectrodes in the infragranular layers of the primary auditory cortex and were than trained on a classical conditioning task where a tone was

paired with a mild foot shock. The training consisted of 10 - 30 pairings and subjects began showing signs of conditioned fear within 5 - 10 trials which continued throughout the training process. Receptive fields and best frequencies of auditory cortical neurons were found prior to training and compared to receptive fields following classical conditioning. Results showed that responses to the CS increased while responses to the best frequency decreased, thus showing a tuning shift towards the CS making it the new best frequency. Responses to all other frequencies decreased while responses to the CS increased. This increase in response to the CS and decreased response to the best frequency showed that learning induced receptive field plasticity is associative and reflects learning through experience.

Weinberger and Bakin (1998) also found that direct NBM stimulation promotes long lasting receptive field plasticity in the auditory cortex in a classical conditioning paradigm. This study was conducted by stimulating the NBM after a presentation of a tone. Another group of animals received the same treatment of NBM stimulation without pairing with a tone. This training schedule was used to imitate typical schedules of classical conditioning. Receptive fields were measured

after training and were then compared to the receptive fields before training. This study found that NBM stimulation paired with tone produced CS specific receptive field plasticity in the auditory cortex similar to that in behavioral training. As with behavioral training, there was a greater response towards the CS and a weaker response to the previous best frequency as well as other frequencies. Also, animals that did not receive the paired tone presentation with NBM stimulation showed no CS specific receptive field plasticity. This CS specific change in receptive fields due to the tone and NBM stimulation pairing showed that NBM activity is important in the development of receptive field plasticity.

According to Ingles and Fibiger (1995), there is substantial evidence suggesting that cholinergic neurons in the basal forebrain play a role in arousal. Using microdialysis and biochemical detection (HPLC) techniques, they found that the cholinergic neurons in the nucleus basalis, which release ACh into the neocortex, played an important role in cortical arousal activity. Microdialysis is an in vivo sampling method used to determine the extracellular concentration of neurotransmitters including ACh in the brains of behaving animals; once samples are

collected from the behaving animal, they are quantified by an electrochemical process known as high-performance liquid chromatography (HPLC). Ingles and Fibiger (1995) established a baseline level of ACh release when their rats were at rest. The animals were than presented with one of four different stimuli for 20 minutes each. The four stimuli included an intermittent buzzer sound that was on for 30 s and then off for 30 s, a flashing white light, pepper-mint soaked swab, and a nylon brush which was used to stroke the fur on the back of the animal's neck.

Results showed that ACh release in the cortex and hippocampus increased in response to each stimulus type that was presented to the animal. These results suggest that ACh plays an important role in arousal. This study also found that ACh release in the cortex differed significantly depending on the type of stimulus that was presented to the animal, suggesting that ACh release maybe differentially regulated.

Cortical Acetylcholine Release in Associative Learning

According to Acquas, Wilson, and Fibiger (1996), basal forebrain cholonergic neurons with projections to

the frontal cortex and hippocampus are involved in responding to behaviorally relevant stimuli, which suggests that these neurons are important to arousal and attentional processes. These experimenters examined ACh release using microdialysis techniques in three groups of rats; a habituation, novel stimuli group, and conditioned fear group. The rats within the habituation group were extensively exposed to light and tone stimuli during training sessions before microdialysis testing. The rats within the conditioned fear group were also trained in the same manner as those within the habituation group except that the light and tone stimuli were paired with footshock. Finally, the rats within the novel stimuli group were not presented with any stimuli until the day of microdialysis testing.

Results showed a significant increase in the release of ACh into the frontal cortex and hippocampus in the conditioned fear and novel stimuli groups but not in the habituation group. Further, the rats in the conditioned fear and novel stimuli group exhibited several arousal and fear related behaviors. These findings support the idea that ACh plays a significant role in arousal and attentional processing but also provides evidence that the

same cholinergic neurons in the basal forebrain may also be activated by behaviorally relevant conditioned stimuli.

Butt and colleagues (Butt, Testylier, & Dykes 1997) conducted another microdialysis experiment exploring the relationship between ACh release and learning and memory. This research provides evidence suggesting that ACh release can be enhanced in regionally specific cortical areas where this enhancement is clearly related to learning. This experiment involved using two groups of rats; a tactile discrimination group and a non-discriminating but food-reinforced control group. Both groups were first habituated to the testing environment. The discrimination group was reinforced with food for making the correct choice in a tactile discrimination task while the control group was reinforced for any choice that they made.

Results for both groups showed an increased release of ACh in the frontal cortex as well as the somatosensory cortex during testing. However, ACh release in the somatosensory cortex of the tactile discrimination learning group was significantly greater than in the control group. Results also showed a significant increase in somatosensory cortical release of ACh as compared ACh release in the frontal cortex of the tactile

discrimination rats when compared to the rats in the control condition. This study provides evidence that ACh enhancement is also associated with learning, and that the pattern of ACh release in different parts of the cortex may differ depending on the specific behavioral task animals are engaged in.

The presentation of auditory stimuli produces electrophysiological activation of primary auditory cortex, and this activation diminishes across habituation trials (Condon & Weinberger, 1991; Westenberg & Weinberger, 1976). It is likely that ACh plays a role in the differential electrophysiological response to repeated auditory stimulation during habituation. As described earlier, ACh released into the primary auditory cortex following NBM stimulation enhances the cortical response to auditory stimulation, and this enhancement is blocked by cholinergic antagonist drugs acting on AI neurons (Metherate & Ashe 1991).

Based on these findings, we believe that the initial increase in primary auditory cortical response to auditory stimulation reflects NBM activation and ACh release onto primary auditory neurons, and that the subsequent decrease in cortical response to auditory stimulation reflects a concomitant decrease in NBM activation and ACh release.

