Establishing a Novel Open Field for Characterizing IL-BLA Circuitry Neurobehavior

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Abstract

Mental disorders are a prevailing issue that impact millions of people across the globe yet aren't well understood. Neuroscientists and psychiatrists have found that these disorders stem from abnormalities within the neural circuitry of the brain. As these circuits are investigated, we can take our knowledge of how key circuits are involved in mental health and develop brain stimulation treatments for disorders. Using rats with neural implants in the infralimbic cortex (IL) and basolateral amygdala (BLA) as translational models of mental illness, we research brain stimulation methods to increase LFP synchrony between these regions. We determined that a new system of measuring neurobehavior was needed for bridging the effects of circuit specific stimulation and coinciding changes in test subject behavior. To do thicoherences, I constructed a novel open field that can record neural data, deliver stimulation, and classify behavior based on position and movement analysis.

Background

Illnesses such as depression, anxiety, and OCD can be traced to abnormal communication within neural circuitry between specific brain regions. In human neuroimaging, it has been shown that abnormal communication within mPFC-amygdala circuitry is responsible for mental disorders based on mood and anxiety[1]. In both human and rodent studies, it has been shown that fear learning and behavior are coordinated with theta brainwave frequencies (4-8Hz) in circuitry between the medical prefrontal cortex (mPFC) and the amygdala. In humans, it has been shown that the dorsal mPFC is active in fear expression and the ventral mPFC is responsible for fear extinction. In translational research with rodents, these regions exhibit the same role as the prelimbic cortex (PL) and the infralimbic cortex (IL) respectively[2]. With this knowledge of circuitry and its involvement in certain mental disorders, our goal is developing brain stimulation treatments that can remedy dysfunctional communication between these brain regions

In our lab, we focus our attention on the circuitry between the IL and the BLA in rats with electrodes implanted in each region. These electrodes measure the cumulative average of regional neuron activity, known as the local field potential (LFP). The LFP is a powerful tool for measuring regional activity and synchrony across regions. Studies have shown that the LFP oscillates to produce brainwaves and influences the excitability of neurons[3]. We can manipulate the LFP through both fixed-interval and oscillation phase dependent delivery of electrical stimulation. The motivation behind LFP manipulation is built on the theory of communication through coherence. Coherence is defined in neuroscience as a measure of the LFP synchrony between two brain regions[4]. It is theorized that neural circuits operate with communication through coherence, meaning cross-regional communication is dependent on synchrony of the LFPs. By recording the LFP in each region and using brain stimulation to synchronize the two LFPs, we can effectively increase the coherence and communication between targeted brain regions.

The brain stimulation methods we use are based upon real-time brainwave phase and frequency calculation algorithms. This is done through Python and MATLAB scripts in combination with recording software Open EPHYS. By obtaining real-time oscillation data from the IL and BLA, we are then able to use closed-loop stimulation to deliver electrical input to the BLA at a specified oscillation phase of the IL. Opposed to open-loop which simply delivers stimulation at a specified time interval, closed-loop allows for precise synchrony of the IL-BLA circuit.

With the tools for modulating circuity coherence involved in mental disorders, our next step is to analyze how increasing IL-BLA coherence affects behavior. It was decided that we would use an open field setup for answering this research question. An open field test is an enclosed environment with a defined center and periphery that is used to evaluate defensive behaviors of test subjects. Subjects can be categorized as 'defensive' or 'non-defensive' based on the percentage of time that they spend in either the center or periphery of the environment. The same type of open field experiment was conducted in 2013 at Columbia University with mice. These researchers analyzed the relationship between activity in the open field and theta-frequency neural activity between the basolateral amygdala and the medial prefrontal cortex[5]. Mice were conditioned to a dark environment for three days and then introduced to a brightly lit open field. These test subjects were classified as 'anxious' in the Open Field if they spent less than 10% of their time within the center of the chamber. The Columbia University study reported clear differences in mPFC-BLA neural activity between categories of mice.

However, the classification of 'anxious' based on activity in the open field is not accurate for using rodent test subjects. Anxiety is a mental process that is associated with higher level cognition and is too complex for rodents[6]. A better classification, which we will be using throughout our open field testing, will be defensiveness. It was hypothesized that as IL-BLA coherence increases in a test subject, they will exhibit less defensive behavior and be less inclined towards the chamber periphery. As a prospective biomedical engineer, I was left with the task of designing and constructing a new open field chamber so that we can investigate the neural mechanisms of fear and safety in freely moving rats.

