

THE EFFECTS OF MEDIAL AMYGDALA LESIONS ON FEAR AND ANXIETY

Rachel Bergman, Eileen Gongon, David Handsman, Saheela Ibraheem, Eileen Jiang, Nikhil Keny, Wendy Wei, Caresse Yan, Annie Yang, Sabrina Zeller, Allen Zheng, Linda Zhong

Advisor: Dr. Graham Cousens
Assistant: Zack Vogel

ABSTRACT

The purpose of this study was to examine the underlying neural circuitries in fear and anxiety related pathways. Three experiments evaluated the effects of medial amygdala lesions on startle potentiation in rats. In Experiment One, olfactory fear-potentiated startle (oFPS) was used to model fear. In Experiment Two and Experiment Three, light-enhanced startle (LES) and allomone-potentiated startle (APS) were used to model anxiety, respectively. The amplitude of the acoustic startle response was used to evaluate the degree of either fear or anxiety in rats. Non-lesion rats and lesion rats were subjected to all three tests. A decreased startle response was observed in lesion rats subjected to both LES and APS while lesion rats exposed to oFPS demonstrated a limited change in startle response. These findings suggest that the medial amygdala plays a significant role in anxiety, but may not be involved in discrete fear response.

INTRODUCTION

The National Institute of Health approximates that 40 million American adults, about 18.1% of the population, have struggled with an anxiety syndrome, such as panic disorder, post-traumatic stress disorder, or obsessive-compulsive disorder, this past year (1). Much of the neural circuitry still remains a mystery. To answer these questions, researchers seek to map the brain's specific anxiety pathways. The present experiment traces the anxiety pathways of lab rats, specifically the role of the medial nucleus in the amygdala.

Although similar, fear and anxiety are two distinct psychological responses. Fear, the instinctual fight or flight symptom, not only manifests in the immediate presence of danger, but also recedes fairly quickly. Anxiety, however, is a response to distant threats. It continues for a sustained period of time in which the senses are augmented and awareness is increased (2). Just as the responses of fear and anxiety differ, so do their underlying neural pathways. The series of experiments seeks to clarify the difference between fear and anxiety pathways. To be able to discern the neurological mechanisms would significantly advance the understanding of the wide array of stress and anxiety disorders affecting America today.

Anatomy

Both fear and anxiety signal pathways pass through the almond-shaped neural structure called the amygdala. Fear-related inputs, such as a conditioned stimulus (CS), send signals to the basolateral nucleus in the amygdala (BLA), where they are in turn sent to the central nucleus to be discharged as physical responses. Therefore, the central nucleus and the lateral nucleus constitute a fear-related system of nuclei. When a CS is given, inputs from that stimulus are

formed in the BLA and memories that associate the CS with the unconditioned stimulus (US) are also formed. Signals are sent to the central nucleus of the amygdala, which sends output in the form of a physical response to fear (2).

Of the amygdala's various regions, the medial nucleus of the amygdala (AMe), the central focus of this study, is the one that is understood the least. The AMe is thought to be involved in the processing of anxiety related stimuli, particularly ones dealing with pheromones and allomones, olfactory signals originating from outside a specific organism.

Several distinct pathways in the brain transmit nasal chemosensory information to the amygdala. The nasal epithelium organ in the nose detects smells and sends signals to the main olfactory bulb (MOB) in the brain. The information is then processed in the cortical nuclei region of the amygdala. The vomeronasal organ, by contrast, detects chemical stimuli such as pheromones and allomones and sends these signals to the accessory olfactory bulb (AOB). From there, signals are sent to the AMe, where the information is processed. Because the MOB receives specific scents that can be conditioned with negative events, it is believed to be part of the fear-related pathway. Since the AOB receives signals from chemicals that cause more instinctive responses, it is believed to be part of an anxiety-related pathway (3).

Startle

In these experiments, fear and anxiety were quantified by measuring the startle and freezing responses in rats. When a rat is exposed to an anxiogenic stimulus, the startle response may magnify. The startle response is an instinctive reaction to some sort of noxious stimulus. It is characterized primarily by muscle contraction, although changes in heart rate and respiration also accompany it (4). It is a good indicator of fear because the magnitude of the startle is easy to measure. The freezing response, characterized by a period of motionlessness usually associated with a much more prolonged stimulus, is a secondary response of anxiety and was used in the experiment involving pheromone-like substances. Both responses are common to most, if not all, mammalian species, including rats. It also serves as the alternative to the fight or flight response. Below, three mechanisms for startle enhancement are described.

Pavlovian Conditioning and Olfactory Fear Potentiated Startle

Ivan Pavlov conducted the first experiments dealing with the learned association of a neutral stimulus with an aversive one. By consistently pairing the sound of a bell with food, Pavlov eventually caused the dogs to salivate at the mere sound of the bell, confirming associative learning. In a similar vein, the present experiment sought to amplify startle by associating an unpleasant shock with a CS in the form of an odor. The introduction of an US in the form of an auditory stimulus incorporated the associative learning of Pavlov and mimicked a previous shock-and-scent association procedure done by Walker, Paschall, and Davis in 2002 (2).

Past neurological experiments have explored the parts of the brain that are involved in FPS. Walker et. al. identified two regions of the amygdala, the basolateral region and the medial nuclei, as key to processing fear (5). They infused two receptors, artificial cerebrospinal fluid and

1,2,3,4-tetrahydro-6-nitro-2,3-dioxobenzoquinoxaline-7-sulfonamide (NBQX), into the basolateral nucleus and concluded that the rats were unable to show fear but were able to learn from conditioning. The role of this region is not an area of dispute among neurologists; however, there is a discrepancy among the role of the AMe in olfactory fear conditioning. Walker's findings indicated that the damage to the medial amygdala seemed to disrupt the FPS, implying that the medial amygdala was involved in fear; although in Paré's experiment, no connection was found between the medial amygdala and oFPS (6). Although AMe damage disrupted oFPS, nothing indicated its role in associating the CS with the US, something that the central amygdala (CeA) controls.

The first experiment of this study emulated Walker and Davis's previous experimental procedures. The experiment attempted to validate the effect of fear on rats by pairing an olfactory CS and a shock to generate heightened startle. In addition, it tried to determine the AMe's role in linking the olfactory senses and FPS by comparing rats that had lesion medial nuclei to control rats. It was predicted that the results of this experiment would be consistent with those of Walker et. al., where there would be less startle in the rats due to the lack of functioning AMe despite the contrasting results of Pare et. al. (5, 6).

Light Enhanced Startle

While the olfactory potentiated startle response paradigm is a model of conditioned fear, the light-enhanced startle (LES) is one of anxiety. Because rodents are largely nocturnal, exposure to bright light magnifies their startle response. Therefore, past scientific studies have used harsh illumination as a model of an anxiety-causing stimulus. Because rats avoid light as a natural instinct, this reaction represents a more distant threat and does not need to be conditioned. A bright light acts as a long-duration stimulus that usually precedes a more intense, short-lasting stimulus (2). Since this process does not involve conditioning, it is a common model of anxiety, opposed to FPS.