Dimyan and Weinberger (1999) support the idea that receptive field plasticity is a division of memory and show that increased responding to a CS+ remained significant for as much as 60 min post training. Dimyan and Weinberger (1999) also provide evidence that associative learning is achieved through activation of the basal forebrain. This study found that activation of the basal forebrain paired with tone caused receptive field plasticity as well as increased responding to the conditioned tone and a decrease in responding to the unconditioned tone. Receptive fields of adult male Hartley guinea pigs were measured and its best frequency was determined. The CS+, tone and basal forebrain stimulation, was chosen outside of the best frequency so that neuronal responding before training was equal to CS- tone. Training consisted of 30 presentations of both the CS+ and CS- with an average inter-trial interval of 2 min. After training, receptive fields were measured for retention at 20, 40, and 60 min.

This study found that responding to the CS+ increased while responding to the CS- decreased, providing evidence of associative learning is mediated by basal forebrain. This experiment bypassed the use of a US and was able to obtain conditioned responding to a CS through basal

forebrain stimulation alone. This demonstrates that associative learning is associated with activation of the basal forebrain.

Basal Forebrain Involvement in Auditory Learning and Memory

According to a study by Weinberger and Bakin (1998), learning based receptive field plasticity is based on classical conditioning. In their study, adult guinea pigs were implanted with microelectrodes in the infragranular layers of the primary auditory cortex and were than trained on a classical conditioning task where a tone was paired with a mild foot shock. The training consisted of 10 - 30 pairings and subjects began showing signs of conditioned fear within 5-10 trials which continued throughout the training process. Receptive fields and best frequencies of auditory cortical neurons were found prior to training and compared to receptive fields following classical conditioning.

Results showed that responses to the CS increased while responses to the best frequency decreased, thus showing a tuning shift towards the CS making it the new best frequency. Responses to all other frequencies decreased while responses to the CS increased. This

increase in response to the CS and decreased response to the best frequency showed that learning induced receptive field plasticity is associative and reflects learning through experience.

Weinberger and Bakin (1998) also found that direct NBM stimulation promotes long lasting receptive field plasticity in the auditory cortex in a classical conditioning paradigm. This study was conducted by stimulating the NBM after a presentation of a tone. Another group of animals received the same treatment of NBM stimulation without pairing with a tone. This training schedule was used to imitate typical schedules of classical conditioning. Receptive fields were measured after training and were then compared to the receptive fields before training.

This study found that NBM stimulation paired with tone produced CS specific receptive field plasticity in the auditory cortex similar to that in behavioral training. As with behavioral training, there was a greater response towards the CS and a weaker response to the previous best frequency as well as other frequencies. Also, animals that did not receive the paired tone presentation with NBM stimulation showed no CS specific receptive field plasticity. This CS specific change in

receptive fields due to the tone and NBM stimulation pairing showed that NBM activity is important in the development of receptive field plasticity.

Bakin and Weinberger (1996) defined receptive field plasticity as physiological memory and found that plasticity due to NBM stimulation also showed physiological memory change due to that stimulation. In this study, Tone was paired with electrical stimulation of the NBM in the adult male Sprague-Dawley rat in order to cause receptive field plasticity similar to that in behaving animals. Receptive fields were measured and the best frequency was determined for each animal. The rats were then separated into a paired and unpaired NBM stimulation groups where a tone outside of the best frequency was used to pair with NBM stimulation in order to see a tuning shift in the paired group. The subjects in the paired group received forty trials of tone paired with NBM stimulation and receptive fields were measured ten, twenty, and thirty minutes after training.

Results showed that tone and NBM pairings caused a long term tuning shift in receptive fields away from the best frequency and towards the conditioned tone making it the new best frequency, while subjects in the unpaired group showed no tuning shift in receptive field.

Cortical plasticity represents physiological memory and is due to learning (Bakin & Weinberger 1996; Dimyan & Weinberger, 1999; Weinberger & Bakin 1998). This learning is achieved with basal forebrain activation or through artificial stimulation of the basal forebrain (Bakin & Weinberger 1996; Dimyan & Weinberger 1999; Weinberger & Bakin 1998). During associative learning, basal forebrain is activated and facilitates plasticity in the cortex through release of ACh from its cholinergic projections to the cortex (Baxter & Chiba 1999; Ingles & Fibiger, 1995). When basal forebrain activation is blocked, no plasticity occurs. However, when basal forebrain is stimulated artificially and paired with a cue, associative responding occurs similarly to responding when a cue is paired with a US (Bakin & Weinberger 1996; Dimyan & Weinberger 1999; Weinberger & Bakin 1998). In other words, the US is not needed in order to form a conditioned response, when basal forebrain is artificially stimulated. These studies provide evidence that basal forebrain activation occurs during associative learning, cortical plasticity and physiological memory.

CHAPTER THREE

THESIS PROPOSAL

Introduction

Recent advances in the use of *in vivo* microdialysis and biochemical detection techniques have allowed new insight into the role of the neurotransmitter ACh released into the neocortex by the cortically-projecting cells of the NBM during learning. For example, Butt et al. (2004) found an increase of ACh release in the rat primary auditory cortex using microdialysis techniques during an auditory classical conditioning task when compared to rats in a non-associative control task where animals do not learn to associate the CS with the US. In this experiment, rats in the conditioning group received one-hour sessions of 60 pairings of a 10 s broad band white noise CS and the delivery of a single sucrose pellet US. Rats received one session per day for three consecutive days. Rats in the random control group also received the 60 CSs and the 60 USs in a one-hour session per day for three days; however, the CSs and USs were presented randomly and independently, such that the CS did not serve as a reliable signal for the US. Both groups went through microdialysis procedures to collect cortical ACh release in the primary auditory

cortex. Conditioned responding was assessed by measuring conditioned approach to the food magazine during the 10 s CS presentations and comparing this response to approach behavior during the 10 s interval preceding each CS plus the during the 10 s CS presentation. As animals learn to associate the CS with the US, the proportion of approach behavior occurring during the CS relative to approach during the pre-CS interval increases.