Objectives

The task of constructing a new experiment chamber came with a handful of basic requirements that needed to be met so that we could conduct Open Field experiments. The biggest necessities that I needed to meet were having an appropriately sized area for the rat to move around in during experimentation, being able to take video recordings of the rat, and being able to simultaneously record neural data. From here, I was also able to incorporate additional features that neuroscience students in our group felt were important as well as my own themes for the design of the chamber.

Table 1: *Needs of the experiment setup and reason behind each requirement.*

User Need	Rationale
Chamber size, center, periphery.	To conduct open field experiments, clearly defined regions are needed to determine when a subject exhibits defensive behavior.
Chamber circular area	A circular open field prevents a subject from simply hiding in a corner rather than having to do some exploration of the environment.

Camera and video analysis software	A camera is needed to record the rat's behavior within the open field and analysis software to produce results.
Neural recording and analysis	Hardware such as neural implants and connecting wires are needed to use the software OpenEPHYS which records electrophysiological data.
Cleaning ease, waste resistant	For reusability, the chamber cannot be damaged by solid or liquid waste produced by rats and must be relatively easy to clean.
White floor	A dark figure (the rat) against a white or light-colored background work best for using video analysis software.
Movability	The chamber will be large and being able to easily move it will be critical in its usability.
Modularity	To account for future directions research may take, a key feature to implement in design is how the chamber can be modulated to fit various experiment needs.
Construction Efficiency	To ensure construction is sound and minimal error is made; including the use of GD&T and prototyping.
Cost minimization	Saving money to reach an end-goal while still paying attention to detail is always a good business practice.

Materials and Methods

Figure 1: Early Prototype of the Open Field chamber. A cylinder constructed with cardboard and duct tape. A light source and camera are suspended from a coat rack and a rat is inside the chamber for preliminary testing. Testing was also conducted in the Behavioral Room to minimize environmental noise sources during recording.

The first step in the process of building a new chamber was determining what design aspects would work best for meeting the outlined needs of the chamber. I accomplished this through constructing prototypes of the chamber and CAD modeling the final design. During prototyping, I used basic material such as cardboard and duct tape to get a feel for what would work best in characterizing the innate defensive behavior of rats.

By doing this prototyping, our group determined that a cylindrical model would be the most appropriate so that test subjects can't simply hide in a corner. The chamber also needed a diameter of approximately 30 inches. We determined that something would need to be suspended above the chamber to hold crucial instruments such as a camera, a light source, and a commutator for neural recording and stimulation wires. From here, I was able to take these design specifications and model them within SolidWorks (Figure 2). In the final model, I decided that a clear plastic cylinder on a square base would work the best for both functionality and modularity.

