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Alekhya Pidugu **April 10, 2024**

Developing and Testing a Video-Based System to Explore the Effects of Induced Epilepsy on Social Housing in Adult Male Mice

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and
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Abstract

Developing and Testing a Video-Based System to Explore the Effects of Induced Epilepsy on Social Housing in Adult Male Mice

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Epilepsy, a neurological disorder characterized by disrupted nerve cell activity leading to seizures, impacts millions worldwide. Despite the pivotal role of mouse models in understanding epilepsy, many studies overlook social influences on seizures. This study investigates how induced epilepsy, utilizing the intrahippocampal kainic acid model, alters behaviors in dually housed mice. Mice were paired, marked, and observed for baseline behavior pre-injection and post-injection for thirty days. DeepLabCuts algorithm and Python were employed to track mouse locations relative to the enclosure and each other. Post-injection, epileptic mice spent less time near the exercise wheel, contrasting with healthy mice ($p = 0.0462$). Both cohorts displayed increased proximity to food post-injection, with epileptic mice showing unexpected behavior approaching malnutrition. Cohort 2 showed no significant differences for epileptic mice pre-and post-surgery. For proximity to the water in cohort 1, both mouse 0 and mouse 1 spent more time at the water bottle post-surgery compared to before ($p < 0.0001$ for both). During the evening, significant differences in time spent together were noted, with reduced social ties observed during the second 10 days, potentially indicating the impact of epilepsy on social interactions. Similarly, during the midnight period, significant differences were noted across various intervals, with mice spending more time together after day 20, indicating minimal disruption by the epileptic mouse. In cohort 2, a similar trend was observed with mouse 0 and mouse 1 spending less time together during noon, evening, and midnight 30 days post-surgery compared to pre-KA days. The increasing distance over time suggests reduced interaction frequency, possibly influenced by the seizures of the mouse. Observations of fighting or caretaking behaviors post-seizures underscore the importance of social dynamics in mouse models of epilepsy. The study provides critical insights into the role of environmental factors in epilepsy research and may inform strategies for epilepsy management in both animal models and humans.

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Figures and Tables

Introduction

Epilepsy is a complex neurological condition characterized by its hallmark symptom of seizures. These seizures are defined as an occurrence of symptoms due to abnormal excessive or overstimulated neuronal activity in the brain, leading to a variety of manifestations that significantly impact the lives of individuals (Fisher et al., 2014). Seizures significantly impact individuals by jeopardizing their physical safety, causing emotional distress and social isolation, hindering educational and occupational endeavors, compromising independence, diminishing the overall quality of life, impairing cognitive function, subjecting individuals to medication side effects, and placing stress on caregivers. Seizures can occur when disruptions in the typical synaptic connectivity between neurons result in an excessive propagation of electrical impulses. According to the Centers for Disease Control and Prevention (CDC), epilepsy affects approximately 3.4 million people in the United States, underscoring its significance as a public health concern (Epilepsy Data and Statistics, 2020).

Seizures can be broadly categorized into two main types: focal and generalized (Lopes et al., 2020). Focal seizures, as the name suggests, originate in just one area of the brain and can occur with or without loss of consciousness. These seizures may manifest through a range of symptoms, from emotional changes to physical twitching, depending on the specific brain region involved. Twitching or convulsions typically involve only one part of the body, such as an arm or leg, or specific muscle groups. Sometimes, focal seizures spread to other parts of the brain leading to generalized seizures with a patient displaying sudden falls, muscle spasms, or loss of consciousness. Generalized seizures tend to involve both sides of the body symmetrically. This means that movements, such as jerking of the arms or legs, occur bilaterally. The distinction between these types is crucial for diagnosis and treatment, as the management strategies for each

can differ significantly (National Institute of Neurological Disorders and Stroke, 2024). The new classification of seizures, as delineated by Kiriakopoulos (2022), provides a comprehensive framework for categorizing seizure types and facilitating accurate diagnosis and treatment strategies. Epilepsy is a condition with a broad spectrum that includes a variety of specific syndromes and conditions, each presenting its own set of unique characteristics and challenges (Kiriakopoulos E, 2022).

Among these, Temporal Lobe Epilepsy (TLE) is a condition characterized by recurrent, unprovoked seizures originating from the medial or lateral temporal lobe (Ko, 2022). TLE is especially noteworthy due to being one of the most common forms of epilepsy and the most challenging to treat (Types of Epilepsy & Seizure Disorders in Children, n.d.). This can manifest through a variety of symptoms, including complex partial seizures and episodes of *déjà vu* or hallucinations. Complex partial seizures, also known as focal impaired awareness seizures, are a type of seizure that originates in a specific area of the brain, in this case, the temporal lobe. These seizures are called "complex" because they affect consciousness or awareness. Despite the progress in medication for treating TLE, 30% of individuals still endure uncontrolled seizures, prompting the consideration of surgery as an alternative treatment. This underscores the critical necessity for ongoing research and innovation in this field (Ryvlin, 2014). This has spurred ongoing research efforts aimed at better understanding and managing this complex form of epilepsy.

In scientific research, the utilization of mouse models is prevalent due to their genetic, physiological, and behavioral similarities to humans. These models offer a valuable tool for studying various aspects of human biology and disease. Notably, the similarities in brain connections between mice and humans provide a foundation for understanding neurological

disorders such as TLE (Strange, 2014). Generalized and absence seizures exhibit similarities and differences in both mice and humans, offering a unique opportunity to explore the underlying mechanisms of these conditions across species (Quigg et al., 1998; Kandratavicius, 2014). In both mice and humans, generalized seizures involve disruption of cortical and thalamic structures, leading to widespread neuronal synchronization and convulsive movements (Marshall et al., 2021). Absence seizures in mice, characterized by spike-wave discharges (SWDs), are thought to be analogous to the generalized spike-and-wave discharges observed in human absence seizures (Wang et al., 2021). By studying these phenomena in mouse models, researchers have gained insights into the mechanisms of epileptogenesis and seizure propagation. It is worth noting that while the hippocampus in humans is located ventrally, its counterpart in mice is positioned dorsally, emphasizing the importance of understanding species-specific anatomical variations when extrapolating findings from mouse studies to human conditions.

In the realm of epilepsy research, three distinct models are commonly used to study different aspects of seizure disorders: the Kainic Acid Model, the Pilocarpine Model, and the Kindling Model. Each model offers unique insights into specific aspects of epilepsy pathology, providing researchers with valuable tools to investigate various seizure types and progression patterns. The Kainic Acid Model is utilized to study and focus on the chronic progression of seizures (Bertoglio et al., 2017). This model induces seizures by administering kainic acid (KA), leading to a gradual escalation of seizure activity and eventual cell death in the brain. Similarly, the Pilocarpine Model is also used to study the chronic progression of epilepsy in TLE research, triggering seizure episodes (Arshad et al., 2020). On the other hand, the Kindling Model is employed to study focal epilepsy and chronic seizure development, requiring electrode implantation in brain regions such as the amygdala or hippocampus for stimulation. Because this

model involves keeping a constant electrode connection to the mouse's hippocampus, it would be difficult to implement in this study since the mouse has a cage mate.

The Kainic Acid (KA) Model was chosen due to its relevance in studying the long-term development of seizures, which aligns with the research focus on understanding changes in behavior over a period of cohabitation. Additionally, the Gross laboratory conducting this study has devised a replicable KA model protocol with a low mortality rate to reliably induce epilepsy in mice (Fernandez et al., 2022). In the KA model, KA was injected into the hippocampus, activating excitatory glutamate receptors, specifically the kainic acid/2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid receptor. This excitatory action leads to seizures originating from limbic systems such as the hippocampus, mirroring the pathological processes observed in TLE. When KA is injected into the mouse brain, beyond inducing seizure progression, it elicits various effects on the neural tissue. One notable consequence is cell dispersion in the dentate gyrus of the hippocampus, reflecting alterations in the structural organization of this brain region. The KA injection also leads to neuronal cell death, a phenomenon closely associated with the development of seizures. This neuronal loss can impact brain function and contribute to the pathological changes observed in epilepsy models. Pathological changes observed in epilepsy models can include alterations in neuronal structure and function, such as aberrant neuronal excitability (Rusina et al., 2021). There may be changes in neurotransmitter systems, synaptic connectivity, and neuroinflammatory processes. This affects the formation of abnormal neuronal networks, which further exacerbate seizure activity. The loss of specific neurons can disrupt cognitive processes, memory, and behavior.

Despite the extensive body of research on KA mouse models, which includes over 6200 articles found on PubMed using specific search terms such as “kainic acid,” “mouse model,” and

"epilepsy" in March 2024, there is a significant gap in the literature concerning the effects of social housing on seizure progression. Many studies tend to focus on individual mice, neglecting the social nature of these animals (Manouze et al., 2019). Mice are inherently social creatures, and studying them in isolation may not fully capture the complexities of seizure development (Shemesh, 2013). A study conducted by Chesner in 2021 showed evidence that social isolation stress can exacerbate the development of epilepsy and other neuropsychological disorders by impacting various neural systems, such as the GABAergic system (Chesner, W., et al., 2021). Imbalances in the GABAergic system can exacerbate epilepsy by heightening neuronal excitability and reducing the seizure threshold. Introducing a social housing component to mouse models could potentially impact seizure progression and outcomes. The presence of a cagemate may introduce variables tied to social interaction, encompassing factors like the duration of time spent together, proximity (including time spent lying next to each other), and potential social behaviors that impact the dynamics of seizure development. This underscores the importance of investigating the effects of social interaction on epileptic mice. There are research studies that highlight the impact of early-life environment, including social factors, on epilepsy development and progression. Studies have demonstrated that early-life social isolation stress, such as single housing, can lead to increased seizure susceptibility and epileptogenesis in rats (Sarkisova and Van Luijtelaar, 2022). Additionally, the study conducted by Pontes Silva and Gama Marques about homeless populations affected by epilepsy demonstrated that social impairment in epilepsy can negatively affect the overall quality of life of individuals with epilepsy, diminishing academic achievement and employment opportunities (Pontes Silva and Gama Marques, 2023). While the search results do not present explicit evidence of social housing improving epilepsy outcomes, they underscore the importance of considering environmental factors, including social

interactions, in epilepsy research. The influence of social environments on seizure susceptibility and progression remains a relevant area for further investigation to understand the holistic impact of social factors on epilepsy outcomes. By exploring the effects of social housing on seizure progression in epileptic mice, researchers can potentially uncover valuable insights into how social interactions may influence epilepsy pathophysiology and treatment responses.

Description of Project

This research project aims to investigate how social housing impacts the development and progression of epilepsy and behavior in male mice, focusing on a social setting rather than socially isolated conditions. A novel dual-housing video monitoring system has been devised to observe male mouse pairs, with each mouse uniquely marked using hair dye to enable individual identification by DeepLabCut (DLC), a specialized computer program. The project began by recording continuous 24/7 video footage using wide-angle cameras to establish baseline behavior for each mouse pair over a three-day period, capturing their activities within the cage environment and providing an understanding of their natural interactions. Following the baseline assessment, one male mouse in each pair underwent KA injection in the left anterior hippocampal region to induce epilepsy. Each cage was equipped with essential amenities such as food, water, and exercise wheels to ensure the mice's well-being and enrichment. Subsequently, the mice were monitored for 30 days post-KA injection to observe the emergence of spontaneous behavioral seizures, allowing for a detailed analysis of how a social housing setting influences epilepsy progression and behavior. The study involved the examination of both epileptic and non-epileptic behavior patterns, focusing on their spatial preferences within the enclosure assessed by the duration spent in specific areas. By correlating these behavioral observations with epilepsy progression, the research aimed to unravel how social housing dynamics impact seizure manifestation and behavioral responses. This comprehensive analysis provided insights into the effects of social contact on individuals with epilepsy, potentially informing optimal living arrangements for patients with this condition. Given the demonstrated similarities in neural circuitry, neurotransmitter systems, and behavioral responses between mice and humans in various studies, these findings hold significant translational implications. Furthermore, this

study explored whether cohabitating with an epileptic partner influences behavior and interactions, mirroring aspects observed in human epilepsy scenarios. By elucidating these intricate relationships in a controlled mouse model setting, this research bridged gaps in understanding the social dimensions of epilepsy and paved the way for tailored interventions that consider the holistic impact of social environments on epilepsy outcomes.

Specific Research Aims

Aim 1: Develop, refine, and validate a dual-housing mice enclosure alongside a 24/7 video monitoring and recognition system to facilitate comprehensive observation and analysis of mouse pairs in a social housing environment.

Aim 2: Measure the influence of seizures on social behaviors in dually housed mice and its repercussions on the normal and stress behaviors of the epileptic and non-epileptic mice. Stress behaviors are classified as a decrease in vital activities such as eating, drinking, and exercising. The distance between the mice will also be measured. If social interaction influences epilepsy development, we hypothesize that dual housing will result in less stress behaviors in the epileptic mouse, whereas the healthy mouse will show more stress behaviors.

Hypothesis

Given the social nature of mice, it is hypothesized that epileptic mice housed in pairs may demonstrate reduced stress behaviors due to social companionship. This study seeks to unravel the intricate dynamics between social interactions and epileptic progression, shedding light on how living conditions can impact seizure development and behavioral responses in a paired mouse setting. The social interaction provided by paired housing could potentially mitigate stress levels in epileptic mice. The hypothesis suggests that the presence of a companion will lead to decreased stress levels in epileptic mice, potentially resulting in delayed or reduced seizure activity. Conversely, the non-epileptic mouse may demonstrate heightened stress behaviors as a result of cohabitating with an epileptic partner. The hypothesis further speculates that the living conditions and social dynamics within the shared housing environment could influence the behavioral patterns of the non-epileptic mouse, highlighting the potential interplay between social context and individual responses in a paired mouse setting.

Proposed Methodology and Sourced Materials

Animals:

The study was conducted using adult C57Bl6J black mice procured from Jackson Laboratories. The mice were 10 weeks old at the start of the experiment. Mice arrived in groups of five and were mostly littermates. This is important to note as it facilitated the pairing with minimal fighting. Mice pairings were done by randomly pairing mice from a group to avoid fighting. The experimental design entails the utilization of a total of eight male mice: four pairs of male-male mice (4 cohorts). Each pair/ cohort was housed in an enclosure equipped with essential amenities, including standard food pellets provided by the Division of Animal Resources husbandry, water, and an exercise wheel. During the experiments, mice had ad libitum access to food and water. The experiments were conducted in accordance with protocols reviewed by the Emory Institutional Animal Care and Use Committee (PROTO2017000826).

Tagging:

Mice were lightly anesthetized using isoflurane vapors (2-3%) and placed on a stereotaxic frame, following which their fur was dyed according to an established protocol (Ohayon et al., 2013). Specifically, mouse 0, which is the mouse that was induced with epilepsy, was marked with a single dot on the stomach side (Figure 2), while mouse 1, which is the nonepileptic healthy mouse, was marked with a head-to-tail stripe along its body (Figure 1). This distinctive marking scheme enables individual mouse identification within the shared enclosure, irrespective of their positioning or orientation (Ohayon et al., 2013). The dye (L'Oreal Paris Feria Platinum Bounce Bond Care Lightening System) was prepared according to the manufacturer and applied using a cotton tip. After 5 minutes, the dye was washed away using warm saline. The fur was dried using clean gauze, and the mouse was placed on a warming pad for 30-45 minutes before being

returned to its cage. Both mice underwent fur dyeing to ensure equal exposure to the dye chemicals, thereby mitigating the potential for the dye pattern to act as a confounding factor in assessing subsequent behavioral responses.

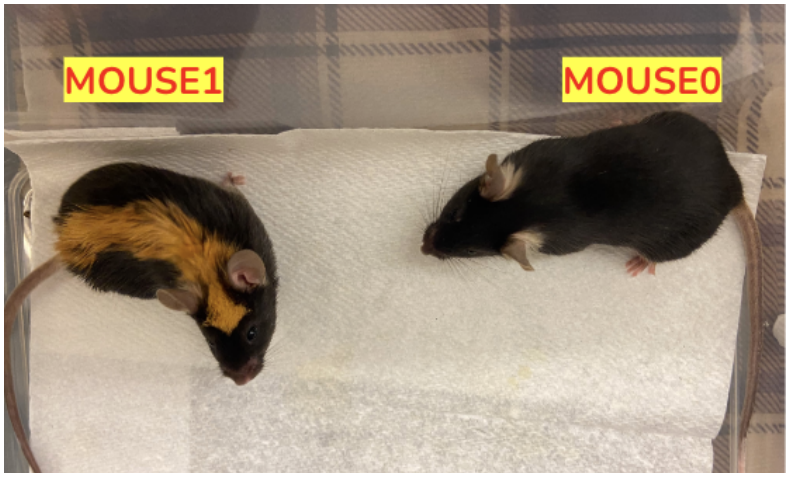

Figure 1: Picture of Mouse 1(nonepileptic) and Mouse 0 (induced epilepsy) view from the top

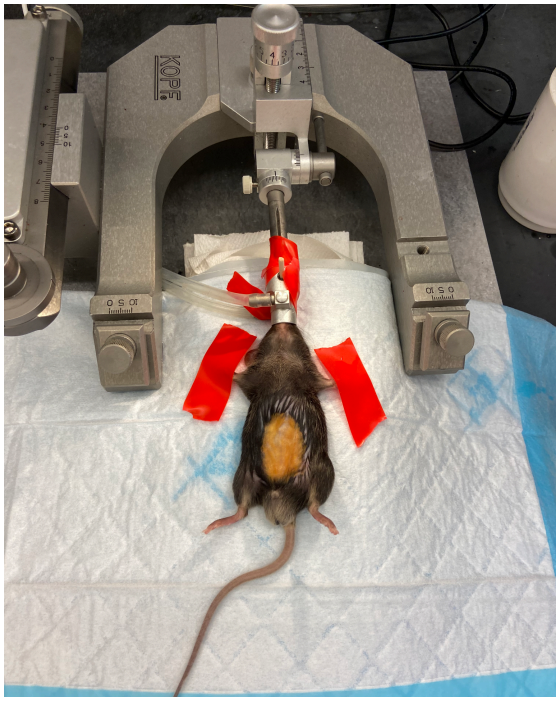

Figure 2: Mouse 0 being dyed using a stereotaxic frame

Mouse Enclosure:

To create the enclosures, a 12-inch X 12-inch X 12-inch clear plexiglass box was purchased from Amazon (link: https://rb.gy/dgpoz). The hardware was placed on the top of the box (Figure 3). A hole in the center on the top side was created to place the camera. A rectangular hole was created on the top of the box for an LED light to be angled into the enclosure.

Additionally, a metal 12 x 12 pan was added at the bottom of the cage. A small elliptical hole was created for the spout of the water bottle to slide in. Two nuts were drilled above it to hold a wire to secure the bottle. A rectangular piece was cut out to accommodate the food dispenser, leaving a small space at the top (Figure 4). To address this, an additional piece of plexiglass was added at the top to prevent mice from escaping through this opening. Moreover, an exercise wheel was securely anchored down in the cage using a strong magnet to prevent the mice from pushing it and potentially causing disruptions within the enclosure.

Before the use of the enclosure, the Emory Division of Animal Resources verified the mouse enclosure met the required standards and approved its use. Figure 5 shows the final enclosure in the mouse room.

Part A: Ethernet Connection

The Ethernet connection plays a role in enabling the mouse enclosures to record data 24/7 through an IP address. This connection provides a network link, allowing data transfer and continuous monitoring of the mice within the enclosures (Figure 3).