Results from the Butt et al. (2004) study showed that rats in the conditioning task showed more conditioned responding and greater increases in cortical ACh release in the primary auditory cortex when compared to the random control group. These results demonstrated that increased cortical ACh release from the basal forebrain is associated with learning in behaving animals and is not just a phenomenon of mere stimulus exposure such as is experienced in the random control group.

It is not known which brain system mediates the differential release of ACh in the auditory cortex of rats response to auditory stimuli that either predict important events, as in the classical conditioning group, or in response to the same auditory stimuli that lack predictive significance, as in the non-associative control group. However, some research suggests that conditioned

responding is attained through selective attention to the CS (Mackintosh, 1974). Additionally, many studies have found that the medial prefrontal cortex (mPFC) is responsible for selective attention (Apparsundarum et al., 2005; Dalley, Cardinal, & Robbins, 2004; Kozak, Bruno, & Sarter, 2006; Sarter, Givens, & Bruno, 2001). Selective attention-demanding tasks cause an increase in cortical ACh release, demonstrating the involvement of the basal forebrain in attention (Himmelheber, Sarter, & Bruno, 2000). Consequently, a potential relationship between mPFC, basal forebrain, and neocortical release of ACh is suggested. An anatomical basis for this relationship has been described by Zaborszky et al. (1997), who have demonstrated that not only does the basal forebrain cholinergic system send projections to the cortex, the prefrontal cortex sends descending projections onto basal forebrain neurons themselves. Zaborszky et al. (1997) show that the principal projections to the NBM originating in the frontal cortex include cells in the mPFC, in addition to cells in the agranular insular cortex (AIC). This anatomical arrangement between mPFC/AIC and the NBM within the basal forebrain may provide the basis for modulating cortical ACh release in associative learning situations.

Sarter, Givens, and Bruno (2001) call the process of sustained attention a "top-down" process, where attention is activated through direct connections from the prefrontal cortex to the cholinergic basal forebrain. According to Sarter, Givens, and Bruno (2001), the cortical cholinergic inputs maintain sustained attention performance by sensitizing sensory inputs in the cortex. The prefrontal cortex accomplishes this through its connections to the basal forebrain, which in turn sends its inputs to the cortex. These inputs to the cortex from basal forebrain support sustained attention as well as increases sensory processing (Sarter, Givens, & Bruno, 2001). In agreement with this view, Apparsundaram et al. (2005) found that rats performing a cognitive vigilance task showed an increase in choline transporters (an indirect measure of cholinergic function) in the mPFC when compared to control rats.

Prefrontal involvement in mediating attention is also suggested by data from lesion experiments. Lesions of the rat prefrontal cortex impair performance on a variety of attentional tasks (Dalley, Cardinal, & Robbins, 2004; Kozak, Bruno, & Sarter, 2006). Specifically, lesions of the cortical cholinergic inputs have been associated with decreases in ACh and decreased ability on an attentional

performance task in rats (Kozak, Bruno, & Sarter, 2006). Behavioral impairments such as perseveration, impaired choice accuracy, and slower latency to respond correctly have all been associated with lesions of the medial prefrontal cortex (Dalley, Cardinal, & Robbins, 2004). These behavioral impairments indicate that selective attention to visual stimuli depends on the mPFC (Dalley, Cardinal, & Robbins, 2004).

The purpose of the current research experiment was to find out whether the frontal cortical regions of the mPFC and AIC exert a modulating influence on cortical ACh release during associative learning. To test this hypothesis, rats received ibotenic acid lesions of the mPFC and AIC, or received sham lesions where the cortex was not damaged. Next, these two groups of rats were tested in an appetitive Pavlovian conditioning task using an auditory CS and food US. During training, ACh samples were collected from the auditory cortex in order to assess the extent of learning-induced ACh release in the two groups.

Hypotheses

Based on the theoretical arguments of Sarter, Givens, and Bruno (2001), and on the anatomical findings of

Zaborszky et al. (1997) suggesting potential involvement of the mPFC and AIC in modulating NBM function, it was hypothesized that combined mPFC/AIC lesions would prevent the learning-induced increase in ACh release in the primary auditory cortex that occurs in normal animals. Consequently, it was predicted that type of lesion (mPFC/AIC vs. sham lesion) would affect learning-induced ACh release, with the mPFC/AIC lesion rats releasing less ACh than the sham lesion rats. It was further hypothesized that, although the normal pattern of learning-induced ACh release is expected to be prevented by mPFC/AIC lesions, behavioral acquisition of the CR is expected to occur normally. Consequently, it was predicted that type of lesion (mPFC/AIC lesion vs. sham lesion) would not affect CR acquisition.

CHAPTER FOUR

METHODS

Introduction

Rats with bilateral ibotenic acid lesions of the mPFC and AIC and sham-operated rats were tested in a Pavlovian classical conditioning task using an auditory CS and food US. During training, ACh samples were collected from the primary auditory cortex using in vivo microdialysis methods in order to assess the extent of learning-induced ACh release in the two groups. ACh quantification was achieved using high pressure liquid chromatography and amperometric detection techniques.

Methods

Guidelines for Animal Use

The following procedures involving research animals meet the requirements set by the Society for Neuroscience, the American Psychological Association, the National Research Council, and the California State University, San Bernardino (CSUSB) Animal Care and Use Committee.

Experimental Design

A between-subjects experimental design was used to test the proposed hypothesis. The independent variable was

type of lesion. This independent variable is a qualitative, categorical variable with two levels: mPFC/AIC lesion vs. sham lesion. Two dependent variables were measured: ACh release in the auditory cortex and the CR ratio scores.

Subjects

Subjects were 7 adult male Long-Evans rats (Harlan Sprague-Dawley, Indianapolis, IN) weighing approximately 300 g were placed on a food-deprivation schedule to reduce weight to 85% free-feeding weight. Rats were handled and habituated to the experimenters and testing environment to reduce stress during microdialysis testing. Rats were randomly assigned to each treatment so that there were 4 rats in the lesion group and 3 rats in the sham control group.

Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg ip; Sigma, St. Louis, MO) and placed in a stereotaxic frame (Kopf Stereotaxic Instruments, Tujunga, CA). The scalp was incised and rats in the mPFC/AIC lesion group were infused with ibotenic acid (Sigma, 0.06 m 2.4 µl over 3.5 min) bilaterally into the mPFC (coordinates AP + 3.5mm, + 2.2mm and ML $\pm$ 0.6 mm relative to bregma, DV - 3.2 mm relative to dura) and AIC (coordinates AP +2.7mm

and ML ± 4.0mm relative to bregma, DV - 4.4 mm relative to dura, and AP + 3.7mm and ML ± 3.7 mm relative to bregma, DV - 3.6 mm relative to dura)through 26-gauge stainless steel canulae. Rats in the sham lesion group received similar infusions of sterile saline (Roffman et al., 2000).

The incision was cleaned and sutured and rats were administered an antibiotic to guard against infection. Rats were closely monitored and cared for over the following 10 days before they underwent a second surgery for microdialysis probe guide implantations.

Allowing 10 days for recovery following the lesion, rats underwent a second surgery. Again, rats were anesthetized with sodium pentobarbital and placed in a stereotaxic frame. The scalp was incised and craniotomies were made in the left hemisphere over the primary auditory cortex at -4.8 mm posterior to Bregma and 2.7 mm lateral to midline (Paxinos & Watson, 1997). The left primary auditory cortex was located by mapping evoked potentials (1-1000 Hz, 1000x, DAM-50H, WPI, Sarasota, FL) in the temporal lobe (needle point stainless steel electrode) to clicks (100 µs, ~90dB SPL, at 0.1Hz) presented to the contralateral (right) ear via a calibrated miniature speaker. Mapping began at the above mentioned reference

point (AP - 4.8, L 2.7; Paxinos & Watson, 1997) and continued until site was found that had the highest amplitude (200-400 μV) response having at least two positive-to-negative components (7-12 ms, P1 and 18-27 ms, N1; 32-60 ms, P2 and 60-104 ms, N2, respectively). Probe guides were inserted into the primary auditory cortex at a 26° angle, extending away from midline, such that the microdialysis probe came to rest in the primary auditory cortex. Probe guides were secured using dental acrylic anchored to the skull via three small screws.

The incision was cleaned and sutured and rats were administered an antibiotic to guard against infection. Rats were closely monitored and cared for over the following 2 days before behavioral testing and microdialysis sampling.

Apparatus

Testing was conducted in computer-controlled operant chambers (Coulbourn, Lafayette IN) equipped with a speaker connected to a white noise generator, a 5 W flashing (2 Hz) cue light, a pellet dispenser, and a food magazine with an infrared photo beam mounted across the opening of the magazine to detect snout entry, as well as an overhead infrared photo beam set above the chamber to detect movement. The presentation of auditory or visual stimuli,

the delivery of sucrose pellets (45 mg, Formula F; P. J. Noyes, Lancaster, NH), and the recording of snout entries was achieved by computer interface.

Behavioral Procedures

Rats in both the mPFC/AIC lesion and sham lesion groups received a one-hour session of CS-US pairing for four consecutive days. Each session consisted of four blocks of 15 trials per day for a total of 240 trials for the four days. The CS-US pairings were separated by an average inter-trial-interval (ITI) of 40 s. The CS was a 10 s broadband white noise (86 dB) and the US was sucrose pellets (45 mg) delivered into the food magazine.

Gross motor measurements were also recorded throughout training in both groups by infrared movement detectors to measure any possible differences between groups.

Microdialysis Procedures

Prior to placing rats in the testing environment, microdialysis probes were inserted into the guide and were continuously perfused (1 µl/min) with artificial cerebrospinal fluid (aCSF) containing 155.0 mM NaCl, 27.5 mM $NaHCO_3$, 2.4 mM KCl, 0.5 mM KH_2PO_4, 1.1 mM $CaCl_2$, 0.8 mM $MgCl_2$, and 1.0 mM glucose, at pH 7.0 (see Himmelheber, Sarter, & Bruno, 2000). After rats were transferred to the

testing chamber, ACh levels were allowed to equilibrate for 3 h prior to collecting baseline ACh samples. This lengthy equilibration procedure has been shown to be adequate to allow changes in ACh release associated with insertion of the microdialysis probe, or caused by transfer from home cage to testing environment (see Bruno, Sarter, Arnold, & Himmelheber, 1999). Beginning after the equilibration time, a 1 h baseline period commenced, with ACh samples being collected every 30 min. Finally, the conditioning, with ACh samples again being collected every 30 min for 1 h. Microdialysis samples were immediately frozen in liquid nitrogen and stored at -80 C until assayed.

Acetylcholine Assay and Quantification Procedures

Quantification of ACh in the dialysates was accomplished using high-performance liquid chromatography with electrochemical detection (HPLC/ED; ESA, Chelmsford, MA). Briefly, using a sodium phosphate mobile phase, a pre-column enzymatic reactor was used to oxidize choline and reduce H_2O_2 in the dialysate sample prior to separation of choline and ACh by a C-18 carbon polymer column. Post-column hydrolysis of ACh was achieved using an enzymatic reactor containing covalently-bound acetylcholinesterase and choline oxidase. ACh was

hydrolyzed to acetate and choline, and choline oxidized to H_2O_2 and betaine. Subsequent electrochemical detection of H_2O_2 was achieved using a peroxidase-wired glassy carbon electrode at a potential of -200 mV (Himmelheber, Sarter, & Bruno, 2000).