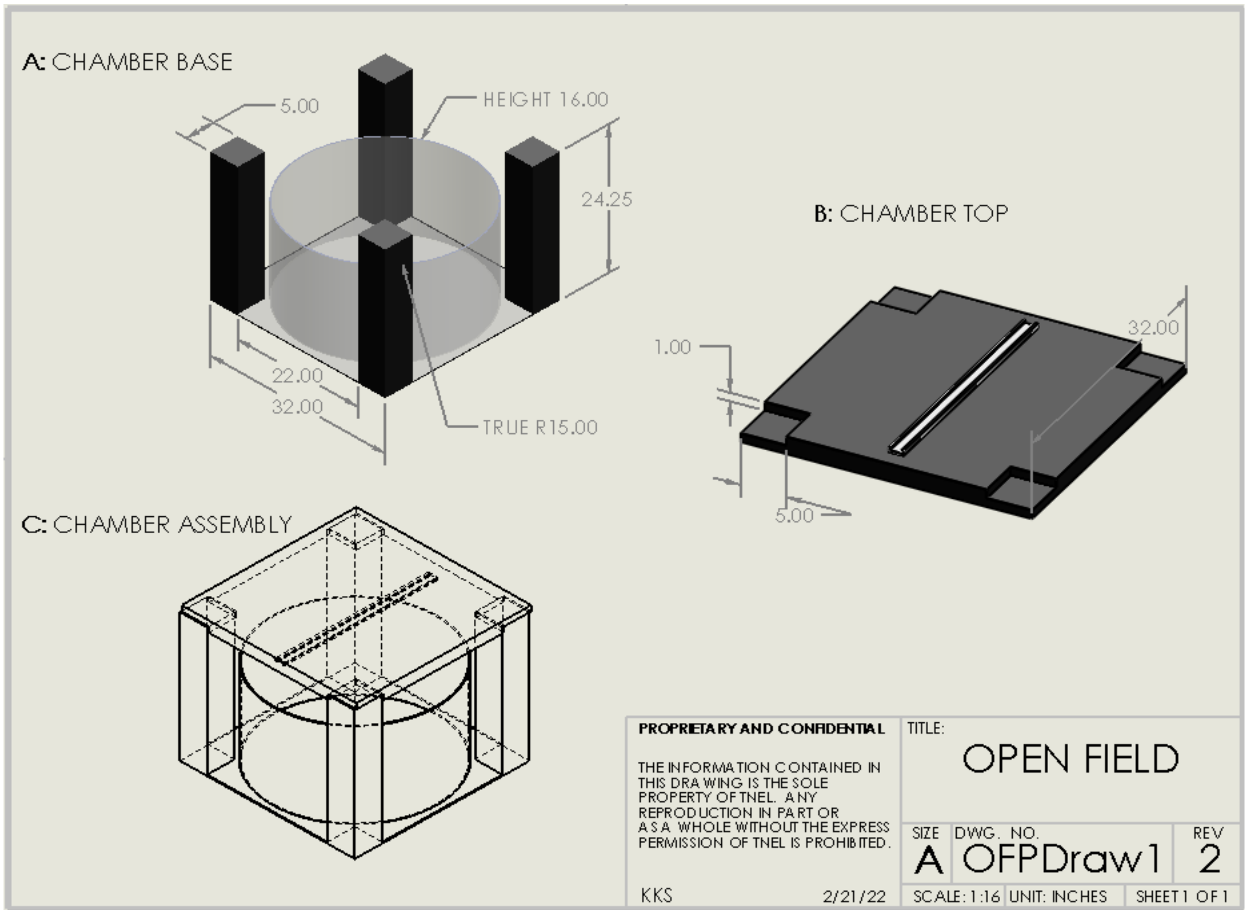

Figure 2: *SolidWorks assembly drawing of the open field.* ***A)*** *The base of the chamber; a clear cylinder placed on a square base with side supports in each corner.* ***B)*** *The proposed chamber top with grooves to fit the base's supports and a built-in track system for holding technology needed for experimentation.* ***C)*** *The complete proposed chamber with the base and top.*

Prior to construction, we decided that the top was not necessary on the enclosure and that the best design for mounting the experimental accessories (i.e., a 'track system') would be a simple rail. Once the final design of the chamber was decided, construction began in Rapson Hall at the University of Minnesota. Rapson Hall, home of the College of Design, houses a materials shop with a wide variety of supplies and a workshop with a healthy spread of hardware and fabrication equipment. The material used for the complete construction of the chamber included HDPE plastic, PETG plastic, acrylic, chipboard, poplar wood, screws, wheels, and wood glue.

