DISTINCT ROLES OF THE MEDIAL AND CENTRAL NUCLEUS OF THE AMYGDALA IN UNCONDITIONED AND CONDITIONED FEAR

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ABSTRACT

Pre-clinical and clinical data suggest that the amygdala plays a role in the detection of emotional events and in the production of fear responses. The amygdala is composed of distinct nuclei that may serve different functional roles in the modulation of fear. The present study examined the roles of the medial (MeA) and central (CeA) nucleus of the amygdala in unconditioned and conditioned fear. Following bilateral ibotenic acid lesions of the MeA or CeA, rats were exposed to cat odor, an unconditioned fear stimulus. In comparison with sham-operated controls, rats with MeA lesions exhibited significant deficits in cat odor-induced unconditioned fear as indicated by a significant reduction in the duration of freezing and avoidance and an increase in the frequency of contact with the cat odor stimulus. In contrast, excitotoxic lesions of the CeA had no significant effects on cat odor-induced unconditioned fear. To examine the role of the MeA and CeA in conditioned fear, rats with similar fiber-sparing lesions of the MeA and CeA were exposed to foot-shock. Conditioned freezing was measured in the immediate post-shock period and a retention test administered after 24-h. Results indicated that MeA lesions had no reliable effects on contextual fear conditioning as indicated by no significant differences in freezing between lesion and control groups in the immediate post-shock period and in the retention test. In contrast, CeA lesions produced significant deficits in freezing occurring in the post-shock interval and in the retention test. Together, these results suggest that the MeA, but not the CeA, plays a role in the mediation of predator odor-induced unconditioned fear. In contrast, the CeA, but not the MeA, appears to play a role in fear conditioning to a context paired with electric foot-shock.
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CHAPTER 1: INTRODUCTION

Anxiety disorders are common fear-related psychiatric illnesses. As a result, considerable attention is focused on how the brain normally processes fear and how brain abnormalities may predispose an individual to develop psychopathology. One brain region that has attracted widespread attraction is the amygdala because of its involvement in the detection of emotional events and the production of fear and anxiety responses (LeDoux, 2000; Pitkanen, Savander, & LeDoux, 1997). In humans, amygdala damage impairs the ability to recognize emotional expressions associated with fear (Morris et al., 1996). In addition, brain scans show that the amygdala is highly active in overanxious children and veterans with post-traumatic stress disorder (Pissiotta et al., 2002; Thomas et al., 2001). These studies suggest that a neurobehavioral analysis of the amygdala may offer insights into the pathogenesis of anxiety disorders.

Historical Perspective

In 1939, Kluver and Bucy reported that temporal lobotomies in monkeys produced a loss of fear to a variety of fear-inducing stimuli such as humans, gloves, or stuff animals. This loss of fear was accompanied by a reduction in neophobia, hypersexuality, visual agnosia, and oral tendencies. This behavioral phenomenon is termed the Kluver-Bucy syndrome (Kluver & Bucy, 1939) and is the first animal study linking a brain region with fear. Subsequent nonhuman primate studies involving the destruction of specific nuclei in the temporal lobe suggested that amygdala damage was a major factor underlying the Kluver-Bucy syndrome (Horel, Keating, & Misantone, 1975). The fear-reducing effects stemming from amygdala damage were further demonstrated to occur in rats exposed to a
cat (Blanchard & Blanchard, 1972). Thus, across species, the amygdala appears to play an important role in the elicitation of fear.

The Role of Specific Amygdala Nuclei in Unconditioned Fear

The amygdala consists of several discrete nuclei including the lateral (LA), the basolateral (BLA), the basomedial (BMA), the central (CeA), the cortical (COA), the medial (MeA), and the posterior nucleus (PA). Among these various nuclei, the MeA has long been associated with emotionality. Early research reported that lesions of the MeA increased tameness in dogs (Fonberg, 1965) and produced deficits in passive avoidance in rats (Pellegrino, 1968) and cats (Ursin, 1965). In contrast, electrical stimulation of the MeA facilitated the occurrence of innate defensive responses in cats (Magnus & Lammers, 1956) and dogs (Fonberg, 1963).