Part B: Raspberry Pi

The Raspberry Pi 5 (www.raspberrypi.com) was purchased from Amazon and served as a supercomputer in this project. The Raspberry Pi 5 created a wireless server that works with the Ethernet network connection to save the information to an IP address. In this context, the

Raspberry Pi is instrumental in saving data and video recordings onto our lab server for data collection and analysis (Figure 3).

Part C: Power source

The power source is a component that facilitates the connection between the Raspberry Pi and the ethernet connector (Part A) as well as the camera (Part D). This converter ensures communication between these components, enabling data transfer and synchronization essential for recording and monitoring activities (Figure 3).

Part D: Camera with Infrared Capabilities

The camera utilized in this project was purchased from Amazon (MakerFocus Raspberry Pi 4 Camera 5MP OV5647). The camera is equipped with infrared capabilities, allowing for enhanced visibility of the mice during nighttime recording sessions. This feature enables clear imaging even in low-light conditions, ensuring continuous monitoring (Figure 3).

Part E: LED Light

The LED light serves a specific function during daytime and nighttime operations by illuminating the cage when ambient lighting is low. This targeted illumination ensures optimal visibility for the camera to capture clear footage of the mice's activities during recording sessions, enhancing the quality of recorded data and observations (Figure 3).

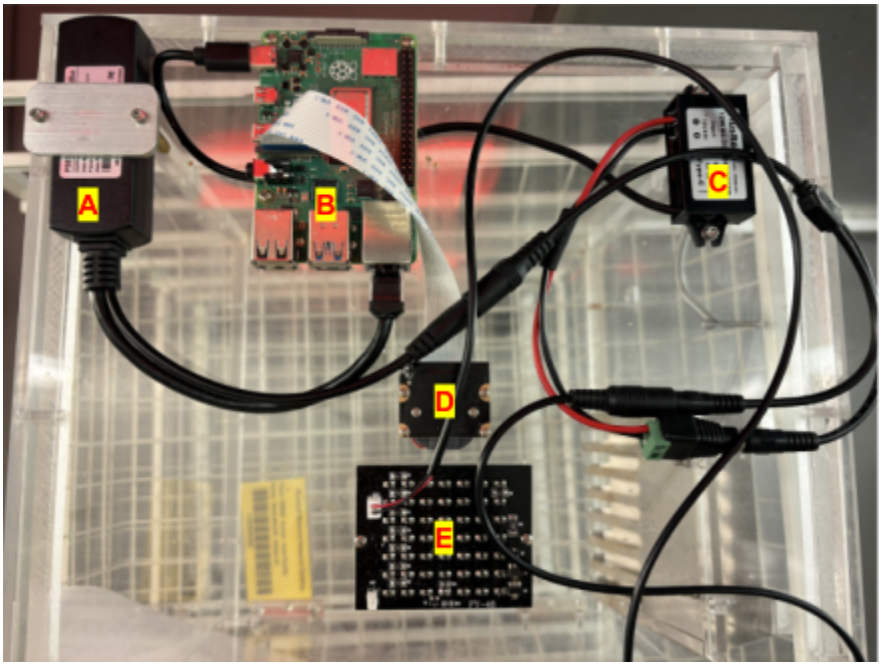

Figure 3: Top View of Enclosure In Order to Record Mice, A - ethernet connection, B - Raspberry Pi, C - Power source, D - Camera with Infrared capabilities, E - LED light

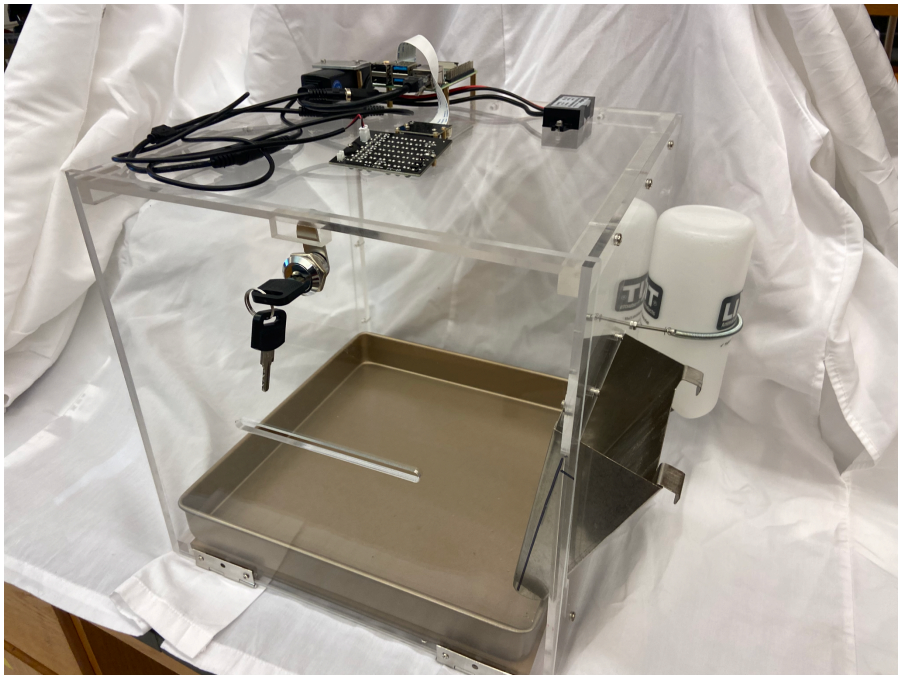

Figure 4: Mice Enclosure with installed food dispenser, water bottle, and pan

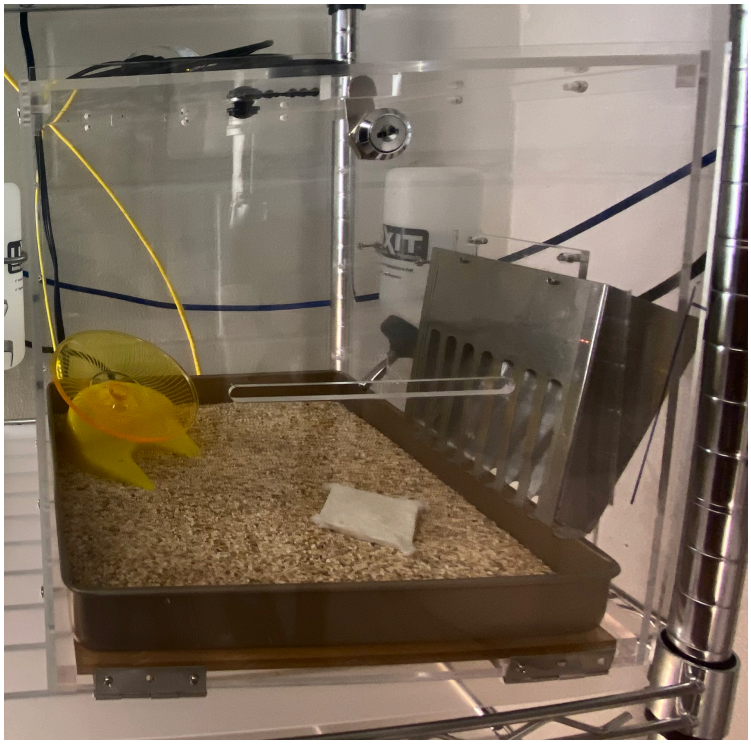

Figure 5: Enclosure in mice room with installed food dispenser, water bottle, wheel, and bedding

Video recording:

Baseline behavior will be acquired for three days before KA injection and 30 days post KA injection. Figure 6 illustrates the appearance of the videos captured during the daytime, while Figure 7 portrays their depiction during the nighttime hours.

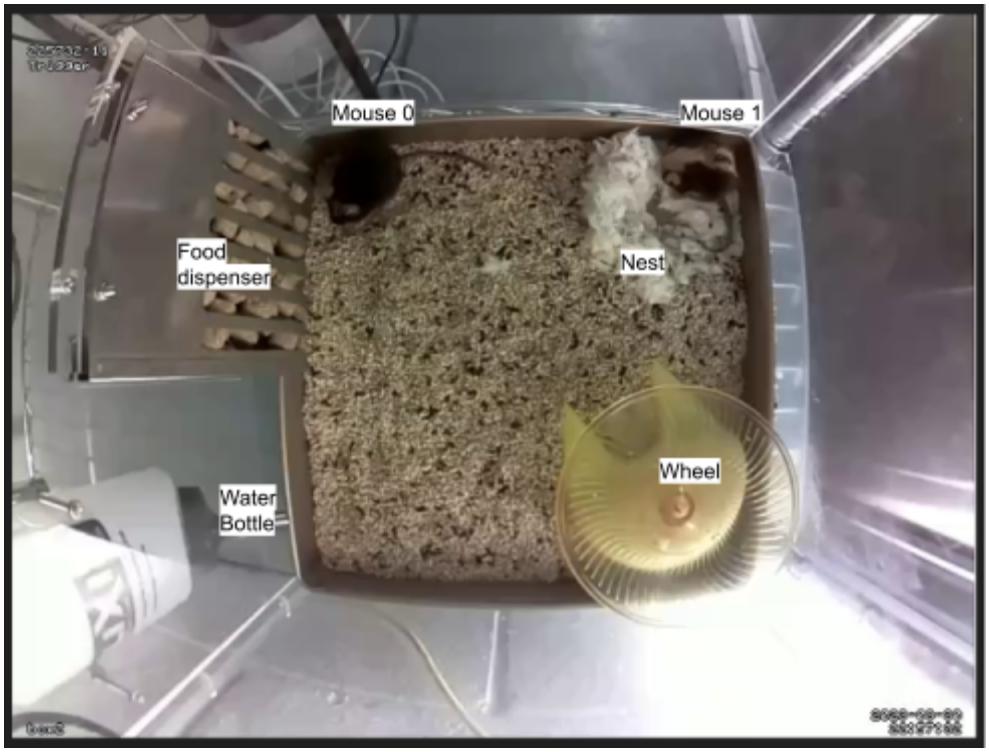

Figure 6: Video Recording in the Day-Time

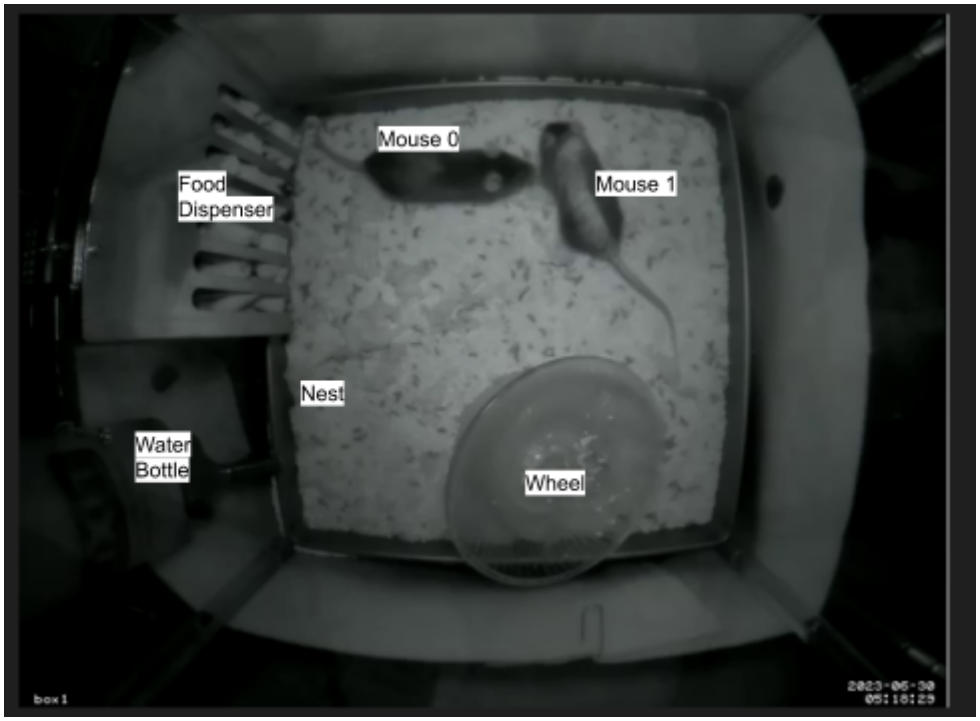

Figure 7: Video Recording in the Night-Time using Infrared Light

Video Monitoring and Tracking:

The DeepLabCut software was used to develop an algorithm aimed at training for the purpose of tracking and distinguishing between the mice within the enclosure. DeepLabCut is a software used for multi-animal projects, enabling the tracking and differentiation of individual animals within an enclosure. It employs deep neural networks and transfer learning techniques for 2D and 3D markerless pose estimation (Figure 11). The software allows for the creation of a program capable of monitoring behaviors like detecting seizures with minimal training data. This markerless tracking system uses deep learning to estimate animal poses without physical markers, making it suitable for both lab settings. The latest version addresses challenges in tracking social animals by developing novel network architectures and assembly algorithms to identify individual animals accurately (Lauer et al., 2022)

DeepLabCut is known for its accuracy in detecting key points without requiring large datasets, making it a valuable tool for pose estimation in animal behavior recognition (Mathis et al., 2018). It has been widely used in various studies analyzing behaviors such as self-grooming in mice and social interactions among animals.

Coding the Video Monitoring Algorithm:

The algorithm development and training processes were conducted using Python within the Anaconda prompt environment. The DeepLabCut library was imported for this purpose, along with the necessary dependencies:

```
import deeplabcut as DLC
from glob import glob
```

The configuration file, essential for defining the project parameters, was specified as follows:

```
cfg = 'D:/PaintedMice/Aquamouse2_Painted/config.yaml'
```

Subsequently, a multi-animal training dataset was created using the configuration file:

```
dlc.create_multianimaltraining_dataset(cfg)
```

Videos of the mice were then fed into the DeepLabCut program for analysis. Initially, a set of five random videos either from box 1 or box 2 were defined as 'vids':

```
dlc.analyze(cfg, vids)
```

Outlier frames, where the program displayed low confidence in accurately plotting the mice, were extracted using the following code:

```
dlc.extract_outlier_frames(cfg, vids, epsilon=20)
```

Figure 9 depicts a frame that was accurately tracked by the program with no corrections needed. Figure 10 shows an example of a frame that needs to be corrected, as mouse 1 is not identified. The extracted outlier frames were manually corrected within the DeepLabCut software by accurately plotting key points on the mice. Figure 11 shows which body parts are marked on each mouse. These points included the nose, mid-head, left head, right head, left ear, right ear, ear center, neck, left shoulder, right shoulder, body, left hip, right hip, tail, and tail tip. Figure 8 shows the interface for plotting and correcting the body points in the DeepLabCut software. After correcting all mistakes in the outlier frames, the program's new training was evaluated:

```
dlc.evaluate_network(cfg, plotting=False)
```

Each training iteration involved feeding the program five new videos and repeating the entire process. This process was repeated a total of 24 times throughout the project.

Upon completion of training iterations, the '`dlc.analyze(cfg, vids)`' command was executed for all videos to generate '`el.h5`' files. These files were utilized for data analysis in Jupyter Notebook to further analyze and interpret the results. Figure 17 summarizes the training steps.

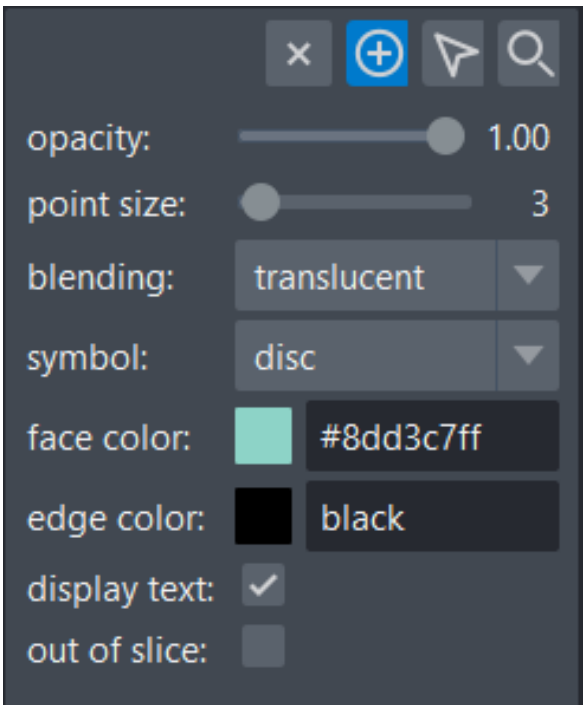

Figure 8: DeepLabCuts software to outlier frames

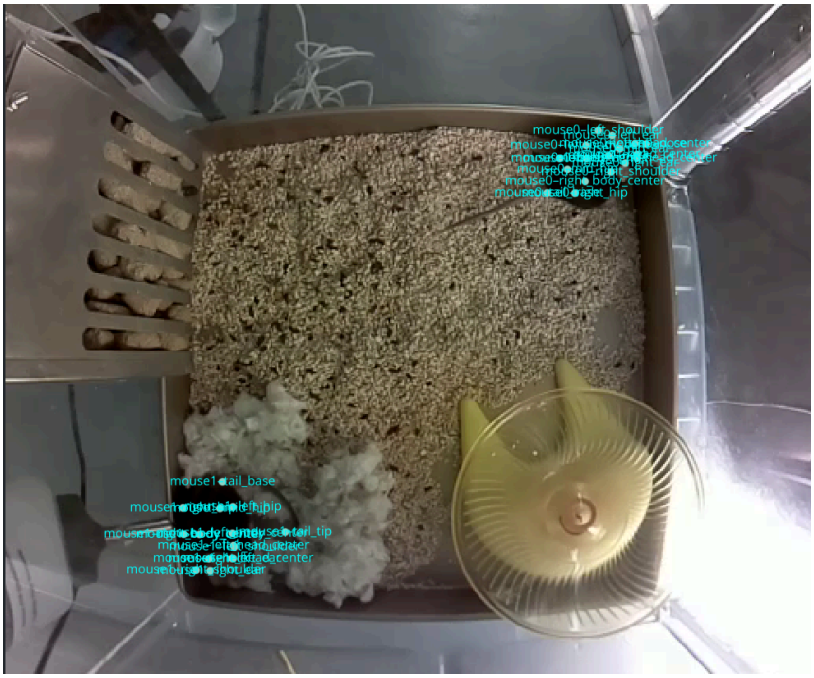

Figure 9: Example frame with correct mouse body part points

Figure 10: Example frame with incorrect mouse body part points. Only the body parts of mouse 0 are identified properly. Not a single body part of mouse 1 is identified in the bottom left corner of the enclosure.

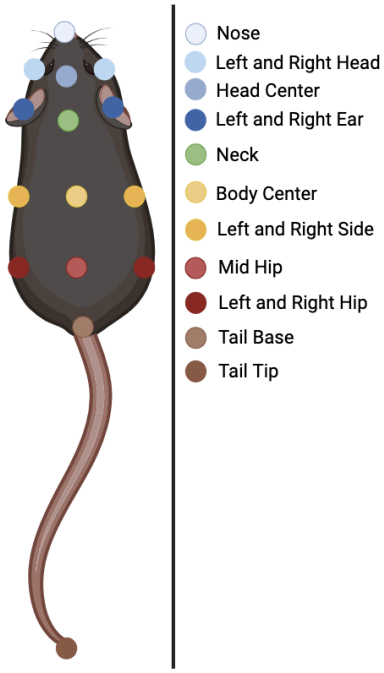

Figure 11: Body parts and locations of the mouse that are marked in DeepLabCut. Schematic created with Biorender (biorender.com).

