PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOLOGY

INNATE FEAR MODELING THROUGH PREDATOR ODOR EXPOSURE IN AWAKE RODENTS – AN FMRI STUDY

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Spring 2018

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biology
with honors in Biology

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ABSTRACT

Observing and interpreting brain activity is a complicated science. Pinpointing distinct regions of the brain responsible for certain emotions is an ongoing effort. Stress and innate fear are noteworthy emotions for their implications in human health. Utilizing a unique, awake-animal fMRI paradigm, I here attempted to contribute to the current understanding of brain activity associated with innate fear. Several questions were asked relating to how innate fear activated the brain and how exposed rats would respond to innately (unlearned) fearful stimuli. An innately fearful odor (fox urine) was administered to the rats and the resulting BOLD activation was observed during imaging, while the behavioral responses were observed over a week later. Exposure to fox urine resulted in altered BOLD signals in six regions of the brain: the retrosplenial cortex, the pituitary gland, the amygdala, the hypothalamus, the bed nucleus of the stria terminalis, and the anterior cingulate cortex. Behavioral responses of rats exposed to fox urine in the so-called Elevated Plus Maze indicated trends for elevated levels of anxiety, reminiscent of human Post-Traumatic Stress Disorder (PTSD). To conclude, further experimentation with a greater sample size was deemed necessary for several future exploits. These potential further inquiries include obtaining more information about PTSD, the retrosplenial cortex, as well as remaining unanswered questions about fMRI and behavior.

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ACKNOWLEDGEMENTS

I would first like to thank all the readers of this thesis: my research advisor Dr. Nanyin Zhang, PhD, Professor of Biomedical and Electrical Engineering; my honors advisor Dr. Bernhard Lüscher, PhD, Professor of Biology and Biochemistry and Molecular Biology; and my faculty reader Dr. Yingwei Mao, PhD, Associate Professor of Biology. Following this I would like to thank all members of the Zhang lab, as I would not have been able to conduct the research necessary for this thesis without both the intellectual and monetary support of the lab. Specifically I would like to thank Pablo Perez and David Dopfel. I thank Pablo for laying down the initial groundwork for the study as well as teaching me the basic principles of many procedures. I thank David for his assistance with conducting many of the later experiments as well as for his input on the work required to complete this thesis.

Additionally I would like to acknowledge the following publishers for sharing their copy rights for the following figures included within this thesis:

Elsevier

- "Fear induced neuronal alterations in a genetic model of depression: An fMRI study on awake animals" (Figures 2, 6, and 13)
- "Brain responses to symptom provocation and trauma-related short-term memory recall in coal mining accident survivors with acute severe PTSD" (Figure 5)
- "Imaging unconditioned fear response with manganese-enhanced MRI (MEMRI)". (Figure 7)

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- A rat brain MRI template with digital stereotaxic atlas of fine anatomical delineations in Paxinos space and its automated application in voxel-wise analysis (Figure 3)

Last of all I would like to thank my family and friends for being unendingly supportive throughout the entirety of my educational career.

INTRODUCTION

Background

Blood-Oxygen-Level-Dependent Functional Magnetic Resonance Imaging, also known as BOLD fMRI, is a method to observe hemodynamic brain activity. When brain cells known as neurons interact with each other in functional networks it is possible for their interactions to be observed as both electrical signaling and resulting hemodynamic signaling. These measurements can be initiated by an electrophysiological event known as a Local Field Potential (LFP) in one region of the brain. LFPs summarily have an effect throughout adjacent segments as each individual neuron communicates with its neighbors. While the hemodynamic signals are believed to be indicative of neural activity, the observation of said hemodynamic signals may lag the observation of neuronal activity signals by up to eight seconds. This depends on the signal frequency utilized in fMRI, with a higher lag time associated with a higher gamma-range frequency (Schölvinck et al., 2010). BOLD fMRI is currently the optimal way to observe brain function, as other supplementary procedures for monitoring the brain such as EEGs are not the most efficient in terms of viewing three-dimensional space. Another option, optical imaging procedures only reveal hemodynamic activity already revealed by BOLD (Logothetis et al., 2001).

The exact relationship between electrical neuronal signals and hemodynamic signaling is currently being researched. The latter of these was previously mentioned to be the form observed in BOLD fMRI. In a 2010 study performed by Blankenburg et al., it was hypothesized that BOLD fMRI functions by interpreting both of these signals together, and derives the bulk of the image from the power disparity between these frequency components at both high and low frequencies (Kilner et al., 2010).

With this information in mind, recent experiments have begun interpreting the significance of brain activity in several locations within the brain. Activation of certain regions is thought to correspond

with different functions. Next to primitive procedures such as lobotomies (partitioning off lobes of the brain and noticing what character traits went missing) these observed "activation spikes" serve as the basis for understanding that certain regions of the brain are responsible for different actions. An example relevant to this study is a paper by Gianaros and McEwen that has observed activation in the hippocampus, amygdala, and regions of the prefrontal cortex indicating likely involvement with the conscious experience of stress (McEwen and Gianaros, 2010). In addition, recent studies such as those by Tovote et al. have described the circuitry involved in fear activation. In fact, they claim "recent evidence indicates that emotional states correspond to the functional states of defined neuronal circuits within and between various brain regions" (Tovote et al., 2015). The brain regions they specifically cite include the amygdala, the medial prefrontal cortex (mPFC), and the hippocampus.

One important factor to take into account however is that at this point in time neuroscience is still a developing scientific endeavor. The brain is a difficult organ to understand, as it interacts with every other body system be they autonomic or somatic (involuntary versus voluntary). Further experimentation is required if scientists ever hope to entirely understand the almost immeasurable amount of intricacies that are integral to neurological function. More evidence is required to fully conclude claims such as those presented by the aforementioned papers. This experiment hopes to contribute to this ever-growing pool of neurological knowledge by making use of BOLD fMRI.

The purpose of this experiment is to forcibly induce stress and fear in rats, with the goal of using BOLD fMRI to interpret how their brains respond to said stress. "Innate Fear Modeling" is the chosen terminology for this procedure, as the induced fear will stem from a species-inherent aversion to scents of a natural predator to the rat. The individual organism will never have encountered the predator before. Our experiment is being conducted in order to obtain a more robust understanding of the processes indicative of innate fear in the brain. Secondly, we wish to contribute to clarifying the connection between BOLD fMRI signaling and the interpretation of how the brain responds. There are many questions worth asking. First, what specific portions of the brain are activated by the fear stimulus? Second, are activation

pathways contained within these affected brain regions altered by fMRI? Third, when exposed to predatory odor, do the resultant variations in brain activity actually predict behavioral outcomes – in this case, apprehension and fear?

We decided that the optimal way to study these questions would be to construct and utilize an olfactometer device. This device was used in order to administer the scent of the natural predator animal, in this case a fox, to the rats within the fMRI machine. By spraying the scent of a predator into the fMRI machine and having the rat constrained inside, the animal was forcibly exposed to a stressor in an environment in which it could be observed without being able to physically react. The anxiety-like behavioral consequences of exposure to fox urine were recorded 7-10 days after exposure by means of an Elevated Plus Maze (EPM). The EPM is an anxiety test that measures the aversion of the rat to a wide-open space versus their preference towards a closed space. EPM combined with BOLD fMRI in this thesis additionally measures to what extent the exposure to fox urine affects this aversion. A fourth question, is how the rat's behavioral tendencies will be modified by the predator stress brought on by the scent of the urine, if at all?

A contentious point is the extent to which rodent brains can be compared to human brains. In defense of this experiment's conclusions with regards to humans, a study by Liang et al. postulates that through resting-state fMRI they were able to observe very clearly discernable topological features of the rat brain that were also present within the human brain (Liang et al., 2011). They make this statement based upon their observations of what they call "community structure" signifying functional connectivity in the awake resting state rat brain. Community structure is something that they state is present within the human brain, and support with evidence from other publications. By "community structure" they mean "cohesive clusters of strongly interconnected nodes" (Schwarz et al., 2008). These nodes are partitioned from the surrounding data based upon their seemingly consistent modularity. Modularity is in turn referencing the belief that certain sections of the brain have uniquely developed to maintain specific functionality in groupings known as "modules". Thus, Liang et al. have theorized that the joint presence

of such a function as “community structure” is indicative of a similarity between rat and human brain functionality. This justifies their aptness for comparison in scientific study (Liang et al., 2011).

This work aims to shed light on the intricacies of the brain and its behavioral learning methods that are not yet fully understood. Our study attempts to acquire more information about not only stress and its effect on the brain, but how the brain functions in general. This is alongside an attempt to elucidate the inner machinations of the current system used for brain activity monitoring.

Innate Fear Modeling

“Innate Fear Modeling” is a descriptive term detailing the focal point of this experiment. Innate Fear Modeling is attempting to model a voxel representation of the brain activity that accompanies innate fear. Innate fear is a specification of the common emotion, fear. Fear has been studied fairly extensively. It has been found that the amygdala is an important area of the brain involved in processing and storing information pertaining to fear as well as the expression of fear (LeDoux, 2000). Injury to the amygdala has also been shown to prevent the sensations of both learned and innate fear (Davis, 1992). This will be covered in further detail below when the concept of varying forms of “fear” is discussed.

It is possible to elaborate on the description of Innate Fear Modeling by looking towards a study by Jesuthasan and Mathuru. They found that an “alarm response” in zebrafish could be examined in order to obtain information about how organisms mentally process what is known as “innate fear” (Jesuthasan and Mathuru, 2008). Innate fear is seen as the biologically evolved sense of apprehension expressed by prey. Prey that possessed this sense of innate fear survived longer and reproduced more often than those who lacked this sense of self-preservation. In accordance with the proposed experiment, the alarm response that they studied originated from the olfactory system. Essentially, once the odor had been

picked up by the fish's olfactory system, a fearful "alarm response" was activated, causing the fish to be more excitable and aware, behaviorally demonstrating innate fear.

Interpretations of Stress

At this point in time it is apparent what broad regions of the brain activate in response to certain feelings. For example the amygdala may broadly respond to fear while the cerebellum may generally activate when coordinated movement comes into play (Allin et al., 2001). But the brain is an intricate organ that is too complicated to be completely understood by these generalizations. Stress, and a specific variation known as "predator stress", is a distinct emotion with an elaborate region of activation. While it is generally understood what sections of the brain are responsible for these emotions, there is a tremendous amount of information yet to be obtained on the more involved aspects of their interpretation.

Stress

Stress can be defined as "an event that challenges an organism and threatens to exceed available coping resources" (Liang et al., 2014). External stress is often the cause of internal biological responses. In humans and animals, such biological responses can include altered production of hormones and neurotransmitters. Examples of hormonal quantities manipulated include catecholamines, corticosteroids, and adrenocorticotropin (Axelrod and Reisine, 1984).

In rat brains, it has been shown that stress can lead to an increase in inhibitory neurotransmitters. For instance, presence of the common neural inhibitor gamma-aminobutyric acid type A (GABA) is increased in the hypothalami and frontal cerebral cortices of rats exposed to acute stress (Acosta and Rubio, 1994). Another study demonstrated that acute stress, present in rats that had undergone the swim

stress test, led to an abundance of steroids that directly correlated with increased GABAA receptor (one of two forms of GABA receptors) functionality (Purdy et al., 1991). The exact reasons for this inhibitory neurotransmitter accumulation are currently under further study. For instance as recently as 2017 a new publication has found that both inhibitory GABA and excitatory glutamate are involved in the response to stress (Houtepin et al., 2017). The inquiries of this thesis however aim to understand what parts of the brain physically respond to stress and signal for the described changes in hormones and transmitters.

As mentioned previously, the regions of the brain that have been hypothesized to have an association with interpreting stress thus far have been the hippocampus, amygdala, and prefrontal cortex (McEwen and Gianaros, 2010). This is supported in studies by both Shin et al. and Hastings et al. Hastings et al. studied the physiology behind major depressive behavior, and found that the stress accumulated from such a disorder resulted in decreased "components of the limbic-cortico-thalamic circuit"; in other words the three aforementioned regions (Hastings et al., 2004). Shin et al. found that these regions of the brain were also somehow involved Posttraumatic Stress Disorder (PTSD), and that patients who suffered from PTSD had increased amygdalar activity and decreased hippocampal and medial prefrontal cortical activity (Shin et al., 2006).

The brain regions impacted by stress are depicted in Figure 1 (Adapted from McEwen and Gianaros, 2010). The regions in the figure have been found to participate in the several cerebral functions listed adjacent to their name. Stress impacts these regions either through increased activity in terms of the amygdala or through decreased activity in terms of the prefrontal cortex and hippocampus.

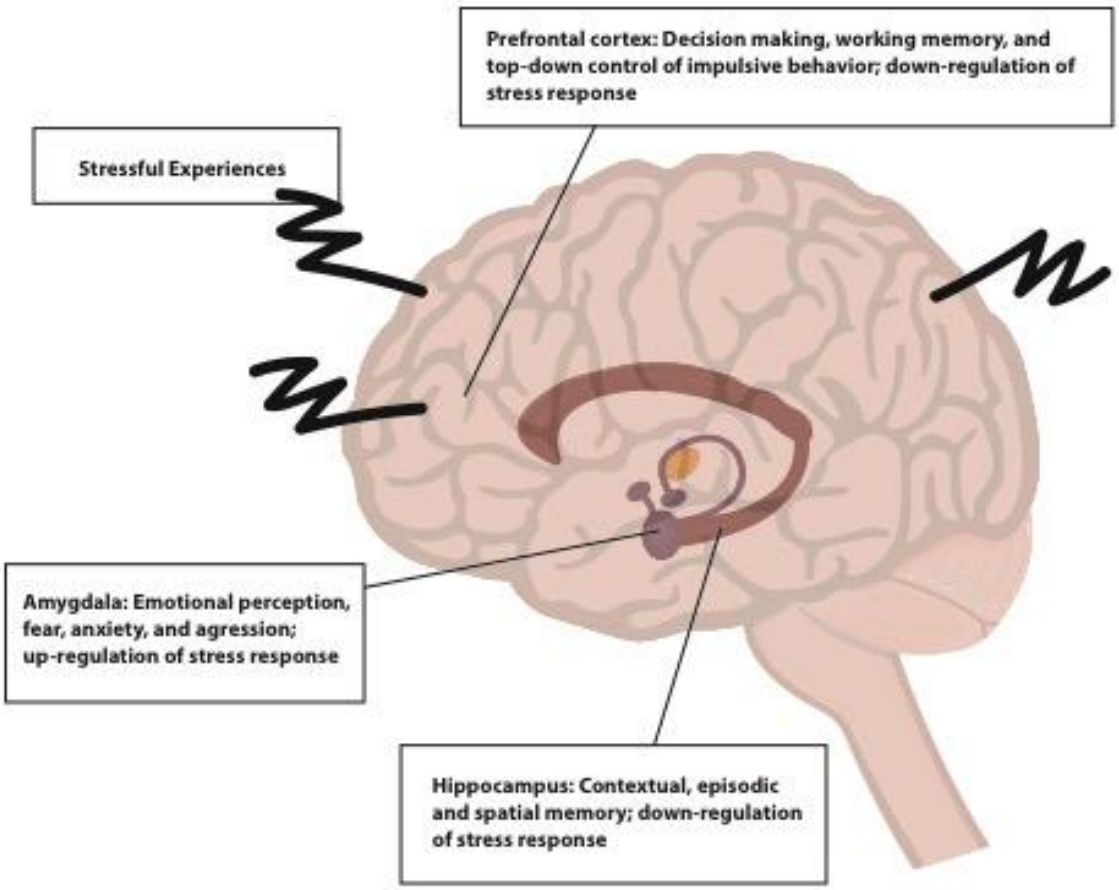

Figure 1: Regions of the Brain Integral to Stress Allostasis, Adapted From McEwen and Gianaros, 2010

As McEwen and G… go on to explain, the circuitry that exists between mPFC, amygdala and hippocampus is influenced by both inflow and outflow signals; signals not only being sent into the brain but also those being dispersed into the other systems of the body by the brain. These signals come in two forms, both neural and hormonal, which are integral in the overall process of allostasis. Allostasis is the way in which the body initially responds to stressors. McEwen describes allostasis in a separate paper as "adaptation in the face of potentially stressful challenges" that makes use of "neural, neuroendocrine, and neuroendocrine-immune mechanisms" in order to maintain homeostasis (McEwen, 2006). Homeostasis is

the process by which the body is consistently attempting to maintain its natural healthy status, and allostasis is a key portion of that process. McEwen then goes on to explain what is known as "allostatic load". Allostatic load is the process by which over stimulated allostatic systems become adjusted to the overstimulation. The systems then compensate by developing abnormal tendencies that result in disease. Allostatic load is likely the cause of anxiety and other affective disorders, a topic that will be covered shortly.