Recent studies have focused on the use of natural unconditioned fear stimuli to study the neural basis of innate defensive behavior. In many studies, cat odor is used to elicit vigorous behavioral, cardiovascular and endocrine responses (Blanchard, Blanchard, Weiss, & Meyer, 1990; Dielenberg & McGregor, 2001). A mapping study showed that when rats were exposed to cat odor, Fos immunoreactivity increased in the MeA and its efferent projection sites such as the hypothalamus and periaqueductal gray (PAG) (Dielenberg, Hunt, & McGregor, 2001). In contrast, Fos protein did not increase in other amygdala nuclei such as the LA, BLA and CeA (Dielenberg et al., 2001), suggesting that the MeA is a component of a neural system underlying innate fear. This specific elevation in Fos expression in the MeA is likely due to the direct inputs of chemosensory information from the accessory olfactory bulb (Luskin & Price, 1983; McDonald, 1998) and main olfactory bulb (Scalia & Winans, 1975) to the MeA. In addition, the MeA
receives indirect chemosensory stimuli from the posterolateral cortical (PLCo) amygdala (Krettek & Price, 1978), anterior olfactory nucleus (AON) (Luskin & Price, 1983) and dorsal endoperiform nucleus (Krettek & Price, 1978). Thus, the MeA is centrally positioned to received olfactory information related to fear.

The extent to which MeA lesions would produce deficits in fear expression to predator odor remains to be determined. However, excitotoxic lesions of the BLA or the LA had no effect on unconditioned fear to the predator odor trimethylthiazoline (TMT) (Wallace & Rosen, 2001), albeit another study reported that the damage to the BLA and LA impaired freezing and avoidance responses to a ball of cat hair (Vazdarjanova, Cahill, & McGaugh, 2001). The basis of these conflicting results is not clear but may be related to the nature of the different odor stimuli or testing methods that were employed.

The role of the CeA in unconditioned fear has also received attention. CeA-lesioned rats did not differ from controls in the heart rate unconditioned response to shock (Sananes & Campbell, 1989). AMPA receptor antagonism in the CeA had no effect on light-enhanced startle, another unconditioned response (Walker & Davis, 1997). Because the rat CeA failed to show increased Fos expression during exposure to cat odor (Dielenberg et al., 2001), the CeA may not be involved in cat odor-induced innate fear. On the basis of these studies, the CeA does not appear to play a prominent role in the expression of unconditioned fear.

The Role of Specific Amygdala Nuclei in Conditioned Fear

In the last two decades, the neurobiology of conditioned fear has been extensively studied using the Pavlovian fear conditioning paradigm. In this animal model, rats receive pairings of a discrete conditioned stimulus (CS), such as a tone, with a noxious
unconditioned stimulus (US), such as a foot-shock. After several pairings, the CS elicits conditioned fear responses (CRs) such as freezing, heart rate and blood pressure increases. Fear conditioning also occurs when a context is paired with shock. Conditioned fear responses to the context are often examined immediately after shock or after 24 h. Freezing in the immediate post-shock interval is a conditioned fear response because testing the rat in a novel context in the post-shock interval reduces freezing (Blanchard & Blanchard, 1972; Fanselow, 1980). Freezing occurring 24 h after shock exposure is another assessment of conditioned fear because rats freeze more in the context paired with shock than in a different context (McNish, Gewirtz, & Davis, 1997).

Two amygdala subsystems appear to have unique roles in fear conditioning (Davis & Whalen, 2001; LeDoux, 2000; Maren & Fanselow, 1996). The basolateral complex of the amygdala, consisting of LA, BLA, and BMA, play a major role in receiving CS and US and modulating their association (Fanselow & Kim, 1994; LeDoux, Farb, & Ruggiero, 1990b; Quirk, Repa, & LeDoux, 1995; Romanski, Clugnet, Bordi, & LeDoux, 1993). Lesions of the basolateral complex of the amygdala disrupt a variety of conditioned fear behavior (Campeau & Davis, 1995; Cousens & Otto, 1998; Kim, Rison, & Fanselow, 1993; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990a; Maren, Aharonov, & Fanselow, 1996). In contrast, the CeA, which receives direct inputs from the basolateral complex of the amygdala and other amygdala nuclei, projects to brain areas involved in the generation of fear responses, such as lateral hypothalamus (LH) and PAG. Therefore, the CeA is thought to be the final output of the amygdala that modulates the expression of learned associations (Davis & Whalen, 2001; Krettek & Price, 1978; LeDoux, 2000; Maren & Fanselow, 1996; Price & Amaral, 1981). Lesions
of the CeA are also effective in disrupting fear CRs (Falls & Davis, 1995; Helmstetter, 1992; Kapp, Frysinger, Gallagher, & Haselton, 1979; Sananes & Campbell, 1989; Weisz, Harden, & Xiang, 1992).