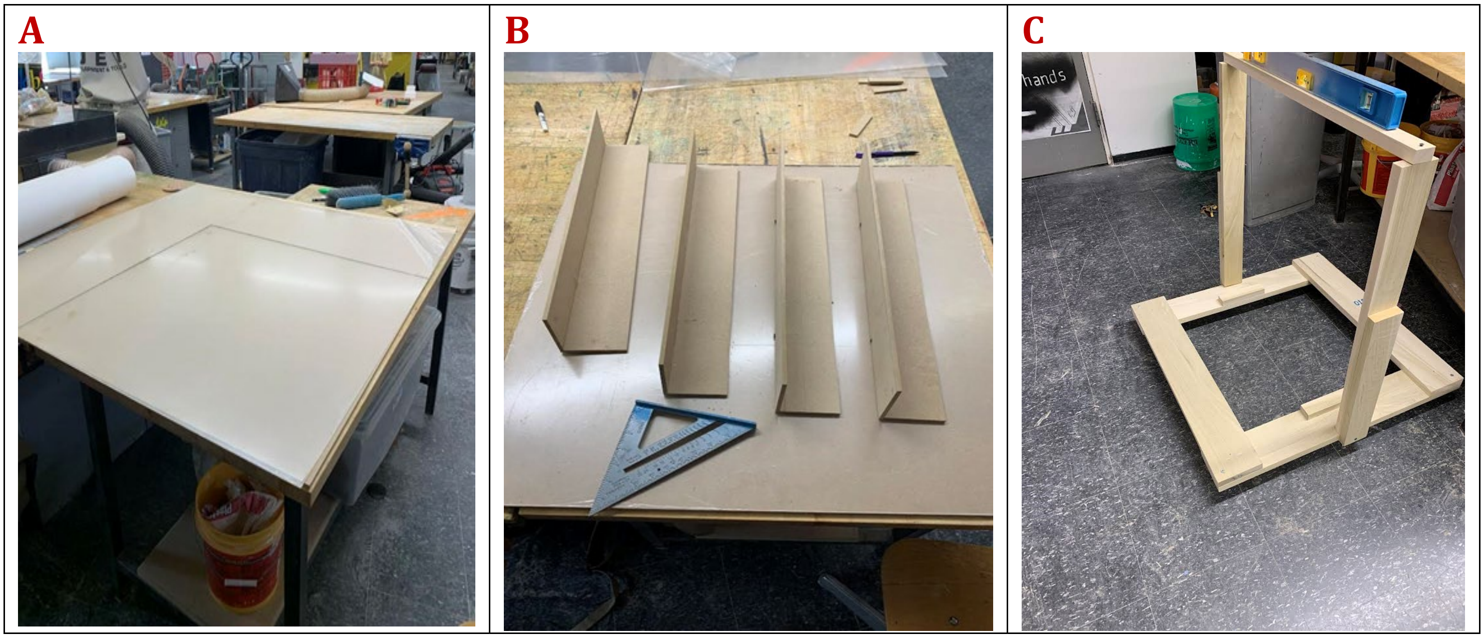

Figure 3: *Construction of the open field in Rapson Hall.* ***A)*** *HDPE plastic and chipboard used for constructing the chamber floor.* ***B)*** *Fully cut HDPE plastic and chipboard for chamber floor as well as additional chipboard assembled into side supports.* ***C)*** *Chamber outer frame and rail constructed from ¾" poplar boards.*

The machining equipment used to complete the construction of the chamber included a table saw, crosscutter, laser cutter, and trimmer. Other basic tools used include a drill with various bits and measuring equipment such as a tape measure, speed square, and level.

Results

The final chamber successfully met all the key features as well as additional features and themes that were identified.

Table 2: *Needs of the experiment setup, rationale, and solutions to each need.*

User Need	Rationale	Solution
Chamber size, center, periphery.	To conduct open field experiments, clearly defined regions are needed to determine when a subject exhibits defensive behavior.	The chamber has a 30" diameter inner cylinder and a 32" outer acrylic boundary. These sizes are sufficient for video analysis software AnyMaze to define a center and periphery given the size of the rat.
Chamber circular area	A circular open field prevents a subject from simply hiding in a corner rather than having to do some exploration of the environment.	30" diameter cylinder allows for circular bottom area.
Camera and video analysis software	A camera is needed to record the rat's behavior within the open field and analysis software to produce results.	A camera mount was printed for the top rail, and AnyMaze was chosen for analyzing the movement of the rat.
Neural recording and analysis	Hardware such as neural implants and cables are needed to use the software OpenEPHYS which records electrophysiological data.	A commutator was put directly into the top rail and a light blue cable runs from the commutator to the rat's implant. Light blue was needed so that it wouldn't interfere with AnyMaze.

