EXAMINING THE EFFECTS OF ACUTE ETHANOL ADMINISTRATION ON THE CONSCIOUS RAT BRAIN

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ABSTRACT

In the United States, over sixteen million adults have a type of alcohol use disorder [1]. With better understanding of a disorder, better treatments can be developed. The purpose of this project is to provide a better understanding of the effects of alcohol on the brain by exploring the acute effects of ethanol on brain functional connectivity in an animal model. Naive male Long Evans rats were injected with either ethanol or saline during an fMRI scan. Data analysis assessed the temporal correlation between brain regions and the rest of the brain, creating maps to compare regional differences in connectivity before and after injection. Acute ethanol administration was seen to produce effects on the conscious brain throughout the reward regions and visual areas. In particular, our data showed increased connectivity between reward and sensory processing centers; decreased connectivity between the thalamus and nucleus accumbens; as well as decreased connectivity between the hippocampus/amygdala and reward regions. These results agree with previous research that has shown that acute ethanol administration increases connectivity between sensory processing and reward centers. Additionally, these results further explain previous research that has shown changes in reward system activity in response to acute ethanol by providing a functional connectivity perspective. In the future, the experiment should be repeated with more subjects to interrogate the validity of results and to improve the statistical significance of the results.
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Chapter 1: Introduction

1.1. Research Significance

While many can enjoy alcohol in moderation, the impacts of alcohol abuse are not trivial, shown in Figure 1. Alcoholism is the fifth leading risk factor for premature death and disability for adults globally. In the United States, over sixteen million adults have a type of alcohol use disorder, and in 2013, fourteen percent of Americans aged twelve to twenty were binge drinkers. Studies have indicated that drinking during adolescences may impair brain development and increased the risk of developing an alcohol use disorder [1].

Additionally, alcoholism does not only affect those who drink. In the United States, thirty percent of driving fatalities, over ten thousand deaths in 2013, involved alcohol impaired drivers [1]. Alcohol is also thought to increase the likelihood of physical and sexual assault [2]. Additionally, alcohol can affect children: in 2012, over ten percent of American children were living with a parent with an alcohol use disorder, and the prevalence of fetal alcohol spectrum disorders is estimated to be between twenty to fifty births per one thousand [1]. Fetal alcohol spectrum disorders can have a wide range of effects on children as they grow including abnormal facial features, decreased height and weight, attention and memory difficulties, intellectual disabilities, as well as heart, kidney, or bone issues [3].

Figure 1. Percent of Americans with substance abuse disorder by drug of choice [4]
On a broader scale, some researchers have found tenets of general addiction which is interrelated between different types of addiction. Drug addiction kills around two-hundred thousand people globally each year and forty-four Americans die every day from a prescription drug overdose [5].

Current treatment options for alcohol abuse are limited and only effective for a subset of the population. Most drug treatments for alcohol use disorders are designed to reduce the effects of alcohol intoxication and do not treat the underlying causes of the addiction. Electrical stimulation as a potential treatment option is restricted in that it non-specifically stimulates neurons in an anatomical region, as opposed to stimulating neurons with specific functions. Behavioral treatments and support groups are effective for some and may only help temporarily [6].

1.2. Experimental Exigence

The purpose of this project is to provide a better understanding of the effects of alcohol on the brain by determining the acute effects of ethanol on brain functional connectivity. A basic principal in biomedical research is that with better understanding of a disease or condition, better treatments can be developed. With a better understanding of the effects of alcohol on brain function, more specific and efficacious treatments can be developed for use in a broader population. In addition to the study of chronic alcohol use, an understanding of the acute effects may give information on questions such as: how do the effects on brain function compare between chronic and acute alcohol use? What are possible mechanisms of action of treatments for acute alcohol intoxication? How do subjects with an alcohol use disorder respond to acute alcohol exposure differently than those without? Can subjects at risk of alcohol use disorders be differentiated by their response to acute alcohol exposure before using alcohol chronically? How do subjects with or at risk of other addictions respond to acute alcohol exposure compared to those without or not at risk of other addictions and can this inform addiction on a larger scale?
1.3. Background on Experimental Procedures

1.3.1. Resting-state fMRI

Blood-oxygen-level dependent contrast (BOLD) -based function magnetic resonance imaging (fMRI) is used for measuring neural activity. Neural activity in specific brain locations is difficult to measure directly in live animals, so fMRI uses blood flow as an indirect measurement of neural activity. This technique is advantageous over other imaging methods for its non-invasiveness and large field of view. In the 1970s, major advancements in MRI technology were made and applications of MRI were being discovered. The first major breakthrough of fMRI was realizing that blood flow to a region of the brain is correlated with neural activity of that brain region. The second major development was made by Seiji Ogawa and Ken Kwong in the early 1990s by recognizing that these changes in blood flow can be recorded with MRI because of hemoglobin’s magnetic properties and changes in its magnetism when it is bound versus unbound to oxygen [7].