Histology

Upon completion of behavioral testing, rats were euthanized by lethal dose of sodium pentobarbital (80 mg/kg, ip; Sigma, St. Louis, MO) followed by cardiac perfusion with 0.9% saline ending with 10.0% formalin. Brains were extracted and placed in a 10.0% formalin and 30.0% sucrose solution for 48 hrs prior to freezing and sectioning. Sections (60 μm) were stained with thionin and examined to verify probe placement.

Data Analyses

To test the behavioral hypotheses, the number of CRs (i.e., snout entries into the food magazine) during the CS and during the pre-CS interval were used to calculate CR percent ratio (nose-pokes during CS /nose-pokes during CS + pre-CS interval x 100). CR ratio scores were analyzed by an analysis of variance (ANOVA) for the mixed design where the between subjects variable was type of lesion and the within subjects variable is day of testing. To test the neurochemistry hypotheses, the absolute amount of ACh

released (fmol/15μl sample) in the primary auditory cortex during testing were expressed as a percent of baseline release, where the mean of the four baseline samples were used in calculating testing-induced ACh levels. ACh data for day four of testing was analyzed by an ANOVA for between subjects. A significance level of $p = .05$ was adopted to conclude statistical significance.

CHAPTER FIVE

RESULTS AND DISCUSSION

Results

Lesion Verification

All brains were sectioned and stained for verification of microdialysis probe placement (see Figure 1) as well as mPFC/AIC lesion placement (see Figure 2). Microdialysis probes were reliably placed within the layers of primary auditory neocortex, consistent with the observation of auditory evoked potentials acquired during probe implantation. Lesions were accurately placed in all mPFC/AIC lesion rats, with damage to the majority of these areas according to the standardized rat brain atlas.

Behavioral Results

Pavlovian conditioning was assessed in terms of CR ratio scores (number of nose-pokes into food cup during CS divided by the number of nose-pokes during 10 s preceding CS plus the number of nose-pokes during the CS), which were analyzed and compared between groups and within groups (i.e., across days). There were no significant differences in CR ratio scores between groups ($p > .05$). Further analysis revealed that there were significant

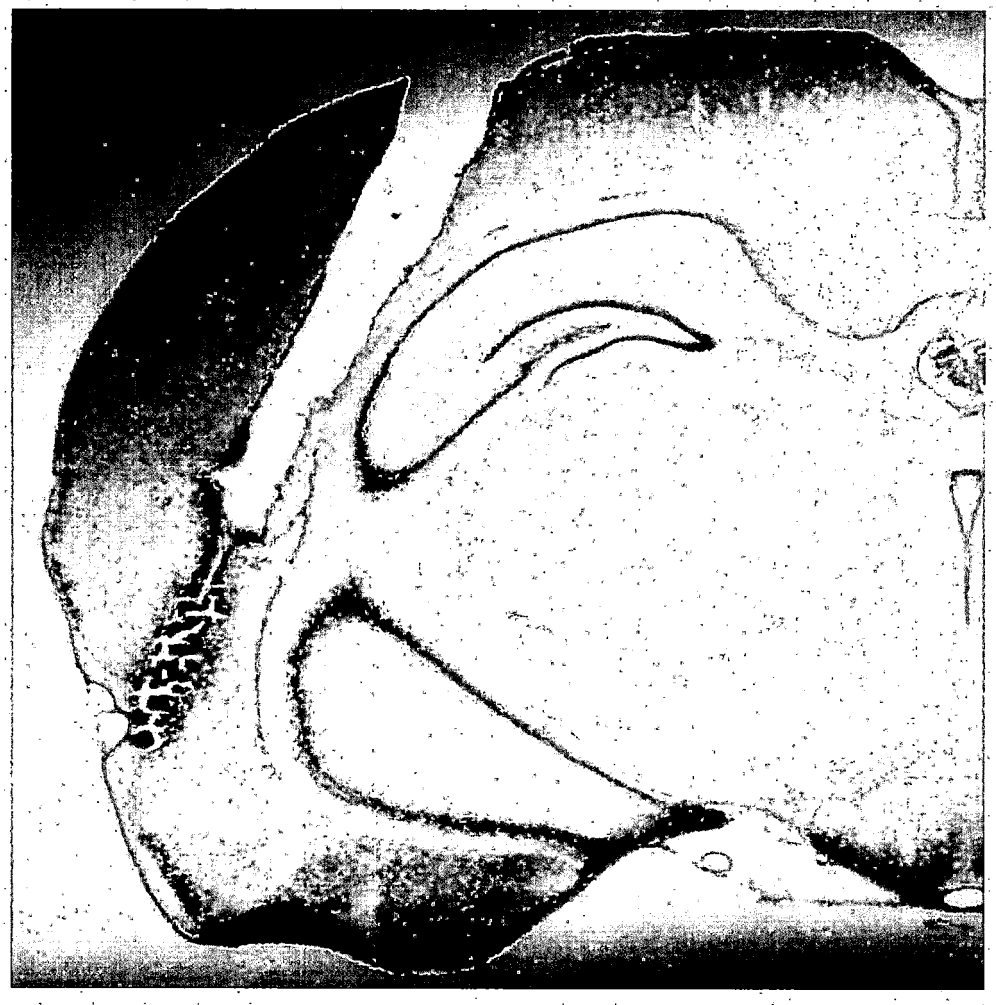

Figure 1. Microdialysis Probe Placement in the Primary Auditory Cortex. Probes were located within the layers of the neocortex in areas electrophysiologically identified as being responsive to auditory stimulation using auditory evoked potential methods.