In contrast, there is little information on the role of the MeA in fear conditioning when foot-shock is used as the US. One study reported that electrolytic MeA lesions failed to disrupt auditory fear conditioning (Nader, Majidishad, Amorapanth, & LeDoux, 2001). Unlike the LA, CeA, and BLA which receive nociceptive input from cortical and subcortical brain regions (Bernard, Alden, & Besson, 1993; LeDoux et al., 1990b; McDonald, 1998), and contain foot-shock responsive neurons (Romanski et al., 1993), the MeA is not a component of central pain pathways. Therefore, the MeA may not be involved in fear conditioning when shock is used as the US. However, another study examining the patterns of Fos expression suggested that the MeA is involved in the association of the CS (odor) and the US (shock) in olfactory fear conditioning (Schettino & Otto, 2001). The role of the MeA in other types of fear conditioning such as contextual fear conditioning remains to be determined.

**Specific Aims**

The purpose of this thesis research is twofold. The first aim is to test the hypothesis that the MeA plays an essential role in the modulation of innate or unconditioned fear when activated by olfactory stimuli of a threatening nature. To evaluate the functional specificity of the MeA in unconditioned fear, the CeA was also examined because of its hypothesized role in conditioned but not unconditioned fear (Walker & Davis, 1997). To achieve this aim, fiber-sparing lesions were made in the rat MeA or the CeA. After recovery, rats were tested for unconditioned fear by exposing them to cat odor. It is
hypothesized that MeA, but not CeA, lesions will impair the expression of unconditioned fear induced by predator odor.

The second aim is to determine the extent to which the MeA plays a role in the expression of conditioned contextual fear. In this study, rats with fiber sparring lesions of the MeA or CeA were exposed to the widely used electric foot-shock conditioning procedure. Freezing occurring in the shock box was measured in the immediate post-shock period as well as in a retention test administered after 24-h. Although it is hypothesized that the CeA will have a major influence on conditioned freezing, the involvement of the MeA in fear conditioning to a context paired with foot-shock has not been previously explored. Thus, this second experiment will provide novel information on the role of the MeA in facilitating the expression of contextual fear.
CHAPTER 2: EXPERIMENTAL PROCEDURES

Animals

Male Long-Evans rats weighing 275-300 g at the time of surgery were used. Rats were bred at the University of Hawaii Animal Facility from a stock obtained from Charles River Laboratories (Raleigh, NC, USA). Animals were singly housed in polycarbonate cages and maintained on a 12-h/12-h light/dark cycle with lights on at 0600 h. Each cage was provisioned with food, water, and a layer of Sani-chips. Effort was made to minimize the suffering and the number of animals used. The research was conducted in accordance with the principles and procedures outlined in the NIH Guide for the Care and Use of Laboratory animals.

Surgery

Each animal was deeply anesthetized with a mixture of ketamine hydrochloride (100 mg/kg, IP) and xylazine (20 mg/kg, IP) prior to mounting in a stereotaxic frame. Ibotenic acid lesions were made with a 28-gauge stainless steel cannula connected to a microliter syringe by polyethylene tubing. The microliter syringe was driven by an infusion pump. Ibotenic acid (Sigma-Aldrich, St. Louis, MO, USA) was dissolved (10 μg/μl) in 0.1 M phosphate buffer saline (PBS, pH 7.4) and infused into either the MeA or CeA at a rate of 0.1 μl/min. The injection cannula was left in place for an additional 4 min before withdrawal. Each MeA was infused with PBS or ibotenic acid (2.0 μg) using the following flat-skull coordinates: A-P = -2.1 mm from bregma, M-L = ±3.2 mm, D-V = -9.1 mm from the skull surface. The CeA was infused with PBS or ibotenic acid (1.0 μg) using the following flat-skull coordinates: A-P = -1.5 mm from bregma, M-L = ± 4.2
mm, D-V = -7.9 mm from the skull surface. Animals were allowed to recover for six to seven days before testing.

**Experiment 1: Role of the MeA and CeA in unconditioned fear**

The unconditioned fear test was conducted in an elongated rectangular apparatus (100 X 12 X 50 cm) constructed of three white Plexiglas walls, a clear Plexiglas front wall to allow videotaping, and an opened top. The test box rested on a white Plexiglas base.

A terry cloth-wrapped wooden block (9 X 9 X 2 cm) was used to present cat odor. The block was placed on the bedding of the cat overnight and rubbed several times against the cat’s body immediately before testing. The cat odor block was placed on the floor at one end of the test apparatus.