In the late 2000s, researchers helped define resting-state fMRI, fMRI in the absence of a task, noting that regions of the midline of the brain are synchronized and that eight brain networks appear. The connectivity of these resting state networks is also thought to be able to predict the coordination of the same brain regions while performing a task requiring the use of those brain regions [8]. Resting-state fMRI examines the temporal correlations between regions of BOLD signals at low-frequencies (<0.1 Hz). Common methods to analyze resting-state fMRI data include: seed-base analysis, independent component analysis, graph methods, clustering and multivariate pattern classification. In this study, a seed-based analysis was used. The brain is divided into sections called seeds or regions of interest, and the correlation between one seed and the rest of the seeds over time is calculated. The correlation between two seeds is conceptualized as the connectivity between the two seeds. With this analysis method, the default mode network was discovered, which is a network of brain regions active while subjects are at rest, and this network is deactivated when subjects are performing a task [9].
1.3.2. Awake Animal Imaging

An animal model is used in this study because in general, studies with animal models allow more research method flexibility and more control over confounding variables than human studies. With human subjects, the effects of ethanol can vary with situational factors such as drinking rate, concentration of alcohol being consumed, or food intake before or during alcohol consumption. Additionally, biological factors can create differing effects from alcohol including sex, age, genetics, or the level of pre-exposure to alcohol. The use of naive, male animal subjects raised in the same environmental conditions and injected with the same ethanol concentration eliminates most of these confounding variables [8]. Using animals also allows for future manipulations of this study to be conducted easily, such as subjecting rats to additional conditions before or after acute imaging or changing the subjects from those not naive to alcohol.

In the past and still continuing today, researchers performing imagining studies on animal subjects used anesthetization to reduce animal motion during imaging, especially for MRI uses which require a still subject for a long period of time. This imaging technique may cause no differences in image
production for applications in areas such as orthopedics, however brain function is dramatically affected by consciousness and anesthetic agents. In 2012, Zhifeng Liang, Jean King, and Nanyin Zhang from University of Massachusetts Medical School presented a solution they had developed for this challenge by creating a procedure for the imaging of awake rodents using a restraint method [10].

A key feature of the procedure is acclimation. Acclimation is designed for the rats to become accustomed to the methods used for MRI scanning. This procedure benefits the rats by allowing them to slowly adjust to the restraint methods to reduce stress, and benefits the researcher by ultimately training rats to minimize movement in the scanner which improves data quality. The rats are restrained by the restrainer used during scanning, and subjected to the scanner noise, lighting, and smell of the room for increasing amounts of time over a period of seven days. By the last day of acclimation, there are noticeable differences in the rat’s behavior while restrained and the quality of the imaging results improves [10].

![Figure 3. Rat in head restrainer for acclimation](image)

1.4. Review of Previous Studies

1.4.1. Human Studies

One study by Bjorka and Gilman in 2014 reviewed research on alcohol’s impact on the brain, including a few papers on the acute effects of alcohol on functional connectivity. One paper the reviewers
analyzed used acute intravenous injection of 10% ethanol that maintained a blood alcohol level of approximately 600 mg/L. This study found that compared to the placebo group, the experimental group showed a significant increase in connectivity in a network of the auditory and anterior cingulate cortices as well as in the brainstem. Another paper reviewed used oral administration of alcohol to reach an approximate 64 mg/dl blood alcohol concentration. This paper found that compared to the control, visual cortex connectivity was directly correlated with blood alcohol concentration. The reviewers interpreted these results by arguing that because some addiction theories propose that cues usually associated with an addictive substance/behavior become a motivation for the addictive substance/behavior by themselves, the reported effect of alcohol increasing connectivity in sensory processing centers and between sensory processing and reward learning centers may help foster the associative learning between cues and an addictive substance/behavior [8].

A different study gave subjects an oral dose 0.65 grams of alcohol per kilogram of their body weight in 10 minutes, then performed resting state fMRI. They had three groups of subjects: a control group, a low breath alcohol content group, and a high breath alcohol content group. They assessed the connectivity between a brain region and the posterior cingulate cortex/precuneus, which is known to communicate with a variety of brain networks and is involved in awareness, an example image is shown in Figure 4. Their results showed that for the high breath alcohol content group compared to the control, connectivity to the posterior cingulate cortex/precuneus was decreased for the left parahippocampal gyrus, involved in memory encoding and retrieval, left fusiform gyrus, involved in recognition, and left superior frontal gyrus, involved in speech processing, and connectivity was increased for the left occipital lobe, involved in visual processing, right superior parietal lobe, involved in spatial orientation, right superior frontal gyrus, involved in self-awareness, and the right cerebellum, involved in coordination. Their results showed that for the high breath alcohol content group compared to the low breath alcohol content group, connectivity to the posterior cingulate cortex/precuneus was decreased for the left cerebellum, involved in visuospatial processing, the right hippocampal gyrus, involved in memory encoding and retrieval, the right superior frontal lobe, involved emotion regulation, and the right fusiform
gyrus, involved in recognition. Increases in connectivity were seen in the left medial and superior frontal lobe, involved in planning and self-awareness, the right cingulate gyrus, involved in emotion formation, and the right cerebellum, involved in visual attention [11].