Cleaning ease, waste resistant	For reusability, the chamber cannot be damaged by solid or liquid waste produced by rats and must be relatively easy to clean.	HDPE plastic was used for the floor, which is nonreflective, easy to wipe waste off, and easy to sanitize.
White floor	A dark figure (the rat) against a white or light-colored background work best for using video analysis software.	White construction paper sits underneath the HDPE floor panel. This color can be changed by removing the HDPE.
Movability	The chamber will be large and being able to easily move it will be critical in its usability.	Wheels were mounted onto the bottom frame of the chamber
Modularity	To account for future directions research may take, a key feature to implement in design is how the chamber can be modulated to fit various experiment needs.	The inner plastic cylinder can be removed, and a square area can be used. The floor color can be changed by lifting the plastic floor. The top rail can accommodate a variety of tools using 3D printing.
Construction Efficiency	To ensure construction is sound and minimal error is made, including the use of GD&T and prototyping.	Geometric Dimensioning and Tolerancing (GD&T), also known as worst case scenario tolerancing, was used in all construction aspects; 3D printing, laser cutting, and mechanical assembly.
Cost minimization	Saving money to reach an end-goal while still paying attention to detail is always a good business practice.	Total project cost was approximately $150 dollars, including material and machinery costs.

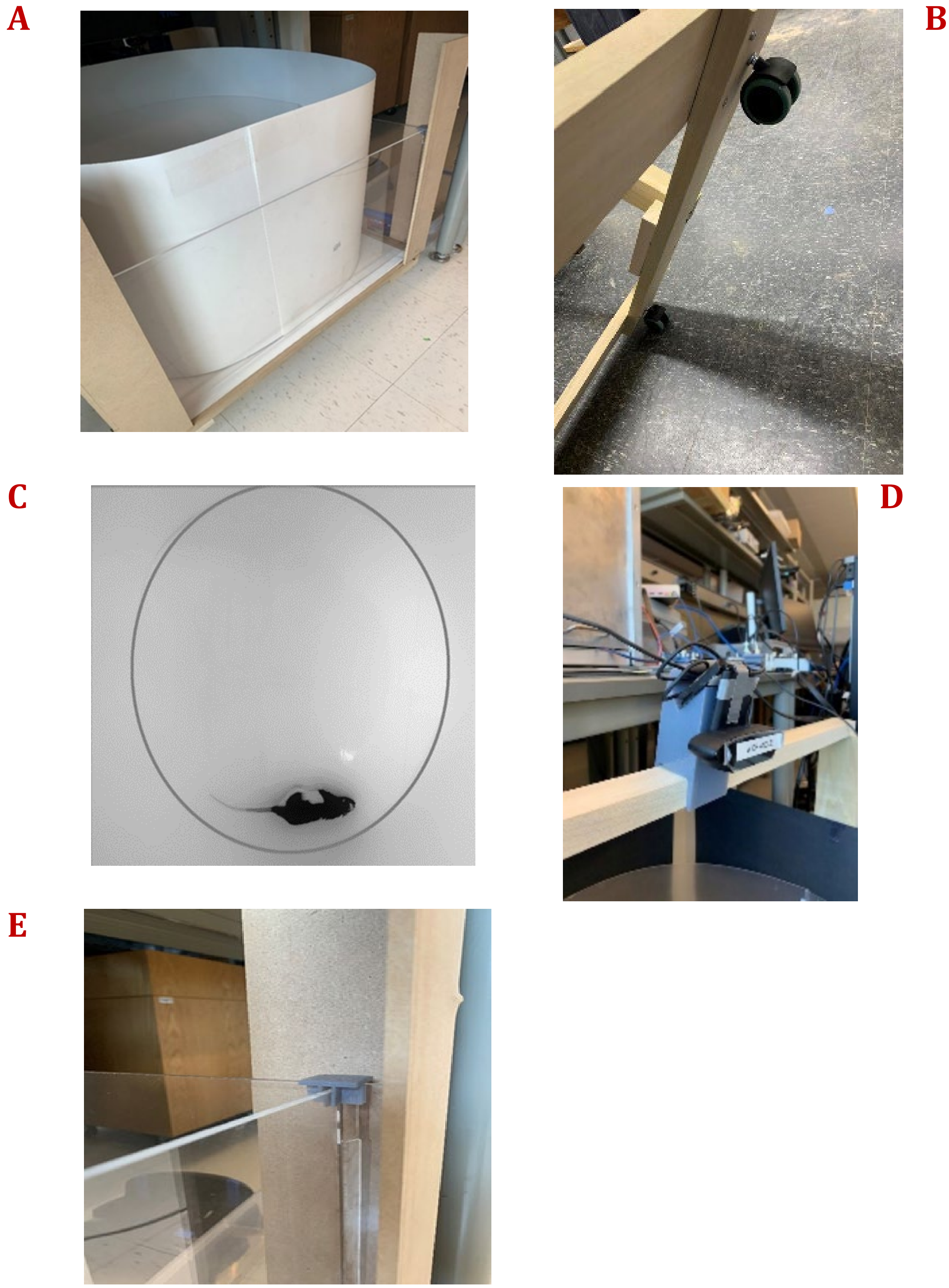

Figure 4: *Completed construction of the open field.* ***A)*** *General view of the chamber with acrylic walls and the inner cylinder.* ***B)*** *Desk chair wheels mounted to the bottom of the outer frame of the chamber for greater mobility.* ***C)*** *Image taken from chamber camera during initial behavioral testing with the new chamber to validate its design. Test subject is shown moving along the periphery of the chamber, which suggests a safety response.* ***D)*** *3D printed wide angle camera holder mounted to the chamber top rail.* ***E)*** *3D printed corner joint for the friction-fit laser cut acrylic outer walls.*

Discussion