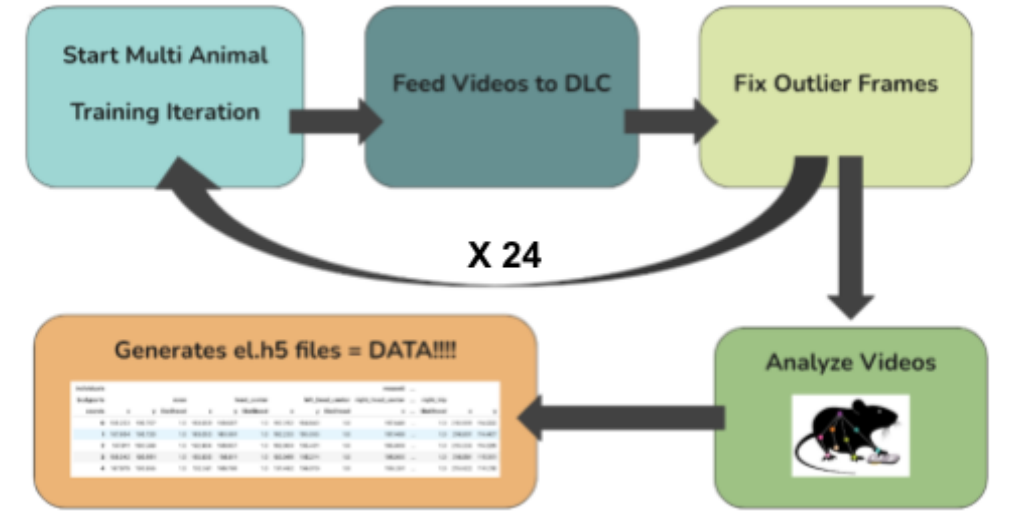

Figure 12: Graphic to summarize the training process. The first three steps were each repeated 24 times in this study to reach an implementable algorithm stage.

Kainic acid injections:

Figure 13: Timeline for KA injections and Animal Tagging with Dye

Mice were anesthetized using isoflurane, placed in a stereotaxic apparatus, and received a unilateral injection of (KA) in the hippocampus (Figure 14). The mice were injected with 100 nl of a 20 mM kainic acid monohydrate solution in the left hippocampus at the following coordinates relative to bregma: AP: − 2.0 mm; ML: − 1.25 mm; DV: − 1.6 mm. The injection was performed using a pulled glass pipette attached to a Nanoject 2 (Drummond Scientific, Broomall, PA 19008). After lowering the glass pipette to the appropriate depth, it was left there for three minutes so the tissue would stabilize around the pipette. Then the injection was performed with 50.4 nl boluses administered every 10 seconds at a speed of 23 nl/sec. Following injection, the pipette was kept in place for five minutes before being retracted, and the wound was closed using tissue adhesive (Fernandez, 2022). The intrahippocampal injection of kainic acid induces lesions and neurodegeneration that mimic hippocampal sclerosis observed in human TLE patients. KA disrupts the balance of excitation and inhibition in the hippocampus, inducing

a period of status epilepticus and ultimately leading to the appearance of spontaneous recurrent seizures (Fernandez et al., 2022). Only mouse 0 in each cohort undergoes the surgical procedure. Figure 13 shows a timeline of the experiment for one cohort.

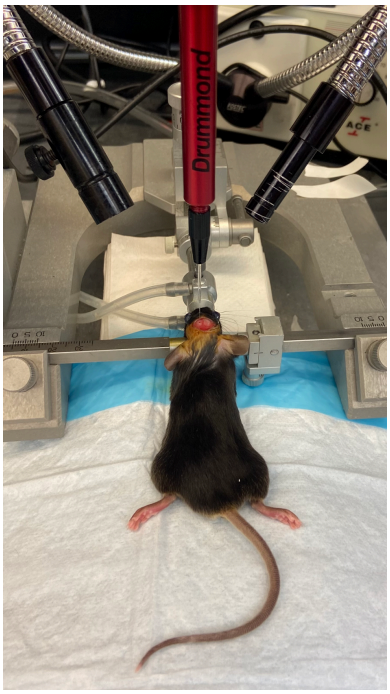

Figure 14: KA injection

Post-mortem Pathology:

Epileptic mice in each cohort were perfusion-fixed using paraformaldehyde (intracardiac injection of paraformaldehyde, 4%). Then, brains were placed in a sucrose solution (10%) and then sectioned using a cryostat. Sections throughout the hippocampus were mounted on glass slides and Nissl stained. KA induced hippocampal damage was confirmed by microscopy.

Behavioral Analysis of epileptic and healthy mice:

The number and the duration of the seizures of the epileptic mouse were extracted from the video data using a markless pose detection algorithm. Seizure outcome (latency to first spontaneous

seizure, seizure frequency, and seizure duration) was studied in these dual-housed mice. The behavior of the non-epileptic mouse was also studied to understand how the disorder in a companion may affect the behavior of a healthy mouse. For each mouse, time spent eating, sleeping, exercising, or grooming was compared to baseline over time. The following of the epileptic and nonepileptic mice were observed:

- Which mouse made the nest
- Time spent next to each other
- Time spent eating food
- Time spent drinking water
- Time spent on the exercise wheel
- Occurrence of Seizures

The cage was partitioned into distinct zones to assess specific behaviors exhibited by the mice (Figure 15). The yellow rectangle signifies the area where mice are considered to be eating, the red circle indicates where they are considered to be drinking water, and the blue circle denotes where they are considered to be exercising. Additionally, the distance between the mice is monitored. A mouse was deemed to be engaging in a particular behavior if it remained within the corresponding zone. For instance, if the nose of mouse 1 was positioned within the yellow or red zone, it would be interpreted as eating or drinking water, respectively.

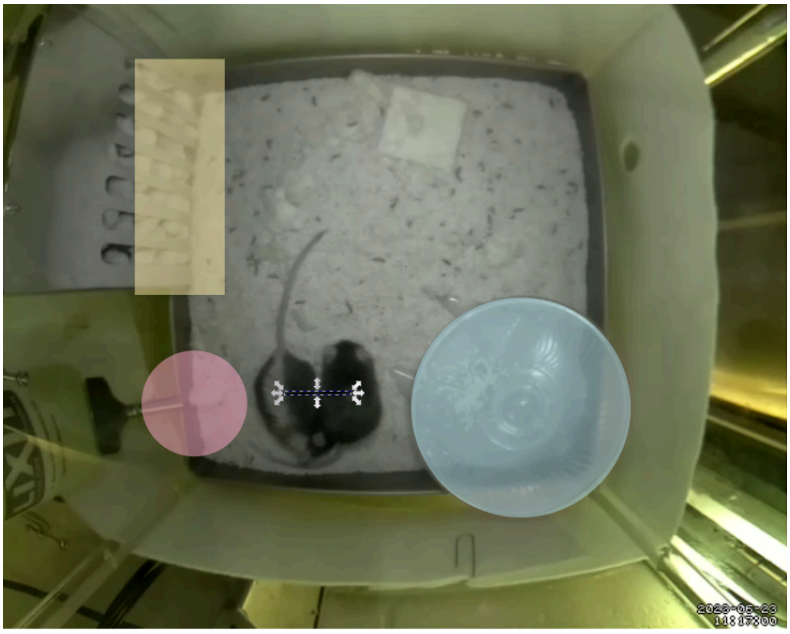

Figure 15: Boundaries in the enclosure to classify mice behaviors. Blue circle - exercising, red circle - drinking water, yellow rectangle - eating food, distance between mice

Statistical analysis:

The statistical analysis was performed on the el.h5 files using Python within Jupyter Notebook. The files were categorized into different groups to analyze the behaviors of the mice housed together throughout the 30 days and before the KA injection. Wilcoxon Rank Sum Test was performed for behaviors (exercise, eating, and drinking) between mouse 0 and mouse 1. For this data set, there were a total of 107,826 data points. Wilcoxon Rank Sum Tests were also performed for the distance between the mice before the KA injection, during the first 10 days, the second 10 days, and the last 10 days. For these data sets, there was a minimum of 10,000 points and a maximum of 44,000 points.

The attached code files in the appendix provide the analytical procedures and methodologies employed in processing the data from the videos. The code was written in Python on Jupyter Notebook.

Results and Analysis

In Cohort 1, epileptic seizures were observed in mouse 0 (Figure 16) alongside confirmed hippocampal sclerosis. For Cohorts 2 through 4, hippocampal sclerosis in the dentate gyrus was observed through nissl staining. It is imperative to note that while Cohorts 1 and 2 were successfully saved for analysis, unfortunately, Cohorts 3 and 4 encountered technical and storage limitations, leading to the unavailability of their corresponding videos for analysis. Additionally, the data collection for this particular cohort spanned across the Thanksgiving and Winter breaks, during which my physical presence in the lab was not feasible to oversee their progress due to the holiday period. Moreover, it's important to acknowledge that the lab computer serves multiple researchers, each utilizing it for their individual projects.

Figure 16: Mouse 0 from Cohort 1 Experiencing a Seizure

We conducted a comparative analysis of the distance of mouse 0 and mouse 1 from the exercise wheel before and after the administration of KA injection in two separate cohorts. In Cohort 1, we observed a significant increase in the distance between mouse 0 and the exercise wheel after KA injection compared to baseline before the KA injection ($p = 0.0462$), whereas no significant difference was found for mouse 1 ($p = 0.1378$). Additionally, there were no significant differences in the distance between mouse 0 and mouse 1 during the baseline time period ($p = 0.7822$); however, there was a notable increase in distance from the wheel in mouse 0 compared to mouse 1 post-injection ($p = 0.0021$). Similarly, in Cohort 2, both mouse 0 and mouse 1 displayed significant increases in distance from the wheel following KA injection ($p < 0.0001$ for both). Prior to KA injection, mouse 0 was closer to the wheel compared to mouse 1 ($p = 0.0087$), yet post-injection, this dissimilarity diminished ($p = 0.4341$) (Figure 17).

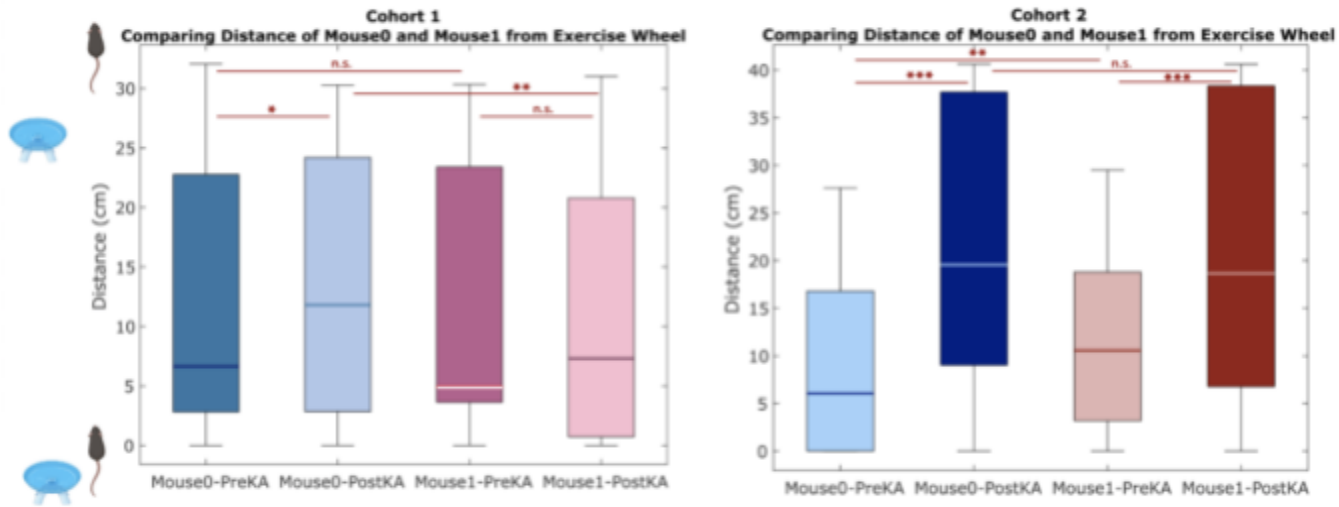

Figure 17: Distance of mouse 0 (epileptic) and mouse 1 (non-epileptic) from the exercise wheel before and after the KA injection in Cohort 1 (left panel), and Cohort 2 (right panel). Mouse 0 is depicted in blue, and mouse 1 is depicted in red.

We performed the same analysis of the distance of mouse 0 and mouse 1 from the food dispenser in Cohort 1 and Cohort 2. In cohort 1, significant decreases were observed in the distance from the food dispenser in mouse 0 and mouse 1 post-KA injection compared to their respective pre-injection distances ($p < 0.0001$ for both). Prior to injection, mouse 0 was closer to the food dispenser than mouse 1 ($p = 0.0017$), and this discrepancy persisted post-injection ($p = 0.0003$). Similarly, in Cohort 2, mouse 1 exhibited a significant decrease in the distance from the food dispenser post-injection compared to pre-injection levels ($p = 0.0007$). However, no significant differences were observed for mouse 0 post-KA injection compared to its pre-injection distance ($p = 0.4084$). Notably, mouse 0 was closer to the food than mouse 1 before the surgery ($p = 0.0466$), but mouse 1 was closer compared to mouse 0 after the KA surgery ($p = 0.0042$)(Figure 18).

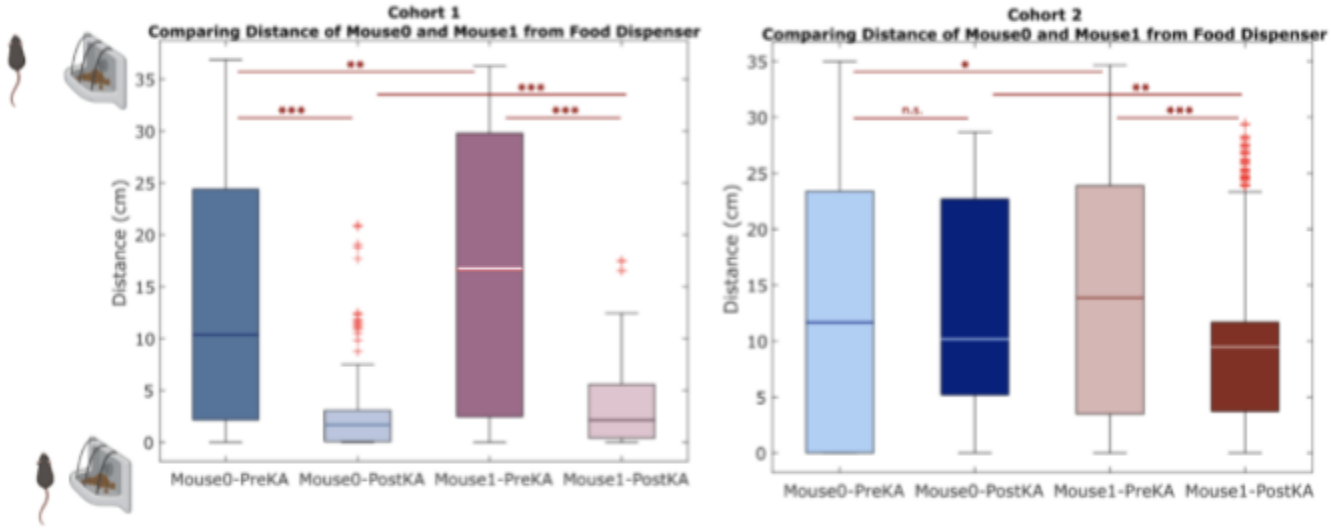

Figure 18: Distance of mouse 0 (epileptic) and mouse 1 (non-epileptic) from the food dispenser in Cohort 1 (left panel), and Cohort 2 (right panel). Mouse 0 is depicted in blue, and mouse 1 is depicted in red.

Once again, we conducted the Wilcoxon Rank Sum test to evaluate the distance between mouse 0 and mouse 1 from the water bottle across both Cohort 1 and Cohort 2. In Cohort 1, mouse 0 and mouse 1 were closer to the water bottle after the KA surgery compared to before ($p < 0.0001$ for both). There were no significant differences between mouse 0 and mouse 1 before the KA injection ($p = 0.1068$), whereas mouse 0 was closer than mouse 1 after the KA injection ($p = 0.0250$). In Cohort 2, mouse 0 and mouse 1 were further away from the water bottle after the KA surgery compared to before the surgery ($p < 0.00001$ for both). No significant differences were observed between mouse 0 and mouse 1 before KA injection ($p = 0.3373$), while mouse 1 was closer to the water than mouse 0 post-injection ($p = 0.0015$) (Figure 19).

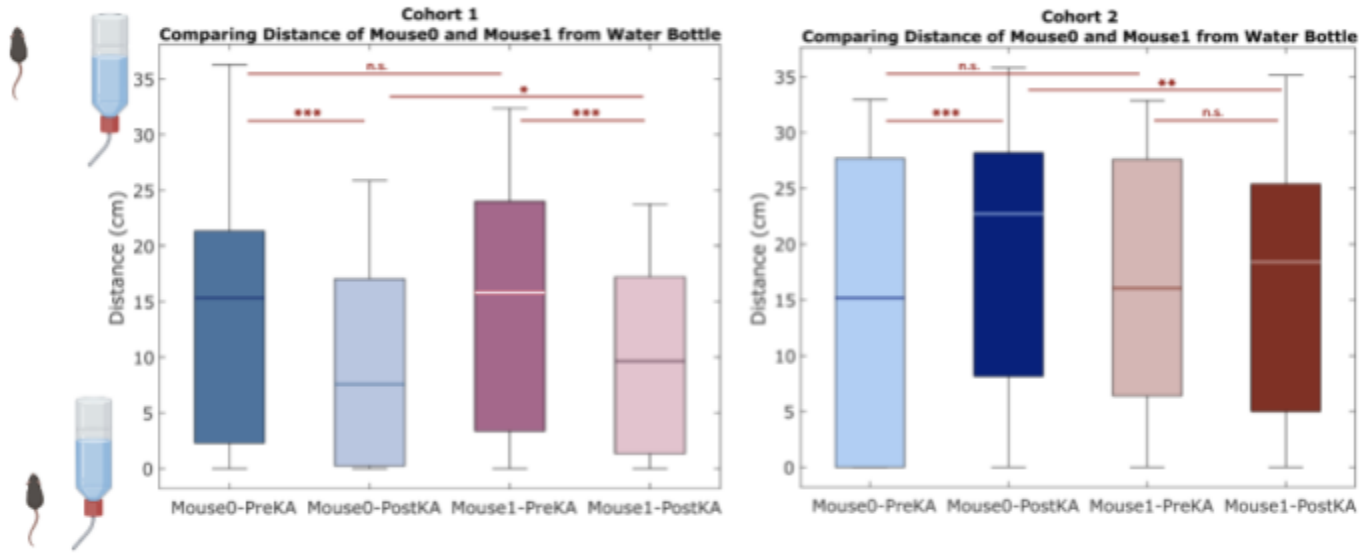

Figure 19: Distance of mouse 0 (epileptic) and mouse 1 (non-epileptic) from the water bottle in Cohort 1 (left panel), and Cohort 2 (right panel). Mouse 0 is depicted in blue, and mouse 1 is depicted in red.

An additional analysis was conducted to calculate the duration spent within specific boundaries of the enclosure. Figure 20 illustrates the proportion of time allocated by mouse 0 and

mouse 1 from cohort 1 toward spending time near the food, water, or exercise wheel. The graphs show the time allocation of spending time in these boundaries contrasting with the representation of spatial proximity from the enclosure boundary as illustrated in the box plots above. The pie chart of mouse 0 shows that it spent the majority of time eating and drinking, while mouse 1 spent the majority of time engaging in different behaviors.