Behavioral testing consisted of two phases – a pretest phase in which cat odor was not present and a test phase involving cat odor presentation. In the pretest phase, rats were adapted to the apparatus over a 2-day period. On each pretest day, rats were placed in the apparatus at the opposite end where the cat odor block would be located. The duration of each pretest adaptation period was 10-min.

On the test day, the cat odor block was placed at one end of the apparatus and each rat was then placed at the opposite end. The behavior of the rat was videotaped for a 10-min period. The cat odor block was replaced with a fresh cat odor block after testing two animals.

The two phases of this experiment were conducted under red light illumination between 1000 – 1200 h. After each test, the apparatus was cleaned with 5% alcohol.

The behavioral measures that were analyzed from the videotape included: freezing duration (in sec) – a stationary posture characterized by cessation of body movements...
except those required for respiration; avoidance duration (in sec) – avoidance was scored when all four paws of the rat were kept in a 33.3 cm length segment of the apparatus that was measured starting from the opposite end wall where the cat odor stimulus was presented; rearing frequency – number of upright stances with raised forelimbs; stretch attend frequency – number of approaches or stationary postures involving a flattened back and stretched neck orientation to the cat odor block; contact frequency – number of times the rat touched the cat odor block.

Experiment 2: Role of the MeA and CeA in contextual fear conditioning

Contextual fear conditioning tests were conducted in a box (25.3 X 20.3 X 22.6 cm) constructed of white Plexiglas sides and top, and a clear Plexiglas front wall to allow video recording. Scrambled electric foot-shock was delivered via the stainless grid floor. Tests were conducted between 1000 – 1200 h. After 2 min in the shock box, 3 electric foot shocks (1.0 mA, 1 s duration) were delivered at 20-sec intervals. The behavior of the rat was videotaped for a 10-min period that commenced after the last shock. Twenty-four hours later, rats were re-exposed to the shock box and videotaped for a 10-min period.

The duration of freezing was scored as described in the cat odor test. All tests were conducted under fluorescent ceiling lighting and the shock box was cleaned with 5% alcohol after each test.

Histology

After behavioral testing, rats were overdosed with sodium pentobarbital and perfused intracardially with physiological saline and 10% formalin. Brains were extracted and kept in 10% formalin followed by 20% sucrose-formalin for cryoprotection. Frozen
sections were cut at 50 μm and every fourth section was mounted throughout the extent of the lesion and stained with thionine. The location and size of lesions were determined with the aid of a rat brain atlas (Paxinos & Watson, 1998).

Data analysis

In experiment 1, independent two-tailed t-tests were used to analyze behavioral differences produced by MeA and CeA lesions. In experiment 2, two-way repeated measures ANOVA tests (group x test) were used to investigate the behavioral alterations produced by MeA and CeA lesions during the immediate post-shock period and in the retention test occurring the next day.
CHAPTER 3: RESULTS

Experiment 1: Role of the MeA and CeA in unconditioned fear

Histology

Of seventeen MeA-lesioned rats, eight animals incurred bilateral MeA damage extending from coronal section –2.80 to –3.30 mm from bregma (Fig. 1 & 3). Nine rats were excluded from the study because of misplacement or infringement on the CeA. In addition to the MeA, several animals had slight damage to the surrounding region including the BMA, COA, PA, periamygdaloid cortex, intercalated nuclei of the amygdala, and anterolateral part of the amygdalohippocampal area. No animals had lesions extending into the CeA or BLA. A total of eight sham-operated and eight MeA lesioned rats were used in the behavioral analysis.

Of eighteen CeA-lesioned rats, eight animals incurred bilateral CeA damage extending from coronal section –2.12 to –2.56 mm from bregma (Fig. 2 & 3). Ten rats were excluded from the study because of extensive damage to the BLA. Minor damage to adjacent tissue may include the lateral globus pallidus, interstitial nucleus of the posterior limb of the anterior commissure, amygdalostriatal transition area, amygdaloid intramedullary gray, commissural stria terminalis, intraamygdala division of the bed nucleus of the stria terminalis and ventral portion of the caudate putamen. The LA and BLA were also partially damaged. The border between the CeA and posterior dorsal part of the MeA (coronal section –2.56) also received minor damage in several cases. A total of eight sham-operated and eight CeA-lesioned rats were used in the behavioral analysis.
Figure 1. The extent of MeA lesions for the 8 animals used in the data analysis. The shading represents the region with neuronal loss and presence of gliosis. The posterior distance from the bregma is indicated in the number to the right. The sections are adapted from Paxinos and Watson, 1998.
Figure 2. The extent of CeA lesions for the 8 animals used in the data analysis. The shading represents the region with neuronal loss and presence of gliosis. The posterior distance from the bregma is indicated in the number to the right. The sections are adapted from Paxinos and Watson, 1998.
Unconditioned fear responses