Using the FPS and LES paradigms, Walker and Davis in 1997 distinguished the neurological pathways between each (7). They injected rodents with NBQX, an AMPA (α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate) receptor antagonist. These receptors are located on both the CeA and the bed nucleus of the stria terminalis (BNST), and help to facilitate the transmission of neural impulses. When infusions of the NBQX were given to the CeA, only FPS was prevented; when given to the BNST, only LES was prevented. This experiment clearly showed that the CeA is involved in fear-elicited responses while the BNST is involved in those of anxiety. Despite the knowledge that anxiety and fear follow different neurological pathways, the function of the AMe is still largely unknown and unsubstantiated through experiment.

The goal of the LES experiment is to discover the relationship between the AMe and fear and/or anxiety related pathways in the brain. Because the AMe is connected to the BNST, it is expected to have some role in anxious behavior. Thus, lesion rats are expected to demonstrate a decrease in startle behavior in the presence of a bright light.

Allomone Potentiated Startle

The third experiment involves allomone potentiated startle (APS). Allomones are pheromone-like substances. Pheromones are chemicals that are excreted by members of a species that are picked up by members of the same species. When they are detected involuntarily by the vomeronasal organ, they elicit a variety of different responses depending on the type of pheromone released. Some pheromones serve as a warning and incur a fight or flight response when detected while others are used for reproductive purposes. Allomones belong to a very similar class of substances. These, however, are excreted by a member of one species and detected by a member of another species. Allomones are common to predator-prey relationships. A predator usually involuntarily excretes the pheromone-like substance, which, when picked up by the prey, elicits a fear response to the allomone. For example, rats, when exposed to cat allomones, will respond by freezing.

Experiments show that rats express increased neuronal activity in the AMe after exposure to cat collars that had been worn by cats compared to rats exposed to clean cat collars (8, 9). This activity was measured by the expression of a gene known as c-Fos, which produces a protein that appears as noticeable black spots or protein product when viewed under a microscope, allowing neural activity to be relatively quantifiable. The exposure to worn cat collars also produced relatively large amounts of c-Fos expression in the AOB (8).

Such findings question whether or not the AMe is involved in triggering freezing responses in rats exposed to pheromones and allomones and whether or not it plays a big role in triggering sustained fear responses in general. Using the results of past experiments on rats exposed to cat allomones as a basis, it is predicted that normal rats that have been exposed to cat allomones will freeze considerably more than the rats with lesions to the AMe since the medial amygdala is hypothesized to play an essential role in the expression of fear triggered by the presence of pheromone-like substances.

Hypothesis

The most critical aspect of all these experiments is the AMe lesions. By using rats that do not have working medial nuclei, these experiments were able to effectively show what types of fear responses (olfactory fear potentiated startle, light enhanced startle, and/or pheromone/allomone potentiated freezing and allomone potentiated startle) in which the AMe is involved. If it is involved in any of these pathways, then there would be a much weaker response to the stimuli if there is any response at all.

There are several reasons why the medial amygdala is expected to be involved in anxious behavior. Firstly, it is known to have connections to the AOB, which is involved in processing pheromone input. The AMe then processes this scent information and sends it to other regions in the brain that control the anxious response. Secondly, the AMe has connections to the bed nucleus stria terminalis (BNST), which is known to control aspects of anxiety (9). Thirdly, the AMe is connected to the La and Ce, which are known to be involved in conditioned fear.

The first experiment involving olfactory fear potentiated startle was predicted to show less of a change in startle response, using the experiment by Walker et. al. as a basis. The second experiment dealing with LES was also predicted to show less of a startle response because light is believed to increase anxiety in rats, and it is predicted that the AMe is a key player in the anxiety response. The final experiment dealing with rats exposed to cat allomones was also expected to reduce the startle response using the Dielenberg et. al. study as a basis.

METHOD

Subjects

Twelve male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing around 250-300 g (prior to surgery) were housed individually in clear plastic cages in a temperature- and humidity-controlled vivarium maintained on a 12-hour light cycle. Each rat had unlimited access to standard rat chow and water. All procedures were approved by the Drew University Institutional Animal Care and Use Committee and are in accordance with current U.S. Public Health Service Guidelines.

Apparatus

Startle Apparatus

Control, light and allomone enhanced startle experiments used two identical custom-built startle systems (Fig. 1). Each device consisted of a ventilated, sound attenuated cubicle (39.5cm×15.5cm×27.5cm) containing a steel grid cage (16.5cm×9.0cm×9.0 cm; Med-Associates, St. Albans, VT) mounted on a white acrylic base with a cylindrical accelerometer (model 338B35; PCB Piezotronics, Depew, NY). The front wall and the ceiling were constructed from clear Plexiglas, and the side walls were also constructed from white acrylic.

The outer, Plexiglas cubicle itself was equipped with a fluorescent bulb (C, Fig. 1) on the ceiling and ventilated by negative pressure using a variable-speed exhaust blower situated in an adjacent room that produces the background noise level of 67dB that drowned out external sounds. The variable-speed exhaust fan was connected to the vents (B, Fig. 1) on the inside on the inside of the Plexiglas cubicle that cleared the enclosure of excess odors and scents not relevant to the experiment. The high-frequency speaker (F, Fig. 1) in the rear wall of the enclosure delivered white noise pulses at 75dB, 85dB, 95dB and 105dB. There was no background illumination in any of the experiments.

The cylindrical accelerometer embedded in the Plexiglas base of the animal chamber records the subject's motor activity. Voltage output proportional to vertical chamber displacement was amplified 100X by a signal processor (model 482A20; PCB Piezotronics), digitized at 2kHz, and recorded 2000 times a second to a dedicated microcomputer equipped with an A/D processing card (model PCI-6070E; National Instruments Corp., Austin, TX) and physiological data acquisition software (Recorder, Plexon, Inc., Dallas, TX). Recorder was used to measure the startle response of the rats during both the baseline and light enhanced startle testing. Corollary stimulus trigger events were sent through a separate recording channel and

were saved for purposes of time-locking accelerometer output to auditory pulses. Trigger events were isolated using OfflineSorter software (Plexon, Inc.).

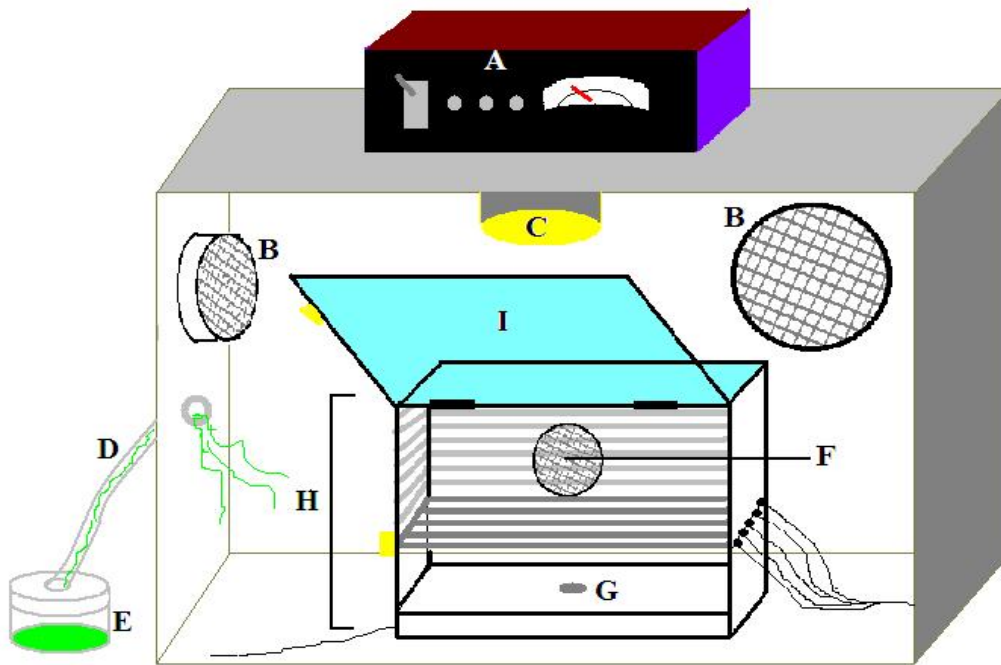


Figure 1 Acoustic Startle Chamber

A) Precision Animal Shocker, B) Vents, C) Light, D) Odor Tube, E) Scent Reservoir, F) Speaker, G) Accelerometer, H) Startle Chamber, I) Chamber Door