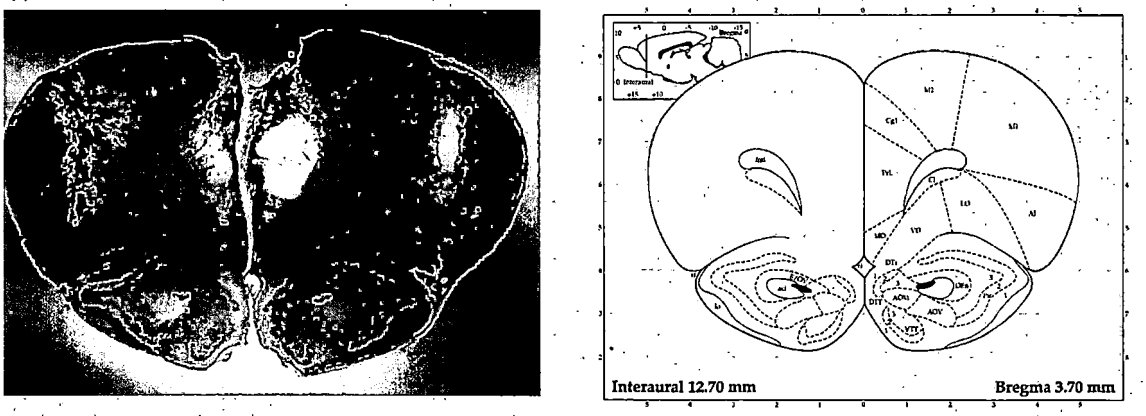

Figure 2. Typical Ibotenic Acid Lesions of the Frontal Cortex. Lesions damaged both the mPFC and AIC bilaterally as shown on the left, where these regions were confirmed anatomically using the corresponding illustration from a rat brain atlas as shown on the right.

differences across days. Conditioned responding increased across days for rats in both the prefrontal lesion group and in the sham control group ($p < .05$, see Figure 3).

Gross motor responses were analyzed using a repeated measures ANOVA. This behavioral measure was analyzed for several reasons. First, the mPFC/AIC lesion cannula track travels through primary motor cortex. Consequently, potential differences in motor activity between lesion and control rats may be attributed to backflow of ibotenic acid into the primary motor cortex. Secondly, because ACh levels are known to correlate with movement, potential group differences in the amount of movement exhibited during testing were assessed. No significant differences in motor behavior were found either between groups or across days ($p > .05$, see Figure 4).

Neurochemistry Results

The percent of baseline ACh release on day four of conditioning procedures was analyzed and compared between prefrontal lesion and sham control groups (see Figure 5). There was a significant difference in auditory cortical ACh release between groups. Testing-induced ACh release in the prefrontal lesion group was significantly less in the sham lesion control group compared to the prefrontal lesion group ($F_{(1,5)} = 40.13$, $p < .001$).

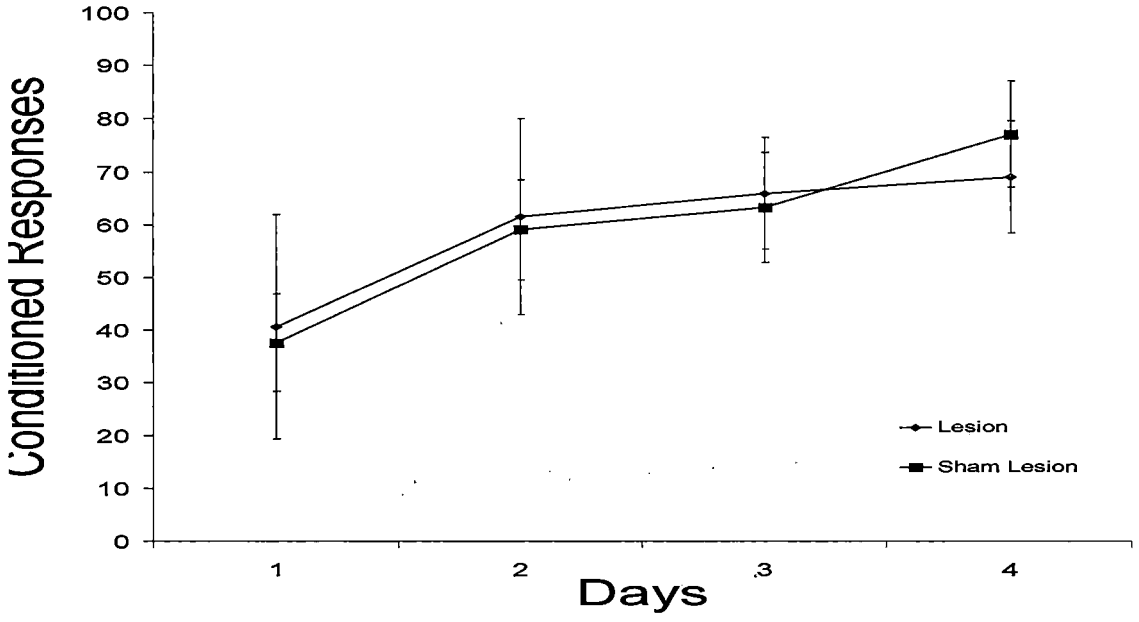

Figure 3. Conditioned Responding in the Frontal Cortex Lesion Group and Sham Lesion Control Group During Classical Conditioning. Conditioned responding was calculated as a percent ratio score between the number of nose-pokes into the food cup during CS presentation divided by the number of nose-pokes during the 10 s preceding CS presentations plus the number of nose pokes during the CS multiplied by 100. The rate and level of conditioned responding did not differ between the mPFC/AIC and sham lesion groups.