In comparison to sham-operated rats, bilateral MeA lesions produced a significant reduction in cat odor-elicited unconditioned fear. MeA-lesioned rats showed significantly less freezing duration, $t(14)=4.59$, $p<.001$ (Fig. 4A), and avoidance time, $t(14)=2.16$, $p<.05$ (Fig. 4B). In addition, MeA-lesioned animals made more contact with the cat odor block, $t(14)=4.59$, $p<.001$ (Fig. 4C), and showed elevated levels of
rearing, t (14)= 4.69, p < .001 (mean ± SE control frequency = 14.1 vs. mean ± SE MeA frequency = 47.5). No reliable group difference was found in the number of stretched attention, t (14)= 0.69, p > .05 (Fig 4D).

![Graphs showing effects of MeA lesions on freezing duration, avoidance duration, contact frequency, and stretched attention frequency.](image)

**Figure 4.** Effects of MeA lesions on freezing duration (A), avoidance duration (B), contact frequency (C), and stretched attention frequency (D) in the unconditioned fear test. * p < .05, *** p <.001, significantly different from sham-operated rats, t-test. N=8 per group.

No significant group differences were found between sham operated and CeA-lesioned rats in freezing duration, t (14)= 1.13, p > .05 (Fig 5A), avoidance duration, t (14)= 1.23, p > .05 (Fig 5B), contact frequency, t (14)= 0.82, p > .05 (Fig. 5C) and stretched attention frequency, t (14)= 1.27, p > .05 (Fig 5D). In addition, no significant group difference, t
(14)= 1.53, p > .05, was found in rearing frequency (mean ± SE sham group = 10.1 ± 5.6 vs. mean ± SE CeA lesioned group = 27.6 ± 10.0).

Figure 5. Effects of CeA lesions on freezing duration (A), avoidance duration (B), contact frequency (C), and stretched attention frequency (D) in the unconditioned fear test. N=8 per group.

Experiment 2: Role of the MeA and CeA in contextual fear conditioning

Histology

Of twenty MeA-lesioned rats, eight animals had bilateral damage to the MeA. Twelve animals were excluded because of misplacement or infringement on the CeA. The majority of rats had MeA lesions in coronal sections that encompass regions –2.3 to –3.3 mm from bregma (Fig. 6). The extent of MeA lesions was similar to that in experiment 1. In addition to the MeA, several animals may have some damage to the
surrounding area including the BMA, COA, PA, intercalated nuclei of the amygdala, and anterolateral part of the amygdalohippocampal area. No animals had lesions in the CeA or BLA. A total of eight sham-operated and eight MeA-lesioned rats were used in the behavioral analysis.

Of twenty-four CeA-lesioned rats, eight animals incurred bilateral CeA lesions extending from coronal sections \(-2.12\) to \(-2.56\) mm from bregma (Fig. 7). The extent of CeA lesions was similar to that in experiment 1. Sixteen rats were excluded because of unilateral damage to the CeA or extensive damage to the BLA. Minor damage to adjacent tissues may have included the BMA, the lateral globus pallidus, interstitial nucleus of the posterior limb of the anterior commissure, amygdalostrial transition area, amygdaloid intramedullary gray, main part of the intercalated amygdaloid nucleus, intraamygdala division of the bed nucleus of the stria terminalis, and ventral portion of the caudate putamen. The LA and BLA were also partially damaged. The border between the CeA and posterior dorsal part of the MeA also received very minor damage in two cases. A total of eight sham-operated and eight CeA-lesioned rats were used in the behavioral analysis.
Figure 6. The extent of MeA lesions for the 8 animals used in the data analysis. The shading represents the region with neuronal loss and presence of gliosis. The posterior distance from the bregma is indicated in the number to the right. The sections are adapted from Paxinos and Watson, 1998.
Figure 7. The extent of CeA lesions for the 8 animals used in the data analysis. The shading represents the region with neuronal loss and presence of gliosis. The posterior distance from the bregma is indicated in the number to the right. The sections are adapted from Paxinos and Watson, 1998.
Contextual fear responses

Although there was a trend for reduced immediate post-shock and retention test freezing following MeA lesions, no significant effects were found, $F(1, 14) = 4.30, p > .05$ (Fig. 8). In addition, there was no significant group $\times$ test interaction, $F(1, 14) = .36, p > .05$. However, there was a significant main effect of test, $F(1, 14) = 8.80, p < .01$. MeA-lesioned and sham-operated rats showed less conditioned freezing in the retention test than in the immediate post-shock period.