The claims made in the aforementioned figure by McEwen and Gianaros about the functions performed by the amygdala, hippocampus, and prefrontal cortex are supported by other publications. The amygdala has been found to be imperative in stress response, as well as fear and fear conditioning. The basolateral nucleus of the amygdala handles aversive learning and the central nucleus is responsible for defensive reflexes (Balleine and Killcross, 2006). A study by Corcoran and Maren supports the findings that the hippocampus is indeed involved in the downregulation of the stress response. They injected rats with a chemical known as muscimol, a $GABA_A$ receptor agonist that served to inhibit dorsal hippocampal function. This resulted in rats that did not experience fear extinction (Corcoran and Maren, 2001). Extinction is cited as "the loss of learned performance that occurs when a Pavlovian signal or an instrumental action is repeatedly presented without its reinforcer" (Bouton, 2002). For the purposes of this study, the distinct extinction type pertinent is fear extinction. Fear extinction involves losing a taught skill or reaction through learning that the fear-inducing stimulus is not representative of a real danger. If a rat were to be over-exposed to a signal/odor that cues for the eventual presence of a predator but were to never actually come into contact with said predator, the animal would begin to believe that the scent was actually not associated. To compensate, its fear would summarily subside. Similarly, it has been noted that "destruction of the ventral medial prefrontal cortex… blocks fear extinction" (Milad and Quirk, 2002). This study claims that extinction is actually the result of a new memory being formed, as opposed to a loss of preexisting memories. Thus when they destroyed the prefrontal cortex region of the brain (believed to be associated with extinction but not associated with forming the innate fear) the extinction

was prevented and the intended response to the stimuli remained. Many scientists have supported this theory, even the infamous behavioral scientist himself Ivan Pavlov in his book "Conditioned Reflexes" (Pavlov, 1927). However the methods through which the prefrontal cortex affects fear are currently fairly up in the air. It was theorized that brain-derived neurotrophic factor (BDNF) had a hand in its processes, but it was found that BDNF does not actually have an impact on fear extinction (or innate fear either), only learned fear (Choi et al., 2010). Recently however, it has been theorized that fear extinction is likely prevented by stress. Recovery from a fear-inducing traumatic event is delayed and made more difficult by the presence of stress (Maren and Holmes, 2016). Therefore, if the function of the hippocampus or PFC were to be prevented (down-regulators of stress), it could potentially lead to excess stress, and secondarily PTSD.

But simply stating that stress is a result of activity in the aforementioned regions is an oversimplification. Functional connectivity, time-dependent brain activation in different sections of the brain, is a key component of how the brain interacts as a whole (Heuvel and Pol, 2010). An integral part of the brain's makeup that contributes to functional connectivity are limbic pathways. Limbic pathways are neural circuits in the brain that deliver signals between multiple regions of the brain. Affective disorders, colloquially known as "mood disorders" such as depression and anxiety, have been determined to be caused by "limbic dysfunction and hypothalamo-pituitary-adrenocortical (HPA) axis dysregulation" (Herman et al., 2005) due to the aforementioned allostatic load. Anxiety, an affective disorder that will be discussed to a greater extent, is a result of prolonged stress and how it modifies the brain, especially in terms of behavioral responses. Herman et al. explain how stress has specifically been seen to activate the HPA, therefore leading to the dysregulation that results in affective disorders. This HPA activation is inhibited by properly functioning hippocampus and anterior cingulate cortex interference. This is because in response to stress, the human body – and other animal bodies for that matter – produces the hormone cortisol. While Herman et al. found that limbic systems specifically had a low amount of direct interaction with HPA effector neurons, they found that "hippocampal, cortical and amygdalar efferents"

communicate with the “stria terminalis, hypothalamus and brainstem”. All three of which have a connection to corticotropin releasing hormone neurons (Herman et al., 2005). Corticotropin is a hormone that stimulates the adrenal cortex to release further amounts of cortisol into the body.

A review by Frodl and O’Keane supports the information posed by Hermen et al. In the review, it summarizes that excessive stress (signaled through cortisol) causes damage to the brain. Brain structural damage that results in affective disorders like major depression often occur in the HPA axis as well as the hippocampus (Frodl and O’Keane, 2012). The reasoning behind this structural modification is known as the “glucocorticoid cascade hypothesis”. This hypothesis theorizes that prolonged stress diminishes the portion of the brain responsible for inhibiting glucocorticoid release. This is a result of an over-exposure of glucocorticoids. So the presence of glucocorticoids ultimately leads to more glucocorticoids in a feed-forward cascade (Sapolsky et al., 2002). This is dangerous and will promote further symptoms of affective disorders.

Therefore, stress may have a wider range of effect on the brain than can be easily predicted. This is because the hippocampus, amygdala, and prefrontal cortex have been shown as “activated” or “deactivated” in response to stress. However there are other important regions of the brain that activate or deactivate as a result of producing the stress response and influence how that response is perceived (hypothalamus, brainstem, anterior cingulate cortex, HPA axis, etc.).

Further complicating scientific understanding of how the brain interacts, and returning to the limbic pathways discussed above, a study by Adamec et al. in 2005 suggested that stress induced changes in behavior were a result of “long term potentiation-like changes” in limbic pathways of the right hemisphere (Adamec et al., 2005). They found that what they called, “long lasting potentiation” (synaptic connections that stayed strong resulting in sturdy memories, shortened to LLP) that would result in anxiety, was present in limbic pathways that traveled through the right ventral angular bundle (VAB) synapsing in the basolateral amygdala (BLA). This pathway was described as an excitatory pathway that played a role in “contextual fear conditioning” (Adamec et al., 2005). In addition they also found that

there was a defensive behavioral response that resulted from stress promoting LLP in a pathway travelling from the amygdala to the lateral periaqueductal gray (LPAG). Their findings suggested that the activity in this pathway was increased and formed an LLP that lasted significantly longer in the right hemisphere of the rat brains tested in response to predator stress. Their conclusion was that the central amygdala and this right hemispheric pathway to the LPAG are involved in defensiveness and anxiety as a result of stress.

Based on this information, we hypothesize that the regions of the brain that are activated by the forced exposure to a stressor are a combination of two groupings. One being the regions of the brain that respond to stress initially, and another being the regions of the brain that activate as a result of this response. The regions that respond initially include the hippocampus, amygdala, and prefrontal cortex. The hypothalamus, brainstem, HPA axis, and anterior cingulate cortex are part of the post-response activation. We also hypothesize that the limbic pathways through the right hemisphere discussed in the previous paragraph would be quite difficult to see in this experiment. This is because the data that Adamec et al. acquired was neuronal. As mentioned, electrical synaptic signaling is not the form of signaling observed in BOLD fMRI; rather hemodynamic signaling is. These signals do work in tandem, with hemodynamic signaling often appearing up to eight seconds after the electrical neuronal signaling (Schölvinck et al., 2010). There is a strong possibility that an electrical pathway so minute would be near imperceptible hemodynamically. So while there is merit in commenting on the presence of such signaling, it will likely not have any impact on the results found in this experiment.

Predator Stress

An important distinction worth mentioning is the differentiation between interpreting stress in general and interpreting stress as a product of exposure to predatory odors. Stress as a result of predatory influence on prey is known specifically as predator stress. Predator stress often leads to behavioral responses, measured by experiments such as the aforementioned Elevated Plus Maze (EPM). The

hypothesis proposed in our experiment is that a more anxious and apprehensive behavioral response will be seen in rats exposed to prolonged predator stress. Predator odor exposed rats that are placed in the EPM will likely spend a greater amount of time hiding within the confines of the closed-arm section of the maze, where rodents tend to feel safer and less explorative. This hypothesis is based upon a common agreement interspersed throughout predator stress literature that gained some traction from a 1993 study performed by Adamec and Shallow. Another paper in support of this hypothesis' validity is a review of several anxiety experiments where EPM was integral to the overall conclusion. Despite some discrepancies EPM is cited as "one of the most commonly used animal models of anxiety" (Hogg, 1996). Adamec and Shallow utilized EPM to test behavioral responses of rats who had been exposed to predatory cats for long periods with no method for the rats to escape or to avoid the predator. They tested the rats with varying time between exposure and EPM, and found that from anywhere between one to twenty-one days, the behavioral anxiety effect produced from exposure to the cat would be apparent (Adamec and Shallow, 1993). This study went on to theorize that the anxiety response displayed by rats that underwent predator stress was seemingly analogous to the human disorder known as PTSD. Their findings when compared to similar studies performed on PTSD patients stated that in both cases, "exposure to a potentially life-threatening event leads to generalized anxiety" (Adamec and Shallow 1993). As mentioned previously it has been found that the amygdala is extremely important in fear expression, but it is also implicit in retaining memories of fearful events (McGaugh, 2004). PTSD and affective disorders are based upon the fact that the amygdala interprets a jarring event and the brain latches onto that experience. Providing further evidence from humans, a study conducted on people who had experienced PTSD from their time in the Vietnam War found that their brains displayed increased regional cerebral blood flow to the right amygdala and less blood flow to the medial frontal gyrus (Shin et al., 2004). But specifically relevant to this thesis is another study by Yehuda and LeDoux that comments on the evidence for the translational network of functionality that results in PTSD. "Translational" is referring to the notion that similar pathways and responses occur in both rats and humans (Yehuda and

LeDoux, 2007). Therefore our experiment makes sense in promoting future inquiries about human brain health and performance. Interestingly, the Yehuda and LeDoux study explains the traumatically stressful event in psychological terms of an unconditioned stimulus (trauma) that provokes an unconditioned response (distress), leading to a change in mental state. The change in status primes the brain to avoid newly conditioned cues that revive the sensations of the trauma (posttraumatic stress). This is analogous to what occurs in fear conditioning of rats, a method that has been shown to display measurably similar neurological responses to that of humans.

If behavioral information obtained from rats can in fact be compared to humans in an attempt to understand tragedy-agitated human behavioral idiosyncrasies, the relevancy and usefulness of this proposed thesis study using fox urine is even more apparent. This thesis aims to not only contribute to an understanding of brain activity but also an understanding of how organisms behaviorally respond to manipulations in stress.

Acclimation

Acclimation of rats prior to fMRI exposure is essential to performing adequate studies of neurological activity, as it "lowers stress-induced physiological indices" (King et al., 2005). This is especially true with regards to stress. It is paramount to ensure that the rats are not stressed prior to or during the study from solely being placed within the confines of the fMRI machine itself. The fMRI machine is a dark and claustrophobic space in which the rats are constrained within a body-tube structure, preventing their movement as loud cacophonous sounds reverberate around them. The purpose of the immobilizing body-tube structure is to ensure that motion is minimized, a prerequisite for proper MR imaging. This is problematic as immobilization is also a cause of duress for most animals. If an organism were to be exposed to an MRI machine without any previous experience, between the stress evoked by the lack of motion and the din of the radiofrequency coils, it would be impossible to observe brain activity

that resulted solely from predator stress. In addition, there would be no intervals alternating between stressful and resting periods, an integral component of observing stress by highlighting disparities between the two statuses. The animal would be agitated for every moment it was confined within the machine and there would be no resting state brain activity to compare the monitored periods of stress to. Ergo without acclimation there does not currently exist a method for observing stress and innate fear through awake resting state BOLD activation. According to Tenney et al., acclimation can serve to reduce several rat reactions known to interfere with fMRI imaging including "body temperature, motor movements, heart rate, and plasma corticosterone levels" (Tenney et al., 2004). King et al. also found that respiration rate and heart rate declined down to normal levels by the third acclimation session (King et al., 2005).

Interpretations of Scent

In a similar 2011 study by Huang et al., a basis for what could constitute as a rat neutral scent was laid out. Their study aimed to perform innate fear modeling on two strains of rats, Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL). The first, FSL, represented a rodent model of depression. They evoked innate fear through a predator odor known as trimethylthiazone (TMT) (Huang et al., 2011). Much like our study, it was necessary for the Huang et al. study to find a control scent to be compared to the predator odor. This is so that the regions of the brain undergoing changes in their BOLD activation can be described as undergoing that change due to fear and not to olfactory activation. Their findings corroborated with previous experiments to state that lemon was a neutral scent that rats could indeed smell. They noticed several regions that underwent a BOLD signal change in both strains of rats that they were testing. Another experiment by Harmon et al. used lemon as a neutral odor in conditioning rats to prefer a stimulus associated by scent (Harmon et al., 2009). Along the same lines, Paredes-Ramos et al.

used the neutral lemon scent as well as a neutral almond scent to condition sexual partner preferences. The preference was based on the scent conditioning that accompanied juvenile play with potential mates, despite both scents being neutral (Paredes-Ramos et al., 2011). Returning to the Huang et al. study, it concluded that there was no significant BOLD activation change in regions of the brain associated with fear for the rats undergoing the exposure to the lemon scent alone. As far as what regions of the brain actually responded to scent, the paper included a helpful figure (Figure 2), claiming that these regions were activated and summarily examined: olfactory bulb, prefrontal cortex, insular cortex, cortical nucleus of amygdala, basolateral nucleus of amygdala, central nucleus of amygdala, bed nucleus of the stria terminalis (BNST), and thalamus.