The final product successfully met the key needs of the Open Field setup and met the desired additional characteristics that were identified. Most importantly, the bedrock necessities of having a large enough chamber and being able to use video analysis were clearly met as seen in **Figure 8**. Additional characteristics requested by members of our group were also successfully met. The chamber bottom is a non-reflective easy-to-clean clear material which can be taken out of the chamber. The color of the floor can be changed by removing the HDPE floor and placing different construction paper on the chipboard base layer. The frame of the chamber is also on wheels to allow easy mobility to an appropriate environment for conducting experiments such as the Behavior Room. These additional characteristics all allow for ease of use by experimenters and help account for animal and human factors in this type of experiment.

Modularity of the chamber was a priority in my design, and it was well met in all aspects of the final product. The inner cylinder and plastic flooring are all removable to allow for a variety of uses for the chamber. The top rail is a simple design, and holders for technology such as a camera, light source, speaker, etc., can be easily designed and printed with SolidWorks. The thorough prototyping, modeling, and sound construction practices I used in the development of the chamber proved to yield an optimal product without having to cut corners to account for flaws.

Geometric Dimensioning and Tolerancing (GD&T) was engineering design technique construction of the chamber. In this technique, the intended dimensions and their 'worst case scenario' manufacturing tolerances are assumed when creating each part of an assembly. This ensures that when the individual parts form the assembly, no part will be too big or too small for the assembly to work even if the worst possible error occurs in each part of production. This was most important in laser cutting the friction fit acrylic walls and 3D printing the camera holder and acrylic corner joints.

A noteworthy theme that was well met in this project was cost minimization. Disregarding the cost of cardboard used in prototyping and materials for 3D printing, the total cost of constructing my open field chamber was approximately $150. When compared to similar chamber available online for purchase, this is significantly less than what can be spent on a similar product. A similar product would cost around $1300-$2000 (**Figure 5**). For reference, my chamber is a 76.2cm diameter clear cylinder or 81.2cm x 81.2cm clear square based on use.