Freezing Behavior Apparatus

The freezing behavior trials were conducted in the behavioral chamber (A, Fig. 2), a sealed Plexiglas chamber (31.75 cm x 33.02 cm x 35.24 cm) ventilated on the left side with air holes. This behavioral chamber was enclosed by the testing chamber (B, Fig. 2), a sound reduction chamber (63.50 cm x 36.20 cm x 16.50 cm) with a viewing window (C, Fig. 2). The testing chamber was equipped with a 6 W light bulb (D, Fig. 2) in the front right corner to illuminate the testing chamber and allow for freezing behavior observations. A webcam (E, Fig. 2) was placed in the back right corner of the testing chamber and connected to a laptop where freezing behavior was observed and scored through Matlab. A hole was present (F, Fig. 2) on the right wall of the testing chamber through which a video camcorder (G, Fig. 2) recorded the freezing behavior testing for reanalysis. The containers (H, Fig. 2) containing either gauze or cat hair were placed against the center of the right wall of the Plexiglas chamber. The gauze filled container served as a control with no odor or allomone. The cat hair filled container held the cat hair for the experiment. A vent (I, Fig. 2) located on the right wall of the testing chamber drew out allomones and odors between sessions of testing.

The program Matlab (The Mathworks Inc., Natick, MA) was used to record the time periods for freezing and nonfreezing behavior and to create time stamps for when containers

were introduced into or removed from the testing chamber. The resulting information was stored using Microsoft Excel (Microsoft Corporation) for statistical analysis.

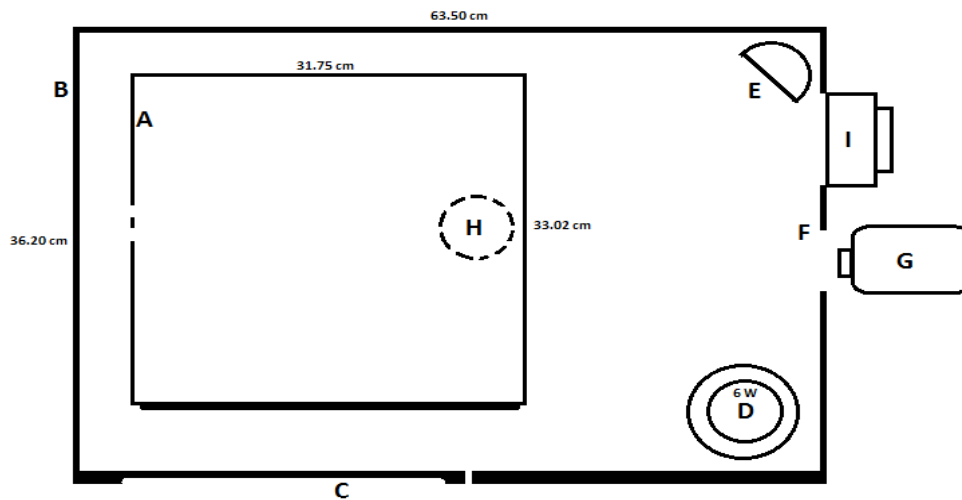


Figure 2 Freezing Behavior Chamber

A) Behavioral Chamber; B) Testing Chamber; C) Window; D) Light bulb; E) Webcam;
F) Video Camcorder opening; G) Video Camcorder; H) Gauze/Cat hair Container; I) Vent

Procedures

Medial Amygdala Lesions

Twelve 250-300 g rats had surgery performed for the purpose of this experiment; two rats had sham surgeries done to the medial amygdala, while four received actual lesions. Surgery commenced with an atropine injection (1 mL of 1 mg/mL) into the intraperitoneal cavity, which suppressed respiratory secretion. The main anesthetic sodium pentobarbital was then given to the rat (50 mg/mL, 1 mL of anesthesia per kg of rat). Once the anesthesia had taken effect, the surgical area of the head was shaved, wiped, and cleansed with 91% isopropyl alcohol. Additional anesthesia, isoflurane, was given by dipping a cotton swab into the solution and placing the cotton swab under the rat's nose for inhalation. The rat was then placed in a standard stereotaxic surgery apparatus with its head immobilized by tooth and blunted ear bars, and an incision was made from the frontal bone to the parietal bone on the mid-sagittal plane. Using bregma to plot skull coordinates (3 mm posterior to bregma, and then 3.3 mm measured on either side of bregma), a blunt tip needle was drilled through the skull to form a channel through which the microsyringe was lowered. A 0.5 mL injection of ibotenic acid (pH 7.4, 1 mg/mL) was made slowly over a 1 minute 40 second period (approximately .3 mL/minute) and 2 minutes were given for the drug to diffuse in the tissue before the microsyringe was pulled out.

Ibotenic acid is an excitotoxin that destroys cell bodies and leaves passing fibers intact. It activates glutamate receptors and essentially excites cells with these glutamate receptors to death. The activated glutamate receptors makes affected cells fire a vast amount of action potentials, causing calcium and sodium ions to flow into the cell which in turn results in the

influx of water into the cell. The cells in the medial amygdala are therefore killed, with only minimal physical damage done to the optic tract and surrounding tissues.

Sham lesions were performed in the same manner as the lesion surgeries, except that no ibotenic acid was injected into the medial amygdala of the rats' brains, (although the injector needle was lowered 7 mm into the brain). Sham lesions were made in order to rule out the possibility that changes in data were not a result of brain cell damage or surgery trauma, and were in fact a direct consequence of damage to the medial amygdala.

Rats were allowed a one-week recovery period before further experimentation to prevent surgical trauma from affecting data.

Baseline Procedure

Test rats were placed in the startle chamber, and after a 5-minute acclimation period, 41 startle stimuli pulses (75, 85, 95, and 105 dB) were given at 30 second intervals. The pulses were presented in a random order, with each intensity value emitted exactly ten times. The final pulse was disregarded for data purposes. This baseline startle was conducted over a course of two days. Each rat was placed in the same box for both the baseline and light-enhanced startle tests, in order to prevent any minor discrepancies in the environment of the two different boxes from affecting the data.