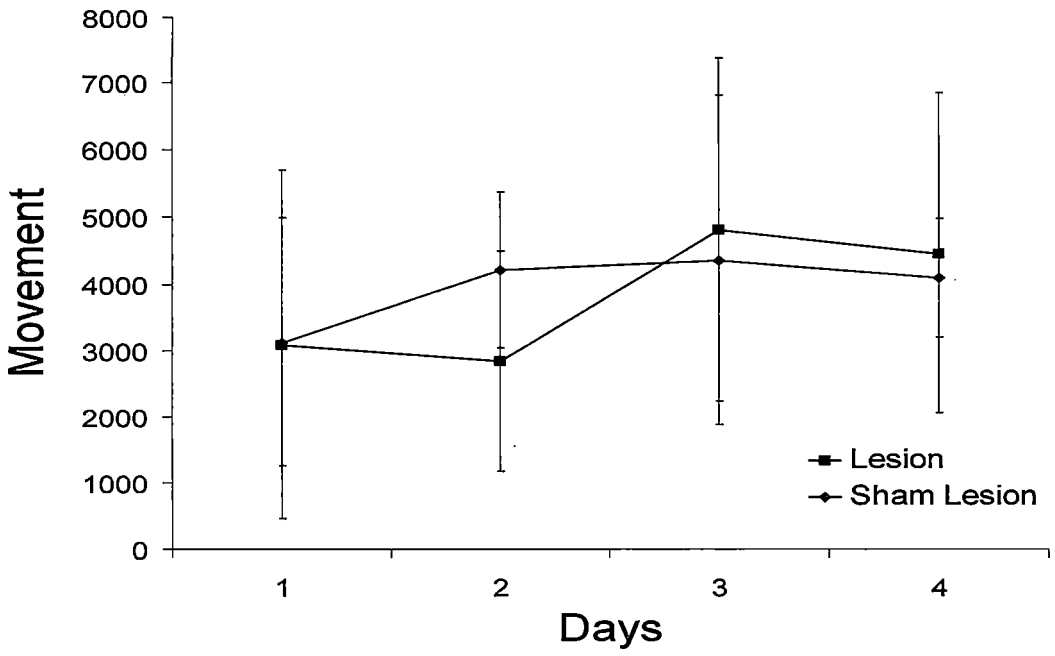

Figure 4. Movement in the Frontal Cortex Lesion Group and Sham Lesion Control Group During Testing. The number of units of movement (activation of occurring throughout the 1 h of testing did not differ between the mPFC/AIC and sham lesion groups.

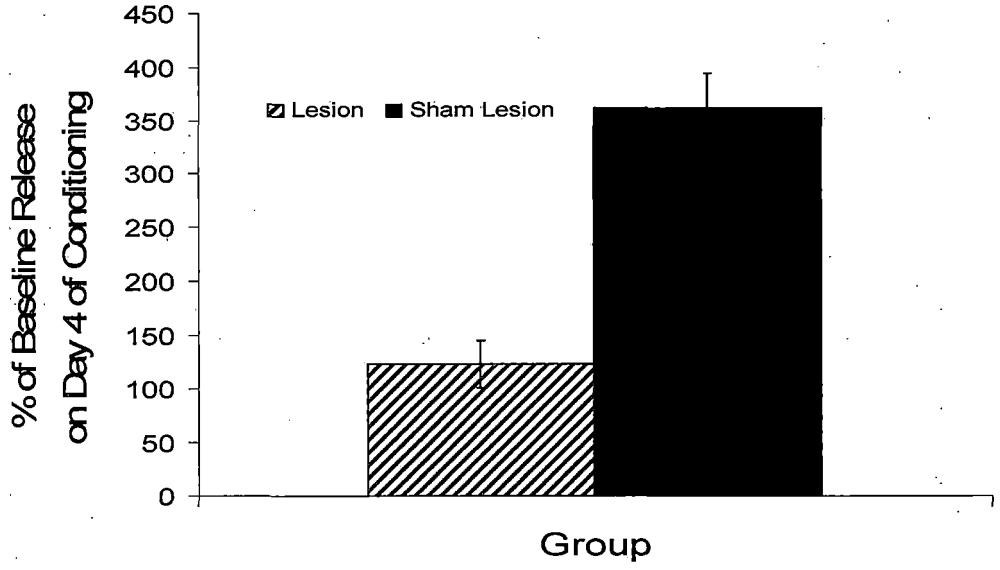

Figure 5. Acetylcholine Release During Classical Conditioning in the Frontal Cortex Lesion Group and Sham Lesion Control Group. There was a significant difference in acetylcholine release between the frontal cortex lesion group and the sham lesion control group; rats in the frontal cortex lesion group showed significantly less acetylcholine release compared to the sham lesion control group ($p < .05$).

Discussion

The findings from this experiment confirmed the hypothesis that frontal cortex lesions would prevent the enhanced ACh release normally seen in the primary auditory cortex in rats learning in an auditory Pavlovian conditioning paradigm; auditory cortical ACh release in the sham lesion control group was significantly greater than release in the frontal cortex lesion group. Despite the difference in auditory cortical ACh release, both groups acquired conditioned food cup approach behavior; there were no statistical differences in the CR measure. Also, there were no between group differences in gross motor abilities, suggesting that the lesion did not affect the animal's ability to move to the food cup. Consequently, the differences in ACh release can not be attributed to differences in movement potentially caused by mPFC/AIC lesions. Instead, results suggest that the mPFC and AIC exert a modulatory influence on the cortically-projecting cells of the NBM during associative learning.

This study provided evidence that the medial prefrontal cortex is important for mediating learning-induced release of ACh during Pavlovian conditioning. The results from this experiment indicate that lesions of the

prefrontal cortex compromise the system that is normally active during learning about behaviorally-relevant, predictive stimuli. Lesions of the prefrontal cortex prevented the learning-induced increases of ACh release normally seen in rats undergoing classical conditioning, although ACh release was slightly increased during training as compared to quiet baseline conditions. It is argued that this small amount of release represents sensory stimulation-induced ACh release, rather than learning-induced release. Collectively, these results demonstrate that lesions of the mPFC/AIC prevent learning-induced ACh release but not sensory stimulation-induced ACh.

Previous studies have shown an increase of ACh release in relevant sensory cortices during learning (Acquas, Wilson, & Fibiger, 1996; Butt, Testylier, & Dykes 1997, Butt et al., 2004). These increases of ACh due to either learning or to NBM stimulation were accompanied by auditory cortical plasticity, where the tone used in training as a CS acquired more cortical territory as a result of experience (Bakin & Weinberger, 1996; Dimyan & Weinberger, 1999; Weinberger & Bakin, 1998). The current findings are consistent with these other studies in that

Pavlovian learning was associated with increased ACh levels in the primary auditory cortex.