Figure 4. Axial view of the resting state connectivity map of brain regions to the posterior cingulate cortex/precuneus, hotter colors indicate higher connectivity [11]

Another study orally administered alcohol to subjects to reach a blood alcohol concentration of 0.5 g/L. Compared to the control results of pre-alcohol scan, decreased connectivity was found in the left temporal fusiform cortex, involved in facial recognition, and in the sub-callosal complex which is involved in the networks for cortical structures, the limbic system, thalamus, hypothalamus, and brainstem nuclei. The results are shown in Figure 5, with hotter colors indicating decreased connectivity. Again by subtracting the post- and pre-alcohol scan data, connectivity was seen to be reduced in the left inferior temporal gyrus, involved in visual processing and recognition [12].

The authors of this study interpreted these results as an indication that acute alcohol decreases connectivity in brain regions involved in emotional responses and visual discrimination processing. The sub-callosal complex is known to be involved in brain reward circuitry and mood regulation and increases in connectivity of this circuit could explain the effects of alcohol as a reward cue and the changes of mood associated with alcohol intoxication. The decreased connectivity of the inferior temporal gyrus and temporal fusiform cortex was interpreted to correspond to the behavioral change of alcohol intoxication of
blurred vision and difficulty recognizing objects and faces. However, the authors cautioned the interpretation of the results because there could be other factors involved in changing brain blood flow besides functional connectivity [12].

**Figure 5. Default mode network comparison of pre- and post-alcohol scans with decreased connectivity as hotter colors, highlighting the sub-callosal complex A. Coronal view B. Sagittal view C. Axial view D. Volume rendering [12]**

The results from these three human studies lead to the expectation that acute alcohol administration leads to changes in reward regions, visual and motor processing centers, and sensory interpretation centers.

1.4.2. Animal Studies

One animal study looked at the acute effects of ethanol by using fMRI and brain alcohol pharmacokinetics on rats. They used tail vein cannulation to inject 0.75 grams per kilogram body weight of ethanol in their experimental group. Their fMRI results showed that compared to the control group, brain regions activated in the experimental group were regions in the dopaminergic mesolimbic pathways: the prefrontal cortex, the cingulate cortex, the caudate putamen, and the nucleus accumbens, shown in Figure 6 [13]. The researchers measured percentage changes in cerebral blood volume (CBV) in specific brain regions over time. The left side of the figure shows, for each region, the maximum signal change
(Max S%) as a peak-to-peak percentage change in the cerebral blood volume signal. The right side of the figure shows, for each region, the percent area under the curve (AUC%) of change in CBV vs. time. The prefrontal cortex is involved in higher order brain functions like decision making, planning, and personality, the cingulate cortex is involved in emotion processing, learning, and memory, the caudate putamen is involved in learning, and the nucleus accumbens is involved in motivation, pleasure, and learning. Because of the functions of these brain regions, they are all believed to relate to addiction through reward learning [14]. Since these subjects had never been exposed to alcohol before the study, increased activity in these brain regions during acute administration of ethanol could indicate that the rats are learning and processing the rewarding effects of acute alcohol exposure.

In summary, this past study analyzed forebrain activity after acute ethanol administration by fMRI and proton magnetic resonance spectroscopy because previous research has found that differences in ethanol uptake kinetics lead to differences in drinking behavior. They found activation of reward regions and the cingulate cortex, suggesting that these regions may change functional connectivity after acute ethanol administration.

![Figure 6. Axial slices of rat fMRI data showing activation in mesolimbic pathways [13]](image)
The purpose of this project was to provide a better understanding of the effects of alcohol on the brain by determining the acute effects of ethanol on brain functional connectivity. This study used an animal model, acclimated to awake imaging, with resting-state fMRI to measure functional connectivity changes after acute ethanol administration. The results from prior studies led to the expectation that increased functional connectivity would be observed after acute ethanol administration between reward regions and sensory processing centers. Additionally, previous work has shown that acute ethanol increases activity in reward regions, so it is also expected that functional connectivity would change as well, however the functional connectivity changes in reward regions after acute ethanol exposure have yet to be examined. Accordingly, this study measured functional connectivity changes after acute ethanol administration in four regions of interest in the reward and sensory areas: the nucleus accumbens, the ventral tegmental area, the caudoputamen, and the visual areas.
Chapter 2: Methods

2.1. Subjects

The subjects used were male Long Evans rats ranging from 264 – 275 g. The rats were naive, meaning that they had no prior exposure to ethanol or MRI scanning. Eight rats were used in total in two groups of four: a control and an experimental group.