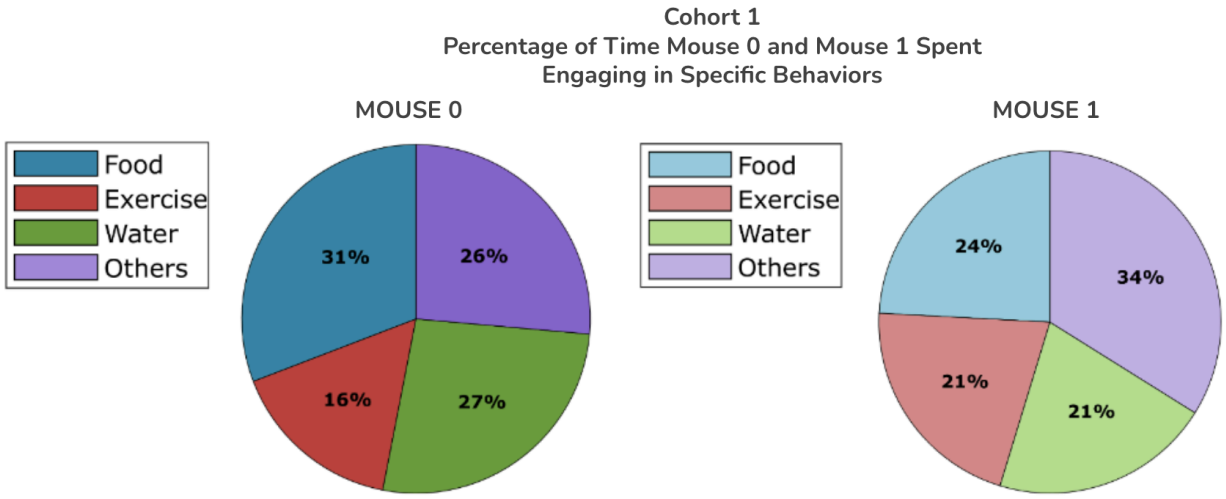

Figure 20: Pie graph depicting the percentage of time mouse 0 and mouse 1 from XCohort 1 spent participating in behaviors such as exercising, eating, and drinking. This data is from after the KA injection.

Figure 21 illustrates that within Cohort 2, both mice predominantly allocated their time towards various behaviors beyond solely eating, drinking, and exercising activities.

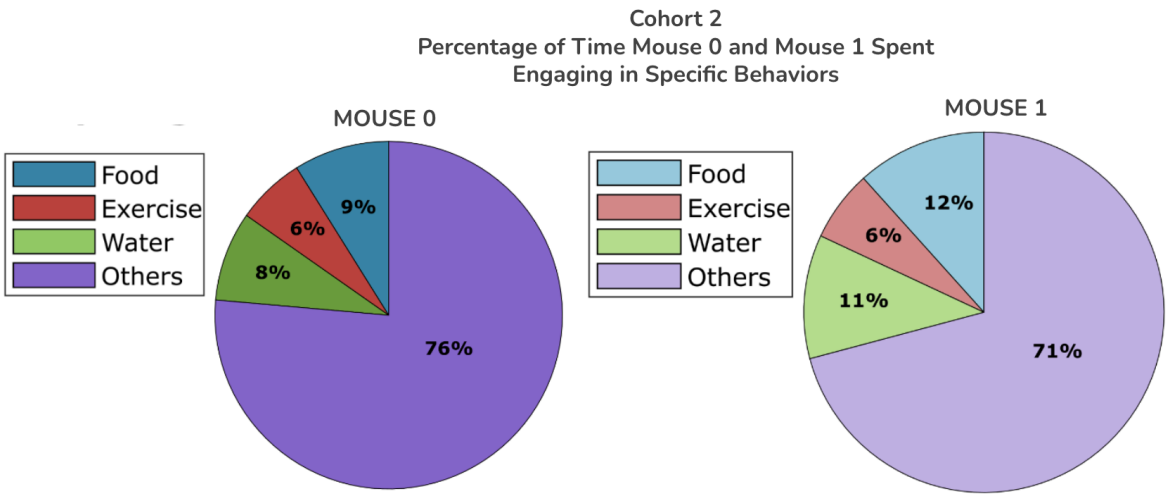

Figure 21: Pie graph depicting the percentage of time mouse 0 and mouse 1 from Cohort 2 spent participating in behaviors such as exercising, eating, and drinking. This data is from after the KA injection.

Next, we compared the distance between mouse 0 and mouse 1 before the KA injection and throughout the 30 days of cohabitation in both Cohort 1 and Cohort 2. Utilizing a Wilcoxon Rank Sum test, we scrutinized data from the three days preceding the KA injection (pre-KA), as well as from the first 10 days after the KA injection (D10), days 11-20 after the KA injection (D20), and days 21-30 after the KA injection (D30) from each time period. Each time period was further subdivided into distinct intervals around noon, evening, and midnight. The noon time period included videos from 11:00 a.m. to 1:00 p.m. The evening time period included videos from the hours of 4:00 p.m. to 8:00 p.m. The midnight hours included videos from the hours of 11:00 p.m. to 1:00 a.m. These specific times were selected based on the varying behaviors exhibited by the mice throughout the day, influenced by their circadian rhythm. At noon, the mice are predominantly sleeping and tend lay next to each other. Midnight typically marks their peak activity period. The evening hours encompass a blend of these behaviors. Notably, at 7 p.m., the lights in the mouse room are turned off. For the evening intervals, there was a

significant increase in distance between the mice observed in various comparisons: D10 vs D20 ($p = 0.0463$), D10 vs D30 ($p = 0.0015$), pre-KA vs D10 ($p = 0.0007$), pre-KA vs D20 ($p = 0.0118$), and pre-KA vs D30 ($p = 0.0001$). There was a significant decrease in distance during D20 vs D30 ($p = 0.0458$). In the noon intervals, while no significant differences were found between D20 and D30 ($p = 0.6526$), D10 and D20 ($p = 0.1249$), and D10 and D30 ($p = 0.8794$), significant decreases in distance were observed between pre-KA and D10 ($p = 0.0017$) as well as pre-KA and D20 ($p = 0.0064$), and pre-KA and D30 ($p = 0.0130$). During the midnight intervals, notable decreases in distance emerged during D20 vs D30 ($p = 0.0010$), pre-KA vs D10 ($p < 0.0001$), pre-KA vs D30 ($p < 0.0001$) and a slight increase in the distance during D10 vs D20 ($p = 0.0242$). However, no significant differences were observed for D30 vs D10 ($p = 0.0821$) and pre-KA vs D20 ($p = 0.1425$) (Figure 22).

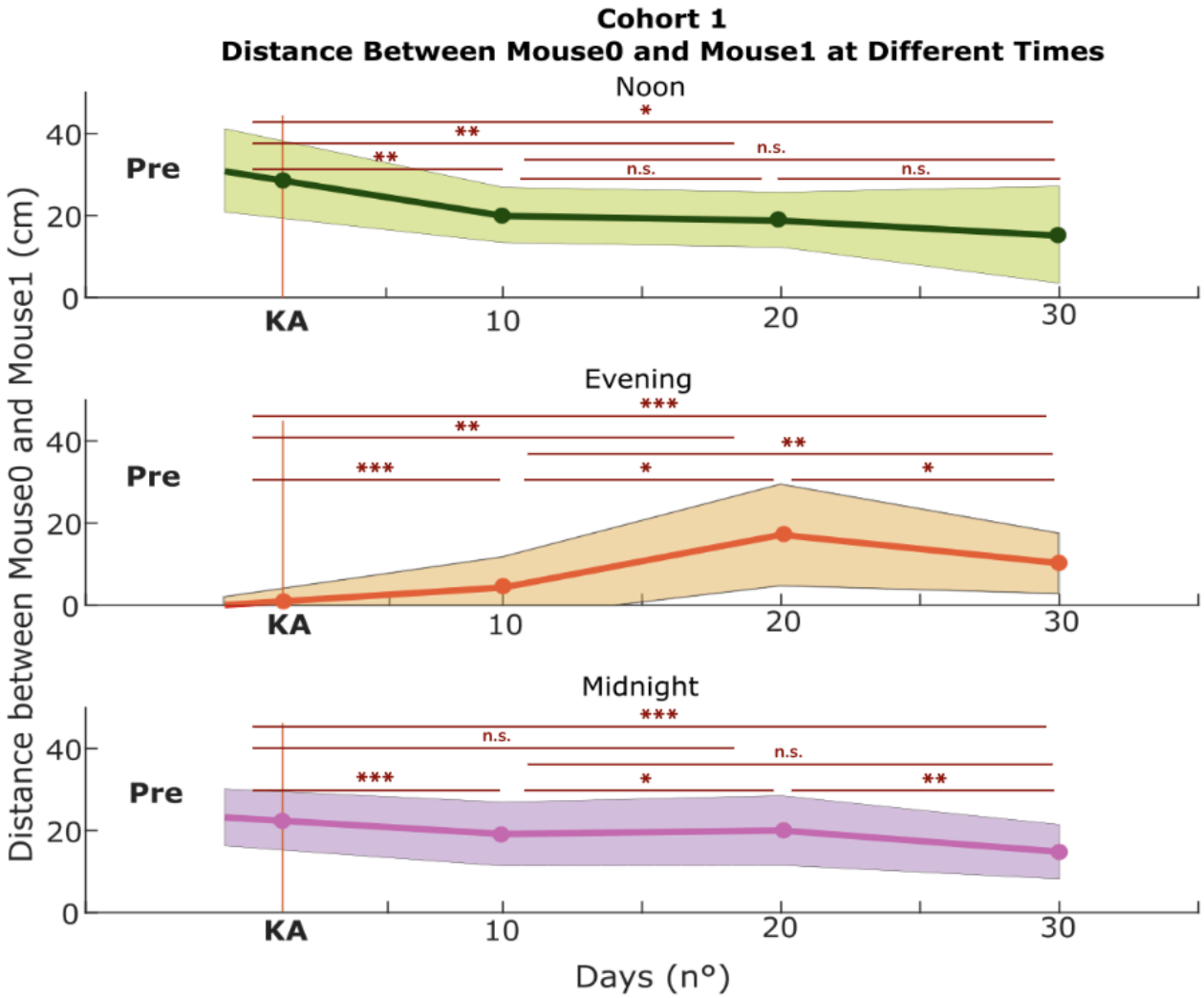

Figure 22: Distance between mouse 0 and mouse 1 from cohort 1 across the 30 days in the enclosure at different times during the day. The mice were analyzed during hours around midnight, noon, and evening.

Once again, for Cohort 2, the distance between mouse 0 and mouse 1 throughout the 30 days in the enclosure was analyzed in the same way as Cohort 1. During the midnight intervals, significant increases in distance between the mice were noted between different time periods: D20 vs D30 ($p = 0.0080$), D10 vs D20 ($p = 0.0156$), and D10 vs D30 ($p = 0.0026$). Furthermore, significant increases were observed between pre-KA and D10 ($p = 0.0420$) as well as between pre-KA and D20 ($p < 0.0001$), highlighting changes in nocturnal activity across the cohabitation

period and in response to KA injection. Similarly, for the noon intervals, substantial increases were detected: D20 vs D30 ($p < 0.0001$), D10 vs D20 ($p < 0.0001$), and D30 vs D10 ($p < 0.0001$). However, significant decreases in distance were evident between pre-KA and D10 ($p < 0.0001$) as well as between pre-KA and D20 ($p = 0.0001$). During the evening intervals, significant increases in the distance were observed across different time periods: D20 vs D30 ($p < 0.0001$), D10 vs D20 ($p < 0.0001$), and D10 vs D30 ($p < 0.0001$). However, no significant differences were found between pre-KA and D10 ($p = 0.1046$). For the pre-KA and D20 group, there was a significant increase in distance between the mice ($p < 0.0001$) (Figure 23).

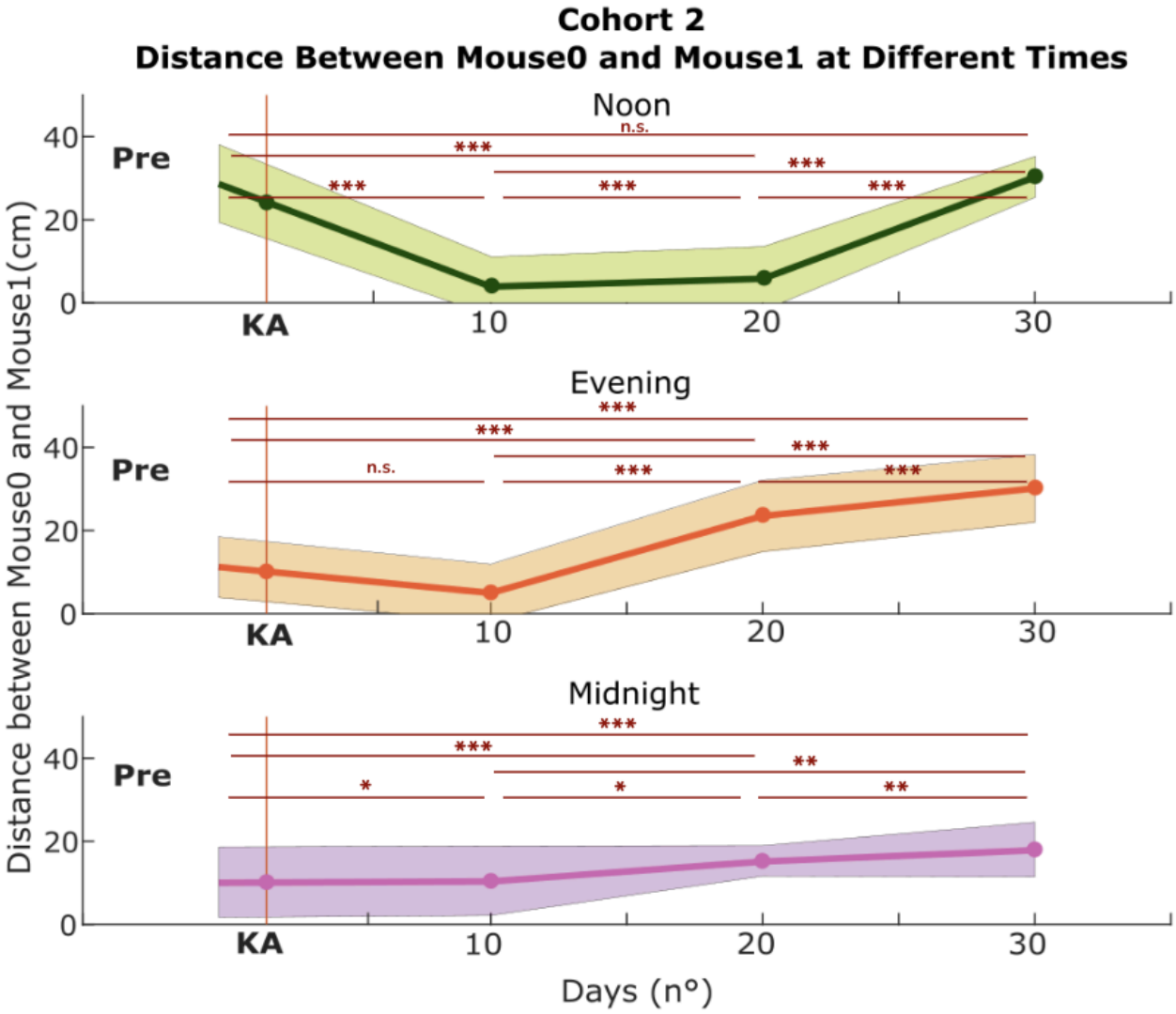

Figure 23: Distance between mouse 0 and mouse 1 from cohort 2 across the 30 days in the enclosure at different times during the day. The mice were analyzed during hours around midnight, noon, and evening.

Discussion

For this particular study, the KA model was chosen due to its relevance in investigating chronic seizure progression, which aligns with the research focus on understanding behavior. Chronic seizures differ from acute seizures in their prolonged nature and potential for recurrent episodes, highlighting the importance of studying their underlying mechanisms for effective treatment strategies. While the Kindling model also demonstrates chronic seizure progression, its invasive nature and constant need for electrode attachment pose practical challenges in a mouse pair setting. The presence of a paired mouse could interfere with electrode placement during grooming or other interactions, making the KA model a more feasible choice for this study.

It is important to note that due to technical and storage limitations, the videos from cohort three and cohort four were unfortunately not stored, rendering them unavailable for analysis. The high demand for resources such as computer storage, with multiple researchers recording mice and utilizing mouse models for various projects and experiments, further contributed to the strain on available storage capacity. This limitation underscores the challenges faced in data management and highlights the need for robust storage solutions in future studies to ensure comprehensive data capture and analysis.

Upon analysis, the algorithm generated 'el.h5' files containing comprehensive data on the coordinates of mouse 0 and mouse 1 and their body parts throughout the recorded videos. The videos from all the days during the pre-KA surgery days of Cohort 1 were grouped together for cohesive analysis. Subsequently, all of the videos from the days post-KA surgery videos were grouped together. The grouped data contains all the coordinate points and locations of both mouse 0 and mouse 1. The same methodology was then employed for Cohort 2, ensuring a comparative analysis across different experimental conditions and mouse cohorts. Given the extensive volume of videos and the time constraints limiting a comprehensive analysis of all

files, a systematic approach was adopted to select specific hours and videos. This method ensured the inclusion of footage capturing mice both pre- and post-KA surgery. Additionally, videos were carefully chosen to cover various timeframes, including daytime recordings, nighttime footage using the infrared camera, periods of mouse activity, and instances when the mice were at rest. It was critical to keep the pre- and post-KA data distinct in order to comparatively analyze the change in the mice's behaviors.

Our findings on the effect of social housing on epileptic and non-epileptic mice provide insights into these animals' behaviors change over a 30-day period. The significance of these results, as illustrated in Figure 17, Figure 18, and Figure 19, shows the impact of social housing on vital behaviors such as movement (exercise wheel), eating, and drinking, which are crucial indicators of animal well-being.

In cohort 1, depicted in Figure 17, it is evident that the epileptic mouse spent a significant amount of time away from the exercise wheel after the KA injection ($p = 0.0462$), whereas there was no difference for the healthy mouse ($p = 0.1378$). There was no significant difference between mouse 0 and mouse 1 before the surgery ($p = .7822$), but there was after the surgery ($p = 0.0021$). Similar results can be seen in Cohort 2. Mouse 0 spent less time at the wheel after the surgery ($p < 0.0001$). In this Cohort, mouse 1 also spent less time at the wheel after the surgery ($p < 0.0001$). This disparity in behavior highlights the potential influence of epilepsy on the activity levels of mice. It is a possibility that the epileptic mouse exhibited reduced exercise activity on the wheel due to the occurrence of spontaneous seizures compared to its non-epileptic state. In one study, early voluntary physical exercise was shown to delay seizure onset and lessen severity in an epilepsy-prone synapsin II knockout mouse (SynIIKO) (Ahl et al., 2019). In humans, the effect of exercise remains unclear. A study conducted by Van Den Bogard (2020)

found that exercise does not generally increase seizure frequency in people with epilepsy. However, Kamel (2014) found that exercise-induced seizures may be more common in patients with TLE (Kamel, 2014). In Cohort 2, the healthy mouse also spent less time on the wheel, which could be attributed to the epileptic mouse's seizures potentially disrupting its inclination to engage in wheel activity.