Experiment 1: Olfactory Fear Potentiated Startle

The oFPS procedure was performed by Drew University lab assistants, but was reported here to supplement the results drawn. We took the data from a previously performed oFPS test. Specimens were sorted into matched groups based on the averages of the startle response amplitudes from the third baseline session. The oFPS test was based on the procedure outlined (Cousens, Blumenthal, and Skewbecz, under review). Each rat underwent a twenty-seven minute conditioning session of six odor-shock pairings: a four second odor was released and co-terminated with a 0.5mA shock of 0.5sec. The rats were counterbalanced across boxes and session times to counterbalance and account for discrepancies.

The day after conditioning, each rat underwent a startle test session based on Paschall and Davis's "long interval" procedures. Each session began with a five minute acclimation period which was followed by 75 95dB pulses. Beginning at the 31st pulse, odor was released every fourth pulse thereafter.

Experiment 2: Light Enhanced Acoustic Startle

Each experiment run consisted of three successive sessions of sound pulses. Using the same sound intensities as those of the baseline, the first session consisted of the five minute acclimation period followed by the 41 pulses. The next session consisted of another five minute period followed by 41 pulses, and the last session went straight to the 41 pulses without the five minute period in between. For light-enhanced startle measurements, the light was turned on during the second session, and switched off in the following session, creating a

Dark>Light>Dark situation. The other half the rats received three sessions, or Dark>Dark>Dark. Dark>Light>Dark and Dark>Dark>Dark sessions ran concurrently.

To counterbalance and account for discrepancies between testing boxes and sessions, the box that was used for the Dark>Light>Dark session was switched each time. Specimens were randomly selected for one of the three testing groups: the control group without any lesions; the sham lesion group that received surgery, but no injection; and the rats with legitimate lesions. Rats were never used in the LES experiment more than once, to prevent habituation and skew data. The cages were cleaned following a complete three-part session to restore a consistent smell to the cage and to remove any fear pheromones.

Experiment 3: Effects of Predator Allomone Exposure

Freezing Behavior

The experiment testing the freezing behavior of rats exposed to cat hair (allomone) was split into six periods of five minutes each. During the periods, the behavioral chamber alternated between being empty and holding a container within. For period 1, the behavioral chamber was empty as the rat adjusted to the new environment without any exposure to odor or allomone. At the beginning of the second five-minute period, the gauze filled container was placed into the behavioral chamber to serve as a control with no odor or allomone. At the start of the third period, this container was removed from the behavioral chamber. Beginning the next period, the cat hair filled container was placed into the behavioral chamber and the allomone was allowed to spread for five minutes. At the start of the fifth period, the container was removed from the behavioral chamber. For the final five-minute period, the gauze filled container was reintroduced into behavioral chamber. After this period, the trial for the rat was complete. In summary, the periods for the experiment were empty>gauze>empty>cat hair>empty>gauze. Four rats were tested in total, with two trials conducted each day over two days.

After initially scoring the freezing behavior of the rats in this manner, the rats were re-scored using the videos from the video camcorder, which recorded the entire procedure. This allowed for assessment of reliability of the data.

Allomone Potentiated Acoustic Startle

A procedure similar to that used for testing light enhanced startle response was used to test the effect of cat hair allomones. This experiment consisted of two successive sessions of sound pulses. Each session consisted of the five-minute acclimation period followed by the 41 pulses and used the same sound intensities as those of the baseline. For cat hair startle measurements, cat allomones were introduced into the testing chamber by spreading cat hair below the startle chamber between the first and second sessions. For the control setting, nothing was introduced into the testing chamber, while it still opened. Between each pair of tested rats, the containers within which the experiment was conducted were both cleaned, so that all of the rats would encounter relatively the same conditions at the start of the trial. Each of the 8 rats experienced both the control and experimental settings. The first four rats were sham rats, while the last four were lesion rats. The results from each group were compared to find the influence of

the medial amygdala on the startle response of the lesion rats. The cages were cleaned following each complete two-part session to restore a consistent smell to the cage and remove any cat hair and fear pheromones. Also, the box into which the cat hair was introduced was alternated, to ensure that data was not skewed by any anomalies in one of the boxes.

RESULTS

Olfactory Fear-Potentiated Startle

Sham and lesion rats were tested for olfactory fear potentiated startle. The mean startle amplitude was calculated for each of the rats for the first thirty pulses with no odor, the pulses with odor, and 30s, 60s, and 90s after the odor was released.

Sham and Lesion Rats

In both groups, the rats' startle amplitude started high at the beginning of the session without odor and their amplitude slowly went down (Fig. 3). Once the odor was released, both groups' startle amplitude sharply increased. As time passed, their amplitudes lowered again.

Lesion rats begin the session with a significantly higher startle than the sham rats and over time, the lesion rats' amplitudes leveled with the sham rats. When the odor was released, the lesion rats' startle rose higher than the sham rats. Though the amplitudes decreased over time, the sham rats returned to no-odor levels while lesion rats stayed more elevated.

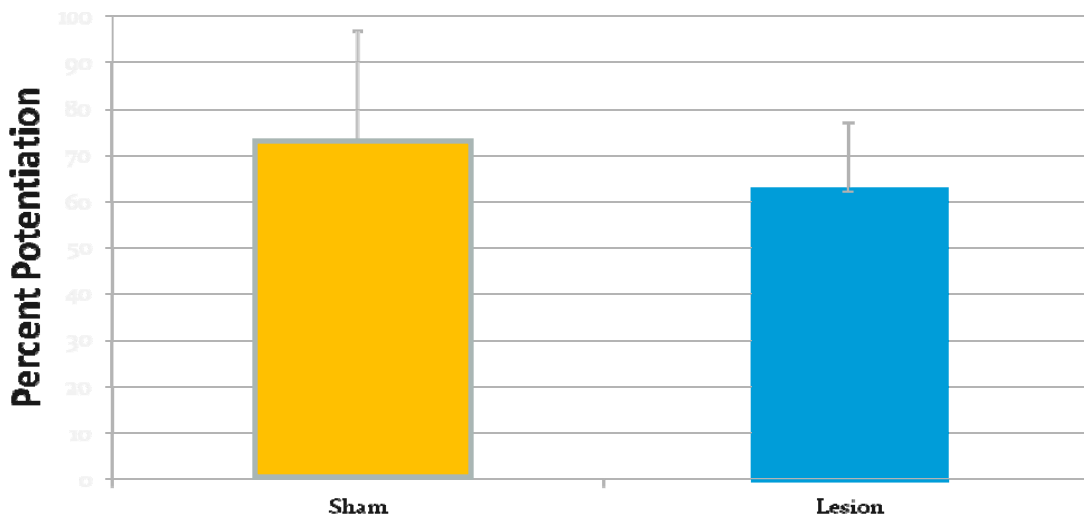


Figure 3 Sham and lesion rats underwent OFPS testing. Their mean startle amplitudes were graphed at pulses with and without odor.

Light-Enhanced Startle

Three sets of groups were used to test for light-enhanced startle; one group containing rats that had not undergone surgery, to establish the procedure, one group of rats with lesions, to

test the effect of medial amygdala lesions, and one group of rats with sham lesions, to ensure that trauma from surgery did not affect the data. For each rat, the mean startle amplitude was calculated at each of the four decibels (75, 85, 95, 105 dB). The effects of light were evaluated by calculating the percent change in startle between the first phase and the second phase of both the dark-light-dark (DLD) trial and the dark-dark-dark (DDD) trials.