2.2. Acclimation

Acclimation is a procedure designed for the rats to become accustomed to the methods used for MRI scanning. This procedure benefits the rats by allowing them to slowly adjust to the restraint methods, and benefits the researcher by ultimately training rats to move less in the scanner which improves data acquisition. Both the experimental and control rats were acclimated in the same manner. Acclimation is a seven-day procedure: on the first day the rats are restrained for fifteen minutes, thirty minutes on the second day, forty-five minutes on the third day, and sixty minutes on days four, five, six, and seven. Below is a protocol describing the details of the acclimation procedure:
1. Twenty to thirty minutes before restraining the rat, remove the rat from its cage and apply the local anesthetic lidocaine to both sides of the rat’s cheeks and above its nose, shown in Figure 7. Since these areas are in direct contact with the restrainers used later, the cream minimizes the rat’s discomfort. Place the rat back in its cage.

![Figure 7. Lidocaine application (A) Lidocaine cream (B) Areas on rat’s face to apply cream](image)

2. Turn on the oxygen valve by the oxygen gasket by turning the lever from the horizontal to vertical position, as shown in Figure 8.

![Figure 8. Oxygen valve, highlighted in orange](image)
3. Turn the dial on the isoflurane container from off to the 4 setting, seen in Figure 9.

![Figure 9. Isoflurane container in off position](image)

4. Turn the flow rate knob from 0 to 1 liters per minute, shown in Figure 10.

![Figure 10. Flow rate meter and knob, highlighting the knob and 1 liter per minute mark](image)

5. Rotate the valve for the anesthetization box to the open position, parallel to the tubing, shown in Figure 11.

![Figure 11. Tubing valves, highlighting the anesthetization box valve in blue in the open/parallel position and the nose cone valve in orange in the closed/perpendicular position](image)
6. After putting paper towels on the bottom of the anesthetization box, take the rat from its cage and place it in box, shown in Figure 12. Keep the rat in the box for about 5 to 8 minutes, waiting for the rat to become completely unconscious. Signs of unconsciousness are slightly labored breathing and a flaccid body and tail. Monitor the rat while it is being anesthetized and remove it from the box once unconsciousness is apparent to avoid over-anesthetization.

![Figure 12. Anesthetization box with paper towels on the bottom](image)

7. Turn on the nose cone valve and turn off the anesthetization box valve labeled in Figure 8. Take the rat out of the box and lay it on the table ventral side up, shown in Figure 13. Immediately place the nose cone over the rat’s nose.

![Figure 13. Rat laying ventral side up with nose cone on](image)

8. Cut about 5 inches of the 2 cm wide tape for wrapping the rat’s arms and legs. While keeping the nose cone on, wrap the tape once around one arm/leg, then wrap multiple times around both arms/legs, shown in Figure 14. Fold over about 1 cm of the end of the tape piece over itself on the sticky side so that the tape is easy to remove after the acclimation.

![Figure 14. Rat with arms and legs taped](image)
9. Flip the rat over so that it is laying dorsal side up, while keeping the nose cone on. Choose a primary head restrainer from the lab bench that fits your rat, this may require some trial and error. An example is shown in Figure 15.

![Figure 15. Primary head restrainer](image)

10. Push the restrainer over the rat’s head with the curved end of the restrainer facing downwards. This may require slightly forceful pushing, but if there is too much resistance, switch to a larger head restrainer so the rat is not injured. Ensure that both the rat’s top and bottom sets of teeth are over the bite bar, seen in Figure 16 (A). If not, use the tweezers to move the rat’s teeth over the bar.

When the restrainer is on, adjust the rat’s orientation so its head and shoulders are straightly aligned with the restrainer, see in Figure 16 (C). Alignment is important so the rat is comfortable, the rat does not escape, and so quality images can be acquired.

Try to keep the nose cone on as much as possible when placing the primary head restrainer to prevent the rat from awakening.

![Figure 16. Placement of the primary head restrainer (A) Rat with teeth over bite bar (B) Tweezers for teeth manuvering (C) Rat with primary head restrainer on](image)
11. Take a model coil from the lab bench, shown in Figure 14A and 14B. Slide the rat’s head with the primary head restrainer into the model coil from the angle shown in Figure 17. Slide until the primary head restrainer can no longer move forward.

Again, try to keep the nose cone on as much as possible to prevent the rat from awakening.