Similarly, in Figure 18, differences in behavior regarding proximity between epileptic and non-epileptic mice further emphasize the impact of epilepsy on these essential behaviors. Regarding the distance to the food dispenser, in both cohorts, the epileptic mouse and the nonepileptic mouse have different behaviors. In Cohort 1, mouse 0 and mouse 1 spent significantly more time near the food after the KA injection ($p < 0.0001$ for both). Mouse 0 spent a significantly larger portion of its time near the food dispenser compared to mouse 1 after the surgery ($p = 0.0017$ and $p = 0.0003$, respectively). It was unexpected that the epileptic mouse would exhibit increased proximity to the food source as it approached the end stage of malnutrition and dehydration at the end of the 30 days. This suggests that the mouse spent more time near the food dispenser but did not actually consume food or nutrients. The mouse exhibited a general avoidance of movement, suggesting a possible lack of energy to reach and consume food. For Cohort 2, there was no significant difference between the epileptic mouse before and after the surgery and before the surgery ($p = 0.4084$). However, mouse 1 spent more time near the food area after the surgery ($p = 0.0007$). Before the surgery, mouse 0 spent more time near the food dispenser ($p = 0.0466$). However, after the surgery, mouse 1 spent more time near the food compared to mouse 0 ($p = 0.0042$). This observation aligns with previous findings, as mentioned, wherein mice with chronic epilepsy tend to consume less food and water toward the end of the 30-day period (Freund et al., 1971).

The next analysis for the mice's proximity to the water bottle (Figure 19) also shows changes in vital behaviors. In cohort 1, similar to the analysis regarding the food dispenser, mouse 0 and mouse 1 spent more time at the water bottle after the surgery compared to before ($p < 0.0001$ for both). There was no significant difference between the mice before the surgery ($p = 0.1068$), but after the surgery, mouse 0 spent more time next to the water ($p = 0.0250$). In cohort 2, mouse 0 spent less time from the water bottle after the surgery ($p < 0.0001$), but mouse 1 did not have a significant difference ($p = 0.9369$). There was also no difference between mouse 0 and mouse 1 before the surgery ($p = 0.4084$), but mouse 0 spent less time near the water bottle after the surgery compared to mouse 1($p = 0.0042$). These findings are in accordance with the hypothesis and the observed behavior of mouse 0.

The variations observed in the distance between mouse 0 and mouse 1 across the initial 10 days, days 11-20, and days 21-30 signify dynamic changes in behavior over time. Notably, significant differences were noted between these time intervals, indicating evolving patterns in behavior that may be influenced by social interactions within the housing environment. Figure 22 shows how, in Cohort 1, there was an overall significant decrease in the amount of time spent together during the noon time period during the 30-day time period ($p = 0.0130$). The mice are the least active during this time, usually sleeping or lying beside each other. The results show that the mice spend more time together, possibly due to the social bonds that have developed over the 30 days. These results suggest that the chronic epilepsy of mouse 0 in Cohort 1 does not affect the sleeping and lounging behaviors between mouse 0 and mouse 1. However, during the evening, there was a significant increase in distance between the mice and then a decrease in time spent together during the 30-day time period. It is crucial to note that chronic epilepsy typically manifests approximately 15 days after the KA injection. Moreover, the evening time is

when the lights turn off in the mouse room, so these videos included when the mice were either still asleep and/or just becoming active and awake. The figure suggests that they tended to spend less time next to each other during the second 10 days. This suggests that as the epilepsy developed, there were less significant social ties between the mice. Meanwhile, in the pre-KA and the first 10 days, the mice spent more time with each other. During the midnight time period, which is when the mice are most active, there was a significant decrease in the time spent together between pre-KA to the first 10 days, the first 10 days to the second 10 days, and the second 10 days to the last 10 days. The figure depicts that the mice spent more time together after day 20, suggesting that the epileptic mouse did not have a significant effect on the healthy mouse and did not disrupt their time spent together. Especially notable during the last 10 days, when the mice spent more of their time together, is the strengthening of social ties over the 30-day period. This suggests that, at a certain point, the influence of epilepsy became less significant on their social interactions in Cohort 1.

In Cohort 2, Figure 23 shows a notable trend, with mouse 0 and mouse 1 spending less time together during noon, evening, and midnight 30 days after the surgery compared to the pre-KA days. As the trend shows, the distance increased as the days went on. This suggests that perhaps the mice did not want to interact as frequently when they were active, possibly due to the seizures of mouse 0. During the noon, there is an increase in the amount of time spent and a decrease in the distance between the mice with each other during the first 10 days. This outcome is anticipated, given the strengthening of social bonds during periods of rest when the mice lie and sleep next to each other. However, as chronic epilepsy symptoms typically emerge around day 15, there is a noticeable spike in distance starting around day 20, which continues to escalate. During the evening, there is an initial trend of the mice spending more time next to each

other until approximately day 10, after which the distance between them begins to increase. This pattern remains consistent during the midnight period as well, albeit to a lesser extent. This observation is likely attributed to the fact that mice are typically more active during this time, engaging in individual activities and interacting with others less frequently. Overall, in Cohort 2, it is evident that the social bonds between the mice are disrupted, most likely due to the seizures experienced by mouse 0, resulting in less time spent together.

The relevance of this data lies in its examination of how social housing conditions impact the mouse model, particularly in the context of epileptic and healthy mice cohabitating. While it may seem intuitive that an epileptic mouse would exhibit poorer health outcomes compared to its healthy counterpart, the significance of this data extends beyond mere health assessments. The key takeaway from these findings is the identification of behavioral changes attributable to social housing, specifically when a cage mate has chronic temporal lobe epilepsy. Furthermore, our observations indicate that as chronic epilepsy advances, it not only influences the behavior of individual mice but also affects their collective interaction time. The contrasting patterns observed in Cohort 1 and Cohort 2 shed light on the complex interplay between epilepsy and social behavior in mice. In Cohort 1, there was an initial increase in distance between the mice, indicative of disrupted social bonds possibly influenced by the onset of epilepsy symptoms. However, towards the end of the 30-day period, there was a notable decrease in distance, suggesting a potential adaptation or adjustment in social interactions despite the persistent presence of epilepsy. Conversely, in Cohort 2, a different trend emerged, with a decrease in distance observed initially, followed by an increase over time. This fluctuation in distance suggests a dynamic response to the presence of epilepsy, where social bonds may initially strengthen before being potentially disrupted by the progression of the condition. Overall, these

findings underscore the multifaceted nature of the relationship between epilepsy and social behavior, highlighting the need for further investigation to elucidate the underlying mechanisms driving these complex dynamics.

These findings align with previous research, such as Manouze (2019), which highlights the impact of housing conditions on stress levels, seizure frequency, and overall well-being in rodents. This study dual-housed male mice together for a pretest period of one week and then a post-test period of four weeks. The study found that isolated male mice have increased stress behaviors and that socially housed male mice have less frequent seizures.

The following observations are not computationally analyzed but rather observations made by watching the videos. An observation in Cohort 3 was the occurrence of fighting among the mice following seizures in the identified mouse 0. The injuries sustained by mouse 0 were severe enough to necessitate early euthanization. This behavior could stem from various factors. One possible reason is the introduction of a new mouse 0 after a day of separation due to the demise of the original mouse 0 paired with mouse 1 post-surgery. Another plausible explanation could be that mouse 1, which did not exhibit seizures, experienced stress and a defensive response upon witnessing the seizures in mouse 0. This scenario sheds light on the complex dynamics of social interactions among mice pairs and underscores the variability in behavioral responses. Furthermore, it is essential to consider additional perspectives and theories regarding the observed fighting behavior. One plausible theory could be related to changes in social hierarchy dynamics within the group following the introduction of a new member. Research has shown that male mice form stable dominance hierarchies (Williamson et al., 2016). Introducing a new dynamic within the group may have triggered territorial or dominant behaviors that lead to conflicts. Additionally, stress-induced responses triggered by witnessing seizures in a group

member may have led to heightened aggression levels among certain individuals. Exploring these diverse theories can provide valuable insights into understanding how social dynamics and stress responses influence behavioral outcomes in experimental settings involving mice models of epilepsy.

In Cohort 1, an observation was made where the healthy mouse (mouse 1) actively relocated the epileptic mouse (mouse 0) to the nest it had constructed, as mouse 0 was nearing death from chronic epilepsy, dehydration, and malnutrition. Typically, when a mouse dies, its cagemate consumes it; however, in this instance, the healthy mouse transports the dying mouse to the nest. This behavior could be attributed to several factors. One possible explanation is the social bonds developed during their 30-day cohabitation period. Studies have demonstrated that familiar males, particularly those raised together from weaning, exhibit reduced levels of aggression compared to unfamiliar males (Dewsbury, 1988). Notably, mouse 0 and mouse 1 were littermates in all of the cohorts. Furthermore, the social connections established over the 30-day period may have prompted the healthy mouse to provide care and grooming for the epileptic mouse. The observed changes in behavior underscore the importance of considering housing conditions in preclinical research interpretations. By elucidating how epilepsy affects behaviors and how social housing influences these patterns, this study contributes to a deeper understanding of the complex interplay between neurological conditions and environmental factors on animal well-being.

Several limitations were encountered during the course of this study. Firstly, the computational resources used to develop the algorithm for video analysis were constrained, as the computer available was slow and often shared among multiple researchers in the lab. Although the introduction of a supercomputer towards the project's conclusion helped alleviate

some of these issues, time constraints hindered the comprehensive analysis of all videos. Additionally, the algorithm primarily focused on tracking the mice within the cage, lacking the capability to ascertain whether they were near the food source or engaging with the exercise wheel. Further refinement of the algorithm is necessary to incorporate features such as monitoring mouse behavior and exercise activity directly. Furthermore, the complex social hierarchies inherent in mouse colonies posed challenges, with behaviors differing notably between cohorts. Given that this study only included two cohorts, it's imperative for future research to encompass a broader range of cohorts to generalize findings effectively. These limitations underscore the need for continued refinement of methodologies and resources to enhance the depth and accuracy of experimental observations.

Conclusions

The development and implementation of a multi-animal video monitoring system enables precise tracking of mice within their cages and provides insights into their interactions with one another. It is important to acknowledge that this study exclusively examined interactions between an epileptic mouse and a wild-type mouse rather than interactions between two wild-type mice. The observations of grooming, sleeping proximity, and cohabitation among partners have illuminated the social dynamics within the enclosure, emphasizing the importance of social context in shaping their behaviors.

Moreover, our study has revealed connections between social interactions and seizure progression in mice. By examining how social bonds influence seizure outcomes, we have identified potential avenues for further research into modifying housing systems to better support mice welfare and accurately replicate their natural behaviors. These advancements not only enhance our understanding of animal behavior but also hold promise for improving research outcomes by creating environments that promote the well-being and natural behaviors of the subjects.

In conclusion, the development and application of the multi-animal video monitoring system have expanded our ability to study mice behavior and explore the intricate relationship between social interactions, housing conditions, and health outcomes. By striving to create environments that mirror natural settings as closely as possible, we can achieve better outcomes for our research subjects while advancing our knowledge in behavioral sciences and neurology. This research can shape future studies aimed at optimizing experimental conditions to ensure ethical treatment and accurate representation of animal behaviors in scientific investigations.

Significance

The prevalence of epilepsy in the United States, as stated by the Centers for Disease Control and Prevention, underscores the significant impact of this condition on individuals' daily living conditions and quality of life. Epilepsy is associated with a mortality rate 2-3 times higher than the general population and increases the likelihood of other chronic conditions like depression and anxiety. Seizures and the fear of experiencing them can limit individuals' ability to work, drive, or participate in social activities (Facts & Statistics About Epilepsy, n.d.). With approximately 1.2% of the population affected by active epilepsy in 2015, translating to around 3 million adults and 470,000 children nationwide, the burden of this neurological disorder is profound (Epilepsy Data and Statistics, 2020). Epilepsy not only poses challenges in performing daily tasks but also presents ongoing hurdles despite advancements in treatment modalities, with many patients grappling with recurrent seizures and associated complications.

While animal models have been pivotal in advancing epilepsy research and therapeutic development, there exists a significant gap in understanding the complex behavioral patterns and literature regarding social housing effects on seizure progression. The influence of companionship and social relationships on seizure dynamics remains an understudied area. Existing research discusses outcomes where individuals with epilepsy often lead isolated lives, potentially impacting their stress levels and overall well-being negatively (Stauder, 2020). This isolation can inadvertently exacerbate stress levels, highlighting the interconnectedness between social factors and disease progression. In other extreme cases, people with epilepsy need 24/ 7 monitoring or even get dogs to detect seizures before they happen, as these seizures are unpredictable. Models of epilepsy in mice are recreated, but the social environment of the patients and caregivers is not constructed in these models.

As most animal studies on epilepsy are conducted in isolation settings, this overlooks the potential influence of the disease course on social interactions. Studying the social behavior of mice in this study provides insights into how companionship may influence seizure propagation and how seizures impact social interactions. This study's exploration of how seizure propagation influences social behavior in mice not only offers valuable insights into enhancing the health outcomes of epilepsy patients but also has the potential to inform the development of more precise and effective mouse models, particularly in the realm of seizure studies.

By exploring the relationship between social elements and seizure patterns in mice, this study has the potential to enhance and fine-tune mouse models used in epilepsy research. This could lead to advancements in our comprehension of the condition and open doors for more effective therapeutic approaches in the future.

Moreover, the study's findings may extend to elucidating the reciprocal impact of epileptic patients on the stress levels and mental health of their partners, as suggested by Norup (2013). By unraveling these intricate dynamics, opportunities arise to enhance the quality of life not only for individuals with epilepsy but also for their partners. Ultimately, this research endeavor stands poised to bridge critical knowledge gaps, paving the way for more holistic approaches to epilepsy management that encompass social dimensions for improved patient outcomes and well-being.

Future Directions and Implications

Moving forward, there are several avenues for enhancing the training and capabilities of the algorithm used in this study. While the algorithm was trained 24 times, there is potential for further refinement through additional training iterations to improve accuracy in plotting the mice's positions and behaviors. By extending the training process, the algorithm can evolve to better discern and interpret mouse behaviors, including more accurate seizure detection.

In future studies, it is imperative to incorporate male-female mouse models, as this research solely focused on cohorts of male-male interactions, to comprehensively understand the gender-specific dynamics influencing seizure progression and behavioral responses. Considering the differences between male-male and male-female interactions among mice could offer valuable insights into how social behaviors influence seizure progression and behaviors in both epileptic and non-epileptic mice. Understanding these gender-specific nuances in social interactions may shed light on how to tailor experimental setups to better capture the complexities of seizure development and behavioral responses. Exploring how these dynamics might vary in different social bonds, such as between mothers and pups, would offer further intriguing insights into the interplay between epilepsy, social behavior, and specific relationships.

In addition to the behavioral and computational analysis, incorporating cortisol measurements through blood samples of the mice throughout the 30-day period can also offer insights into assessing stress levels among the mice.

In this study, the KA mouse model was utilized due to chronic seizure progression. Moving forward, the Pilocarpine and Kindling seizure models could also be considered for studying how mouse behavior and seizure progression may change using these models.

The implications of this research extend beyond the realm of animal studies, with potential translational impacts on human epilepsy management. Insights from this study hold

promise in informing interventions that enhance living conditions for individuals with epilepsy, mitigating social isolation, and potentially preventing caregiver burnout. By addressing these social dimensions, there is a prospect of improving health outcomes and overall well-being for both individuals with epilepsy and their caregivers.

Future directions could involve exploring advanced machine-learning techniques to enhance the algorithm's capabilities in behavior recognition and seizure detection. Incorporating real-time monitoring systems and automated alerts for seizure events could revolutionize epilepsy research by providing timely interventions and personalized care strategies based on individualized behavioral patterns.

Investigating the molecular pathways underlying social interactions and their impact on seizure susceptibility could open new avenues for targeted therapeutic interventions. By studying the intricate interplay between social factors, gender-specific behaviors, and seizure progression, this research sets the stage for a more comprehensive understanding of epilepsy pathophysiology and personalized treatment approaches tailored to individual needs.

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In [1]:
```
import pandas as pd
import h5py
import numpy as np
import os
import matplotlib.pyplot as plt
from scipy.stats import ttest_ind
from scipy.stats import shapiro
from scipy.stats import mannwhitneyu
import seaborn as sns
```

In [2]:
```
pip install openpyxl
```

```
Requirement already satisfied: openpyxl in c:\users\shared_grosslab\anaconda3
\lib\site-packages (3.1.2)
Requirement already satisfied: et-xmlfile in c:\users\shared_grosslab\anaconda
3\lib\site-packages (from openpyxl) (1.1.0)
Note: you may need to restart the kernel to use updated packages.
```

In [3]:
```
# Get the path to the user's home directory
home_directory = os.path.expanduser('~')

# Set the working directory to the desktop directory
desktop_directory = os.path.join(home_directory, 'Desktop')
os.chdir(desktop_directory)

# Print the current working directory to verify
print("Current working directory:", os.getcwd())
```

```
Current working directory: C:\Users\shared_grosslab\Desktop
```

In [4]:
```
file_paths = [
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d05_h14m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d05_h15m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d05_h16m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d05_h23m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d06_h00m17s02_utcDLC_
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    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d06_h10m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d06_h12m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d06_h13m17s02_utcDLC_
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    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d06_h16m17s02_utcDLC_
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    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d07_h01m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d07_h10m03s10_utcDLC_
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    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d07_h14m03s10_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d07_h15m03s10_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d07_h16m03s10_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d07_h23m03s10_utcDLC_
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    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d23_h11m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d23_h12m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d23_h13m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d23_h14m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d23_h15m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d23_h16m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d23_h23m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d24_h00m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d24_h01m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d24_h11m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d24_h12m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d24_h13m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d24_h14m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d24_h15m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d24_h16m27s06_utcDLC_
```

```
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d24_h23m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d25_h00m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d25_h01m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d25_h10m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d25_h11m27s06_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d03_h15m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d03_h16m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d03_h17m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d03_h18m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d03_h19m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d03_h20m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d03_h21m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d03_h22m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d03_h23m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d05_h00m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d05_h01m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d05_h10m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d05_h11m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d05_h12m17s02_utcDLC_
    r"C:\Users\shared_grosslab\Desktop\sample\box1_y2023m09d05_h13m17s02_utcDLC_
]
```

In [167…
```
num_files = len(file_paths)
print("Number of file paths:", num_files)
```

```
Number of file paths: 107
```

In [5]:
```
combined_data = pd.DataFrame()
```

In [165…
```
confidence_threshold = 0.80

for file_path in file_paths:
    # Read data from each file into a DataFrame
    df = pd.read_hdf(file_path).droplevel(0, axis=1)

    # Calculate the mean of the likelihood values across all levels
    mean_likelihood = (df.loc[:, (slice(None), slice(None), 'likelihood')] > c

    # Filter rows based on the mean confidence (likelihood) values
    if mean_likelihood > confidence_threshold:
        combined_data = pd.concat([combined_data, df], ignore_index=True)

# Drop rows containing NaN values
combined_data = combined_data.dropna()
```

```
Index([], dtype='object')
```

Mouse 0 - body and mouth

In [151…
```
body0 = df['mouse0']['body_center'][['y','x']]
```

In [152…
```
mouth0 = df['mouse0']['nose'][['y','x']]
```

Mouse 1 - body and mouth

In [153…
```
body1 = df['mouse1']['body_center'][['y','x']]
```

In [154…
```
mouth1 = df['mouse1']['nose'][['y','x']]
```

Within Bounds of Exercise Wheel

In [155…
```
# the y coordinate is first so it is [y,x]
exercise_center = np.array([313,437])
exercise_radius = 83
```