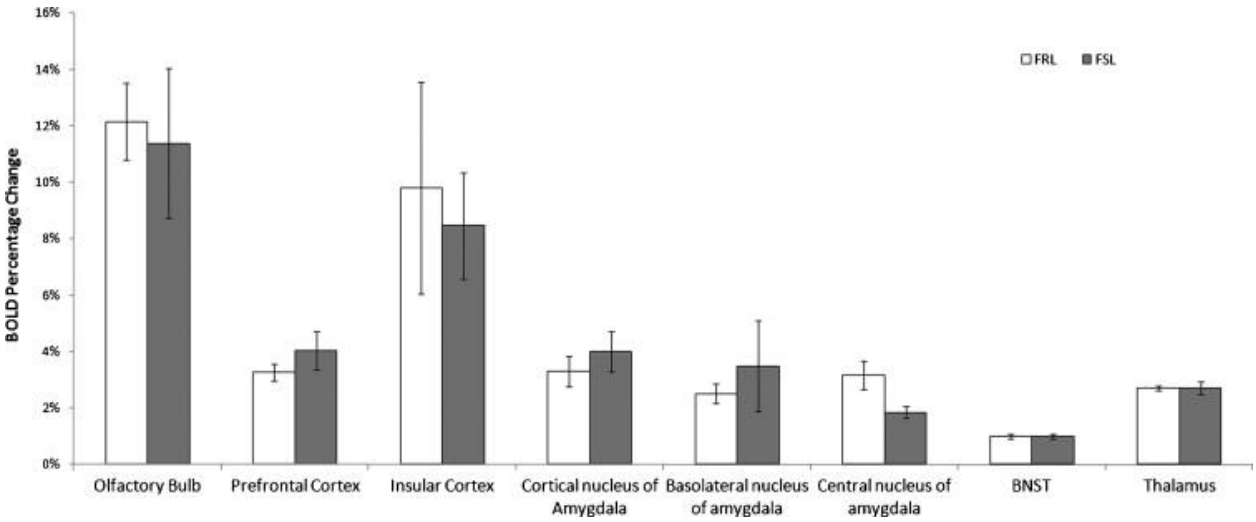

Figure 2: Regions of the Brain Activated by Olfactory Observation of Neutral Lemon Odor, Taken From Huang et al., 2011

Visualizing the Brain

In order to simply and swiftly analyze the data collected, an understanding of how the brain appears in BOLD fMRI is beneficial. This section has been constructed to compare previously found

anatomical and BOLD imaging data to the data retrieved by this study. This is in an effort to demonstrate that the conclusions made by this thesis are valid and supported by previous studies.

Interpretive figures below (Figures 3 and 4) demonstrate common regions of interest in the rat brain for interpretation of this thesis' results.

Figure 3: Regions of Interest in the Rat Brain, Taken From Nie et al., 2013

Index in Figure 5	Structural name	Abbreviation	$Index_L$[a]	$Index_R$[a]
1	Retrosplenial granular cortex, b region	RSGb	178	177
2	Retrosplenial granular cortex, c region	RSGc	156	155
3	Retrosplenial dysgranular cortex	RSD	158	157
4	Senondary visual cortex, mediomedial area	V2MM	184	183
5	Senondary visual cortex, mediolateral area	V2ML	186	185
6	Parietal cortex, posterior area, dorsal part	PtPD	180	179
7	Parietal cortex, posterior area, rostral part	PtPR	190	189
8	Primary somatosensory cortex, barrel field	S1BF	150	149
9	Primary somatosensory cortex	S1	182	181
10	Secondary auditory cortex, dorsal area	AuD	166	165
11	Primary auditory cortex	Au1	168	167
12	Secondary auditory cortex, ventral area	AuV	170	169
13	Ectorhinal cortex	Ect	174	173
14	Perirhinal cortex	PRh	176	175
15	Lateral ectorhinal cortex	LEnt	200	199
16	Piriform cortex	Pir	78	77
17	Dorsal endopiriform nucleus	DEn	94	93
18	Amygdalopiriform transition area	APir	208	207
19	Basolateral amygdaloid nucleus, posterior part	BLP	490	489
20	Amygdalohippocampal area, posterolateral	AHiPL	570	569
21	Amygdalohippocampal area, posteromedial	AHiPM	572	571
22	Posterolateral cortical amygdaloid nucleus	PLCo	686	685
23	Posteromedial cortical amygdaloid nucleus	PMCo	688	687
24	Corpus callosum	cc	930	929
	Cingulum	cg		
	Deep cerebral white matter	dcw		
25	Lateral ventricle	LV	934	933
26	Field CA1 of the hippocampus	CA1	870	869
	Field CA2 of the hippocampus	CA2		
	Field CA3 of the hippocampus	CA3		
	Lacunosum moleculare layer of the hippocampus	LMol		
	Oriens layer of the hippocampus	Or		
	Pyramidal cell layer of the hippocampus	Py		
	Stratum lucidum of the hippocampus	SLu		
27	Granular layer of the dentate gyrus	GrDG	982	981
	Polymorph layer of the dentate gyrus	PoDG		
28	Dorsal hippocampal commissure	dhc	1400	1399
	Alveus of the hippocampus	alv		
29	Molecular layer of the dentate gyrus	MoDG	100	99
30	Fimbria of the hippocampus	fi	956	955
31	Lat amygdaloid nucleus	La	976	975
32	Dorsal fornix	df	1078	1077
33	Dorsal 3rd ventricle	D3V	934	933
34	Medial habenular nucleus	MHb	550	549
35	Stria medullaris of the thalamus	sm	605	604
36	Lateral habenular nucleus, medial part	LHbM	552	551
	Lateral habenular nucleus, lateral part	LHbL		
37	Paraventricular thalamic nucleus	PV	2170	2169
38	Centrolateral thalamic nucleus	CL	932	931
39	Lateral posterior thalamic nucleus, mediorostral part	LPMR	556	555
40	Lateral posterior thalamic nucleus, laterorostral part	LPLR	558	557
41	Intramedullary thalamic area	IMA	960	959
42	Dorsal lateral geniculate nucleus	DLG	560	559
43	Intergeniculate leaf	IGL	978	977
44	Ventral lateral geniculate nucleus	VLG	778	777
45	Superior thalamic radiation	str	770	769

46	Optic tract	opt	722	721
47	Subgeniculate nucleus	SubG	952	951
48	Ventral posteromedial thalamic nucleus	VPM	668	667
	Ventral posterolateral thalamic nucleus	VPL		
49	Posterior thalamic nuclear group	Po	554	553
50	Parafascicular thalamic nucleus	PF	546	545
51	Fasciculus retroflexus	fr	548	547
52	Precommissural nucleus	PrC	758	757
53	Periventricular gray	PVG	974	973
54	3rd ventricle	3V	2086	2085
55	A11dopamine cells	A11	452	451
56	Subparafascicular thalamic nucleus	SPF	680	679
	Subparafascicular thalamic nucleus, parvicellular part	SPFPC		
57	Ventral posterior nucleus of the thalamus, parvicellular part	VPPC	682	681
58	Medial lemniscus	ml	866	865
59	Prerubral field	PR	514	513
60	Nucleus of the fields of Forel	F	678	677
61	Zona incerta, dorsal part	ZID	666	665
	Zona incerta, ventral part	ZIV		
62	Subthalamic nucleus	STh	676	675
63	Internal capsule	ic	954	953
	Cerebral peduncle	cp		
64	Posterior hypothalamic nucleus	PH	544	543
65	Gemini hypothalamic nucleus	Gem	874	873
66	Submammillothalamic nucleus	SMT	872	871
67	Supramammillary nucleus, medial part	SuMM	762	761
	Supramammillary nucleus, lateral part	SuML		
68	Mammillothalamic tract	mt	454	453
69	Fornix	f	582	581
70	Lateral mammillary nucleus	LM	876	875
71	Medial mammillary nucleus, medial part	MM	772	771
	Medial mammillary nucleus, lateral part	ML		
72	Medial mammillary nucleus, median part	MnM	970	969
73	Mammillary recess of the 3rd ventricle	MRe	972	971
74	Arcuate hypothalamic nucleus, medial posterior part	ArcMP	512	511
	Arcuate hypothalamic nucleus, lateroposterior part	ArcLP		

Figure 4: Regions of Interest in the Rat Brain Indices, Taken From Nie et al., 2013

However in addition to solely imaging the brain this thesis study images the brain through BOLD fMRI. BOLD fMRI has a very distinct appearance all its own due to how it displays the blood flow activation in a color scale. This color scale ranges from yellow (low activity) to red (high activity) (Clare, 1997).

Figure 5 by Hou et al. has used various orientations of the human brains of PTSD patients to point out approximate locations of specific anatomical segments. Each person, and rat for that matter, does have slightly different anatomy however, and so these images will always only be approximations.

But understanding where to look facilitates easily pinpointing regions of interest. Table 1 has been constructed to efficiently detail the key regions specified in each image sector (Hou et al, 2007).

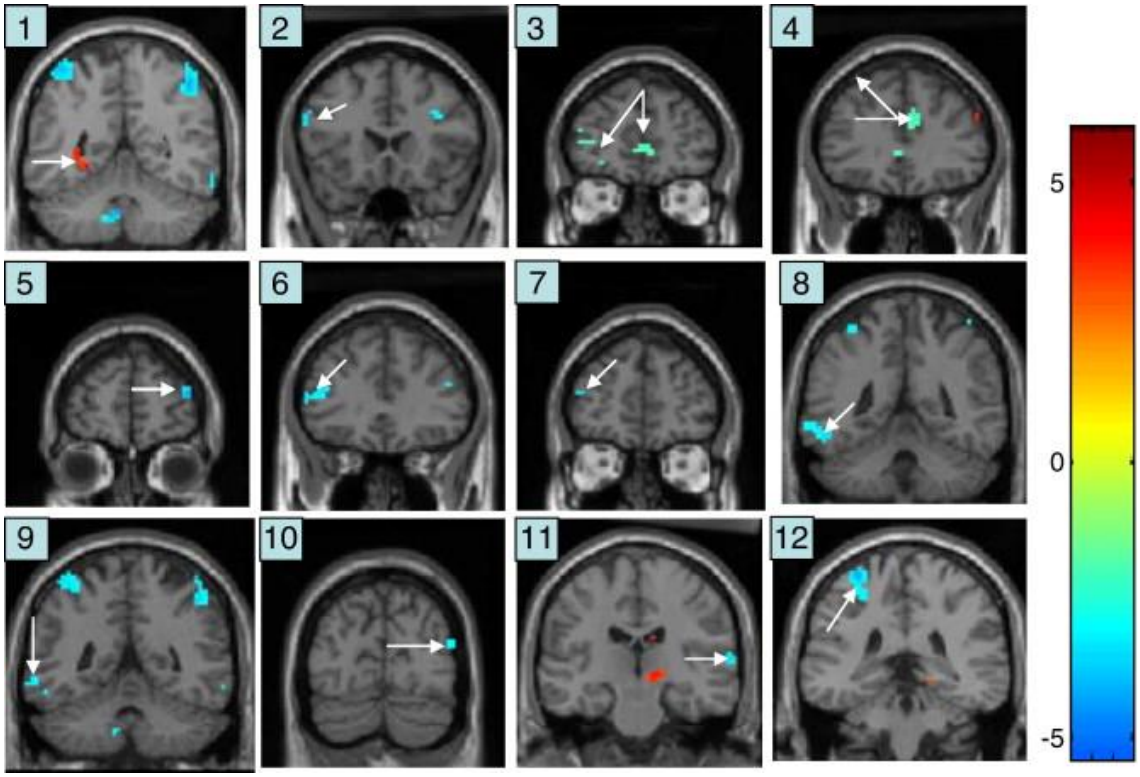

Figure 5: BOLD fMRI Activation Regions of Interest in PTSD Patients, Taken From Hou et al., 2007

Table 1: Hou et al. BOLD fMRI Activation Regions of Interest in PTSD Patients Interpretation

Human Brain Anatomy	
Number	Anatomical Structure
1	Left Parahippocampal Gyrus
2	Left Inferior Frontal Gyrus
3	Right Anterior Cingulate
4	Right Cingulate Gyrus
5	Right Middle Frontal Gyrus
6	Left Middle Frontal Gyrus (1)
7	Left Middle Frontal Gyrus (2)
8	Left Inferior Temporal Gyrus
9	Left Middle Temporal Gyrus
10	Right Middle Temporal Gyrus
11	Right Superior Temporal Gyrus
12	Left Postcentral Gyrus

Returning to the paper by Huang et al., Figure 6 displayed below elaborates upon how regions of the brain relevant to this thesis specifically appear in BOLD fMRI. These images are especially useful in that they show activation as a result of the aforementioned TMT predator in FSL and FRL rats. The designers have helpfully placed both coronal and axial views of the BOLD activated rat brain in addition to sagittal and axial three-dimensional structural images for ease of understanding. The key regions of interest including the amygdala and prefrontal cortex are also labeled (Huang et al., 2011).

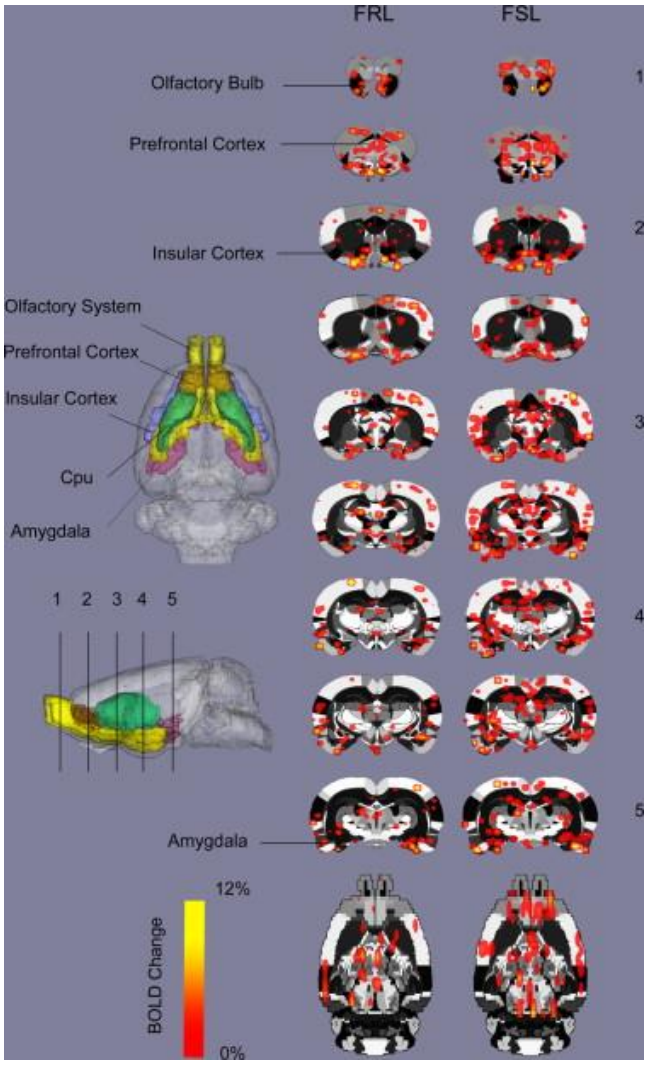

Figure 6: BOLD Percent Brain Activation Maps, Taken From Huang et al., 2011 Pathways of note include the amygdala and the prefrontal cortex for their heavy involvement in stress and fear processing.