Figure 17. Insertion of primary head restrainer into model coil (A, B) Angle of insertion (C) Rat in both primary head restrainer and model coil
12. Once the head restrainer and coil are together, ensure that the front screws are aligned with the holes in the primary head restrainer, then tighten them as much as possible. Next, align and tighten the back screws, shown in Figure 18. Because the back screws push on the rat’s head, do not tighten them as much as possible, but instead only tighten to the point of feeling resistance.

Lastly, drop down the nose piece so that it is putting a small amount of pressure on the rat’s nose, shown in Figures 18 and 19. Then tighten the nose piece screw so the nose piece stays in place.

Figure 18. Screw positions: highlighted in orange are the front screws, highlighted in blue are the back screws, highlighted in pink is the nose piece (A) Front view (B) Top view

Figure 19. Nose piece positioning
13. Place the rat into body tube, tail first, as shown in Figure 20. When putting in the rat, ensure that the front paws are tucked backwards under the rat’s body towards the tail to limit the rat’s ability to move, shown in Figure 21. Align the screws on the bottom of the model coil, shown in Figure 22, with the screw holes shown in Figure 20. Slide the screws into place and tighten them as much as possible. Once the rat is in the body tube, wait for it to wake up before proceeding (5-10 minutes). This is so the rat can have a conscious awareness of their condition to simulate the actual scanning.

14. Place the rat with body tube into the acclimation box head first, shown in Figure 23. Close the doors of the cabinet so the environment is dark to simulate the scanner.
15. Turn on the mp3 player connected to the acclimation box and play the MRI recording, shown in Figure 24.

![Figure 24. Mp3 player with MRI recording](image)

16. Set a timer for the amount of acclimation time required for the day in the procedure you are acclimating on. After the timer has gone off, remove the rat from the acclimation box, and place the rat with body tube back in its cage. Unscrew all of the screws on the model coil and the rat will escape by itself from the setup. Remove the tape from the rat’s limbs.
2.3. Scanning

After the rats were acclimated in the seven-day procedure, scanning began. Both the control and experimental groups were scanned in the same manner. The same restraining procedure was used for scanning as in acclimation except that a radio frequency (RF) -head coil was used in place of the head restrainer. The restrained rat’s body was then covered by a shield, the coil was connected to the scanner, and the set-up was taped into a tube to fit into the scanner to reduce motion, shown in Figure 25.

![Rat set-up for scanning](image)

**Figure 25. Rat set-up for scanning**

Once the rat regained consciousness, he was placed in the scanner. Tuning of the frequency and matching of the impedance of the RF coil was then performed by adjusting the tuning and matching capacitor. Tuning and matching are required because each subject and subject set-up have variable impedances and frequencies and without optimal tuning and matching, image results can appear with high noise and low contrast. A localizer scan was then performed to observe the position of the brain in the scanner and adjust to obtain optimal positioning, shown in Figure 26 (A). In Figure 26 (B), the selection of regions to be scanned is shown, with the red region as an example region not desired for scanning.
Scanning took place in three continuous parts: first a 10 minute, 600-repetition echo-planar imaging (EPI) baseline scan, second a 30 minute, 1800-repetition EPI scan during which the injection was given, and third a 20 minute, 1200 repetition T1-weighted scan to differentiate between anatomical structures.

2.4. Injection

A solution of 0.75 g/kg of ethanol in 2 ml/kg was injected into each rat of the experimental group while the rat was under anesthesia (isoflurane). The control group received the same volume (2 ml/kg) of an isotonic saline solution. The solutions were prepared using a sterile solution preparation technique:

**PPE:**

- Thoroughly wash hands, nails, and arms up to the elbow with antiseptic cleansing agent and water for 30 seconds
- Gloves sprayed with isopropyl alcohol 70% and rubbed thoroughly
Air flow hood use:

- Use isopropyl alcohol 70% to clean the compounding area prior to the beginning of each shift
- Allow alcohol to remain for at least 30 seconds
- Supplies arranged aseptically within the hood to minimize air flow turbulence
- All sterile compounding inside the hood should take place at least six inches into the hood to prevent contamination from the room air

Solution preparation:

- Swab all additive containers with 70% isopropyl alcohol
- Swab the top of the vial with an alcohol prep using firm strokes in a unidirectional sweeping motion at least 3 times
- Open needle packages within hood to maintain sterility

Lidocaine was applied to the tail of the rat and left for fifteen minutes. The tail was secured by Parafilm, shown in Figure 27 (A), and placed in lukewarm water for three minutes to dilate the tail’s blood vessels. A needle coated in heparin was inserted into about 2/3 from the base of the rat’s tail in a peripheral vein, which lie just below the skin on each lateral side of the tail. Needle placement was confirmed by observing blood flow through the catheter lumen, shown in Figure 27 (B). The syringe with the measured amount of either ethanol or saline was then attached to the needle and the set-up was securely taped to the tail, shown in Figure 27 (C).