Rats without Surgery

This group of subjects consisted of six rats. Four of the rats went through the DLD testing, and two went through the DDD testing. The percent change in startle amplitude between the first phase and second phase was calculated for both the DLD test and the DDD test at all four intensities. Rats exposed to the DLD testing on average demonstrated an increase in startle from the baseline dark session to the light session at all decibels. As shown in Figure 4, the rats demonstrated a 14.5%, 62%, 29%, and 31.5% increase in startle response at 75, 85, 95, and 105 dB respectively.

In contrast, rats exposed to the DDD testing demonstrated a 27.5% and 15% increase at 75 and 95 dB only (Fig. 4). These percentages were lower than those demonstrated during the DLD testing. This indicates that the presence of light did indeed induce a greater startle response, and light-enhanced startle was achieved.

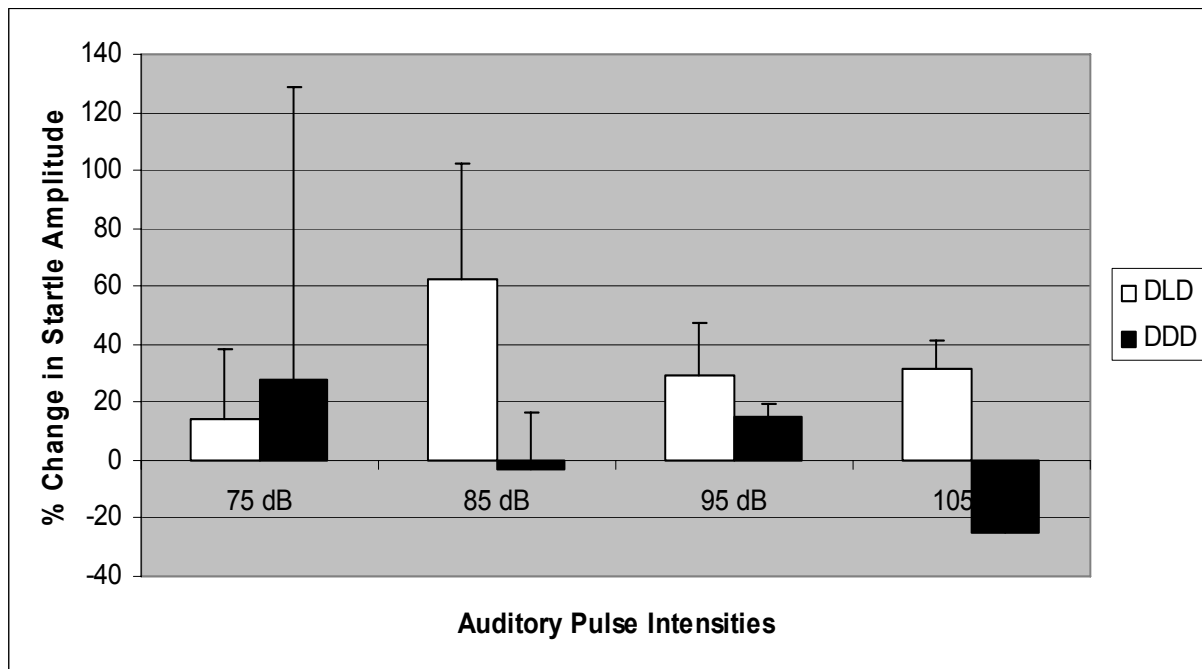


Figure 4 Light-enhanced startle in control rats were exposed to LES testing. The percent startle difference between the first and second phase of both dark-light-dark (DLD) and dark-dark-dark (DDD) sessions were graphed at each dB.

Sham and Lesion Rats

In order to make further comparisons, the possibility of a significant difference in baseline startle response between sham and lesion rats was examined. The initial startle amplitudes of the rats after being subjected to testing for the first time are shown in Figure 5. At each dB level, the data indicated that there was not a substantial difference in startle amplitude between the sham and lesion rats. The data indicates that there is not a significant difference in natural startle response between sham and lesion rats (Fig. 5).

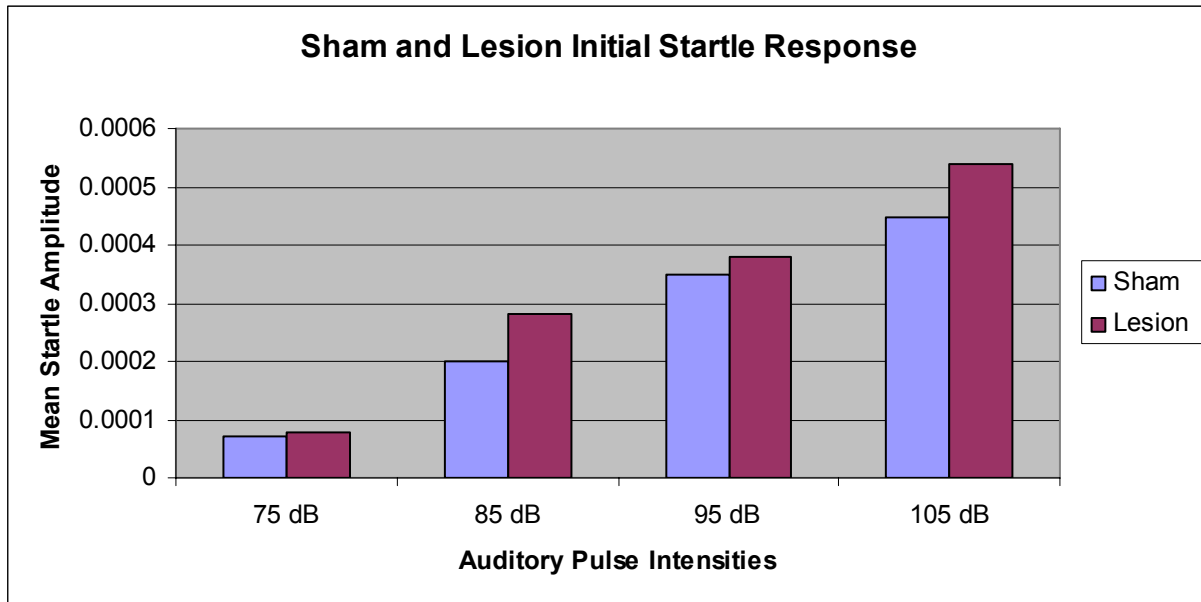
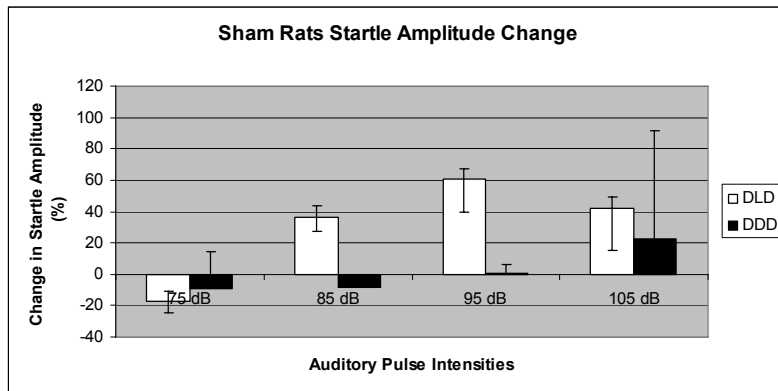