![Graph showing effects of MeA lesions on freezing](image)

**Figure 8.** Effects of MeA lesions on freezing in the immediate post-shock interval and in the retention test administered after 24-h. $N= 8$ per group.

In comparison with sham-operated rats, CeA lesions produced a significant reduction in post-shock freezing and retention test freezing, as indicated by a highly significant group effect, $F(1, 14) = 11.88, p < .01$ (Fig. 9). In addition, both CeA-lesioned and sham-operated rats showed less retention test freezing than post-shock freezing, as indicated by
a significant test effect, $F(1, 14)= 8.70, p < .05$. A significant group x test interaction, $F(1, 14)= 5.19, p < .05$, revealed that CeA-lesioned rats exhibited a significant reduction in both post-shock freezing, $t(14)= 3.34, p < .01$, and retention test freezing, $t(14)= 2.66, p < .05$. Paired t-tests further revealed that sham-operated rats, $t(7)= 2.67, p < .05$, but not CeA-lesioned rats, $t(7)= 1.68, p > .05$, showed a significant decline in conditioned freezing during the retention test.

![Figure 9](image.png)

**Figure 9.** Effects of CeA lesions on freezing in the immediate post-shock interval and in the retention test administered after 24-h. N= 8 per group. * $p < .05$, ** $p < .01$, significantly different from the sham groups.
CHAPTER 4: DISCUSSION

A major result of this study is that bilateral fiber-sparing lesions of the MeA significantly disrupted unconditioned or innate fear behavior to cat odor whereas similar MeA lesions had no reliable effects on contextual fear conditioning. The study also demonstrates that although excitotoxic lesions of the CeA had no significant effects on cat odor-induced unconditioned fear, these lesions produced significant deficits in the facilitation of contextual fear. Together, these data suggest that MeA and CeA have distinct functional roles in the modulation of fear. MeA may preferentially mediate odor-related unconditioned fear behavior; whereas CeA may preferentially mediate conditioned fear behavior induced by an aversive stimulus US.

The role of the MeA in unconditioned fear

Because the MeA is a direct recipient of chemosensory inputs (Krettek & Price, 1978; Luskin & Price, 1983; McDonald, 1998; Scalia & Winans, 1975), one interpretation of this behavioral deficit in fear is that olfactory processing was impaired. However, studies in rats indicate that lesions destroying the entire amygdala do not produce deficits in olfactory discrimination (Cahill & McGaugh, 1990). In other studies, electrolytic lesions restricted to the MeA do not impact the ability of female hamsters to discriminate and recognize odors of different male hamsters (Petrulis & Johnston, 1999). Because the MeA lesions do not appear to disrupt an animal’s ability to smell (Petrulis & Johnston, 1999) it is possible that the MeA may be involved in evaluating the emotional valence of an olfactory stimulus which would then lead to the production of appropriate responses.

Another possible interpretation of the effects of MeA lesions on unconditioned fear is that performance of fear behavior was impaired. However, MeA-lesioned rats exhibited
a high level of freezing in the conditioned fear test. Thus, the behavioral deficits produced by MeA lesions in the unconditioned fear test may reflect a reduction in the emotional and motivational aspects of fear induced by a natural threat.

The MeA is situated in a highly interconnected web of brain regions that appear to play a prominent role in innate defensive behavior. The MeA projects directly to the lateral septum (LS), bed nucleus of the stria terminalis (BNST), anterior hypothalamic nucleus (AHN), ventral medial hypothalamic nucleus (VMH), and periaqueductal gray (PAG) (Canteras, Simerly, & Swanson, 1995). These brain regions are involved in the expression of defensive behavior following stimulation or lesions (Albert & Chew, 1980; Bandler, Depaulis, & Vergnes, 1985; Brandao, Di Scala, Bouchet, & Schmitt, 1986; De Oca, DeCola, Maren, & Fanselow, 1998; Fernandez de Molina & Hunsperger, 1962; Hunsperger, 1956; Milani & Graeff, 1987; Silveira & Graeff, 1992). Moreover, the BNST is hypothesized to play a role in the expression of unconditioned fear behavior (Walker & Davis, 1997). Both the MeA and BNST project to the highly interconnected medial hypothalamus zone including AHN, VMH and the dorsal premammillary nucleus (PMd) which may play a critical role in the organization of innate defensive behavior (Canteras, 2002). PMd lesions have been shown to eliminate innate fear responses to cat odor or cat (Blanchard et al., 2003; Canteras, Chiavegatto, Valle, & Swanson, 1997). Interestingly, these MeA projection sites all show elevated Fos expression in rats exposed to cat odor (Dielenberg et al., 2001). Therefore, the MeA is a key brain region that receives olfactory information and activates a distinct neural system underlying innate fear behavior.
The role of MeA in conditioned fear