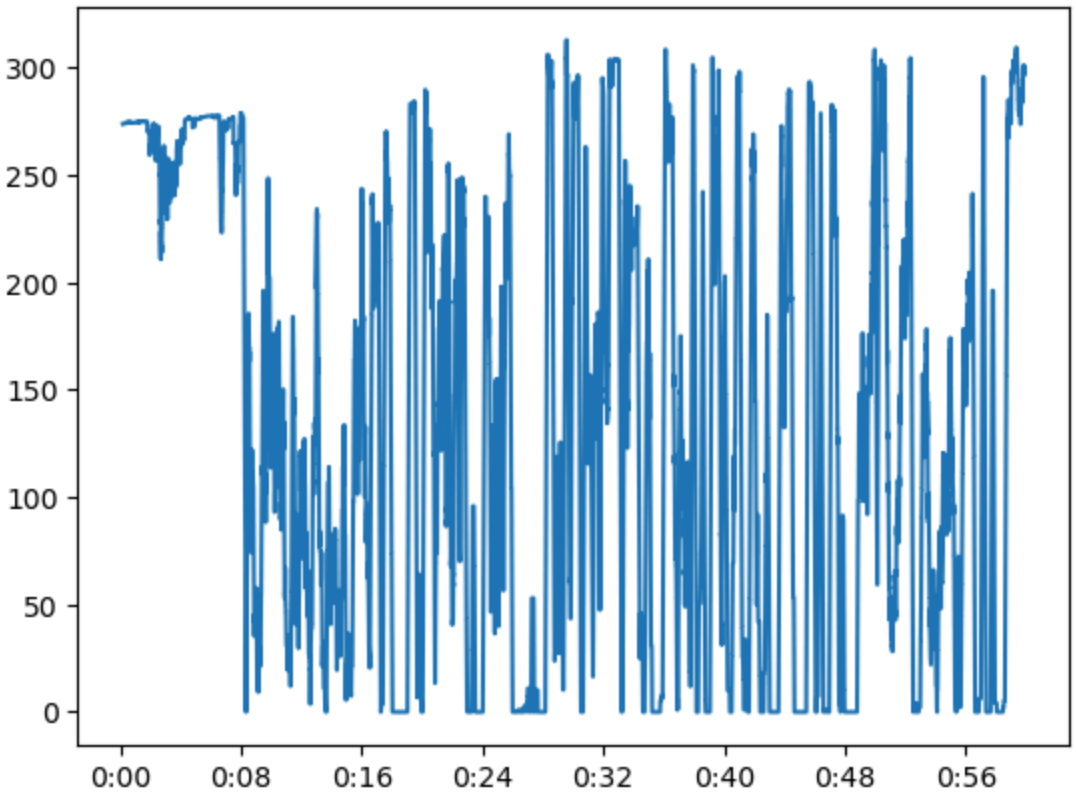
Mouse 0

In [156…
```
exercise_distance0 = (body0 - exercise_center).pow(2).sum(axis=1).pow(1/2)

exercise_within_bounds0=(exercise_distance0 <exercise_radius)

exercise_within_bounds0.mean()
```

Out[156]:
```
0.5316299559471366
```

In [157…
```
num_frames = len(body0 - exercise_center)
time_seconds = np.arange(num_frames) * (1/30)
time_minutes = time_seconds / 60
plt.xticks(np.arange(0, num_frames, 4 * 3600), [f'{int(time // 3600)}:{int((ti
(body0 - exercise_center).pow(2).sum(axis=1).pow(1/2).rolling(300).mean().plot
```

Out[157]:
```
<Axes: >
```



Mouse 1

In [158…
```
exercise_distance1 = (body1 - exercise_center).pow(2).sum(axis=1).pow(1/2)

exercise_within_bounds1=(exercise_distance1 < exercise_radius)

exercise_within_bounds1.mean()
```

Out[158]:
```
0.627693021099003
```

In [159…
```
statistic, p_value = mannwhitneyu(exercise_within_bounds0,exercise_within_boun

# Interpret the result
alpha = 0.05
if p_value < alpha:
    print("Reject null hypothesis: There is a significant difference between g
else:
    print("Fail to reject null hypothesis: There is no significant difference

print("P-value:", p_value)
```

```
Reject null hypothesis: There is a significant difference between groups.
P-value: 0.0
```

In [160…
```
exercise0 = (body0 - exercise_center).pow(2).sum(axis=1).pow(1/2)
```

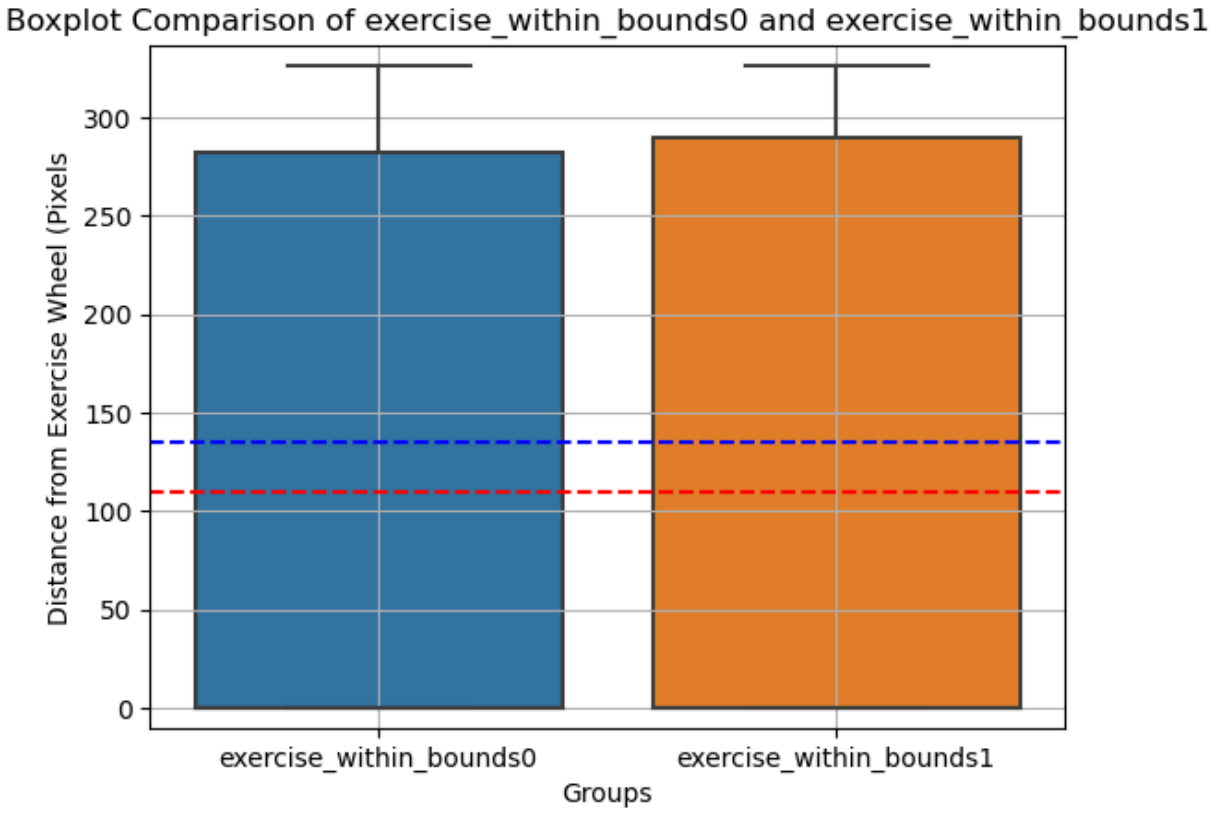
In [161…
```
import os

# Get the path to the user's home directory
home_directory = os.path.expanduser('~')

# Set the working directory to the desktop directory
desktop_directory = os.path.join(home_directory, 'Desktop')
os.chdir(desktop_directory)
```

In [162…
```
# Create DataFrames with the values of exercise0 and exercise1
df_exercise0 = pd.DataFrame({'Exercise0_Values': exercise0})
df_exercise1 = pd.DataFrame({'Exercise1_Values': exercise1})

# Merge the two DataFrames into one
df_combined = pd.concat([df_exercise0, df_exercise1], axis=1)

# Specify the Excel file path
excel_file = 'exercise1NEW.xlsx'

# Export the combined DataFrame to Excel
df_combined.to_excel(excel_file, index=False)
```

In [163…
```
exercise1 = (body1 - exercise_center).pow(2).sum(axis=1).pow(1/2)
```

In [164…
```
data = pd.DataFrame({'exercise_within_bounds0': exercise0,
                     'exercise_within_bounds1': exercise1})

# Create boxplots using Seaborn
sns.boxplot(data=data)
plt.ylim(min(min(exercise0), min(exercise1))- 10, max(max(exercise0), max(exer
plt.xlabel("Groups")
plt.ylabel("Distance from Exercise Wheel (Pixels")
plt.title("Boxplot Comparison of exercise_within_bounds0 and exercise_within_b
plt.grid(True)
```

```
mean0 = sum(exercise0) / len(exercise0)
mean1 = sum(exercise1) / len(exercise1)
plt.axhline(y=mean0, color='blue', linestyle='--', label=f'Mean 0: {mean0:.2f}
plt.axhline(y=mean1, color='red', linestyle='--', label=f'Mean 1: {mean1:.2f}'
plt.show()
```



Within Bounds of Water Bottle

In [26]:
```
waterdish_center = np.array([310,190])
waterdish_radius = 40
```

Mouse 0

In [27]:
```
waterdish_distance0 = (mouth0 - waterdish_center).pow(2).sum(axis=1).pow(1/2)

waterdish_within_bounds0=(waterdish_distance0 < waterdish_radius)

waterdish_within_bounds0.mean()
```

Out[27]:
```
0.5646927892418271
```

In [28]:
```
fig,ax=plt.subplots()
waterdish_within_bounds0.astype(int).plot(ax=ax)
ax.set_xlim([0,1000])
```

Out[28]:
```
(0.0, 1000.0)
```

In [30]:
```
waterdish_distance1 = (mouth1 - waterdish_center).pow(2).sum(axis=1).pow(1/2)

waterdish_within_bounds1=(waterdish_distance1 < waterdish_radius)

waterdish_within_bounds1.mean()
```

Out[30]:
```
0.5219105031300719
```

In [31]:
```
statistic, p_value = shapiro(waterdish_within_bounds0)

# Print the test result
print("Shapiro-Wilk Test Statistic:", statistic)
print("P-value:", p_value)

# Interpret the result
alpha = 0.05
if p_value < alpha:
    print("Reject null hypothesis: Data is not normally distributed.")
else:
    print("Fail to reject null hypothesis: Data may be normally distributed.")
```

```
Shapiro-Wilk Test Statistic: 0.6303646564483643
P-value: 0.0
Reject null hypothesis: Data is not normally distributed.
C:\Users\shared_grosslab\anaconda3\lib\site-packages\scipy\stats\_morestats.p
y:1816: UserWarning: p-value may not be accurate for N > 5000.
  warnings.warn("p-value may not be accurate for N > 5000.")
```

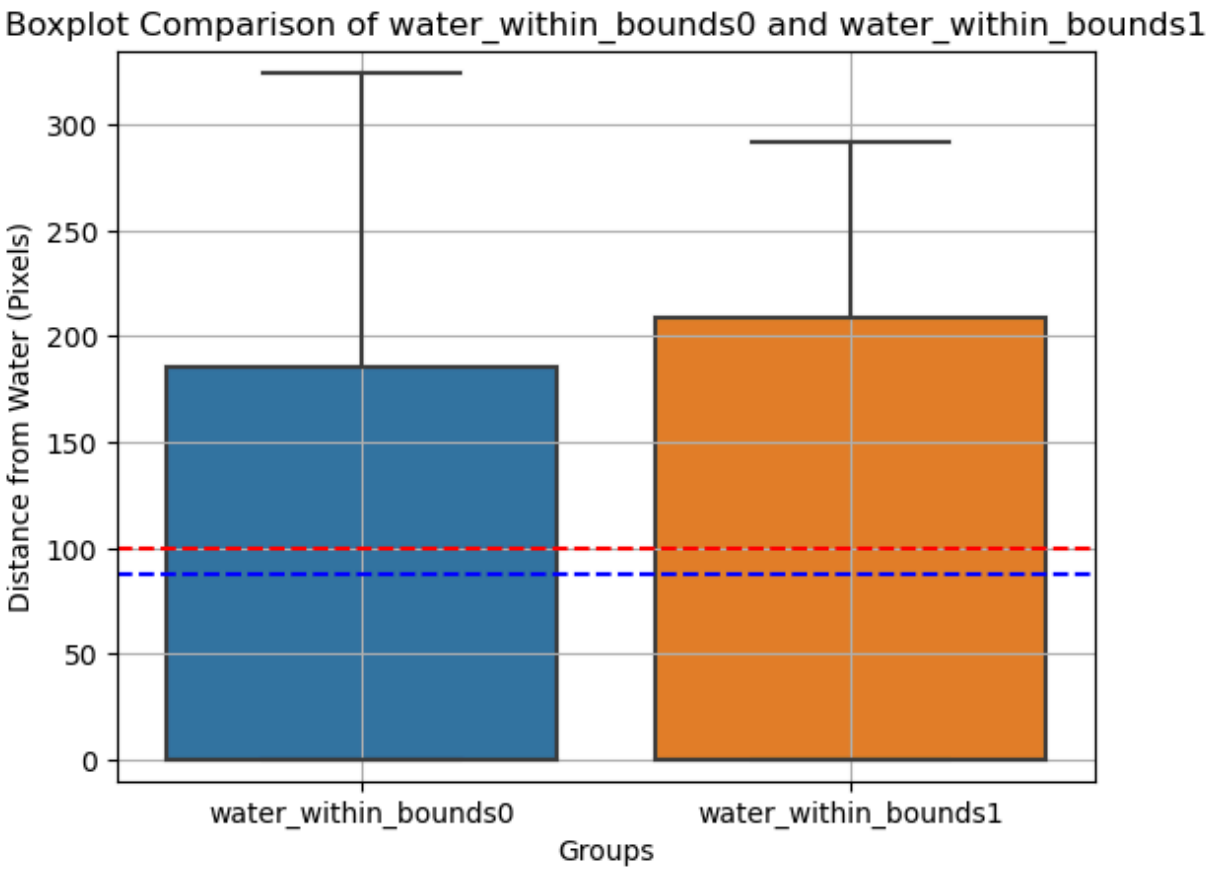
In [32]:
```
statistic, p_value = mannwhitneyu(waterdish_within_bounds0,waterdish_within_bou

# Interpret the result
alpha = 0.05
if p_value < alpha:
    print("Reject null hypothesis: There is a significant difference between g
else:
    print("Fail to reject null hypothesis: There is no significant difference

print("P-value:", p_value)
```

```
Reject null hypothesis: There is a significant difference between groups.
P-value: 1.7528217178764744e-88
```

In [33]:
```
water0 = (mouth0 - waterdish_center).pow(2).sum(axis=1).pow(1/2)
water1 = (mouth1 - waterdish_center).pow(2).sum(axis=1).pow(1/2)
```

In [52]:
```
# Create DataFrames with the values of exercise0 and exercise1
df_water0 = pd.DataFrame({'Water0_Values': water0})
df_water1 = pd.DataFrame({'Water1_Values': water1})

# Merge the two DataFrames into one
df_combined = pd.concat([df_water0, df_water1], axis=1)

# Specify the Excel file path
excel_file = 'water2.xlsx'

# Export the combined DataFrame to Excel
df_combined.to_excel(excel_file, index=False)
```

In [35]:
```
data = pd.DataFrame({'water_within_bounds0': water0,
                     'water_within_bounds1': water1})

# Create boxplots using Seaborn
sns.boxplot(data=data)
plt.ylim(min(min(water0), min(water1))- 10, max(max(water0), max(water1))+ 10)
plt.xlabel("Groups")
plt.ylabel("Distance from Water (Pixels)")
plt.title("Boxplot Comparison of water_within_bounds0 and water_within_bounds1"
plt.grid(True)
mean0 = sum(water0) / len(water0)
mean1 = sum(water1) / len(water1)
plt.axhline(y=mean0, color='blue', linestyle='--', label=f'Mean 0: {mean0:.2f}
plt.axhline(y=mean1, color='red', linestyle='--', label=f'Mean 1: {mean1:.2f}'
plt.show()
```

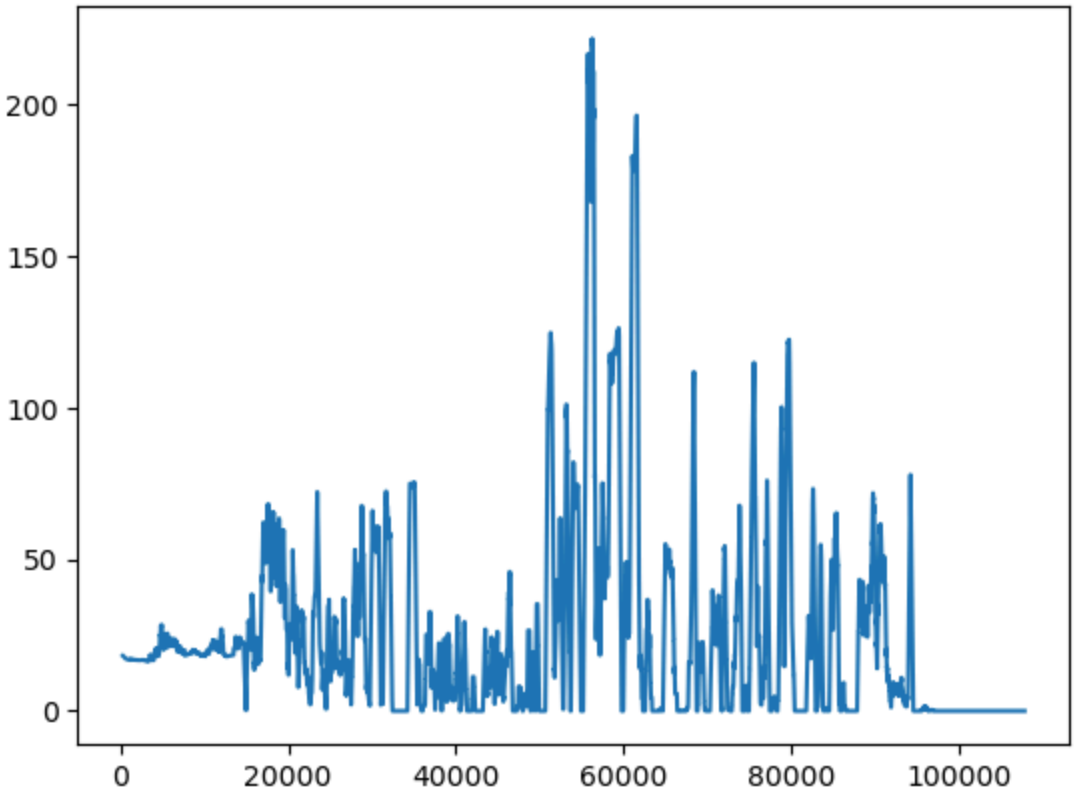


Within Bounds of Food

In [36]:
```
food_center = np.array([139,179])
food_width = 68
food_height = 178
```

Mouse 0

In [37]:
```
food_distance0 = np.abs(mouth0 - food_center)

food_within_bounds0 = np.all(food_distance0 <= [food_height / 2, food_width / 
```