As previously discussed, Butt et al. (2004) demonstrated that ACh release increases in the auditory cortex during auditory classical conditioning, and these increases were related to the development of the CR. The non-associative control group, which received the white noise CS and the sugar pellet US in an unpaired, random sequence did not develop a CR. However, this group did show an increase in testing-induced ACh release approximately 25 percent above baseline levels, although the ACh release of the learning group was approximately 350 percent greater than the non-learning control group. This ACh release in the non-learning control group is argued to reflect sensory stimulation-induced ACh release similar to that found in studies involving sensory stimulation alone (e.g., Acquas, Wilson, & Fibiger, 1996). Although there are low levels of ACh release in response to sensory stimuli, it is the greater amount of ACh release that is seen during learning that most likely contributes to cortical plasticity and reorganization of the neocortical representations of behaviorally relevant conditioned stimuli.

The current experiment is unusual in that it demonstrates an increase in ACh release in auditory cortex in normal animals learning a CR to an auditory CS, but reveals otherwise normal learning in the mPFC/AIC lesion group despite the lesion-induced blockade of that learning-induced ACh release. In other words, results show a neurotransmitter modulation of the sensory cortex during learning that does not appear to be necessary for that learning to take place. Other studies have shown that the auditory cortex is not necessary for auditory classical conditioning (Allen, 1945; Thompson, 1970). Although auditory cortex is not necessary for auditory classical conditioning, it is probably evolutionarily important to encode information concerning the predictability of appetitive or aversive events. The encoding of information in the primary auditory cortex might represent a secondary or "back-up" copy of information that is important. The reorganization and representation of important cues may also be important and is dependent on the cortex when tasks become more difficult such as, during discrimination learning, where responding differently to different auditory cues would lead to the opportunity to avoid shock more often (see Allen, 1945; Thompson, 1970). The opportunity to have information encoded and readily

available in a more complicated situation would be expected to aid in the survival and adaptability of a species.

By eliminating learning-induced cortical ACh release in the auditory cortex, the effects of ACh on the cortex and its modulatory role during learning were probably abolished. These effects normally include cortical plasticity and tonotopic reorganization such that behaviorally significant auditory stimuli tones do not gain representational territory in the auditory cortex (Bakin & Weinberger, 1996; Dimyan & Weinberger, 1999; Weinberger & Bakin, 1998). Although the ACh-dependent modification of the cortical representation of the auditory CS are not necessary to learn in the current auditory conditioning task (see Allen, 1945 and Thompson, 1970), a performance deficit would be expected in mPFC/AIC lesion rats tested in tasks that are dependent on the integrity of the cortex. Such tasks might include differential conditioning or trace conditioning.

Lesions of the NBM, which remove the inputs delivering ACh throughout the neocortex (including sensory cortices) impede a rats' ability to perform in a complex task (Cabrera et al., 2006). This deficit in performance can reflect their inability to act adaptively by using

cortical representations in sensory cortices that would normally be present in animals without lesions.

The prefrontal cortex is a highly interconnected brain region that might exert its effects onto the NBM through many different connections (Sarter, Givens, & Bruno, 2001). The lesion used in the current study consisted of several prefrontal cortical brain regions. Zaborsky et al. (1997) showed that the AIC was the main contributing input from the prefrontal cortical area to the NBM. They also showed that the prelimbic and infralimbic cortices provided some overlapping projections to the NBM and also projected onto the rest of the basal forebrain.

Future studies should focus on the individual contributions of each of these cortical areas to the modulation of the NBM. Although this paper focuses on direct afferent connections from the prelimbic/infralimbic and AIC cortices, these cortices also have indirect pathways by which they may exert their effects. For example, the prelimbic and infralimbic cortices also have direct projections to the amygdala (Gabbot, Warner, & Busby, 2006; Quirk, Likhtik, Pelletier, & Pare, 2003), and the amygdala synapses on the NBM, where it may exert its effects (Sarter, & Bruno, 2000). By lesioning the mPFC/AIC

their influence through direct input into the NBM as well as their indirect input through the amygdala was removed. There are also projections from the amygdala to the mPFC (Gabbot, Warner, & Busby, 2006; Quirk, Likhtik, Pelletier, & Pare, 2003). The existence of this circuitry could be a means by which the amygdala can exert its effects indirectly to the NBM, in addition to its direct connections to the NBM. Future research should focus on locating the exact circuitry that is involved in mediating the learning-induced ACh release enhancement and its blockade by mPFC/AIC lesions observed in the current study.

Top-down regulation modulates sensory information through practice, memory, expectations, and knowledge at all levels of cortical and subcortical inputs (Sarter, Hasselmo, Bruno, & Givens, 2005). The current experiment provides unique evidence that the prefrontal cortical projections to the NBM are able to modulate the processing of sensory information in the primary sensory cortex for audition. The prefrontal cortex appears to be able to exert its effects through modulating the cholinergic ascending system NBM. In this sense, the ascending cholinergic system can be thought of as another component in top-down regulation of learning operated on by the

mPFC/AIC (Sarter, Hasselmo, Bruno, & Givens, 2005). The mPFC/AIC attention system appears to selectively enhance the processing of sensory relevant stimuli and suppress the processing of irrelevant "noise" (see also Sarter, Givens, & Bruno, 2001). This system may selectively augment the processing of learning-relevant sensory cues and contributes to their long-term representation. This augmentation would lead to better representations of CSs and increased receptive field territory devoted to those CSs in primary sensory cortices. In contrast, learning-irrelevant sensory cues would not benefit from such augmentation. Results from the current experiment provide unique evidence that medial prefrontal cortex projections to the NBM are important for mediating ACh release during associative learning, where this circuit might serve as a neurobiological substrate for selective attention.

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