The ability of MeA-lesioned rats to exhibit contextual freezing in the immediate post-shock period as well as in the retention test administered after 24-h indicates that the MeA may not have a prominent role in contextual fear conditioning that required the convergence of contextual and nociceptive information. Because the MeA is not a component of the central pain pathway (McDonald, 1998), it does not seem to subserve the association of context (CS) and foot-shock (US) and modulate the conditioned fear behavior when shock is used as the US.

Although MeA lesions did not produce deficits in conditioned freezing, the MeA is responsive to a diverse range of stressful stimuli. Exposure to a conditioned auditory cue, foot shock, elevated plus maze, air puff, noise, restraint and forced swim often produces an increase in c-fos mRNA or Fos protein in the MeA but not any other amygdala nucleus (Cullinan, Herman, Battaglia, Akil, & Watson, 1995; Duncan, Knapp, & Breese, 1996; Pezzone, Lee, Hoffman, & Rabin, 1992; Rosen, Fanselow, Young, Sitcoske, & Maren, 1998). This increase in c-fos expression suggests that the MeA may have an important role in processing emotional stimuli but may not always be linked to explicit behavioral alterations.

It is also possible that c-fos expression is not a sensitive indicator of brain functions. Other immediate early genes (IEGs) display different patterns of neuronal activity than c-fos and are more closely associated with neuronal activation during specific behavioral displays (Cullinan et al., 1995; Rosen et al., 1998). For example, a study shows that although c-fos expression occurs in the MeA of control and shocked groups, egr-1 (also called zif268, krox-24, TIS-8, NIGFI-A) increases specifically in the LA of rats that
display conditioned freezing. This result suggests that the immediate early gene, egr-1, rather than c-fos, provides a more specific index of neuronal activity during learning and memory (Rosen et al., 1998; Worley et al., 1993).

Although the MeA lesion had no reliable effects on contextual fear conditioning, it may be involved in other types of fear conditioning. For example, MeA lesions made in subordinate rats after social defeat disrupt conditioned avoidance to a dominant rat (Luiten, Koolhaas, de Boer, & Koopmans, 1985), suggesting that the MeA can play a role in the expression of conditioned social fear. Other studies have demonstrated that the MeA plays a role in short-term recognition of individuals (Ferguson, Aldag, Insel, & Young, 2001), and long-term preferences for familiar mates (Demas, Williams, & Nelson, 1997; Kaba, Rosser, & Keverne, 1989; Kirkpatrick, Carter, Newman, & Insel, 1994).

**The role of CeA in unconditioned fear**

The inability of CeA lesions to produce deficits in unconditioned fear is consistent with other studies showing that CeA lesions do not influence the heart rate unconditioned response to shock or that AMPA receptor antagonism in the CeA does not impair light-enhanced startle, an unconditioned response (Sananes & Campbell, 1989; Walker & Davis, 1997). The ineffectiveness of CeA lesions to reduce unconditioned fear cannot be attributed to the size or specificity of the lesion because similar CeA lesions are capable of producing deficits in contextual fear.

The lack of direct olfactory inputs from the olfactory and accessory olfactory bulb to the CeA (McDonald, 1998) may account for the absence of CeA involvement in the odor-related unconditioned fear. The CeA receives olfactory information only indirectly
through intra-amygdalar connections with the COA or MeA (Savander, Go, Ledoux, & Pitkanen, 1996) or through very little projection from other primary olfactory cortical areas (Luskin & Price, 1983). Therefore, it is less likely that the CeA is the key area in the amygdala that processes olfactory information from the US and generates fear responses to the stimulus.

Previous studies suggest that the CeA is the final output to motor regions controlling fear responses (Davis & Whalen, 2001; Fanselow & Kim, 1994; LeDoux, 2000; Maren & Fanselow, 1996). However, the present study demonstrates that cat odor-induced unconditioned freezing occurs even after damage to the CeA. This result suggests that alternative projections such as those stemming from the MeA to the hypothalamus and PAG are sufficient to generate innate fear responses.