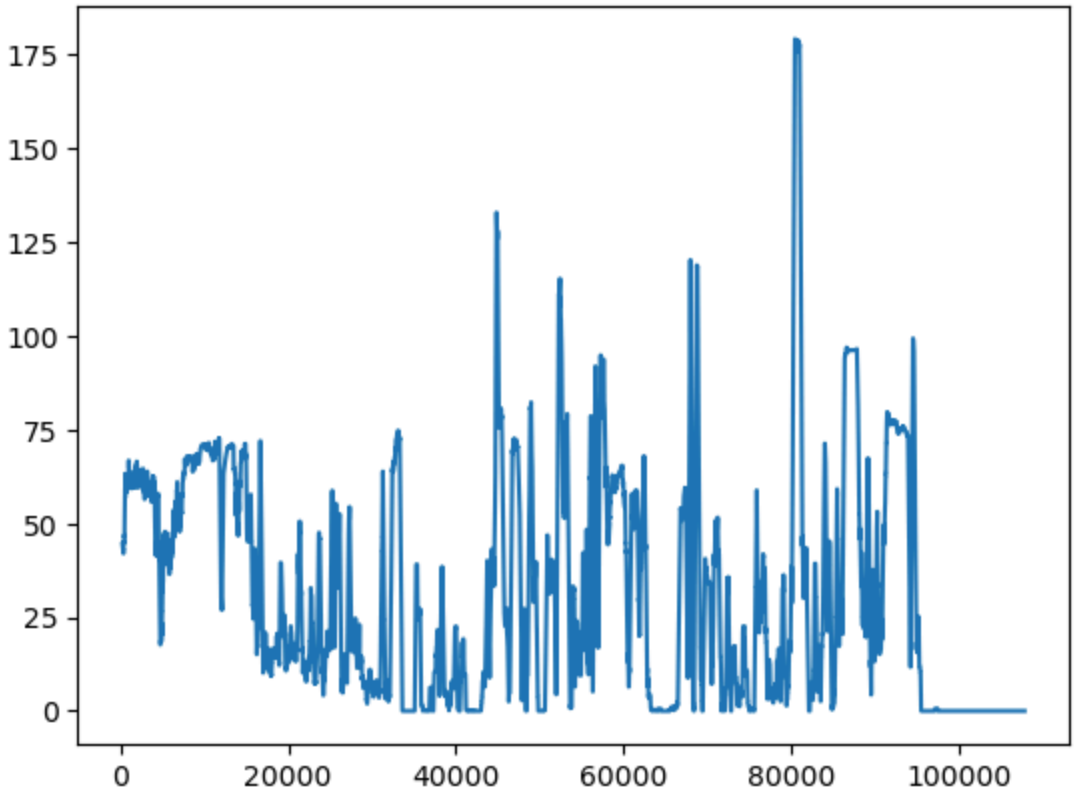


In [41]:
```
food0 = (mouth0 - food_center).pow(2).sum(axis=1).pow(1/2)
```

In [42]:
```
food1 = (mouth1 - food_center).pow(2).sum(axis=1).pow(1/2)
```

In [43]:
```
(mouth1 - food_center).pow(2).sum(axis=1).pow(1/2).rolling(300).mean().plot()
```

Out[43]:
```
<Axes: >
```



Mouse 1

In [44]:
```
food_distance1 = np.abs(mouth1 - food_center)

food_within_bounds1 = np.all(food_distance1 <= [food_height / 2, food_width / 

food_within_bounds1.mean()
```

Out[44]:
```
0.16932993276141897
```

In [45]:
```
statistic, p_value = shapiro(food_within_bounds0)

# Print the test result
print("Shapiro-Wilk Test Statistic:", statistic)
print("P-value:", p_value)

# Interpret the result
alpha = 0.05
if p_value < alpha:
    print("Reject null hypothesis: Data is not normally distributed.")
else:
    print("Fail to reject null hypothesis: Data may be normally distributed.")
```

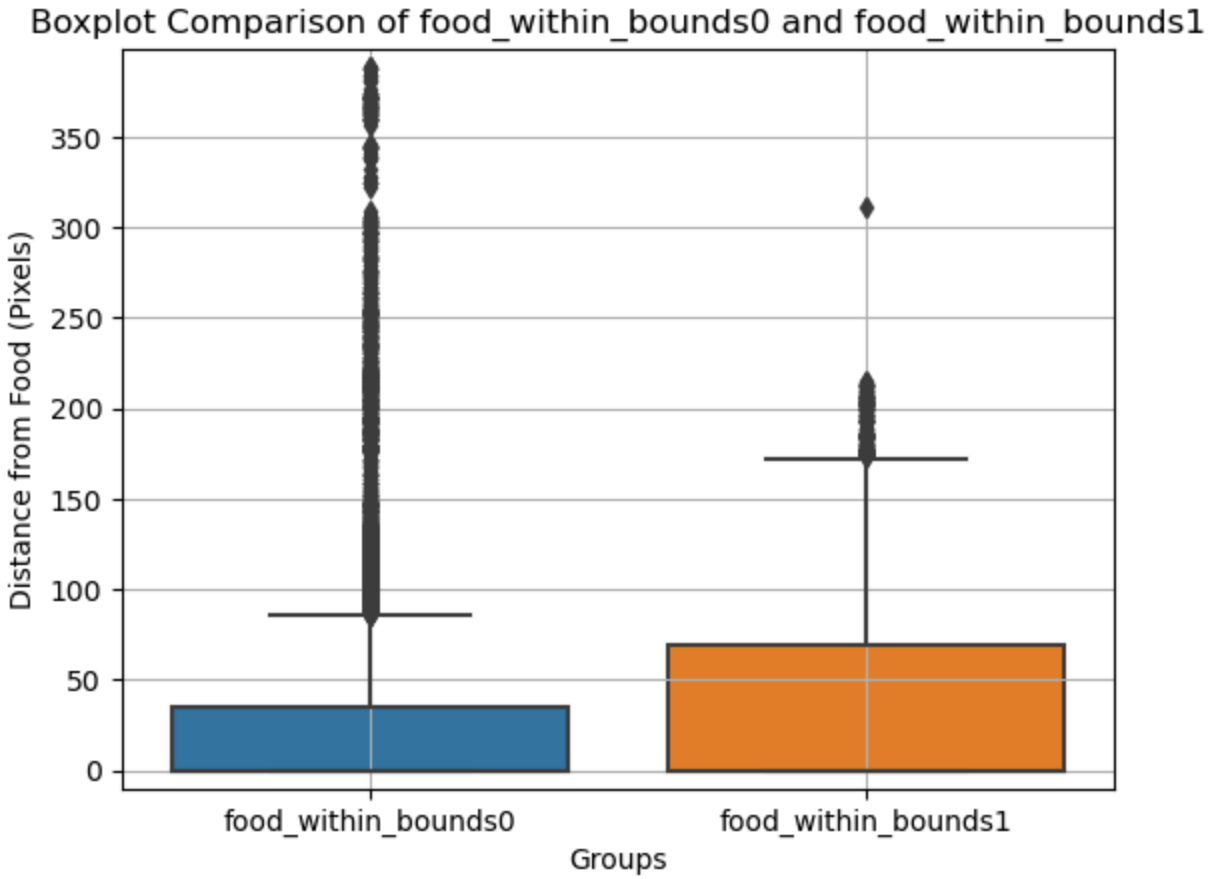
```
Shapiro-Wilk Test Statistic: 0.541311502456665
P-value: 0.0
Reject null hypothesis: Data is not normally distributed.
C:\Users\shared_grosslab\anaconda3\lib\site-packages\scipy\stats\_morestats.p
y:1816: UserWarning: p-value may not be accurate for N > 5000.
  warnings.warn("p-value may not be accurate for N > 5000.")
```

In [46]:
```
statistic, p_value = mannwhitneyu(food_within_bounds0,food_within_bounds1)
```

```
# Interpret the result
alpha = 0.05
if p_value < alpha:
    print("Reject null hypothesis: There is a significant difference between g
else:
    print("Fail to reject null hypothesis: There is no significant difference b

print("P-value:", p_value)
```

```
Reject null hypothesis: There is a significant difference between groups.
P-value: 0.0
```

In [47]:
```
data = pd.DataFrame({'food_within_bounds0': food0,
                     'food_within_bounds1': food1})

# Create boxplots using Seaborn
sns.boxplot(data=data)
plt.ylim(min(min(food0), min(food1)) - 10, max(max(food0), max(food1)) + 10)
plt.xlabel("Groups")
plt.ylabel("Distance from Food (Pixels)")
plt.title("Boxplot Comparison of food_within_bounds0 and food_within_bounds1")
plt.grid(True)
#mean0 = sum(food0) / len(food0)
#mean1 = sum(food1) / len(food1)
#plt.axhline(y=mean0, color='blue', linestyle='--', label=f'Mean 0: {mean0:.2f}
#plt.axhline(y=mean1, color='red', linestyle='--', label=f'Mean 1: {mean1:.2f}
plt.show()
```



In [51]:
```
# Create DataFrames with the values of exercise0 and exercise1
df_food0 = pd.DataFrame({'Exercise0_Values': food0})
df_food1 = pd.DataFrame({'Exercise1_Values': food1})
```

```
# Merge the two DataFrames into one
df_combined = pd.concat([df_food0, df_food1], axis=1)

# Specify the Excel file path
excel_file = 'food2.xlsx'

# Export the combined DataFrame to Excel
df_combined.to_excel(excel_file, index=False)
```

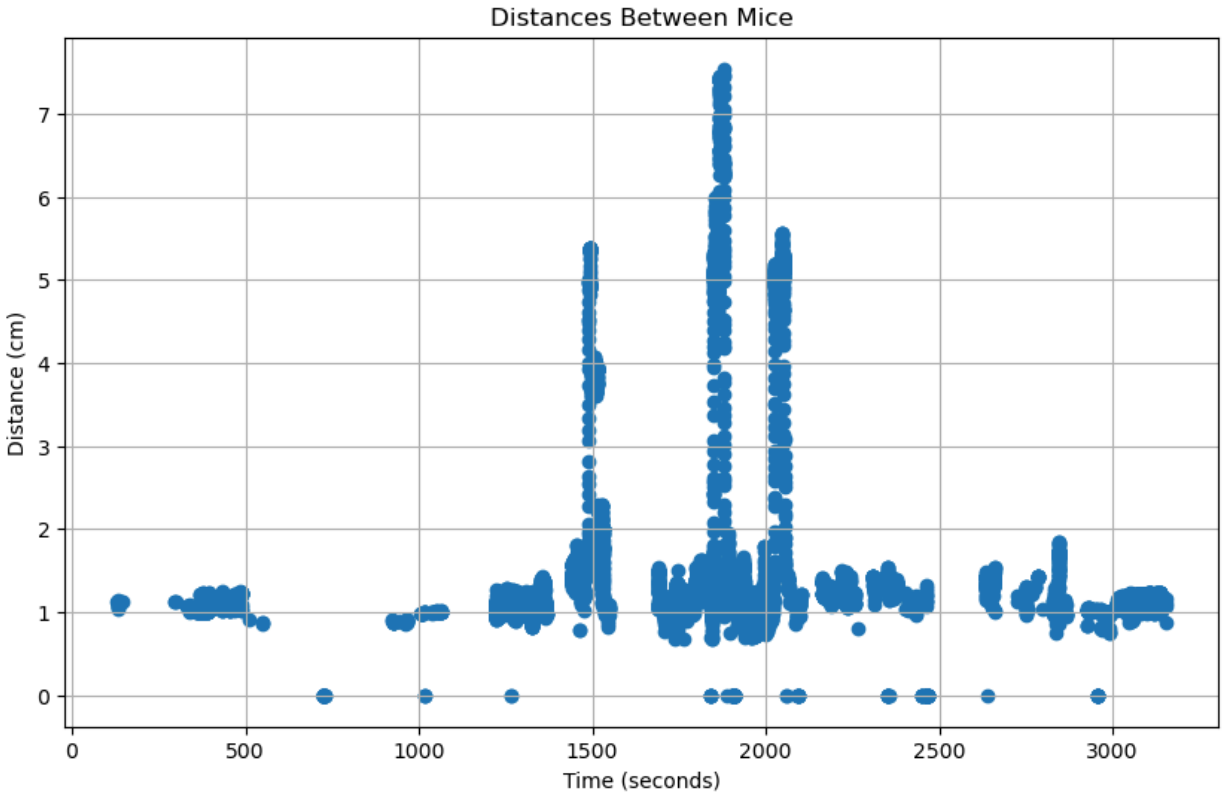
Mouse Together

In [130…

```
# Calculate distances between mouse0 and mouse1 body centers for all instances
distances_between_mice = np.linalg.norm(body0 - body1, axis=1)
```

In [137…

```
# Conversion factor from pixels to centimeters
pixel_to_cm = 0.0264583333

num_frames = len(distances_between_mice)
time_seconds = np.arange(num_frames) / 30  # Assuming 30 frames per second

# Convert distances from pixels to centimeters
distances_in_cm = distances_between_mice * pixel_to_cm

# Plot the distances as a scatter plot
plt.figure(figsize=(10, 6))
plt.scatter(time_seconds, distances_in_cm, marker='o')
plt.xlabel('Time (seconds)')
plt.ylabel('Distance (cm)')
plt.title('Distances Between Mice')
plt.grid(True)
plt.show()
```


Distances Between Mice
Distance (cm)
Time (seconds)
0
1
2
3
4
5
6
7
0
500
1000
1500
2000
2500
3000

In [ ]:

In [48]:
```
import pandas as pd
import h5py
import numpy as np
import os
import matplotlib.pyplot as plt
from scipy.stats import ttest_ind
from scipy.stats import shapiro
from scipy.stats import mannwhitneyu
import seaborn as sns
```

In [91]:
```
pip install openpyxl
```

```
Requirement already satisfied: openpyxl in c:\users\shared_grosslab\anaconda3
\lib\site-packages (3.1.2)
Requirement already satisfied: et-xmlfile in c:\users\shared_grosslab\anaconda
3\lib\site-packages (from openpyxl) (1.1.0)
Note: you may need to restart the kernel to use updated packages.
```

In [109…
```
# Get the path to the user's home directory
home_directory = os.path.expanduser('~')

# Set the working directory to the desktop directory
desktop_directory = os.path.join(home_directory, 'Desktop')
os.chdir(desktop_directory)

# Print the current working directory to verify
print("Current working directory:", os.getcwd())
```

```
Current working directory: C:\Users\shared_grosslab\Desktop
```

In [35]:
```
file_paths = [
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d24_h16m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d24_h17m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d03_h00m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d03_h01m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d03_h11m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d03_h12m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d03_h13m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d03_h14m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d03_h15m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d03_h16m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d03_h23m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d04_h00m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d04_h01m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d04_h11m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d04_h12m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d04_h13m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d04_h14m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d04_h16m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d04_h23m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d07_h10m1
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d07_h12m1
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d07_h13m1
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d07_h14m1
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d07_h15m1
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d07_h16m1
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d07_h23m1
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d08_h00m1
```

```
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d08_h01m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d08_h10m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d08_h11m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d08_h12m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d08_h13m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d08_h14m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d08_h15m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d08_h16m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d08_h23m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d09_h00m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d09_h01m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d09_h10m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d09_h11m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d09_h12m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d09_h13m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d09_h15m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d09_h23m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d15_h00m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d15_h01m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d15_h10m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d15_h11m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d15_h12m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d15_h13m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d15_h14m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d15_h15m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d15_h23m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d16_h00m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d16_h01m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d16_h10m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d16_h11m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d16_h12m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d16_h14m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d16_h15m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d16_h16m
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d17_h01m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d17_h03m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d17_h10m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d17_h11m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d17_h12m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d17_h13m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d17_h14m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d17_h16m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d18_h00m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d18_h01m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d18_h10m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d18_h11m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d18_h14m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d18_h15m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d18_h16m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d18_h23m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d19_h00m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d19_h01m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d19_h10m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d19_h13m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d19_h14m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d19_h15m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d19_h23m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d20_h00m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d20_h01m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d20_h10m(
```

```
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d20_h11m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d20_h12m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d20_h13m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d20_h14m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d20_h15m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d20_h16m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d20_h23m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d21_h00m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d21_h01m(
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d21_h17m1
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d21_h18m1
    r"\\170.140.232.175\aquamice_working\painted20240314\box1_y2023m10d21_h19m1

]
```

In [36]:
```
combined_data = pd.DataFrame()
```

In [37]:
```
confidence_threshold = 0.80

for file_path in file_paths:
    # Read data from each file into a DataFrame
    df = pd.read_hdf(file_path).droplevel(0, axis=1)

    # Calculate the mean of the likelihood values across all levels
    mean_likelihood = (df.loc[:, (slice(None), slice(None), 'likelihood')] > c

    # Filter rows based on the mean confidence (likelihood) values
    if mean_likelihood > confidence_threshold:
        combined_data = pd.concat([combined_data, df], ignore_index=True)

# Drop rows containing NaN values
combined_data = combined_data.dropna()
```

In [38]:
```
df.head()
```

Out[38]:

individuals									
bodyparts	nose			head_center			left_head_center		
coords	x	y	likelihood	x	y	likelihood	x	y	likelihood
0	204.254	18.985	0.990	195.334	17.595	0.992	195.764	11.651	0.994
1	204.168	19.490	0.954	195.014	18.949	0.946	195.448	11.850	0.956
2	204.252	20.010	0.978	194.527	18.807	0.989	195.021	12.416	0.988
3	204.278	19.727	0.977	194.817	18.671	0.986	195.289	12.126	0.986
4	204.056	20.090	0.961	194.637	19.371	0.968	195.255	11.990	0.967

5 rows × 162 columns

Mouse 0 - body and mouth

In [96]:
```
body0 = df['mouse0']['body_center'][['y','x']]
mouth0 = df['mouse0']['nose'][['y','x']]
body1 = df['mouse1']['body_center'][['y','x']]
mouth1 = df['mouse1']['nose'][['y','x']]
```

Within Bounds of Exercise Wheel

In [97]:
```
# the y coordinate is first so it is [y,x]
exercise_center = np.array([313,437])
exercise_radius = 83
```

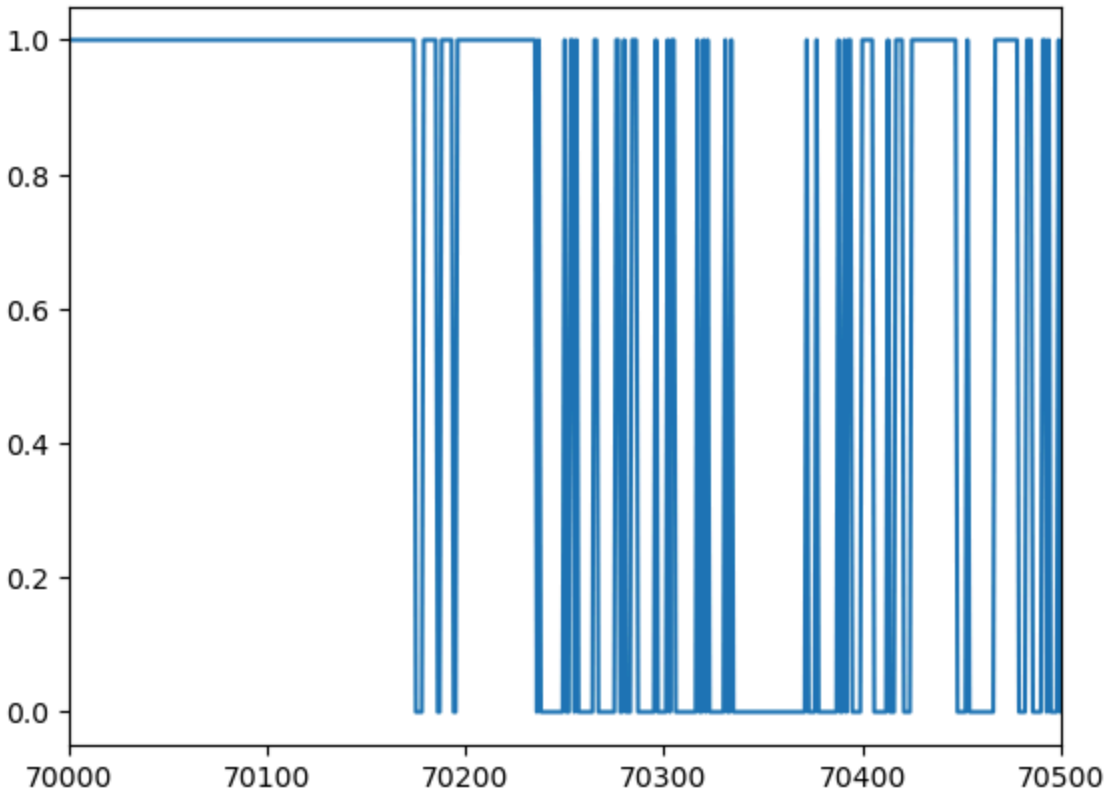
Mouse 0

In [98]:
```
exercise_distance0 = (body0 - exercise_center).pow(2).sum(axis=1).pow(1/2)

exercise_within_bounds0=(exercise_distance0 <exercise_radius)

exercise_within_bounds0.mean()
```