A final study to help understand brain visualization is a study by Chen et al., which looked at imaging unconditioned fear response. Their study was very similar to this thesis', and employed lemon control scent and fox scent to test innate fear. However it differed in that the scents were administered prior to the animals being placed inside of the MRI machine, whereas our experiment exposed the rats to the odors while inside of the machine thanks to its unique olfactometer paradigm. Their paper's overall

conclusion was that “threatening/fearful olfactory scent elicited more activation in the amygdala and hypothalamus” (Chen et al., 2007), but they additionally put together graphs that can assist with mapping where key regions will appear on the result data. Their figure for fox scent activated rat brains is displayed below as Figure 7. Locations of the prefrontal cortex, caudate putamen, cingulate cortex, hippocampus, hypothalamus, and thalamus are clearly defined.

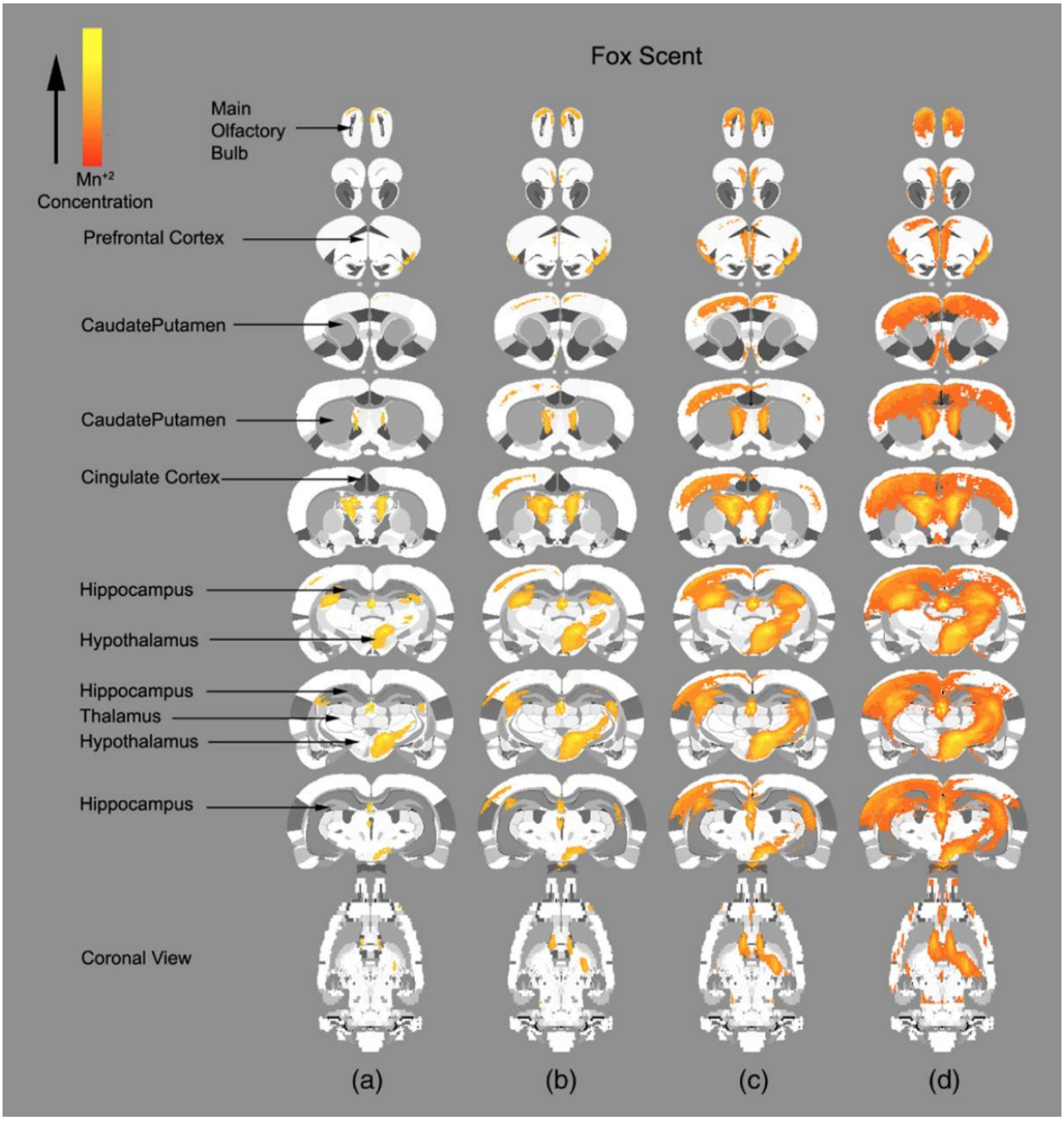

Figure 7: Fox Scent Brain Activation Map, Taken from Chen et al., 2007

Fear Neural Circuitry

The review by Tovote et al (2015) that was briefly mentioned in the introduction touched upon a difference between "regions of activation" and "activation between regions". The authors stated that emotional states are reliant upon signaling that occurs between distinct regions of activation. Anxiety and fear are the two emotional states they specifically mention. Fear is an emotion that is understood to a greater extent because fear is based on a distinct fearful stimulus. Anxiety is more complicated because a defined source or stimulus is often absent. Anxiety may be a result of an ill-defined event that has not yet occurred, but may be "anticipated" because of previous experiences (Tovote et al. 2015).

Their review goes on to explain what is currently understood about the emotional neural circuitry. They claim that amygdalar circuits handle conditioned fear – fear that has been learned due to previous experiences. They elaborate that cortical and thalamic centers send sensory information to the amygdala, and then the central nuclei of the amygdala (CEA) sends projections to the hypothalamus and brainstem in order to enact fearful behavior.

Their review also states that fear learning, important in forming conditioned anxious tendencies, is handled by disinhibitory microcircuits affecting the mPFC. Disinhibition is described as inhibiting an inhibitor, which leads to activation (Karnani et al., 2016). Tovote et al. surmised that output cells in the mPFC are inhibited by parvalbumin-expressing (PV) interneurons, which are in turn inhibited by conditioned stimulus (CS) induced excitation of presynaptic inhibitory neurons (Tovote et al., 2015). Therefore, the output mPFC cells integral to fear learning and anxiety are disinhibited by CS excitation. Organisms learn to respond fearfully to a stimulus and circuitry present in the mPFC responds accordingly. Fear conditioning has been seen to not only involve the mPFC but also the amygdala, thalamus, and hippocampus. Interestingly, Tovote et al. noted that there is even a specific type of oscillatory neuronal activity frequency involved in fear learning known as theta rhythms. Theta rhythms

have a frequency of about 4-10Hz and mediate reciprocal interactions between the mPFC and the basolateral amygdala (BA).

Functional Magnetic Resonance Imaging

An understanding of the various forms of Magnetic Resonance Imaging (MRI) helps to better comprehend the results of this experiment. Functional MRI (fMRI) is a method for imaging the composition of organisms by utilizing magnetic fields, with a greater focus on brain activity, ergo functionality. However, the notion that MRI represents brain function is not a direct outcome of MR itself. The MR signal is a function of blood circulation and oxygen levels, which themselves are thought to be affected by (or to affect) brain function. Blood with a high concentration of oxygen has a more defined MR signal as it possesses a different magnetic field compared to de-oxygenated blood. This is a result of hemoglobin and its transitional structure, as the shape of hemoglobin changes depending on whether it is bound by oxygen or CO2. Therefore, fMRI is thought to report neuronal activity indirectly as a change in blood flow and blood oxygenation that is coupled to neural activation. When the brain is involved in a thought process the local cerebral blood flow changes, and the corresponding magnetic difference between varying oxygen-bound states of hemoglobin is then observable by fMRI.

MRI in general functions through the analysis of hydrogen atoms. When hydrogen atoms in the tissue (of which there are many due to the significant amount of water in human tissue) undergo nuclear magnetic resonance (NMR), their atomic nuclei align with the primary magnetic field generated by MRI technology. This primary magnetic field is known as B0. A net magnetic moment is then created with the overall low-energy parallel positioning of the hydrogen nuclei and proton pointing in the same direction as B0. This moment is excited periodically by radiofrequency (RF) signals emanated by RF coils from the inside of the MRI machine. These RF signals are defined as B1, the secondary magnetic field. The RF

signals cause the hydrogen atoms to momentarily absorb excess energy, allowing them to transition away from their parallel low energy state. It is this higher energy state that can be observed, and the same RF coils that produced the RF pulse will notice the change (*UCSDSOM Center for Functional MRI*). Tissue can then be observed based on two variations of what is known as "relaxation time". T1 is known as longitudinal relaxation time, and describes how long it takes excited protons to reposition their alignment to that of B0. T2 is known as transverse relaxation time, and measures the length of time that is required for the protons to go out of phase with the other nuclei around them aligned with B0 (Preston, 2006).

Blood-Oxygen-Level-Dependent fMRI

A more in-depth understanding of BOLD fMRI is also necessary to comprehend our results. As mentioned above, varying changes in frequency can be presented as BOLD signals that allow for interpretation of activity. Hutchison et al. explains that the rodent brain "is organized into functional networks that can be studied through examination of synchronized low-frequency spontaneous fluctuations (LFFs) of the functional magnetic resonance imaging BOLD signal" (Hutchison et al., 2010). Imaging these functional networks however proves to be challenging. This is because standard fMRI imaging uses specific "regions of interest" to parse out extraneous data. This selectively focuses the image on a very literal region that the examiner is interested in. The article goes on to explain that BOLD fMRI images the entirety of the brain without needing to hypothesize about a prioritized region. It does this through a procedure known as Independent Component Analysis (ICA). ICA is a computational method responsible for defining and allocating subcomponents of a signal that is composed from multiple observable variables. In this case, the "multivariate signal" is brain activity, and the subcomponents are the numerous independent hemodynamic interactions in the brain. ICA functions by a special method known as blind source separation. It is only possible to perform this separation of information based on their undefined sources if the ICA is processing data of a non-Gaussian distribution. The central limit

theorem explains why this reliance on non-Guassian distribution – also known as non-normal distribution – is necessary. The central limit theorem postulates that if you linearly combine random variables that have a normal Gaussian distribution, the newly formed distribution will be more Gaussian by comparison (*Wolfram Mathworld*). Thus it is important for studies using ICA to incorporate the blind source separation on non-Gaussian data, as ICA is not used to search for a normal distribution of signals. ICA is used to look at unbiased raw data of the aforementioned multivariate signals. Therefore, these signals cannot be related to each other and must be statistically independent (ergo, non-Gaussian). Otherwise there would be significant noise or information overlap, and individual regions of interest could not be derived and analyzed. Since brain activity does not emulate a normal distribution, it can cooperate with the processing performed in ICA, and BOLD fMRI can collect the desired information.

Task fMRI

BOLD fMRI is a type of task fMRI. Task fMRI refers to the idea that "activity is larger in a cortical area when subjects are performing a task relevant to that area" (Buracas et al., 2005). Activation levels may differ depending upon the total strength of signaling required or conditioned, as a response to a certain stimulus. This is in contrast to resting state fMRI (rsfMRI). rsfMRI is fMRI performed while the subject is in what is known as a resting state, and is not performing any sort of cerebral task. If no task is being completed, then the brain is being monitored in its resting state. In humans this often means that subjects undergoing rsfMRI will be asked to close their eyes and relax their mind. Such a request is not possible in rats. However if a rat can be acclimated to the conditions inside of an MRI machine, then there should be minimal extraneous activity to interfere with the data collection. rsfMRI attempts to measure functional connectivity. As mentioned previously, a procedure to study functional connectivity through the observation of community structure and modularity was proposed by Liang et al. (Liang et al., 2011). Similar ideas are integral in task based fMRI as well, but with the addition of a specific observable action.

While rsfMRI is useful, what is unique about the awake-state paradigm of this thesis is that brain functionality is not the same when asleep as when awake. This is supported by Liang et al., as well as a study by Horovitz et al., which found that resting state brain function was altered when unconscious, especially in the frontal cortex (Horovitz et al., 2009). Humans undergoing rsfMRI are usually not forcibly knocked unconscious and so if the rat brain were to be studied while the rat was under the effects of isoflurane the results could display minute differences. By having a method through which rats can be imaged whilst awake, restrained but still very much conscious, our data is more relevant to human anatomical imaging. Of course, being awake also allows tasks to be performed inside of the fMRI machine as it is scanning. This method will be explained in greater detail during the experimental procedure portion of this report.

Based upon the information put forth by the Liang et al. study, a theoretical answer to the second posed hypothesis can be stated. Hypothesis two asks if signaling pathways that include regions affected by stress are altered by fMRI. Task fMRI is a common method for observing functional connectivity. Thus, it does not seem likely that there will be any significantly different effects on the stress-activated circuits due solely to the employment of task fMRI. However further comparisons will need to be made in order to determine if there is a blatant difference between data observed in previous non-task fMRI studies and this experiment.

Elevated Plus Maze

The Elevated Plus Maze (EPM) procedure is a commonly utilized and widely regarded method for studying behavioral responses in rats. The EPM is exactly as it sounds; it is an elevated, plus-shaped maze. The maze possesses two “open arms” and two “closed arms”. The time rats choose to spend in each arm is indicative of their mental state. The competency of EPM was discussed previously. The procedure

was additionally validated in a study performed by Pellow et al. where several key features were noted. The features relevant to this thesis are as follows: (1) rats tend to avoid open spaces, i.e. the open arms; (2) when rats are more anxious, they spend an even greater proportion of time in the closed arms; (3) when rats were limited to only the open arms, their stress hormones were significantly more abundant in comparison to rats limited to only the closed; and (4) anxiety-provoking compounds in humans also had an effect on rats, causing them to shy away from the open arms (caffeine for example) (Pellow et al., 1985). The first three explain the ability for EPM to adequately test for behavior, while the final feature proposed in the study is support for the plausibility of relating rat behavior to human behavior.

EPM is a five minute, non-repeatable procedure in a plus-shaped maze containing both open and enclosed arms that is suspended about three feet off the ground. For this experiment the data was collected in a maze with 112 centimeters of open arm space length and 61 centimeters of closed arm space length. The data collected from this experiment culminates in the comparison between open arm time and closed arm time. This ratio between times can help determine how anxious the rat is. A lower ratio of open to closed time often signifies that the rat is more prone to hiding within the confines of the closed arm as opposed to investigating the unknown of the open arms.