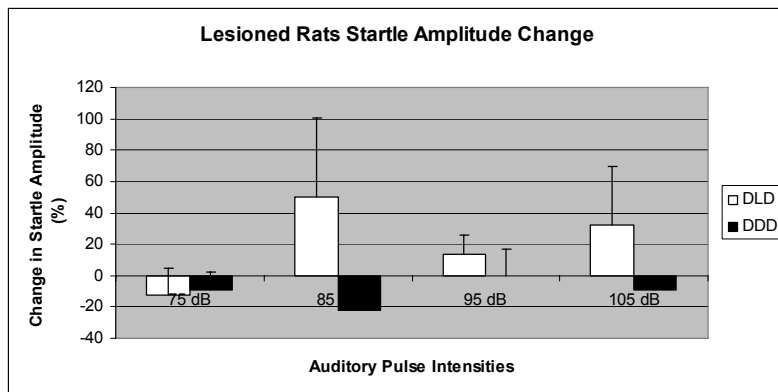
Figure 5 Discrepancies between sham and lesion rats were examined. The initial startle amplitudes of both types of rats after being subjected to testing for the first time are shown.

As shown in Figure 6A, the percent startle amplitude changes in sham rats were then calculated. As expected, there was a general increase in startle response with each increasing dB. DLD testing elicited a greater change in startle response than DDD testing, suggesting that sham rats also experienced light-enhanced startle. Because they did not receive the lesions, the sham rats were also expected to demonstrate an amount of startle similar those of the control rats, which was indicated by the fact that both types of rats exhibited a 30-60% change at 85 and 95 dB.

Differences in percent amplitude change between sham and lesion rats were considered to evaluate the effects of the lesions to the amygdala. As shown in Figure 6, the overall differences in percent amplitude change between the two types of rats were relatively minimal. A substantially lower startle response was demonstrated in the lesion rats than in the sham rats at 95 dB, however, as the response decreased from approximately 60% to approximately 14%. This decrease in percent amplitude change provides some evidence for the fact that lesions in the amygdala diminish light-enhanced startle. However, this conclusion is not substantially reinforced because of the few number of subjects used. Further tests with more subjects must be done in order to achieve more conclusive results.



Panel A



Panel B

Figure 6 Panel A shows the percent change in amplitude between the first and second phases of both the DLD and DDD sessions in sham rats. Panel B shows the percent change in amplitude between the first and second phases of both the DLD and DDD sessions in lesion rats.

After all the data was calculated, further tests were conducted in the laboratory with eight more subjects. The overall results were fairly consistent with the previous data gathered. Figure 7 shows that the percent change in amplitude for sham rats between the first dark session and the light session (D1-L2) of DLD testing was 40.9%, between the first dark session and the final dark session (D1-D3) of DLD testing was 45.1%, between the first dark session and the second dark session (D1-D2) of DDD testing was 4.4% and between the first dark session and the final dark session (D1-D3) of the DDD testing was 15.6%. In lesion rats, the percent change startle amplitude was 22.4% between the first dark session and the light session of DLD testing, 18.6% between the first dark session and the final dark session of DLD testing, -2.8% between the first dark session and the second dark session of DDD testing, and 13.9% between the first dark session and the final dark session of DDD testing.

The higher levels of startle amplitude in the sham rats during the D1-L2 stage compared to those of the lesion rats indicate that sham rats experienced increased level anxiety while the light was on. Furthermore, the startle amplitude in sham rats increased from D1-L2 to D1-D3, suggesting that the rats demonstrated sustained anxiety when the light was turned off. The startle amplitude from D1-L2 to D1-D3 in lesion rats decreased, and was generally lower than that of the sham rats, indicating that the lesion rats did not have sustained anxiety when the light was turned off. The fact that the lesion rats neither experienced the same high levels of startle

amplitude as the sham rats while the light stimulus nor experienced sustained anxiety suggests that lesions to the medial amygdale diminished light-enhanced startle.

Though it is possible that the sham rats experienced some sustained anxiety from D1-L2 to D1-D3 during the DLD testing because of the long duration of time they spent in the startle chamber, it is unlikely that this affected their startle response because the startle amplitude decreased in the lesion rats during the same sessions.

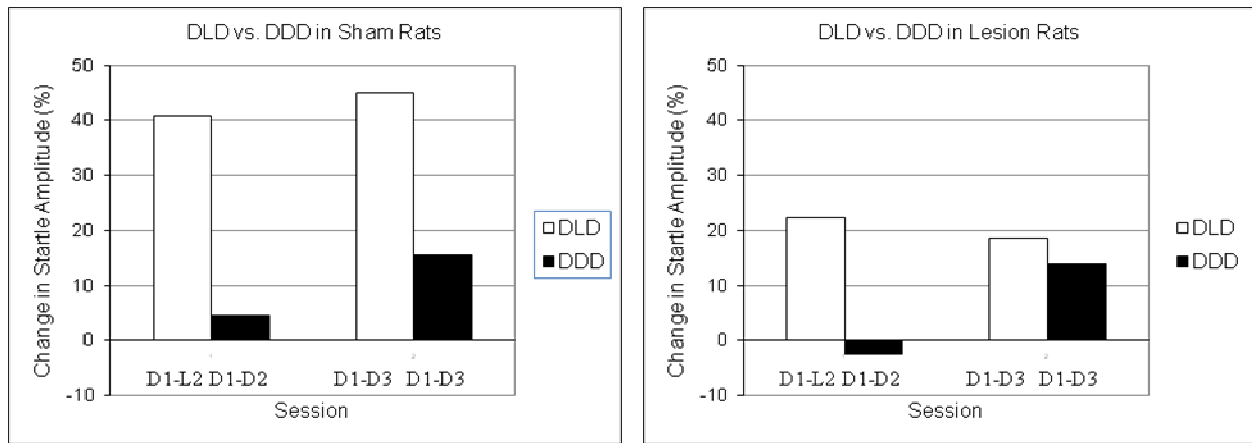


Figure 7 Percent change amplitude startle for DLD and DDD testing in both sham and lesion rats. Percent change is calculated between the first dark stage and second stage, as well as the first dark stage and last dark stage for both tests.

Allomone Enhanced Freezing Response

Data were collected solely for rats that did not undergo surgery in order to assess the presence of a cat-hair-induced freezing response and determine whether the cat hair was anxiogenic. For each rat, the amount of time spent freezing was calculated for each of the six experimental conditions as a percentage of each 300 second interval. Data were collected in this manner for four rats. Means and standard errors were then calculated using data from all four rats and a graphical representation can be seen in Figure 7.