The role of CeA in conditioned fear

The effectiveness of CeA lesions to attenuate freezing in the immediate post-shock interval and in the retention test indicates that the CeA plays a critical role in the modulation of contextual fear conditioning. These data are consistent with previous studies showing that damage to CeA disrupts a variety of conditioned fear behavior including contextual freezing (Kim et al., 1993; McNish et al., 1997; Phillips & LeDoux, 1992), fear-potentiated startle (Hitchcock & Davis, 1986), auditory heart rate conditioning (Kapp et al., 1979), olfactory heart rate conditioning (Sananes & Campbell, 1989), and conditioned blood pressure and respiratory response (Zhang, Harper, & Ni, 1986). Because both contextual and nociceptive information can reach the CeA directly from the hippocampus and nociceptive brain areas, or by way of the BLA (Bernard et al., 1993; Canteras & Swanson, 1992; LeDoux et al., 1990b; McDonald, 1998), the CeA may
effectively subserve the association of context (CS) and foot-shock (US) to modulate conditioned fear.

A widely accepted view is that the CeA is primarily involved in the expression of conditioned fear (Davis & Whalen, 2001; Fanselow & Kim, 1994; LeDoux, 2000; Maren & Fanselow, 1996). However, several lines of evidence indicate that the CeA is not merely a relay structure for behavioral expression. First, there is extensive overlap of somatosensory, visual, auditory, and gustatory cortical inputs in the CeA, suggesting that convergence of sensory information occurs in the CeA (McDonald, 1998). Second, CeA lesions block the suppression of behavior elicited by a conditioned aversive stimulus (CS+) (Killcross, Robbins, & Everitt, 1997) but do not block an animal’s ability to avoid the CS+ by their choice behavior. This finding suggests that the CeA is capable of forming a CS-US association and mediating the expression of conditioned fear responses.

The role of MeA in odor-elicited social behavior

The involvement of the MeA in innate fear underscores the dynamic nature of this nucleus in regulating a variety of odor-elicited social behavior including kin recognition (Ferguson et al., 2001), affiliation (Kirkpatrick et al., 1994), maternal behavior (Fleming, Vaccarino, & Luebke, 1980; Numan, Numan, & English, 1993; Sheehan, Paul, Amaral, Numan, & Numan, 2001), aggression (Shibata, Yamamoto, & Ueki, 1982), and reproduction (Kollack-Walker & Newman, 1995; Lehman, Winans, & Powers, 1980; Takahashi & Gladstone, 1988). The MeA may be a key brain structure that integrates chemosensory and hormonal information, evaluates the valence of the cues, and activates distinct circuitries underlying specific social and sexual behavior. In rodents, the social information is encoded by chemosensory and hormonal signals. Because the MeA
receives direct olfactory and pheromonal inputs (Canteras et al., 1995; McDonald, 1998) and contains steroid-responsive neurons (Simerly, Chang, Muramatsu, & Swanson, 1990), the MeA may be involved in the integration of chemosensory and hormonal inputs (Wood & Coolen, 1997). In addition, MeA lesions do not impair an animal's ability to smell but specifically disrupt odor-elicited responses such as female preference for the male odors (Petrulis & Johnston, 1999) and cat odor-induced innate fear behavior (the present study), suggesting that the MeA plays a specific role in evaluating the information from social cues, rather than serving as a nonspecific relayed station of olfaction. Furthermore, the MeA also plays a role in transferring the processed chemosensory information to brain regions that modulate specific social and sexual behavior. Studies examining neural activation following mating, conspecific intrusion, pup exposure, and cat odor exposure (Dielenberg et al., 2001; Kollack-Walker & Newman, 1995; Sheehan, Cirrito, Numan, & Numan, 2000) all report increased Fos-immunoreactivity in MeA and its projection sites including BNST and LS. However, the patterns of neural activation following sexual or agonistic behavior differ in some MeA projections sites (Dielenberg et al., 2001; Kollack-Walker & Newman, 1995; Sheehan et al., 2000). These studies suggest that the MeA activates distinct neural circuitries underlying specific social behavior. Therefore, the MeA may play an important role in the general organization of sociosexual and defensive behavior.

The diverse roles of the MeA in innate fear and in a variety of social behavior suggest that fear-induced MeA activation may impact other MeA-modulated behavior. On the other hand, MeA activation by social behavior may impact fear levels of an organism. Because fear often arises from social interactions in humans, understanding the neural
mechanism of MeA-modulated innate fear and social behavior may promote an understanding of human anxiety disorder.
CHAPTER 5: REFERENCES


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