The reason that this procedure is non-repeatable is that it tests the rat's level of comfort and exploration in an environment which it is unfamiliar with. If the rat has become accustomed to the environment it will demonstrate certain reactions characteristic of rodent explorative tendencies. A rat that has undergone EPM previously will spend more time in the closed arm portion of the maze. This is because small, enclosed spaces are seen as safer environments for rats to hide from predators in. When a rat investigates the open arms that is because the explorative nature within the rat has overcome the fearful prey inhibitions. If a rat has already explored the open arm in a previous experiment, it would likely feel no impulse to leave the perceived safety of the closed arm regions. Therefore the data collected would not be indicative of any particular behavior. If anything that data would detract from the results of the previous experiments.

EPM can also contribute to analyzing anxiety and PTSD. The Adamec and Shallow paper provides support for this, as well as a study by Cohen and Zohar more recently. Adamec and Shallow used EPM to observe PTSD symptoms between 1 and 21 days after predator exposure (Adamec and Shallow, 1993). Cohen and Zohar found that after a single 10 minute predator scent exposure, some rats expressed symptoms of anxiety/PTSD only 7 days later (Cohen and Zohar, 2006). In our experiment all rats performed the EPM test only 7-10 days after exposure.

Previously in this paper, a fourth hypothesis was proposed that the behavioral response to predator stress would be for expressed behavior to become more anxious. Rats who had been exposed to fox urine would likely spend more time in the closed-arm region of the EPM, as they had developed a sort of apprehension. The exposure period itself requires caution though, so as to not be so long that the rats become adjusted to the scent. This process of fear extinction was also previously discussed. Over time it would be entirely possible that prey could become acclimated to the presence of a predator. The idealized short but frightening exposure should cause the rats to remain apprehensive, knowing that at any moment they may be trapped with the "predator" once again. The third proposed hypothesis under consideration however, was whether or not this behavioral response would be predicted by the individual variations in brain activity during predator odor exposure? In theory this could be possible, if the behavioral response is noted in EPM alongside a unique hemodynamic brain activation during exposure. With that result it would not be ludicrous to propose that the behavioral response could be predicted by the variations in brain activity. This is especially true if controlled rats in the experiment (rats unexposed to predatory odor) neither have the anxious response to EPM nor the specific component of hemodynamic brain activation. If there is a distinct activation and distinct behavioral response noted only in rats that underwent predator stress, it would serve as strong evidence towards hypothesis three.

METHODS

Overview

The methods section has been split into four parts: 1) Acclimation, 2) Olfactometer construction, 3) fMRI Imaging, and 4) elevated plus maze analysis. Acclimation took place over the span of two weeks. Afterwards imaging was conducted. One week or more after imaging, EPM was conducted.

Acclimation Procedure

Rats were placed within a transparent box that was slowly filled with air that was 2-3.5% isoflurane (this value is entirely dependent upon the weight of the rodent – for 150-200 gram Long-Evans strain rats this number was appropriate). Isoflurane is an anesthetic that will put the rat to sleep.. The transparency of the box allowed us to continually monitor the rat's vital signs. Upon any sign of biological systems failure the rat was to be removed from the box and allowed to recuperate. Once anesthetized, the rat was placed in front of a smaller nose cone that pumped a more concentrated stream of isoflurane into their nostrils. Too much exposure to isoflurane is detrimental to animal health and therefore caution and relative haste was employed. Lidocaine topical ointment was applied to the rat's face on both sides as well as the top of its head to prevent skin abrasions when the head restraining equipment was attached. It was important to avoid harming the rats by only applying the lidocaine gently and precisely; coating their eyes could have resulted in vision impairment and other complications.

A two-pronged headpiece was then placed gently over the rat's head, stopping once it reached their shoulders. The rat's upper row of teeth were placed on top of the "bite bar" at the front of the

headpiece, while the bottom row of teeth were made to be placed below and in front of the bar. This was to ensure the rat was secured within the headpiece. The locations of the teeth around the bar made it difficult for the rat to twist its head and escape the restraints. A head restraining half-pipe (representing the actual scanning procedure's birdcage radiofrequency coil) was then put in place surrounding the headpiece. Screws were utilized to keep the pipe attached to the headpiece. An additional nosepiece was lowered and screwed into place in order to prevent the rat from lifting its head above the bite bar. The rat was then turned and its feet were gently taped together so that it was unable to propel itself forward with its back legs. Along with the headpiece and pipe the rat was finally lowered into place within an encompassing clear body tube. Screws were once again integral to holding the rat in place within the tube.

The rat was removed from the isoflurane and allowed to wake prior to the procedure. It was important to monitor the rats breathing for a few moments after they woke up to be satisfied that the rat had made a full recovery from the anesthesia. Then the rat was placed inside of the acclimation box. The acclimation box contained four square holes. Each hole was approximately the same size as the intended fMRI machine bore (in this case the sides were about 3.5 inches). The box also contained a speaker that was made to simulate a variety of sounds heard within the fMRI machine. The standard acclamatory procedure that we developed took about 7 sessions within a ten day to two-week span, detailed in Table 2 below.

Table 2: Acclimation Procedure Summary

Acclimation Procedure Summary							
Session Number	1	2	3	4	5	6	7
Acclimation Length (Minutes)	30	45	60	60	60	60	60

Olfactometer Construction

The olfactometer was made to achieve two functions: (1) to send scented air into the bore, and (2) to send clean air into the bore. This fluctuation of predator odor and no odor was integral to the experiment so that the brain could be observed both while under the influence of the fox urine odor and without it. It additionally served to avoid extinction. The control rats in the experiment used the same mechanism however instead of fox urine they were exposed to a neutral odor (lemon juice). The olfactometer pumped a low airflow of about 0.5 liters per minute of air siphoned through liquid composed of either 1:5 parts fox urine to water, or 1:5 parts lemon juice to water. The components are listed in Table 3 by manufacturer.

Table 3: Olfactometer Components by Manufacturer

Olfactometer Components By Manufacturer				
Manufacturer	Catalog Number	Equipment Name	Dimensions	Number of Units
McMaster-Carr	5195T67	Abrasion-Resistant Polyurethane Clear Tubing for Water	1/4” ID 3/8” OD 10’ Length	3
Purpose:	Flexible material for facilitation of airflow through the olfactometer.			
McMaster-Carr	5195T62	Abrasion-Resistant Polyurethane Clear Tubing for Water	1/8” ID 3/16” OD 50’ Length	1
Purpose:	Flexible material for facilitation of airflow through the olfactometer.			
McMaster-Carr	8547K1	Tube Made from PTFE (Teflon)	1/4” ID 3/8” OD 6’ Length	1

Purpose:	Rigid material made of Teflon. Teflon's natural fluorine barrier prevents adhesion of odor particles, thus avoiding retaining any left over odors after the experiment. The portion of the olfactometer through which both predator odor and clean air traveled.			
McMaster-Carr	5121K161	Chemical-Resistant Barbed Tube Fitting Connector	1/4" ID	4
Purpose:	Connecting pieces to allow for combination of the several forms of tubing present in the olfactometer.			
Qosina	14006	Turn Valve	0.315" OD	3
Purpose:	Used to cut off airflow from segments of the olfactometer, allowing for the alternation of clean air versus odor-exposed air.			
Qosina	61519	6-in-1 Y Connectors	Fits 1/4"-9/16" ID	1
Purpose:	Used to connect airflow from the nearest output into the VWR Flow Meter to maintain a steady and consistent level of airflow.			
VetEquip	304789	Rodent-Sized Nose Cones	0.55" ID	1
Purpose:	Responsible for encircling the rat nostrils and ensuring that the odor administered through the olfactometer reached its intended target.			
VetEquip	921614	Nose Cone Y Adaptors	1/4" ID	2
Purpose:	Used for the differentiation of airflow directed towards the rat; is the point at which the predator odor or the clean air odor is allowed to enter the Teflon.			
Neobits	3529756	VWR Flow Meter ACR (0.4-5 Liters per Minute Flow Rate)	2" OD	1
Purpose:	Used to monitor airflow from the initial point of contact with the air supply. Creates a controllable steady state of airflow.			

Flinn Scientific	AP1558	Gas Generating Bottles, with Plastic Inflow (PI) Tube and Bent Glass Thistle Outflow (BGTO) Tube	PI – 3/8” OD BGTO – 3/8” OD	1
Purpose:	Solution aeration component. Served as the location where air entered and picked up the scent of the predator (fox urine) or control odor (lemon juice).			

Functional Magnetic Resonance Imaging Study

The procedure for fMRI imaging was almost identical to that of the acclimation procedure. The acclimation procedure was intended to replicate the imaging procedure in order to make sure the rats were adjusted to the event. This was to avoid the influence of any unnecessary stress on data collection.

The differences in procedure started with the final step in the setup, in which the rat was placed into a birdcage radiofrequency coil instead of the half-pipe. The rat was then lowered into the clear imaging body tube. A specific open-faced body tube was used for imaging as this tube held a respiratory monitor in place. The respiratory monitor was used to continually examine the rat’s breathing throughout the imaging procedure. The tube was maneuvered into place and the respiratory device was positioned between the screw holes, superior to the prone rat’s head. Finally, a copper coil was placed surrounding the rat’s cranial region. This copper coil served to improve B1+ image quality.

Once the construction had been affixed to the rat, the body tube was loaded into a clear acrylic tube that stretched the length of the fMRI machine bore. An exhaust pipe was attached to

the end of the acrylic tube with the purpose of removing any lingering scents from the inside of the machine. This was so as not to influence future experimentation. Two cables for tuning and matching were then attached to the coil and the tube was pushed further into the bore until it was about 70 centimeters deep. From this point on the Paravision application was utilized to run the experiments. There were several imaging procedure components performed in Paravision detailed in Table 4. Generally the procedure can be surmised as a block fMRI study that exposed rats to fox or lemon odor for 21 minutes at a time. A block design study means that for a certain interval a stimulus is afforded to the rats continuously, as opposed to solely one brief moment of exposure (which is known as "event-related" design) (Tie et al., 2009). In terms of this study the "blocks" were minute long intervals of alternating clean air and fox or lemon scented air over the total span of the 21 minutes exposure. This was to avoid extinction and to provoke potential periods of excitation throughout the scan.

Table 4: Paravision Imaging Procedure Steps and Descriptions

Paravision Imaging Procedure		
Procedure	Length	Description
Tuning and Matching	N/A	Organisms have a specific resonance frequency that changes acquisition of the B0 and RF magnetic fields by changing impedance; tuning and matching impedance accounts for the fluctuations. (Hwang and Hoult, 1998)
Shimming	N/A	The process of combating inhomogeneity in radiofrequency magnetic fields through manipulating the drive magnitudes and phases of the RF coils. (Mao, Smith, and Collins, 2006)

B0 Field Map	N/A	A preliminary scan to acquire initial data on the magnetic vector field.
Echo Planar Imaging (EPI)	10 minutes	A preliminary scan of the rat prior to any influence being conducted on its mental condition. EPI refers to the type of imaging procedure, an efficient version of MRI that takes only seconds to perform a singular spin excitation for quick image clarity (Stehling et al., 1991).
Innate Fear Modeling Fox Urine Exposure Period	21 minutes	Cycling between one minute intervals of no odor clean air and fox urine scented air. Beginning and ending with clean air. The purpose of the cycling is to avoid extinction of the fear response, something likely to occur if the animal was exposed continuously to the odor with no reprieve.
Structural T1 + T2	20 minutes	Post procedure structural information acquisition by radiofrequency signaling.
EPI Post Procedure	10 minutes	Similar reasoning to the purpose of the initial EPI, however with the intent of monitoring any changes undergone during the exposure period.

Elevated Plus Maze Experimental Procedure

The first step in EPM procedure involved powering on the camera for a preliminary image alignment. Once a template file was selected for data collection, the “Grab Background Image” function was selected in order to validate the arena settings. Detection settings were adjusted to make sure that no artifacts were picked up on the scanner. The minimum subject size parameter was often adjusted until there were no outlying presences noticed on the maze. The

"Acquisition" function was then selected which allowed the camera to begin detecting motion. However the scan would not begin until the rat had been placed onto the EPM for a few seconds. This was to avoid collecting unnecessary data and to ensure the rat was present for all five minutes of the study. It was important that each rat in the experiment was placed into the EPM in the same direction each time so as not to bias the data. Rats should be exposed to either the open or closed arm to the same degree so as not to influence their actions on an individual subject basis. After the rat had been placed, to further avoid outside influence, it was preferable for the experimenter to leave the room. The rat may have had a differing opinion on exploration or perhaps behave differently if it was able to see its handler from its place within the maze. The program concluded the experiment automatically once the standard five minutes had elapsed. At this point the rat was removed from the maze and the data was analyzed. The maze was also cleaned with alcohol to remove all scents of the rat prior to the next subject so as to not influence the next rat with lingering odors.

RESULTS

BOLD fMRI Image Data

BOLD fMRI image results were obtained through a series of post imaging session processing of the 10 out of 12 rats that remained valid candidates for imaging. Raw fMRI data was obtained through the Paravision program. Preprocessing of the raw data in MATLAB was followed by alignment of the images in the Medical Imaging Visualization and Analysis (MIVA) program. This improved image quality by aligning the obtained image data from the experimental rats overtop of a standard anatomy model. Standardized positioning allowed for quick and easy comparison between rats, since the anatomical positions of regions of interest were overlapped in three-dimensional space. This new aligned data was then processed once again in MATLAB in order to then compare images between all 10 rats. The overlapping activation sets for the lemon control and fox urine treated rats were observed to have the following outcome, displayed in Figures 8, 9, and 10.