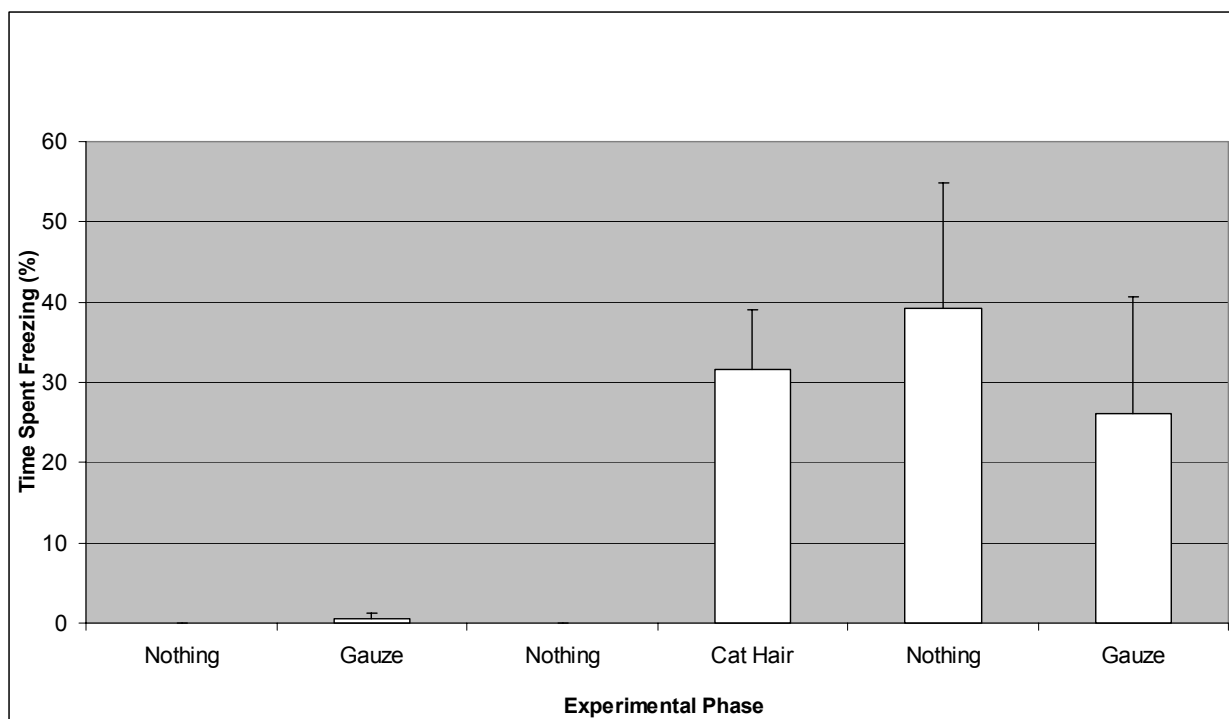


Figure 7 Mean time spent freezing of rats in each of the six conditions of the experiment

The mean percent time spent freezing in the first three experimental conditions was minimal (Fig. 7). This provides evidence that being handled by experimenters and being presented with a glass jar containing gauze did not startle the rat significantly. Therefore, it is likely that subsequent increase in freezing time was due to other experimental factors. When the cat hair was added, the freezing time of the rat dramatically increased, demonstrating its reaction to the allomones. In the final two conditions, the rat continued to freeze, indicating the continued effect of the seemingly anxiogenic cat hair. The decrease in freezing response from conditions four and five to condition six illustrates that the duration of time spent in the chamber was not responsible for the increase in freezing behavior during the last three conditions of the experiment. Because the presence of the allomones increased the freezing response of the rats, it was concluded that cat hair was anxiogenic.

Allomone Potentiated Acoustic Startle

This section consisted of the same six rats used during the light-enhanced startle experiment; four lesion rats and two sham rats. During the initial period when each pulse value was given in the absence of cat hair, the startle response measured was considered the baseline for each subject. For the experimental periods, the change in startle response in the presence of cat hair was measured as a percent change from the baseline. The mean percent change from the baseline of the sham rats to each of the four pulse intensities in the presence of cat hair remained close to zero while the mean percent change calculated from rats in the box without cat hair increased dramatically at 85 dB and steadily decreased at 95 dB and 105dB. These results suggest that cat hair does not potentiate acoustic startle in rats.

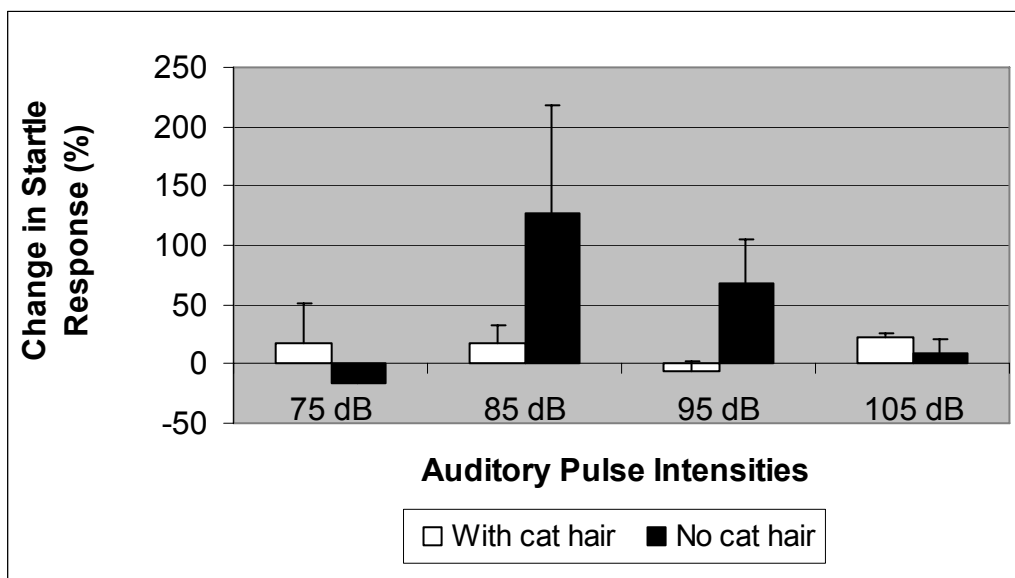


Figure 8 Mean startle response of sham rats with cat hair and without cat hair

A similar trend can be observed with the lesion rats. The lesion rats showed minimal percent change in the startle response from the baseline to the each of the four pulse intensities in the presence of cat hair (Fig. 8). The response of the rats in the empty box increased only for 85 dB and steadily decreased for the subsequent pulses.