Ten minutes into the second portion of the scan, after twenty total minutes of scanning, the solution was injected steadily over two minutes.
2.5. Processing

The data was segmented into three sections: before injection, during injection, and after injection, and the data obtained during the injection was removed. The scans were aligned in all degrees of freedom against a standard anatomy scan using the MIVA program, shown in Figure 28. The background (grey image) was set to a standard Long Evans anatomical scan and the foreground (yellow to red image) was set to the subject being aligned. Anatomical landmarks were aligned between the standard image and the subject in the axial, sagittal, and coronal views by adjusting the translation and rotation of the subject’s brain. Then a preprocessing MATLAB script was used on each EPI scan. This script performed motion correction using the software Statistical Parametric Mapping 12 (spm12) and temporally filtered the time
series of each voxel with a bandpass filter using a lower cutoff frequency of 0.01 Hz and an upper cutoff frequency of 0.1 Hz. The scans were spatially smoothed by averaging pixels with their neighbors to remove high frequency noise, using a Gaussian filter of 0.75 mm full width at half maximum.

A seed based/region of interest analysis was then performed with two additional MATLAB scripts. The first script divided the brain’s voxels into 58 groups called seeds or regions of interests. The BOLD time course of the voxels inside each region was averaged. The time course correlation between each pair of regions of interest, or between a region of interest and a voxel, for each subject was then calculated. The second script averaged the correlation of each region of interest to every other region of interest between subjects. This created maps for each region of interest that show the connectivity between that region of interest and all of the other regions. Two sets of maps were created based on the original segmenting of the data: a set of baseline maps from the data taken before the injection, and a set of maps from the data taken after the injection. For a specific region of interest, these sets of maps were compared to identify differences in connectivity before and after injection.

![Figure 28. MIVA alignment of scans](image)

Figure 28. MIVA alignment of scans
Chapter 3: Results

The aim of the project was to examine the acute effects of ethanol on brain functional connectivity, specifically in reward regions and sensory processing centers. Based on the literature reviewed in section 1.4., four seeds were chosen to examine the changes in functional connectivity between the baseline and post-injection scans: the visual areas (VIS), the nucleus accumbens (NAc), the ventral tegmental area (VTA), and the caudoputamen (CPu). These regions are highlighted in Figure 29 on a standard rat brain anatomy as a reference for the results. As described in section 1.4., the literature review led to the prediction that connectivity would be increased between the visual areas and reward regions (nucleus accumbens, ventral tegmental area, and caudoputamen). Additionally, previous work has showed increased activity in reward regions (nucleus accumbens, ventral tegmental area, and caudoputamen), leading to the question of what functional connectivity changes occur in these regions.

Figure 29. Locations of anatomical regions referenced on standard rat brain

Scanning took place in three continuous parts: a 600-repetition EPI baseline scan, an 1800-repetition EPI scan during which the injection was given, and a 1200 repetition T1-weighted scan. The data was segmented into three sections: before injection, during injection, and after injection. Each rat’s brain was aligned against a standard anatomy, then motion correction, spatial smoothing, and temporal filtering were performed. An example result after this preprocessing is shown in Figure 30. Each image is the BOLD signal in a coronal slice of a rat brain acquired at one time point, staring in the top left and proceeding left to right to the bottom right. The brain was then divided into 58 seeds and the BOLD time
course of the voxels inside each seed was averaged. The time course correlation between each pair of regions of interest, or between a region of interest and a voxel, for each subject was then calculated. The correlation of each seed to every other seed was then averaged between subjects, allowing for the creation of maps showing this correlation between seeds.

Figure 30. Example of preprocessed results showing the BOLD signal at one time point for one subject, units of percent signal change from baseline

Figure 31 shows correlation seed maps of the visual areas. The seed maps show the temporal correlation in connectivity between a seed and the rest of the brain. Each image is a different coronal slice of the rat brain, staring in the top left and proceeding left to right to the bottom right. This map shows the connectivity between the visual areas and the rest of the brain, with hotter colors indicating a positive temporal correlation of connectivity to the visual areas, cooler colors indicating a negative temporal correlation of connectivity to the visual areas, and no color indicating no correlation to the visual areas. Figure 31 (A) shows the resting-state connectivity observed in the baseline scan, before any ethanol was administered. Figure 31 (B) shows the resting-state connectivity after the ethanol was injected, allowing for comparison between the baseline and post-injection states of the rat. Comparison between the baseline and post-injection conditions showed an increased connectivity, a positive temporal correlation, to the nucleus accumbens between the two conditions. Also to note is that minimal/no change in connectivity
was observed between the visual areas and the caudoputamen or ventral tegmental area, since in both maps, these regions showed no correlation to the visual areas.