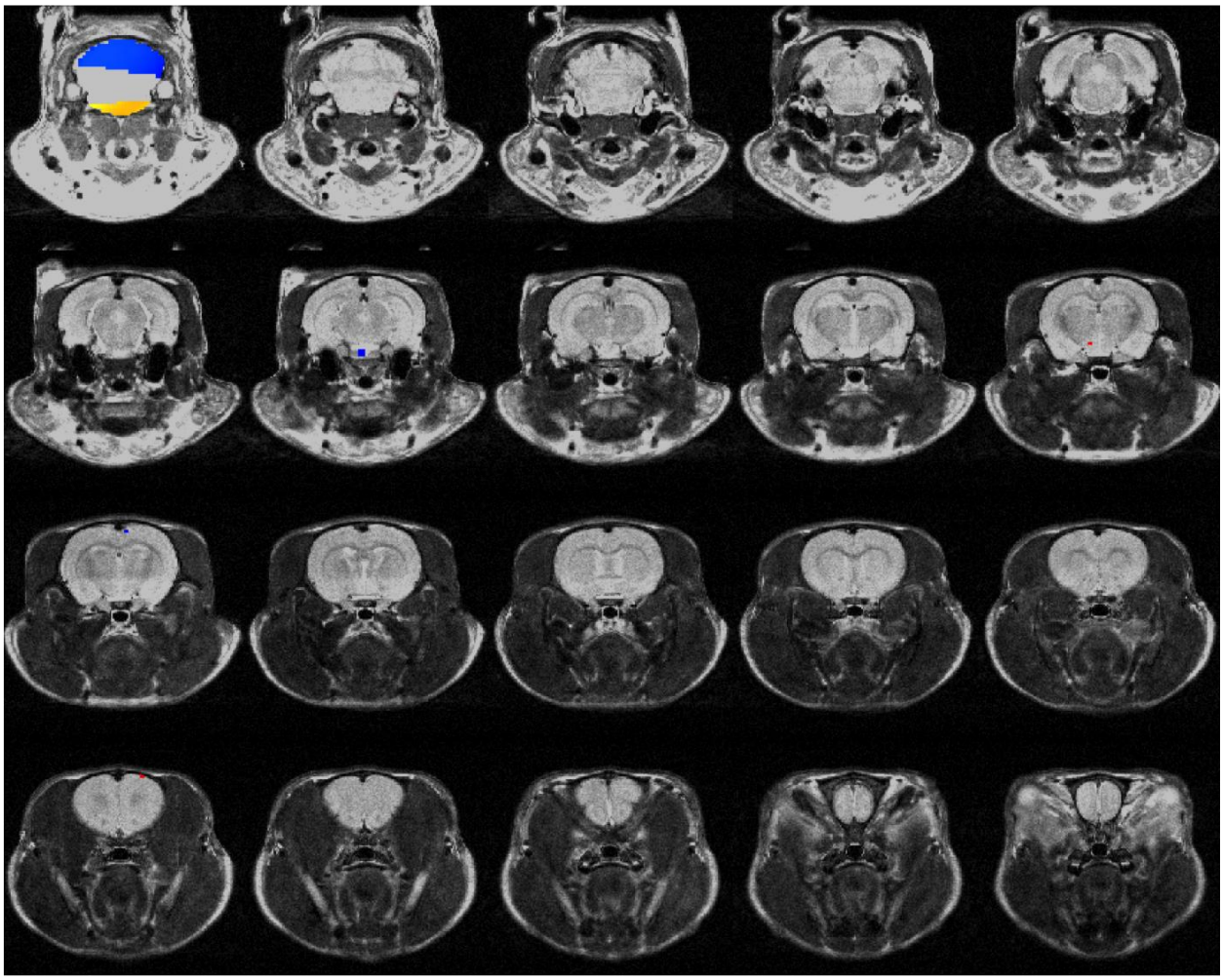

Figure 8: fMRI Group Analysis Imaging Data

This image compares and contrasts the difference between members of the sample group's pre-exposure activation and the lack of activation differences demonstrates that the rats were examined under comparable conditions during the study even prior to odor exposure.

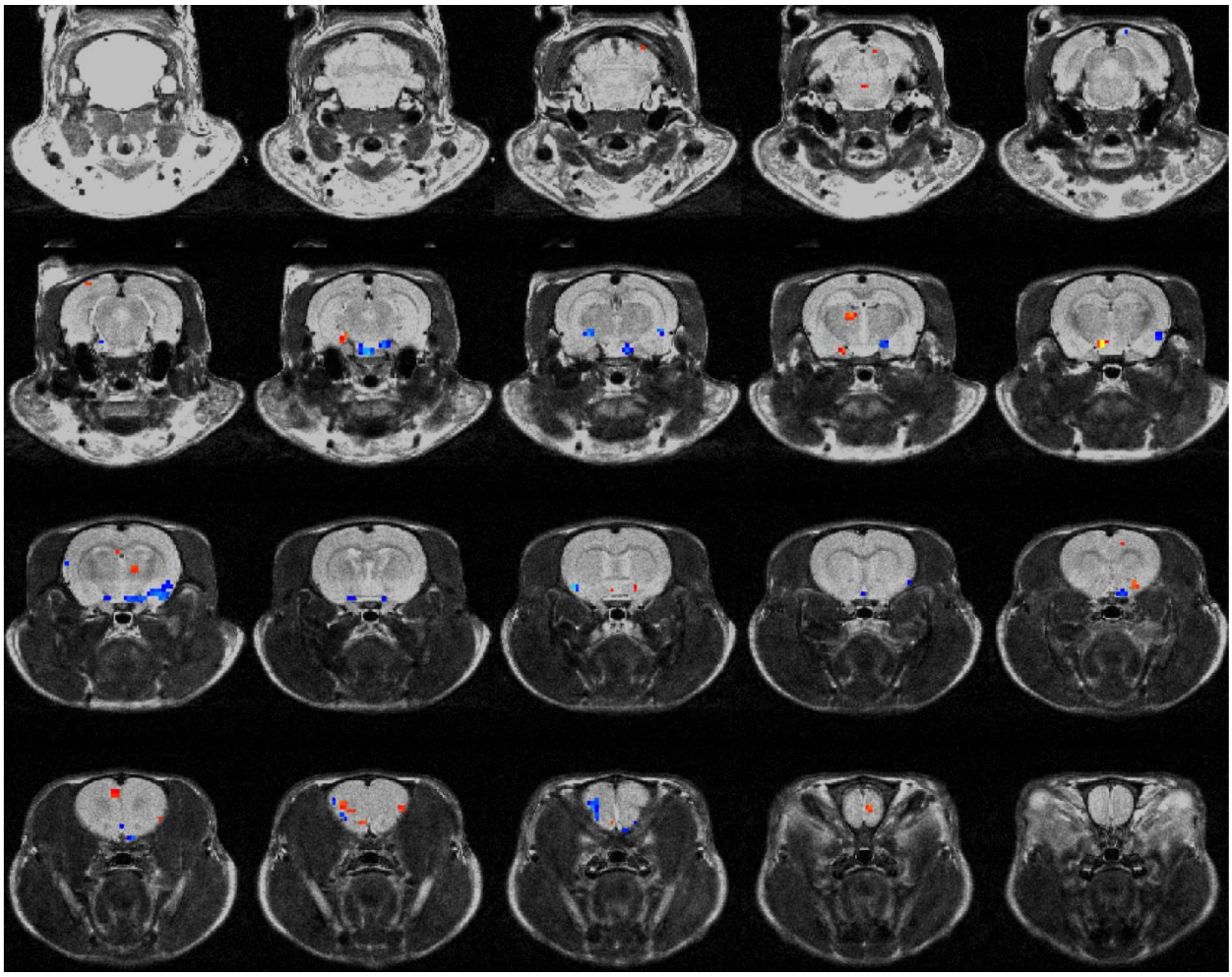

Figure 9: fMRI Effect of Odor vs. Air Imaging Data
This image observes differences in activation between when the rat brain was not being exposed to any stimulant and when it was exposed to either fox urine or lemon scent. It is notable that there is not a tremendous amount of activation of the olfactory bulb, seen in the two bottom right-hand images.

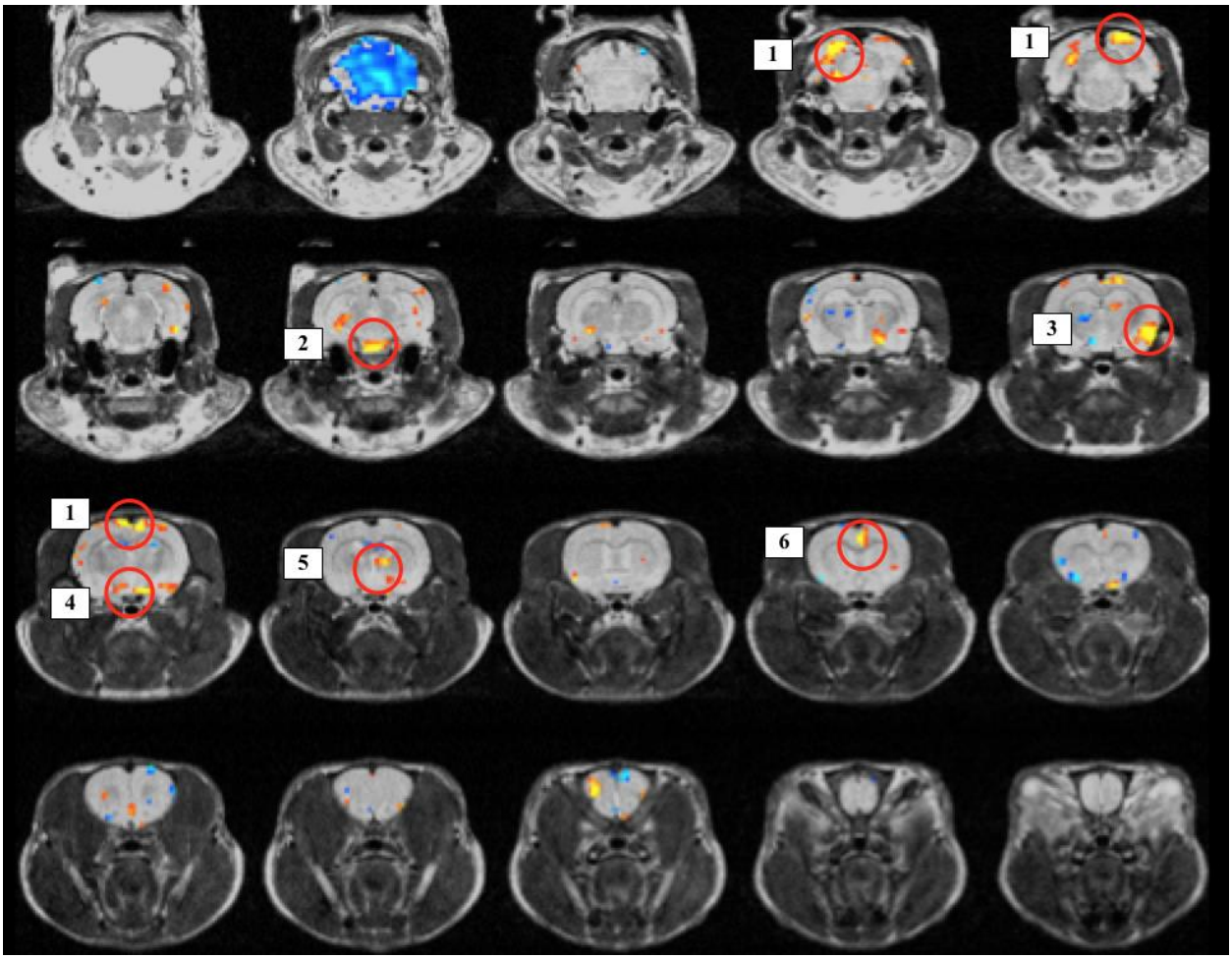

Figure 10: fMRI Effect of Fox Urine Odor Imaging Data
This image examines the specific effect of fox urine odor activation as opposed to the activation present in the control lemon odor exposed rats. Notable regions have been highlighted:

(1) Retrosplenial Cortex (RCC)
(2) Pituitary
(3) Amygdala
(4) Hypothalamus
(5) Bed Nucleus of the Stria Terminalis (BNST)
(6) Anterior Cingulate Cortex (ACC)

Elevated Plus Maze Data

Behavioral results obtained from the elevated plus maze study are listed below in Table 5 and Figure 11.

Table 5: Elevated Plus Maze Results

Elevated Plus Maze Results				
Rat Number	Treatment Administered	Center Open Arm Time (%)	All Open Arm Time (%)	Center Time (%)
1	Lemon	0.36	0.00	6.53
2	Lemon	12.26	10.08	20.16
6	Lemon	12.78	6.18	1.22
9	Lemon	11.01	7.09	12.98
10	Lemon	34.19	47.36	33.27
3	Fox Urine	0.51	0.00	24.91
4	Fox Urine	0.00	0.00	23.16
7	Fox Urine	7.94	6.76	16.62
8	Fox Urine	8.47	5.45	13.80
11	Fox Urine	1.45	0.00	19.20

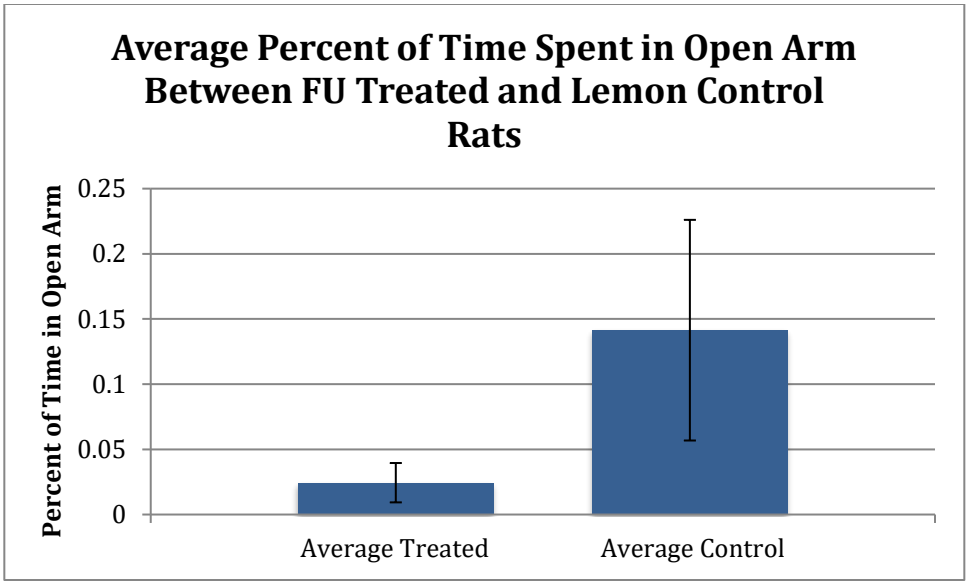

Figure 11: Average Percent of Time Spent in Open Arm Between Fox Urine Treated and Lemon Control Rats Results Histogram

Out of the 10 rats to undergo the trial, 5 were exposed to fox urine and 5 were exposed to lemon. On average the fox urine exposed rats spent only 2.4% of their time in the open arm regions while the control rats spent 14% of their time in the open arms. Using a T-test hypothesis distribution to analyze the 2 groups of 5 rats each, the difference between groups was found to possess a p value score of 0.11. Average treated open arm percent was equal to 0.024, while average control open arm percent was 0.14. Standard error for treated was 0.015 and standard error for control was 0.19.

DISCUSSION

Hypothesis 1: Predator Odor Stress Brain Activation

Results from this thesis experiment (Figure 10) show the regions of the brain influenced by fox urine odor that were activated differently from those influenced by the lemon odor control. These regions included the RCC, pituitary, amygdala, hypothalamus, BNST and the ACC. This observation is very much in line with hypothesis 1's predicted activation regions. The amygdala, hypothalamus, BNST, and ACC were all predicted and summarily observed in the data. The RCC is also closely associated with the hippocampus, another predicted region. Pituitary activation additionally makes a great deal of sense, as the pituitary is responsible for producing adrenocorticotropin (ACTH), a hormone that stimulates the production of the stress hormone cortisol (Aguilera, 1994). The pituitary is also involved in the HPA axis, the aforementioned series of interactions between the hypothalamus, pituitary, and adrenal glands. The HPA axis has been deemed integral in the body's response to stress and as such this is a promising result (Frodl and O'Keane, 2012).