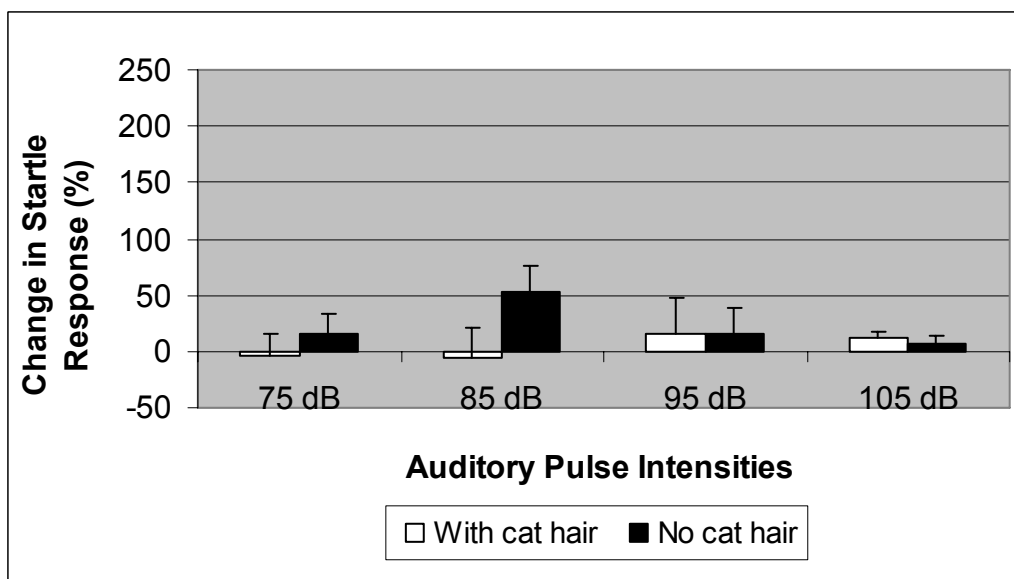


Figure 9 Mean startle response of lesion rats with cat hair and without cat hair

When comparing the mean percent change of the startle response between lesion and sham rats in the presence of the cat hair, it appears that lesion rats show a smaller percent change from the baseline than do the sham rats (Fig. 9). This holds true for each of the decibel intensities aside from the 95dB pulse. The sham rats had approximately a twenty percent increase in startle response from the baseline for each of the four pulse intensities. However, at 95dB, there was a decrease in startle response. In the lesion rats, the response to both the 75dB and 85dB pulses produced a slight decrease in startle response. However, the opposite effect was observed in response to the 95dB and 105dB pulses where slight increase was detected. Although the lesion rats show a generally smaller percent change than sham rats, the large standard error and small sample size make it difficult to draw reliable conclusions.

DISCUSSION

Olfactory Fear-Potentiated Startle

In the OFPS experiment, odor-shock pairings were used to condition the subjects to associate an initially neutral odor with the aversive stimulus of a 0.5 second shock. The startle session tested their fear by evoking startle response using white noise pulses with and without the odor present. The purpose of the olfactory fear potentiated startle procedure is to model fear since, if the odor disappears, the rats quickly returned to normal startle patterns.

It was hypothesized that lesions to the medial amygdala would decrease the startle response of the rats to the odor during testing. However, according to the data from the oFPS procedure, the lesions do not affect olfactory fear potentiated startle. According to the calculations, there was little difference between the percent potentiation of the sham rats and the lesions rats in response to the odor. These results contradicted those of Walker et. al., which stated that the startle response of the rats should decrease due to a connection between the medial amygdala and fear conditioning (5).

An independent sample t-test was done comparing sham and lesion rats by their percent potentiation. The results of the t-test showed that there was no significant difference between the two groups ($t_{11}=0.0234$, $p=0.819$).

Light-Enhanced Startle

The BNST is believed to play a critical role within anxiety-related pathways. Because the BNST receives input from the medial amygdala, it is possible that damages to the medial amygdala would decrease or even negate the amount of anxiety experienced by the brain.

LES is an unconditioned response resulting from a state of uncertainty of potential dangers. Because light causes rats to remain in a sustained state of apprehension, LES is believed to possess anxiogenic qualities (2). Thus the purpose of LES is to serve as a model for further studying anxiety-related pathways and their potential treatments. The lesion rats demonstrated a decreased startle response at 95 dB, affirming our hypothesis that medial amygdala lesions decrease the severity of the startle response. This provides some indication that the medial amygdala is indeed involved in anxiety-related pathways and that future anxiogenic treatments

should focus on targeting this area. Rats exposed to DLD testing demonstrated a greater difference in startle, indicating that LES was successful (Fig. 4).

Allomone Potentiated Startle

Rats have a natural anxiogenic response to allomones from cat hair. In the beginning of experiment 3, the increase in freezing behavior supported our hypothesis that cat hair is anxiogenic. In contrast, the results of the allomone potentiated acoustic startle refuted this theory. However, because the number of rats was small, so the conclusions drawn based on the data may be unreliable.

The objective of the Experiment 3 was to determine whether lesions to the medial amygdala would decrease the acoustic startle response of rats in the presence of cat hair. In order to rule out trauma from surgery as being responsible for any change in startle behavior, sham rats were tested as well. They displayed virtually no percent change from the baseline when exposed to cat hair. This may be explained by excessive ventilation of the startle chambers, which may have caused the concentration of allomones to decrease excessively. In addition, two of the sessions for the lesion rats were eight pulses too short, compared to the other sessions, which may have disrupted the data. Furthermore, histological examinations were not completed to assess the area of the brain that actually received the lesion during the surgery. Finally, the startle response and freezing response may be triggered by different mechanisms in the brain and/or nervous system. The pathway responsible for freezing behavior may be influenced by a large degree to allomones while the pathway responsible for the startle reflex may not be susceptible to influence by allomones.

Despite this, the startle response to cat hair was compared between the sham rats and the lesion rats. The general trend was that lesion rats exhibited a smaller change in startle response with respect to the baseline than did sham rats. However, because of the small sample size and large standard error, conclusions cannot be drawn with certainty.

There are limitations to interpreting these findings. The full effects of the lesions are unknown because we have not evaluated the extent of the lesions and whether or not the medial amygdala was indeed lesion. Histology would need to be performed to determine how effectively the lesions were created.

CONCLUSION

The purpose of this study was to determine the function of the medial amygdala in fear and anxiety related pathway of the brain. Data was ascertained through both a light-enhanced startle test that determined the acoustic startle response of rats exposed to bright light, and an allomone- potentiated startle test that determined the freezing response and acoustic startle response of rats exposed to cat hair. In addition, results from these tests were compared to data from olfactory fear-potentiated startle testing, which modeled fear-potentiated startle in rats. The findings of these tests suggest that the medial amygdala may play a role in the anxiety pathway of the brain; rats that possessed lesions to this part of the brain seemed to startle less during LES

testing, but results gathered during APS testing were not significant. Yet a larger pool of subjects and histology are required to confirm the fact that the medial amygdala does in fact play a role in eliciting anxious behavior.

REFERENCES

- (1) "The Numbers Count: Mental Disorders in America." National Institute of Mental Health. National Institute of Health, n.d. Web. 28 Jul 2010.
- (2) Davis et al. Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology*. 2009; 1-31.
- (3) Mucignat-Caretta C. The rodent accessory olfactory system. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 2010.
- (4) Lang Peter J, Bradley Margaret M, Cuthbert Bruce. 1990. Emotion, attention, and the startle reflex. *Psychological Review* 97: 377-393.
- (5) Walker D.L., Paschall G.Y., Davis M. Glutamate receptor antagonist infusions into the basolateral and medial amygdala reveal differential contributions to olfactory vs. context fear conditioning and expression. *Learning and Memory*. 2005; 12:120-129.
- (6) Paré D., Quirk G.J., Ledoux J.E. New vistas on amygdala networks in conditioned fear. *Journal of Neurophysiology*. 2004; 1-6.
- (7) Davis M, Walker D L, Lee Y. Amygdala and bed nucleus of the stria terminalis: differential roles in fear and anxiety measured with the acoustic startle reflex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 1997; 352: 1675-1687.
- (8) Dielenberg R A, Hunt G E, McGregor I S. 'When a rat smells a cat': The Distribution of FOS Immunoreactivity in Rat Brain Following Exposure to a Predatory Odor. *Neuroscience* 2001; 104 (4): 1085-1097.
- (9) Staples L G, McGregor I S, Apfelbach R, Hunt G E. Cat odor, but not trimethylthiazoline (fox odor), activates accessory olfactory and defense-related brain regions in rats. *Neuroscience* 2008; 151: 937-947.