Figure 31. Correlation seed map of the visual areas, highlighting the nucleus accumbens, caudoputamen, and ventral tegmental area (A) baseline (B) post-ethanol injection

The next region investigated was the nucleus accumbens, shown in Figure 32. Like Figure 31, each image is a different coronal slice of the rat brain and the map shows the connectivity between the nucleus accumbens and the rest of the brain. Figure 32 (A) shows the resting-state connectivity to the nucleus accumbens observed in the baseline scan and Figure 32 (B) shows the resting-state connectivity after the ethanol injection. Comparison between the baseline and post-injection conditions showed a decrease in connectivity between the nucleus accumbens and the thalamus (THAL), hippocampus (HIPP), and amygdala (AMGY). While the baseline map shows a positive correlation to the thalamus, no
correlation is seen after the injection, indicating a decrease in connectivity. Similarly, with the hippocampus, while the baseline map shows a positive correlation throughout the hippocampus, almost no correlation to the nucleus accumbens is seen in the post-injection map. The amygdala and nucleus accumbens also show a decrease in correlation between the conditions indicated decreased connectivity, and the correlation appears to change from negatively temporally correlated to positively correlated.

Figure 3. Correlation seed map of the nucleus accumbens, highlighting the thalamus, hippocampus, and amygdala (A) baseline (B) post-ethanol injection

Another region of the reward system, the ventral tegmental area, was analyzed next. The result is shown in Figure 33, depicting the connectivity between the ventral tegmental area and the rest of the brain. Figure 33 (A) shows the resting-state connectivity to the ventral tegmental area observed in the baseline scan and Figure 33 (B) shows the resting-state connectivity after the ethanol injection. While the
baseline map shows a positive temporal correlation between the ventral tegmental area and the amygdala, the post-injection map shows almost no correlation between these regions. This result suggests that after acute ethanol administration, the connectivity between the ventral tegmental area and amygdala is decreased.

Figure 3. Correlation seed map of the ventral tegmental area, highlighting the amygdala (A) baseline (B) post-ethanol injection

The last region investigated was another component of the reward system, the caudoputamen, shown in Figure 34, depicting the connectivity between the caudoputamen and the rest of the brain. Figure 34 (A) shows the resting-state connectivity to the caudoputamen observed in the baseline scan and Figure 34 (B) shows the resting-state connectivity after the ethanol injection. Comparison between the baseline and post-injection conditions shows that while the baseline map has a mostly positive temporal
correlation between the caudoputamen and hippocampus, the post-injection map shows almost no correlation to this region. This result indicates that connectivity between the caudoputamen and hippocampus regions decreases after acute ethanol administration.

![Figure 34. Correlation seed map of the caudoputamen, highlighting the hippocampus](image)

(A) baseline  
(B) post-ethanol injection

In Figure 35, a summary of the connectivity changes between baseline and post-injection seed maps of the visual areas, nucleus accumbens, ventral tegmental area, and caudoputamen are shown. The figure shows that the most change was observed in the nucleus accumbens. In general, decreases in connectivity were observed between the reward system (nucleus accumbens, ventral tegmental area, and caudoputamen) and other regions of the brain (thalamus, hippocampus, and amygdala). However,
increased connectivity was observed between a sensory processing area, the visual areas, and a component of the reward system, the nucleus accumbens.

![Diagram of neural connectivity](image)

**Figure 35. Summary of connectivity changes between baseline and post-injection seed maps of visual areas, nucleus accumbens, ventral tegmental area, and caudoputamen**

A concern with this type of study is that the results shown in Figure 31-34 are not unique changes in connectivity between the regions indicated, but are in fact a systematic bias of the data acquired between the two conditions. To address this concern, the correlation seed maps of the auditory areas are shown in Figure 36 as a control. Figure 36 (A) shows the resting-state connectivity to the auditory areas observed in the baseline scan and Figure 36 (B) shows the resting-state connectivity after the ethanol injection. If the results were a systematic bias, the connectivity changes observed would be present throughout the rest of the brain, and not be unique connections between regions. Unlike Figure 31-34, comparison between the baseline and post-injection conditions of Figure 36 shows that the connectivity between the conditions did not change in regions that changed connectivity in the previously shown
maps. The auditory areas were chosen as an example, but other seeds such as in the medial nuclei of the dorsal thalamus, the orbital area, and the supplemental somatosensory area also did not show connectivity changes.

Figure 36. Correlation seed map of the auditory areas (A) baseline (B) post-ethanol injection
Chapter 4: Discussion

The goal of the project was to provide a better understanding of the effects of alcohol on the brain by determining the acute effects of ethanol on brain functional connectivity, specifically in reward regions and sensory processing centers. Previous studies indicate that increased connectivity would be observed between reward regions and sensory processing centers, and in this project, the connectivity between the visual areas and reward regions (nucleus accumbens, ventral tegmental area, and caudoputamen) was examined. Prior work has also shown increased activity in reward regions, however the functional connectivity changes in reward circuits after acute ethanol exposure have not yet been studied, so to examine this from a functional connectivity perspective, the connectivity changes in the nucleus accumbens, ventral tegmental area, and caudoputamen were measured.