The activation of the RCC is an interesting observation as it has further implications. At this point in time scientists are still unsure of the exact function of the RCC. Studies have theorized that its function likely has to do with connecting the processes of emotional and episodic memory (Maddock, 1999). More recent work has analyzed the complicated signaling pattern of the RCC and found that it makes these connections through interaction with the prefrontal cortex, occipital cortex, thalamus, and the hippocampus (Vann et al., 2009). Below is a simplified figure adapted from a review by Vann et al. (Figure 12) detailing the episodic memory connections between the RCC and many other observed sections of the brain.

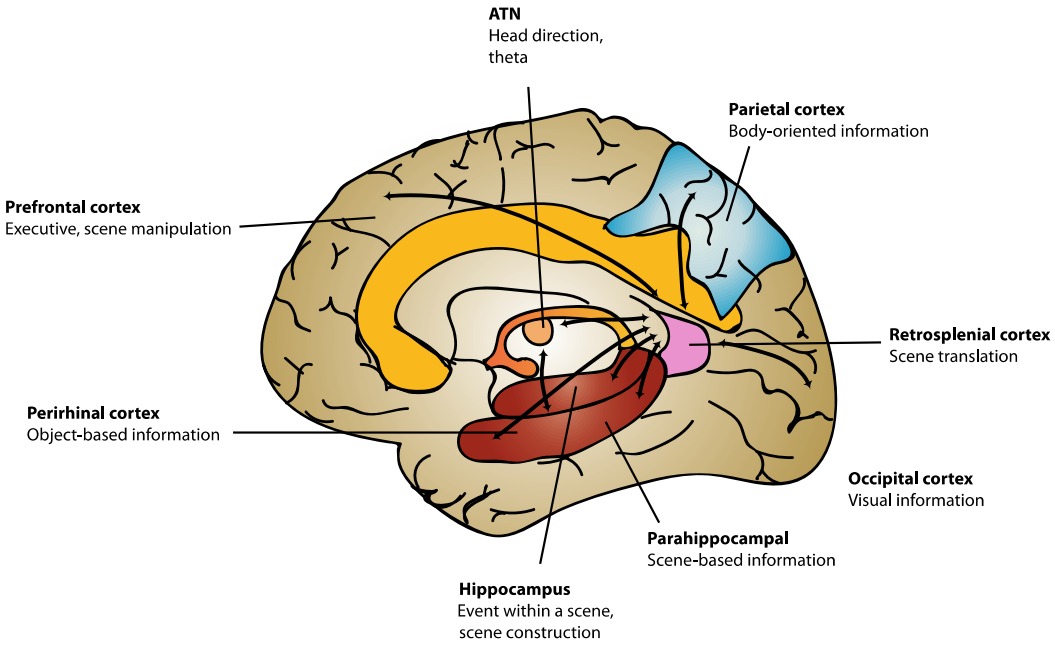

Figure 12: RCC Signaling Circuitry Diagram, Adapted from Vann et al., 2009

Findings that the RCC is involved in connecting emotions and episodic events certainly make sense within the scope of this study. Any rodents who experienced a traumatic event that altered their emotions (leading to PTSD or affective disorders) would experience RCC activation if the RCC is truly responsible for these sorts of actions.

While most of the interesting and substantial results were obtained from Figure 10, Figures 8 and 9 were further sources of information. Figure 8 detailed the group analysis of the rats. Figure 8's scans utilized functions such as a General Linear Model (GLM) analysis to essentially determine whether individual voxel variations were noticed between the group of rats prior to any odor exposure. By breaking down each voxel into singular variable parameter estimates, the MR images are simplified and displayed linearly for comparison (Beckmann et al. 2003). The results displayed by the rats in this

experiment showed that there were not noticeable differences at the start of scanning between the each rat brain's voxels. This was good, as it ensured that each rat was compared from an equal starting point prior to exposure initiation.

Figure 9 portrayed the differences in activation of the rat brains when exposed to either scent (fox urine or lemon) or only air. While minute activation could be observed, the more telling information obtained was from the region that did not activate. The olfactory bulb did not seem to be excessively stimulated by the odors. This seems counterintuitive when the purpose of the experiment was to expose the rats to odor. However the responses from the fox urine exposed rats demonstrated in Figure 10 do certainly verify that the odors were perceived. In concordance with this result, the previously discussed study by Chen et al. also found that between rats exposed to odorless air and rats exposed to lemon and TMT there was no statistically significant change in olfactory bulb activation (Chen et al., 2007). This is a question worth potentially looking into in a further study.

In the study by Huang et al., when they exposed rats to TMT (a component of rat predator odor) they found these regions of the brain to be activated: olfactory bulb, prefrontal cortex, insular cortex, cortical nucleus of amygdala, basolateral nucleus of amygdala, central nucleus of amygdala, BNST, and thalamus. This is demonstrated in Figure 13 below (Huang et al., 2011).

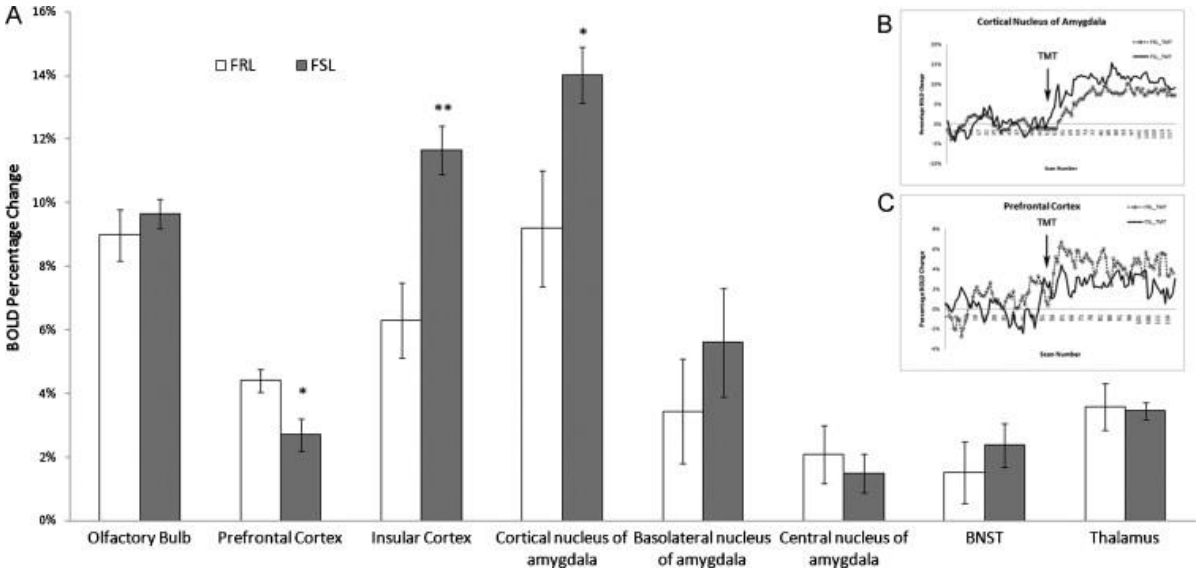

Figure 13: Regions of the Brain Activated by Innate Fear Modeling Using TMT Predator Odor, Taken From Huang et al., 2011

When the information from this graph is compared to Figure 2, it appears as though in TMT exposed rats there was significantly more activation in the cortical nucleus of the amygdala and the BNST. Figure 2 is a lemon scent activation graph from the same study. Additionally the paper states that there was hypoactivation of the prefrontal cortex and hyperactivation of the insular cortex, but only in the translational depression model FSL strain. The demonstrated change in activation is likely to have been a result of the fear response and not simply part of olfaction. This information aligns with our proposition that there is potential for brain activation in response to fear in the stria terminalis, amygdala, and the prefrontal cortex.

In further support of our results Brydges et al. performed a study that assists with understanding BOLD activation in response to conditioned fear stimuli. They claim that fear processing effected the right lateral amgydala, the hypothalamus, the somatosensory cortex, and the granular insular cortex (Brydges et al., 2013). The components are labeled respectively as LA, Hyp, SSC, and GI in Figure 14 down below. Their findings are in unison with the information put forth by the Huang et al. study in terms of the insular cortex and the amygdala, and with this thesis in terms of the hypothalamus and amygdala.

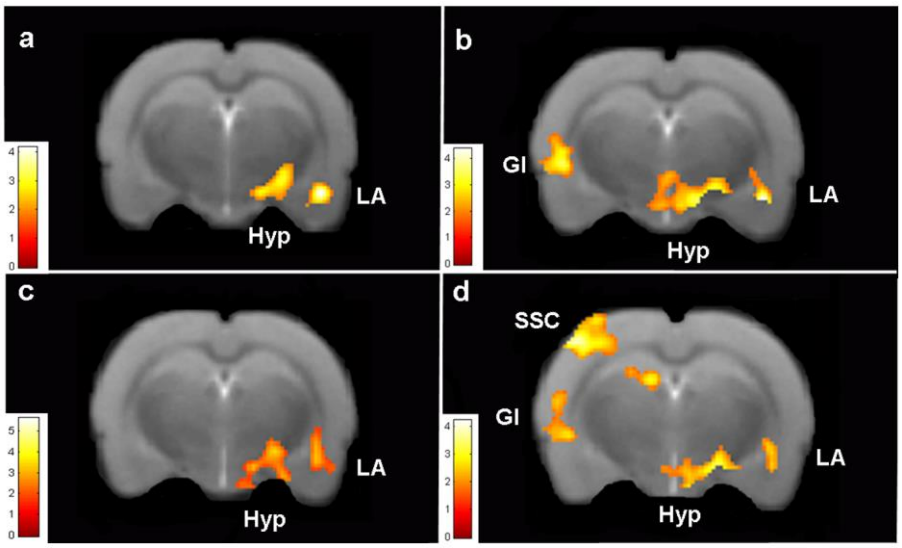

Figure 14: BOLD Activation Images, Taken From Brydges et al., 2013

Comparing the results put forth by these studies to the results obtained in this thesis experiment, it is noticeable that we agree upon the activation (or lack thereof) displayed in the regions such as the hippocampus, hypothalamus, amygdala, BNST, ACC, and the pituitary/HPA axis. The previously proposed hypothesis for this thesis said that known regions of fear activation were the amygdala, hippocampus, and prefrontal cortex. The potential regions of fear activation through limbic circuits and complex signaling patterns were the hypothalamus, brainstem, anterior cingular cortex, and stria terminalis. Meanwhile stress was handled by the amygdala and the HPA axis. These predicted regions were all noticed (with the exception of the brainstem and adrenal glands of the HPA), and with the addition of regions of interest such as the RCC and the pituitary. Technically the prefrontal cortex was not directly observed within this experiment either, however the potential implications of the RCC activation and its intertwined signaling processes with the prefrontal cortex should not be overlooked.

Hypothesis 2: Changes Based on fMRI

Hypothesis 2 stated that it would likely be too difficult to notice the minute signaling that identifies the neural circuitry's interconnected webbing of pathways. Due to the small 10-rat sample size of this experiment, making a claim that notices changes in intricate circuitry would be difficult. Let alone noticing changes between this experiment and other fMRI experiments. More work will need to be done to come up with information for this hypothesis. Though literature such as Adamec et al. (2005) would suggest that any fear-based circuitry fluctuation would be noticed in places such as the ventral angular bundle and the basolateral amygdala (Adamec et al., 2005). But unfortunately with a small sample size allow this thesis to support or refute that ideology. Noise was still a preventative factor in interpreting the imaging data. But noise's affect would be mitigated with greater numbers of rat participants.

Hypothesis 3: Brain Activity Predicting Behavior

While noticing a correlation between activated regions of the brain and apprehensive behavioral responses in rats does indicate that there may be a connection between the brain activity and the behavioral response, the sample size necessary to verify such claims would be fairly large. This thesis has found that at the very least, the RCC, pituitary, amygdala, hypothalamus, BNST, and ACC all activate when a rat experiences predator stress during exposure to an innate fear. We have also found that rats that were exposed to the predator odor were more apprehensive and cautious as a result of the traumatic event. But multiple regression analyses would need to be performed to see what regions of the brain distinctly signaled for the behavioral response if any. This thesis has enough data to confirm that one or more of those six regions of interest is likely concomitant with the affective behavioral response. But once again without a larger sample size it is not possible to state what regions specifically played a role. A future

study must include a larger sample size, and potentially observe that certain regions would activate when exposed to innate fears and the behavioral response would follow. While other times those regions would not be activated by the innate fear and summarily the behavioral response would not follow. If the behavior were not to occur despite the fear exposure, any regions that did not activate were likely responsible for the behavioral response itself. Such a result would provide evidence that those regions possessed roles extending beyond simply becoming activated during stressful encounters.

Hypothesis 4: Behavioral Outcomes

In accordance with the hypothesis, the results found in this study seem to indicate (with close to statistical significance, p value = 11%) that rats who underwent exposure to fox urine developed some kind of posttraumatic stress. They avoided entering the open-arm segment of the elevated plus maze design. The rats that had been frightened by the fox urine were more apprehensive as a result, despite the exposure being at least one week prior for each rat examined, and spent about 1/6 of the time in the open arm segments as the rats exposed to lemon scent. The fox urine exposed rats were still affected by the exposure even after an extended period with no different living conditions from those of the lemon scent group. The fox urine not only invoked an innate fear during the moment of scanning in the rats, but also left a lingering mental status change. This contributes to the validity of this study's comprehensiveness and capability to contribute to the ongoing study of affective disorders and PTSD. This component of the study was supported by aforementioned experiments by Adamec and Shallow as well as Liang et al., 2014.

Conclusion

In summary, the regions of interest found to show unique activation patterns in the fox urine exposed rats compared to the lemon control rats included the RCC, pituitary, amygdala, hypothalamus, BNST, and ACC. These were all mostly supported by previous literature, with the RCC providing intriguing avenues for future research thanks to its under studied role as a complex signaling junction. The lack of olfactory bulb activation when rats were exposed to odors was also an intriguing result worth further investigation.

The implications of this study's findings on lingering anxiety and apprehensiveness after a traumatic event mean that future studies could be conducted with a similar setup and a larger more quantifiable sample size. This would help truly determine the capability of innate fear studies to examine PTSD and other behavioral responses using rodents. Unfortunately this study was limited in its ability to provide irrefutable evidence of the effect of innate fear exposure on prolonged stress due to its small sample size. This small sample size also likely contributed to the near miss of statistical significance of EPM data. It is worth repeating this study with a larger sample, as this information would be invaluable in increasing knowledge about a common disease prevalent in humans. Beyond solely PTSD though, continuing this experiment would also contribute to understanding the effects of fMRI on brain activity. It would also support learning more about the specific signaling methods that lead to apprehensive behavioral responses.

Another avenue for potential future research involves perhaps testing this study with different strains of rats. This thesis was conducted using only the Long-Evans strain. In the previously discussed paper by Yehuda and LeDoux, it is mentioned that we are still unsure as to why certain individuals are more susceptible to PTSD? Not every human who undergoes a traumatic event comes out with PTSD. They state that it would be helpful to potentially monitor aspects such as "phenotypic differences"

between organisms in order to begin understanding what could potentially lead to susceptibility to PTSD and affective disorders (Yehuda and LeDoux, 2007).