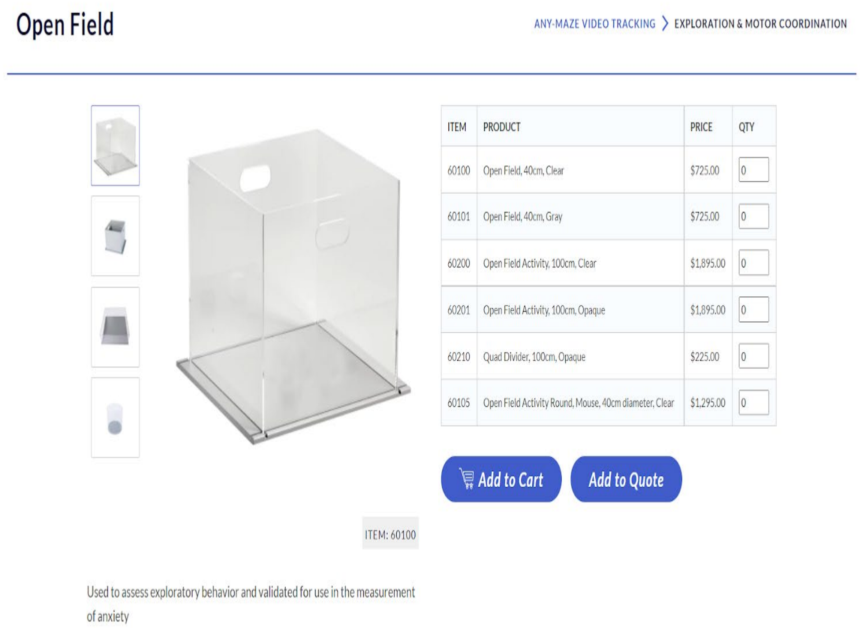

Figure 5: *Open field chamber catalog from AnyMaze.com*

While the construction of the chamber was successful, there are shortcomings to this project. Primarily, the video analysis and neural recording software were not completely paired to analyze position and neural data simultaneously. I was able to configure AnyMaze behavioral tracking software to record the rat's distance from the chamber wall, time stamp each position recording, emit a signal to the acquisition system with every position recording, and verified its accuracy on an oscilloscope. However, further work would be needed to validate that this configuration will properly work with the OpenEPHYS neural recording system. I would also need a custom data analysis script in a program such as MATLAB to combine the data from AnyMaze and OpenEPHYS. The primary reason that this task was not accomplished is that the project was refocused on alternate mechanical prototyping concepts involved in freely moving electrophysiology experiments, such as cable management strategies and a new commutator design. This pivot will enable more consistent recording quality and stimulus delivery as part of the Lab's ongoing work in developing the phase calculation and stimulation algorithm to change brain connectivity. Using proper tools for delivering closed-loop stimulation will support future investigations in neural stimulation for modulating behavioral responses in circuit-based disorders.

I was still able to develop valuable engineering skills throughout this project. I now have an in-depth knowledge of GD&T, prototyping, SolidWorks, 3D printing, and mechanical problem solving that will continue to benefit the lab. These skills are now being applied to projects ranging from tools to keep rats from chewing on cords during experiments to improving aspects of our surgical setup to make working with larger rats easier. One exciting future project is redesigning a nosecone that is used during rat surgeries to deliver anesthesia. Another interesting future project will be designing and constructing tools for conducting empathy research on rats for another researcher.

Conclusion

The objective of this project was to develop a new system of measuring neurobehavior for bridging the effects of circuit specific stimulation and coinciding changes in test subject behavior. I did this by I constructed a novel open field that can record neural data, deliver stimulation, and classify behavior based on position and movement analysis. This new experiment chamber will help develop our understanding of how behavior relates to the neural coherence within infralimbic cortex and basolateral amygdala neural circuitry. This project and future work will continue our research in developing brain stimulation therapies for treating mental disorders.

References

1. Motzkin, J. C. et al. (2015, February 1). *Ventromedial prefrontal cortex is critical for the regulation of amygdala activity in humans*. National Library of Medicine. Retrieved June 27, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4145052/#:~:text=In%20humans%2C%20functional%20imaging%20studies,disrupted%20in%20mood%20and%20anxiety
2. Chen, S. et al. (2021, August 18). *Theta oscillations synchronize human medial prefrontal cortex and amygdala during fear learning*. National Library of Medicine. Retrieved June 27, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8373137/
3. Voytek, B., & Knight, R. T. (2015). Dynamic network communication as a unifying neural basis for cognition, development, aging, and disease. *Biological Psychiatry*, *77*(12), 1089–1097. https://doi.org/10.1016/j.biopsych.2015.04.016
4. Bowyer, S. M. (2016, January 17). *Coherence a measure of the brain networks: Past and present - neuropsychiatric electrophysiology*. Neuropsychiatric Electrophysiology. Retrieved June 27, 2022, from https://npepjournal.biomedcentral.com/articles/10.1186/s40810-015-0015-7
5. Likhtik, E. et al. (2013). Prefrontal entrainment of amygdala activity signals safety in learned fear and innate anxiety. *Nature Neuroscience*, *17*(1), 106–113. https://doi.org/10.1038/nn.3582
6. LeDoux, J., & Daw, N. D. (2018). Surviving threats: Neural circuit and computational implications of a new taxonomy of defensive behaviour.

Nature Reviews Neuroscience, *19*(5), 269–282. https://doi.org/10.1038/nrn.2018.22

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