As seen in Figure 31, the visual areas showed an increase in connectivity to the nucleus accumbens between the baseline scans and post-injection scans. This result agrees with previous studies that have shown after acute exposure to alcohol, connectivity is increased between sensory processing centers and reward centers, even though connectivity was not increased to other reward areas, the ventral tegmental area or caudoputamen [8]. This result may help explain the associative learning of addictive substances and behaviors, associating cues and situations to rewarding effects.

Alcohol intoxication can produce the effect of memory impairment and since the hippocampus is known to be involved in autobiographical memory formation, the disruption of hippocampus activity during alcohol intoxication has been used to explain the memory impairments [15]. These disruptions in the hippocampus may include the results observed, decreased connectivity with the nucleus accumbens and caudoputamen, seen in Figure 32 and Figure 34.

As the amygdala is known to regulate emotional behavior, including anxiety, and is thought to play a role in the decreased fearfulness and stress relieving effects of alcohol [16]. In chronic alcohol users, amygdala volume is known to decrease [17]. The connectivity changes between the amygdala and the reward system, the nucleus accumbens in Figure 32 and ventral tegmental area in Figure 33, may be correlated with these effects.
Figure 32 shows decreased thalamus to nucleus accumbens connectivity after ethanol exposure. This result may help explain previous studies that have shown that ethanol at moderate concentrations can inhibit thalamocortical relay neurons [18]. Additionally, thalamus and nucleus accumbens activity has been shown to be involved in the addictive properties of other drugs, and the connectivity changes observed here may be correlated with this effect [19].

In Figure 36, seed maps of the auditory areas area shown as a control. These maps do not show connectivity changes in the areas that changed in the other maps. Multiple other seed maps of other brain regions follow this example of minimal/no changes in connectivity, supporting the argument that the changes observed in the four regions examined were specific changes to those brain regions, and not a systematic bias of the data acquired between the two conditions.

Some authors who have studied the acute effects of alcohol using fMRI have warned about interpretations of the results. Because of alcohol’s vasoactive properties, changes in cerebral perfusion may affect BOLD signal in ways not correlated to neural activity, such as a globally altered T2* signal or effects of ethanol and metabolites on neural firing. However, a global signal alteration would not affect connectivity changes in specific regions, and the results display specific connectivity changes between specific brain regions, a systematic change of connectivity to a specific region was not observed. Also, in interpreting functional connectivity results, authors emphasize that changes in brain activity cannot be interpreted as directly causal to behavioral changes, but may just be a correlative biological marker of changes [8]. Caution is advised in drawing significant conclusions from these results until more data is obtained, due to the small number of subjects scanned and the removal of data segments due to motion, resulting in a small data set. Based on the previous work of other resting-state fMRI studies including a similar study with on acute nicotine injection, at least twenty rats in each group (experimental and control) are needed to obtain statistically significant results (p-value less than 0.05). Typically, a t-test is performed between the baseline and conditional correlation data for each seed with a significance level of 0.05, allowing for determination of the significant connectivity changes for each seed.
In conclusion, acute ethanol administration was seen to produce effects on the conscious brain throughout the reward circuits and visual areas which may help explain some of the effects of acute alcohol intoxication. In particular, our data showed increased connectivity between reward and sensory processing centers; decreased connectivity between the thalamus and nucleus accumbens; as well as decreased connectivity between the hippocampus/amygdala and reward circuits.
Chapter 5: Future Directions

In the future, the experiment should be repeated with more subjects to interrogate the validity of results and to improve the statistical significance of the results. The same experiment could be repeated with subjects who have prior exposure to ethanol to compare the effects of ethanol after a first exposure and after repeated exposures. Additional experiments could also be performed with an altered design, such as using the same methods to understand the acute effects of other drugs such as nicotine or cocaine, or pharmaceutical agents. The results could be used in conjugation with a chronic study of ethanol use to understand the differences between acute and chronic use, as well as to see the differences in acute use between rats that do and do not regularly self-administer ethanol. Future work could also explore the questions: what are possible mechanisms of action of treatments for acute alcohol intoxication, and can subjects at risk of alcohol use disorders be differentiated by their response to acute alcohol exposure before using alcohol chronically.
REFERENCES


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Penn State Lion’s Pantry, President, February 2013 – December 2016
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Penn State University Health Services, Clinic Intern, September 2015 - April 2016
- Independently completed intakes of patients including taking vitals and asking medical history questions
- Observed and assisted nurses and providers while they saw patients and performed procedures
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Penn Stater Hotel and Conference Center, Banquet Server, May 2015 – August 2015
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