Overall this study was quite effective and intriguing. Now that the setup has been constructed and the methods fully identified, it would be worthwhile to continue the research on a greater scale. More data contributing to the study of neuroscience, both behaviorally and physiologically, would be greatly beneficial.

REFERENCES

Acosta, G.B., Rubio, M.C. (1994). GABAA receptors mediate the changes produced by stress on GABA function and locomotor activity. *Neuroscience Letters*, 176(1), 29-31.

Adamec, R.E., Blundell, J., Burton, P. (2005) Neural circuit changes mediating lasting brain and behavioral response to predator stress. *Neuroscience and Biobehavioral Reviews*, 29(8), 1225-1241.

Adamec, R.E., Shallow, T. (1993). Lasting effects on rodent anxiety of a single exposure to a cat. *Physiology and Behavior*, 54(1), 101-109.

Aguilera, G. (1994). Regulation of Pituitary ACTH Secretion during Chronic Stress. *Frontiers in Neuroendocrinology*, 15(4), 321-350.

Allin, M., Matsumoto, H., Santhouse, A.M., Nosarti, C., Alasady, M.H.S., Stewart, A.L., Rifkin, L., Murray, R.M. (2001). Cognitive and motor function and the size of the cerebellum in adolescents born very pre-term. *Brain*, 124(1), 60-66.

Axelrod, J., Reisine, T.D. (1984). Stress hormones: their interaction and regulation. *Science*, 224(4648), 452-459.

Balleine, B.W., Killcross, S. (2006). Parallel incentive processing: an integrated view of amygdala function. *Trends in Neurosciences*, 29(5), 272-279.

Beckmann, C.F., Jenkinson, M., Smith, S.M. (2003). General multilevel linear modeling for group analysis in FMRI. *NeuroImage*, 20(2), 1052-1063.

Bouton, M.E. (2002). Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. *Biological Psychiatry*, 52(10), 976-986.

Brydges, N.M., Whalley, H.C., Jansen, M.A., Merrifield, G.D., Wood, E.R., Lawrie, S.M., Wynne, S.M., Day, M., Fleetwood-Walker, S., Steele, D., Marshall, I., Hall, J. (2013). Imaging Conditioned Fear Circuitry Using Awake Rodent fMRI. *PLoS ONE,* 8(1).

Buracas, G.T., Fine, I., Boynton, G.M. (2005). The Relationship between Task Performance and Functional Magnetic Resonance Imaging Response. *Journal of Neuroscience*, 25(12), 3023-3031.

Chen, W., Tenney, J., Kulkarni, P., King, J.A. (2007). Imaging unconditioned fear response with manganese-enhanced MRI (MEMRI). *NeuroImage*, 37, 221-229.

Choi, D.C., Maguschak, K.A., Ye, K., Jang, S-W., Myers, K.M., Ressler, K.J. (2010). Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proceedings of the National Academy of Sciences of the United States of America*, 107(6), 2675-2680.

Clare, S. (1997). Functional MRI: Methods and Applications. *Chapter 6.4.5, Image Presentation and Inter Subject Comparisons*.

Cohen, H., Zohar, J. (2006). An Animal Model of Posttraumatic Stress Disorder: The Use of Cut-Off Behavioral Criteria. *Annals of the New York Academy of Sciences*, 1032(1), 167-178.

Corcoran, K.A., Maren, S. (2001). Hippocampal Inactivation Disrupts Contextual Retrieval of Fear Memory after Extinction. *Journal of Neuroscience*, 21(5), 1720-1726.

Davis, M. (1992). The Role of the Amygdala in Fear and Anxiety. *Annual Review of Neuroscience*, 15, 353-375.

Frodl, T., O'Keane, V. (2012) How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. *Neurobiology of Disease*, 52, 24-37.

Harmon, K.M., Greenwald, M.L., McFarland, A., Beckwith, T., Cromwell, H.C. (2009) The effects of prenatal stress on motivation in the rat pup. *Stress*, 12(3), 250-258.

Hastings, R.S., Parsey, R.V., Oquendo, M.A., Arango, V., Mann, J.J. (2004). Volumetric Analysis of the Prefrontal Cortex, Amygdala, and Hippocampus in Major Depression. *Neuropsychopharmacology*, 29, 952-959

Herman, J.P., Ostrander, M.M., Mueller, N.K., Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: Hypothalamo-pituitary-adrenocortical axis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 29(8), 1201-1213.

Heuvel, M.P.V.D., Pol, H.E.H. (2010). Exploring the brain network: A review on resting-state fMRI functional connectivity. *European Neuropsychopharmacology*, 20(8), 519-534.

Hogg, S. (1996). A review of the validity and variability of the Elevated Plus-Maze as an animal model of anxiety. *Pharmacology Biochemistry and Behavior*, 54(1), 21-30.

Horovitz, S.G., Braun, A.R., Carr, W.S., Picchioni, D., Balkin, T.J., Fukunaga, M., Duyn, J.H. (2009). Decoupling of the brain's default mode network during deep sleep. *PNAS*, 106(27), 11376-11381.

Houtepen, L.C., Schür, R.R., Wijnen, J.P., Boer, V.O., Boks, M.P.M., Kahn, R.S., Joëls, M., Klomp, D.W., Vinkers, C.H. (2017). Acute stress effects on GABA and glutamate levels in the prefrontal cortex: A 7T1H magnetic resonance spectroscopy study. *Neuroimage Clinical*, 14, 195-200.

Hou, C., Liu, J., Wang, K., Li, L, Liang, M., He, Z., Liu, Y., Zhang, Y., Li, W., Jiang, T. (2007). Brain responses to symptom provocation and trauma-related short-term memory recall in coal mining accident survivors with acute severe PTSD. *Brain Research*, 1144, 165-174.

Huang, W. Heffernan, M.E., Li, Z., Zhang, N., Overstreet, D.H., King, J.A. (2011) Fear induced neuronal alterations in genetic model of depression: An fMRI study on awake animals. *Neuroscience Letters*, 489(2), 74-78.

Hutchison, R.M. Mirsattari, S.M., Jones, C.K., Gati, J.S., Leung, L.S. (2010). Functional Networks in the Anesthetized Rat Brain Revealed by Independent Component Analysis of Resting-State fMRI. *Journal of Neurophysiology*, 103(6), 3398-3406.

Hwang, F. Hoult, D.I. (1998). Automatic Probe Tuning and Matching. *Magnetic Resonance in Medicine*, 39, 214-222.

Jesuthasan, S. J., & Mathuru, A. S. (2008). The Alarm Response in Zebrafish: Innate Fear in a Vertebrate Genetic Model. *Journal Of Neurogenetics*, 22(3), 211-228.

Karnani, M.M., Jackson, J., Ayzenshtat, I., Hamzehei, S.A., Manoocheri, K., Kim, S., Yuste, R. (2016). Opening Holes in the Blanket of Inhibition: Localized Lateral Disinhibition by VIP Interneurons. *Journal of Neuroscience*, 36(12), 3471-3480.

King, J.A., Garelick, T.S., Brevard, M.E., Chen, W., Messenger, T.L., Duong, T.Q., Ferris, C.F. (2005). Procedure for minimizing stress for fMRI studies in conscious rats. *Journal of Neuroscience Methods*, 148(2), 154-160.

LeDoux, J.E. (2000). Emotion Circuits in the Brain. *Annual Review of Neuroscience*, 23, 155-184.

Liang, Z., King, J., Zhang, N. (2014) Neuroplasticity to a single-episode traumatic stress revealed by resting-state fMRI in awake rats. *Neuroimage*, 103, 485-491.

Liang, Z., King, J., Zhang, N. (2011). Uncovering Intrinsic Connectional Architecture of Functional Networks in Awake Rat Brain. *Journal of Neuroscience*, 31(10), 3776-3783.

Logothetis, N.K., Pauls, J., Augath, M., Trinath, T., Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature*, 412, 150-157.

Maddock, R.J. (1999). The retrosplenial cortex and emotion: new insights from functional neuroimaging of the human brain. *Trends in Neurosciences*, 22(7), 310-316.

Mao, W., Smith, M.B., Collins, C.M. (2006). Exploring the limits of RF shimming for high-field MRI of the human head. *Magnetic Resonance in Medicine*, 56, 918-922.

Maren, S., Holmes, A. (2016). Stress and Fear Extinction. *Neuropsychopharmacology*, 41(1), 58-79.

McEwen, B.S., Gianaros, P.J. (2010). Central role of the brain in stress and adaptation: Links to socioeconomic status, health, and disease. *Annals of the New York Academy of Sciences*, 1186, 190-222.

McEwen, B.S. (2006). Stress, Adaptation, and Disease: Allostasis and Allostatic Load. *Neuroimmunomodulation: Molecular Aspects, Integrative Systems, and Clinical Advances*, 840(1), 33-44.

McGaugh, J.L. (2004). The Amygdala Modulates the Consolidation of Memories of Emotionally Arousing Experiences. *Annual Review of Neuroscience*, 27, 1-28.

Milad, M.R., Quirk, G.J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature*, 420, 70-74.

Nie, B., Chen, K., Zhao, S., Liu, J., Gu, X., Yao, Q., Hui, J., Zhang, Z., Teng, G., Zhao, C., Shan, B. (2013). A Rat Brain MRI Template with Digital Stereotaxic Atlas of Fine Anatomical Delineations in Paxinos Space and its Automated Application in Voxel-Wise Analysis. *Human Brain Mapping*, 34, 1306-1318.

Paredes-Ramos, P., Miquel, M., Manzo, J., Coria-Avila, G.A. (2011). Juvenile play conditions sexual partner preference in adult female rats. *Physiology and Behavior*, 104(5), 1016-1023.

Pavlov, I.P. (1927). *Conditioned Reflexes*, Oxford University Press, London.

Pellow, S. Chopin, P., File, S.E., Briley, M. (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, 14(3), 149-167.

Preston, D.C. (2006). Magnetic Resonance Imaging (MRI) of the Brain and Spine: Basics. http://casemed.case.edu/clerkships/neurology/Web%20Neurorad/MRI%20Basics.htm

Purdy, R.H., Morrow, A.L., Moore, P.H., Paul, S.M. (1991). Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. *PNAS*, 88(10), 4553-4557.

Rosa, M.J., Kilner, J., Blankenburg, F., Josephs, O., Penny, W. (2010). Estimating the transfer function from neuronal activity to BOLD using simultaneous EEG-fMRI. *Neuroimage*, 49(2), 1496-1509.

Sapolsky, R.M., Krey, L.C., McEwen, B.S. (2002) The Neuroendocrinology of Stress and Aging: The Glucocorticoid Cascade Hypothesis. *Sci. Aging Knowl. Environ.*, 2002(38), 21

Schölvinck, M.L., Maier, A., Ye, F.Q., Duyn, J.H., Leopold, D.A. (2010). Neural basis of global resting-state fMRI activity. *Proceedings of the National Academy of Sciences of the United States of America*, 07(22), 10238-10243.

Schwarz, A.J., Gozzi, A., Bifone, A. (2008). Community structure and modularity in networks of correlated brain activity. *Magnetic Resonance Imaging*, 26(7), 914-920.

Shin, L.M., Orr, S.P., Carson, M.A. (2004). Regional Cerebral Blood Flow in the Amygdala and Medial Prefrontal Cortex During Traumatic Imagery in Male and Female Vietnam Veterans With PTSD. *Arch Gen Psychiatry*, 61(2), 168-176.

Shin, L.M., Rauch, S.L., Pitman, R.K. (2006). Amygdala, Medial Prefrontal Cortex, and Hippocampal Function in PTSD. *Psychobiology of Posttraumatic Stress Disorder: A Decade of Progress*, 1071(1), 67-79.

Stehling, M.K., Turner, R., Mansfield, P. (1991). Echo-planar imaging: magnetic resonance imaging in a fraction of a second. *Science*, 254(5028), 43-50.

Tenney, J.R., Duong, T.Q., King, J.A., Ferris, C.F. fMRI of Brain Activation in a Genetic Rat Model of Absence Seizures. *Epilepsia*, 45(6), 576-582.

Tie, Y., Suarez, R.O., Whalen, S., Radmanesh, A., Norton, I.H., Golby, A.J. (2009). Comparison of blocked and event-related fMRI designs for language mapping. *Neuroimage*, 47(2), 207-115.

Tovote, P., Fadok, J.P., and Lüthi, A. (2015). Neuronal circuits for fear and anxiety. *Neuroscience*, 16(6), 317-331.

Vann, S.D., Aggleton, J.P., Maguire, E.A. (2009). What does the retrosplenial cortex do? *Nature Reviews*, 10(11). 792-802.

Weisstein, E.W. Central Limit Theorem. *Mathworld – A Wolfram Web Resource*. http://mathworld.wolfram.com/CentralLimitTheorem.html

What is fMRI?. *Center for Functional MRI*. UC San Diego School of Medicine. http://fmri.ucsd.edu/Research/whatisfmri.html

Yehuda, R., LeDoux, J. (2007) Response Variation following Trauma: A Translational Neuroscience Approach to Understanding PTSD. *Neuron*, 56(1), 19-32.

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EDUCATION

The Pennsylvania State University **University Park, PA**

2014 — 2018 Attended The Pennsylvania State University Schreyer Honors College, May 2018 Graduation. B.S. Biology Neuroscience Option, Minor in History, Honors in Biology. Dean's List (7 Semesters)

EXPERIENCES

The Pennsylvania State University **University Park, PA**

2017 Summer Research Internship under Dr. Nanyin Zhang studying stress and Innate Fear Modeling through predator exposure, monitored via BOLD State fMRI

The Pennsylvania State Milton Snavely Hershey Medical Center **Hershey, PA**

2016 Summer Research Internship under Dr. Qing Yang studying fMRI imaging manipulation via surface coils and dielectric materials, specifically focused on the Temporomandibular Joint. Received co-author status on ISMRM 2017 abstract "Design of Self-Resonance Modes (SRM) of monolithic ultra-high dielectric constant (uHDC) materials and RF Coils for B1 field enhancement"

2015 Summer Internship Funded by Schreyer Honors College shadowing Dr. Peter Waybill in Hershey Medical Center's Cardiovascular Interventional Radiology Unit

2012 — 2014 Pennsylvania Youth Apprentice Program Penn State Hershey Medical Center – Neurosurgery, Neurology, Interventional Radiology Shadowing (300 Hours)

LEADERSHIP AND ACTIVITIES

Organizations Penn State University Schreyer Honors College Student Council; Undergraduate Research Society; Empowering Orphans Organization Penn State Chapter; Scholar Advancement Team for the Schreyer Honors College

ACQUIRED SKILLS

Science Experience with Various Scientific Processes: Polymerase Chain Reaction; Gel Electrophoresis; Simple Mammalian Dissection; Bacterial Culture Growth; Fluid-Flow Spectrometer Analysis; XFdtd Anatomical Simulations; fMRI Imaging Processes Computer Coding in MATLAB, HTML5, CSS3, and Perl

Language Proficiency in the Spanish Language

Healthcare CPR and Aquatic Emergency Care Certification