Out[98]:
```
0.2926474134253334
```

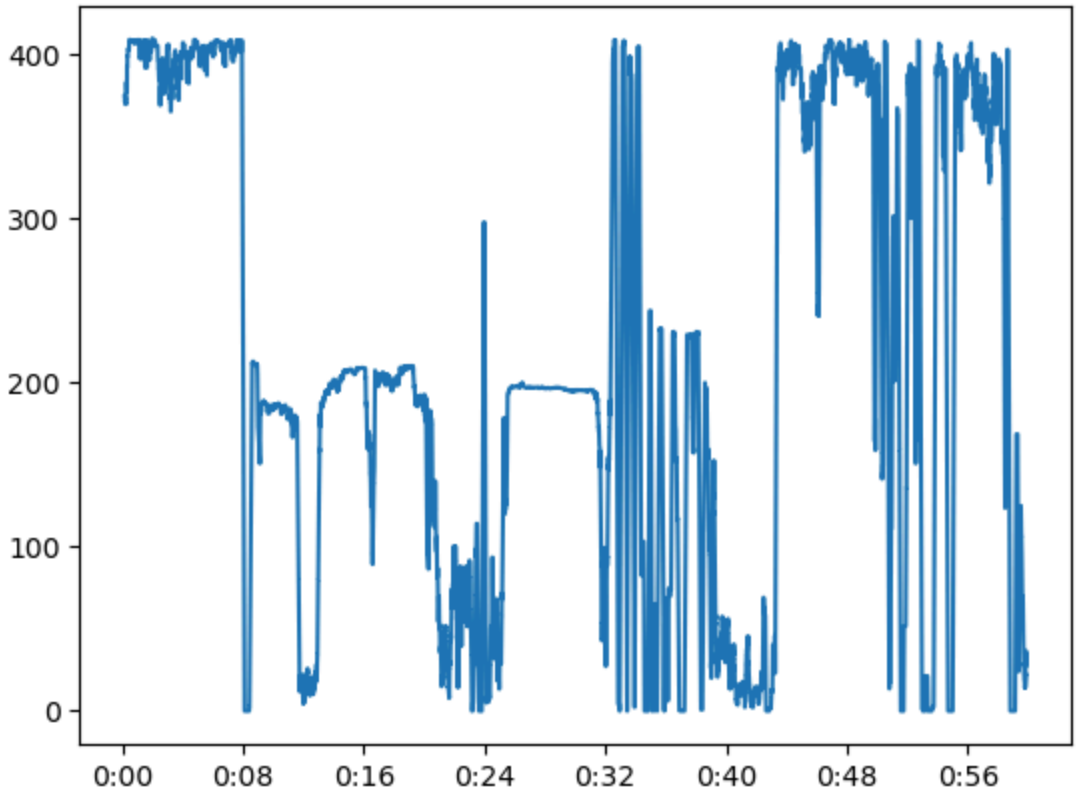
In [99]:
```
fig,ax=plt.subplots()
exercise_within_bounds0.astype(int).plot(ax=ax)
ax.set_xlim([70000,70500])
```

Out[99]:
```
(70000.0, 70500.0)
```



In [100…
```
num_frames = len(body0 - exercise_center)
time_seconds = np.arange(num_frames) * (1/30)
time_minutes = time_seconds / 60
plt.xticks(np.arange(0, num_frames, 4 * 3600), [f'{int(time // 3600)}:{int((ti
(body0 - exercise_center).pow(2).sum(axis=1).pow(1/2).rolling(300).mean().plot
```

Out[100]:
```
<Axes: >
```



Mouse 1

In [101…
```
exercise_distance1 = (body1 - exercise_center).pow(2).sum(axis=1).pow(1/2)

exercise_within_bounds1=(exercise_distance1 < exercise_radius)

exercise_within_bounds1.mean()
```

Out[101]: 0.34614100495242334

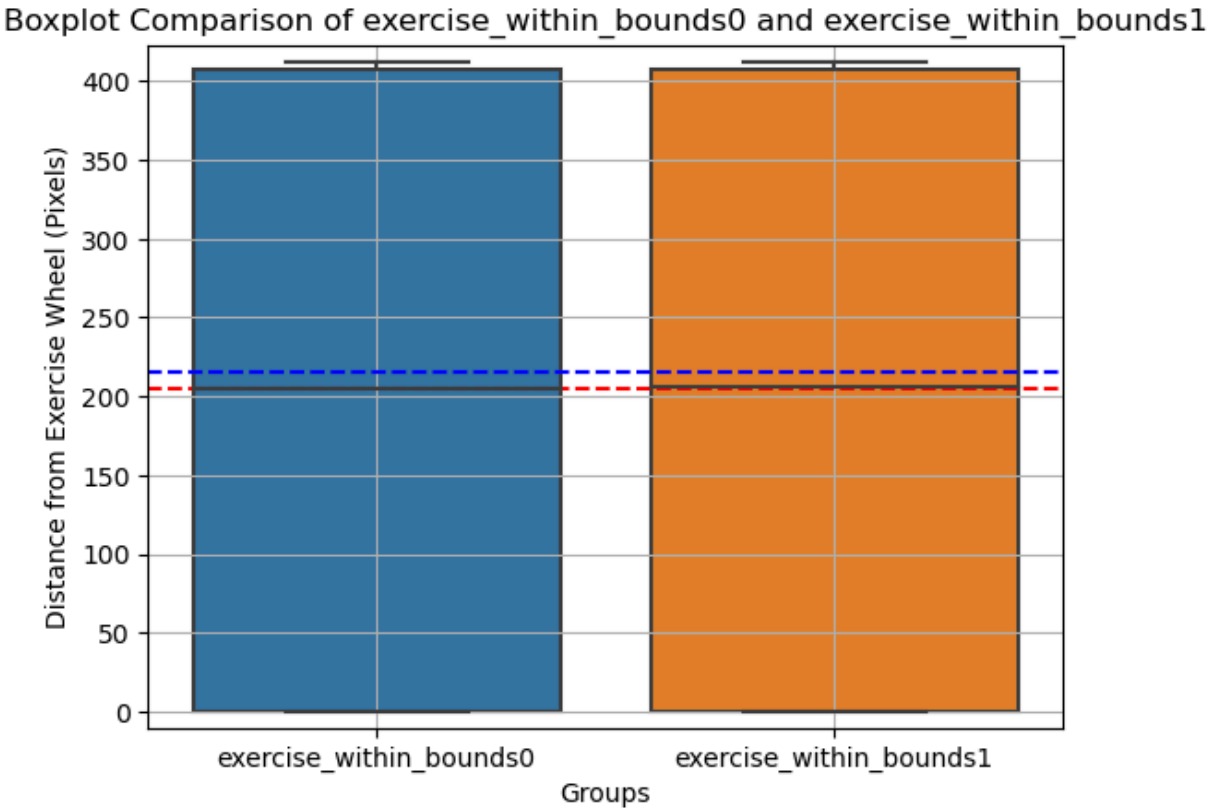
In [102…
```
statistic, p_value = mannwhitneyu(exercise_within_bounds0,exercise_within_boun

# Interpret the result
alpha = 0.05
if p_value < alpha:
    print("Reject null hypothesis: There is a significant difference between g
else:
    print("Fail to reject null hypothesis: There is no significant difference
print("P-value:", p_value)
```

```
Reject null hypothesis: There is a significant difference between groups.
P-value: 2.3295874208870426e-156
```

In [103…
```
exercise0 = (body0 - exercise_center).pow(2).sum(axis=1).pow(1/2)
exercise1 = (body1 - exercise_center).pow(2).sum(axis=1).pow(1/2)
```

In [104…
```
data = pd.DataFrame({'exercise_within_bounds0': exercise0,
                     'exercise_within_bounds1': exercise1})

# Create boxplots using Seaborn
sns.boxplot(data=data)
```

```
plt.ylim(min(min(exercise0), min(exercise1))- 10, max(max(exercise0), max(exer
plt.xlabel("Groups")
plt.ylabel("Distance from Exercise Wheel (Pixels)")
plt.title("Boxplot Comparison of exercise_within_bounds0 and exercise_within_b
plt.grid(True)
mean0 = sum(exercise0) / len(exercise0)
mean1 = sum(exercise1) / len(exercise1)
plt.axhline(y=mean0, color='blue', linestyle='--', label=f'Mean 0: {mean0:.2f}
plt.axhline(y=mean1, color='red', linestyle='--', label=f'Mean 1: {mean1:.2f}'
plt.show()
```



In [111…

```
# Create DataFrames with the values of exercise0 and exercise1
df_exercise0 = pd.DataFrame({'Exercise0_Values': exercise0})
df_exercise1 = pd.DataFrame({'Exercise1_Values': exercise1})

# Merge the two DataFrames into one
df_combined = pd.concat([df_exercise0, df_exercise1], axis=1)

# Specify the Excel file path
excel_file = 'exercise2_value.xlsx'

# Export the combined DataFrame to Excel
df_combined.to_excel(excel_file, index=False)
```

Within Bounds of Water Bottle

In [112…

```
waterdish_center = np.array([310,190])
waterdish_radius = 40
```

In [113…
```
waterdish_distance0 = (mouth0 - waterdish_center).pow(2).sum(axis=1).pow(1/2)

waterdish_within_bounds0=(waterdish_distance0 < waterdish_radius)

waterdish_within_bounds0.mean()
```

Out[113]:
```
0.36459666499731047
```

In [114…
```
waterdish_distance1 = (mouth1 - waterdish_center).pow(2).sum(axis=1).pow(1/2)

waterdish_within_bounds1=(waterdish_distance1 < waterdish_radius)

waterdish_within_bounds1.mean()
```

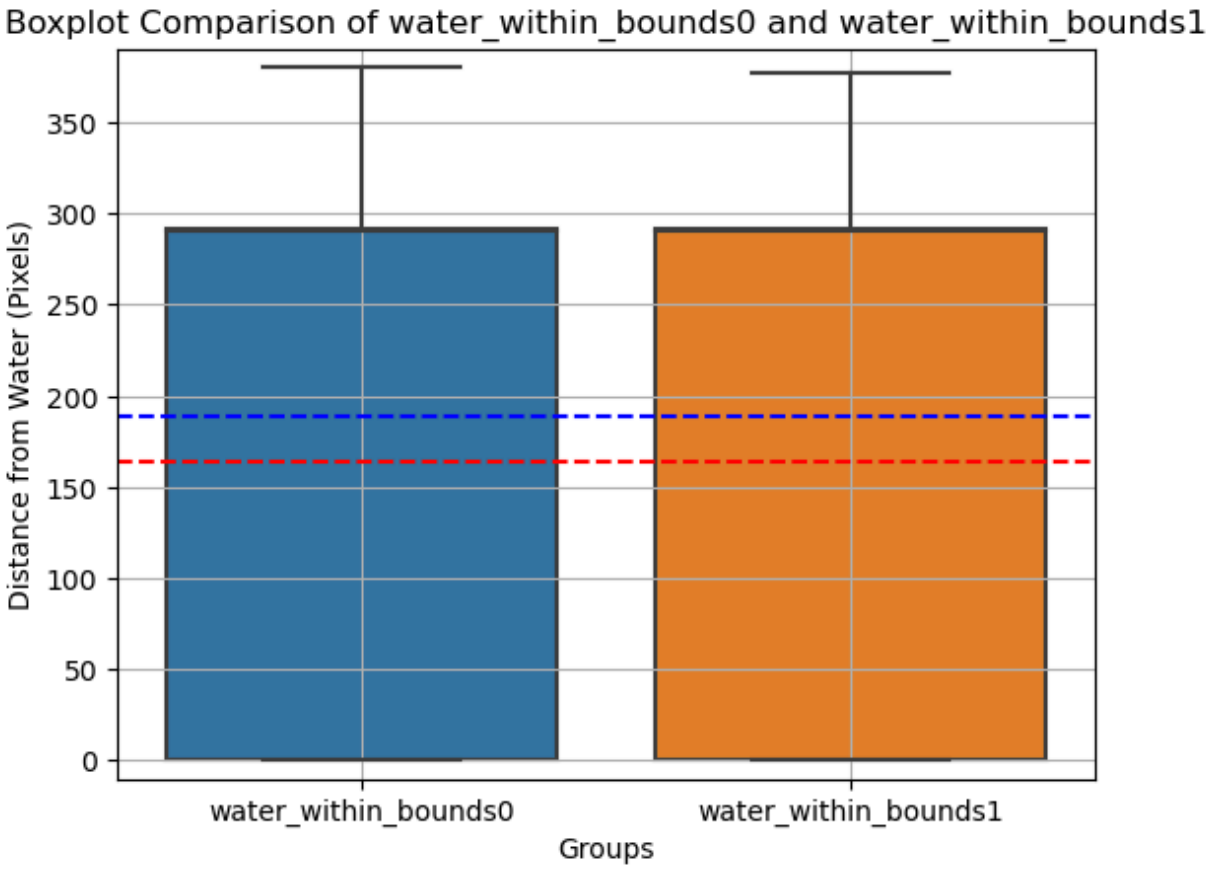
Out[114]:
```
0.4520338322853486
```

In [115…
```
statistic, p_value = mannwhitneyu(waterdish_within_bounds0,waterdish_within_bou

# Interpret the result
alpha = 0.05
if p_value < alpha:
    print("Reject null hypothesis: There is a significant difference between g
else:
    print("Fail to reject null hypothesis: There is no significant difference b

print("P-value:", p_value)
```

```
Reject null hypothesis: There is a significant difference between groups.
P-value: 0.0
```

In [116…
```
water0 = (mouth0 - waterdish_center).pow(2).sum(axis=1).pow(1/2)
water1 = (mouth1 - waterdish_center).pow(2).sum(axis=1).pow(1/2)
```

In [117…
```
data = pd.DataFrame({'water_within_bounds0': water0,
                     'water_within_bounds1': water1})

# Create boxplots using Seaborn
sns.boxplot(data=data)
plt.ylim(min(min(water0), min(water1))- 10, max(max(water0), max(water1))+ 10)
plt.xlabel("Groups")
plt.ylabel("Distance from Water (Pixels)")
plt.title("Boxplot Comparison of water_within_bounds0 and water_within_bounds1"
plt.grid(True)
mean0 = sum(water0) / len(water0)
mean1 = sum(water1) / len(water1)
plt.axhline(y=mean0, color='blue', linestyle='--', label=f'Mean 0: {mean0:.2f}
plt.axhline(y=mean1, color='red', linestyle='--', label=f'Mean 1: {mean1:.2f}'
plt.show()
```



In [118…
```
# Create DataFrames with the values of exercise0 and exercise1
df_water0 = pd.DataFrame({'water0_Values': water0})
df_water1 = pd.DataFrame({'water1_Values': water1})

# Merge the two DataFrames into one
df_combined = pd.concat([df_water0, df_water1], axis=1)

# Specify the Excel file path
excel_file = 'water2_values.xlsx'

# Export the combined DataFrame to Excel
df_combined.to_excel(excel_file, index=False)
```

Within Bounds of Food

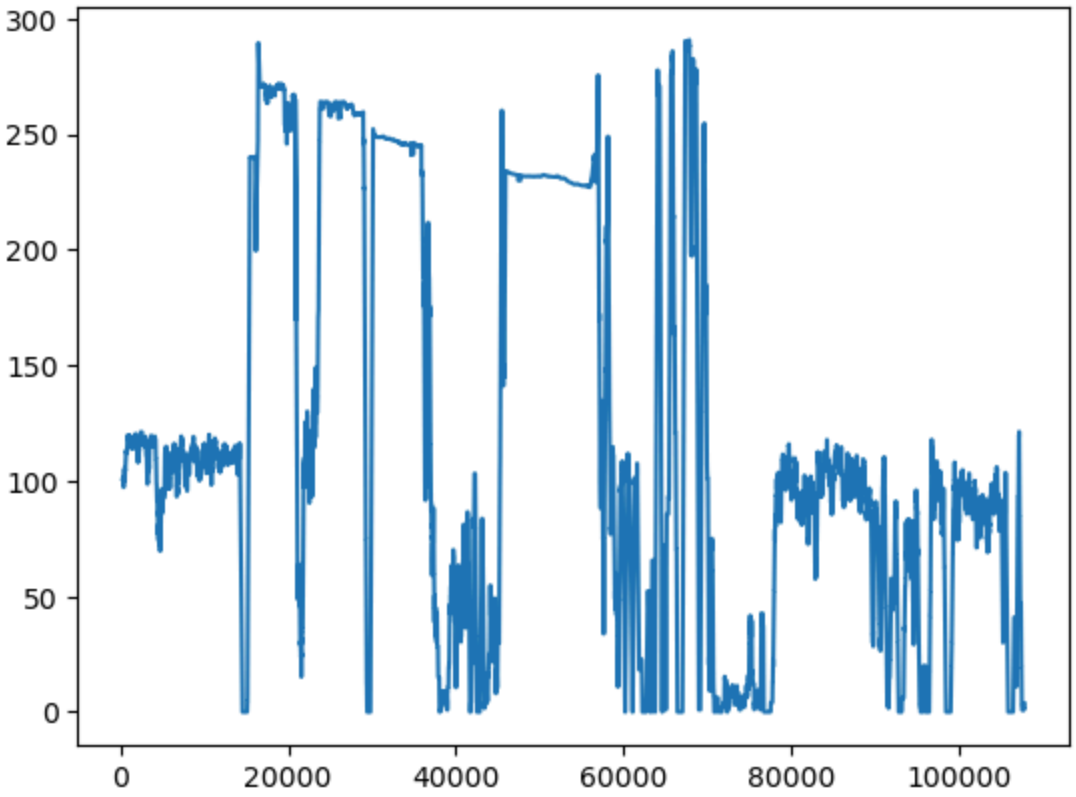
In [119…
```
food_center = np.array([139,179])
food_width = 68
food_height = 178
```

Mouse0

In [120…
```
food_distance0 = np.abs(mouth0 - food_center)

food_within_bounds0 = np.all(food_distance0 <= [food_height / 2, food_width / 
```

In [121…
```
(mouth0 - food_center).pow(2).sum(axis=1).pow(1/2).rolling(300).mean().plot()
```

Out[121]: <Axes: >



In [122…
```
food_distance1 = np.abs(mouth1 - food_center)

food_within_bounds1 = np.all(food_distance1 <= [food_height / 2, food_width / 2

food_within_bounds1.mean()
```

Out[122]: 0.01547864151503348

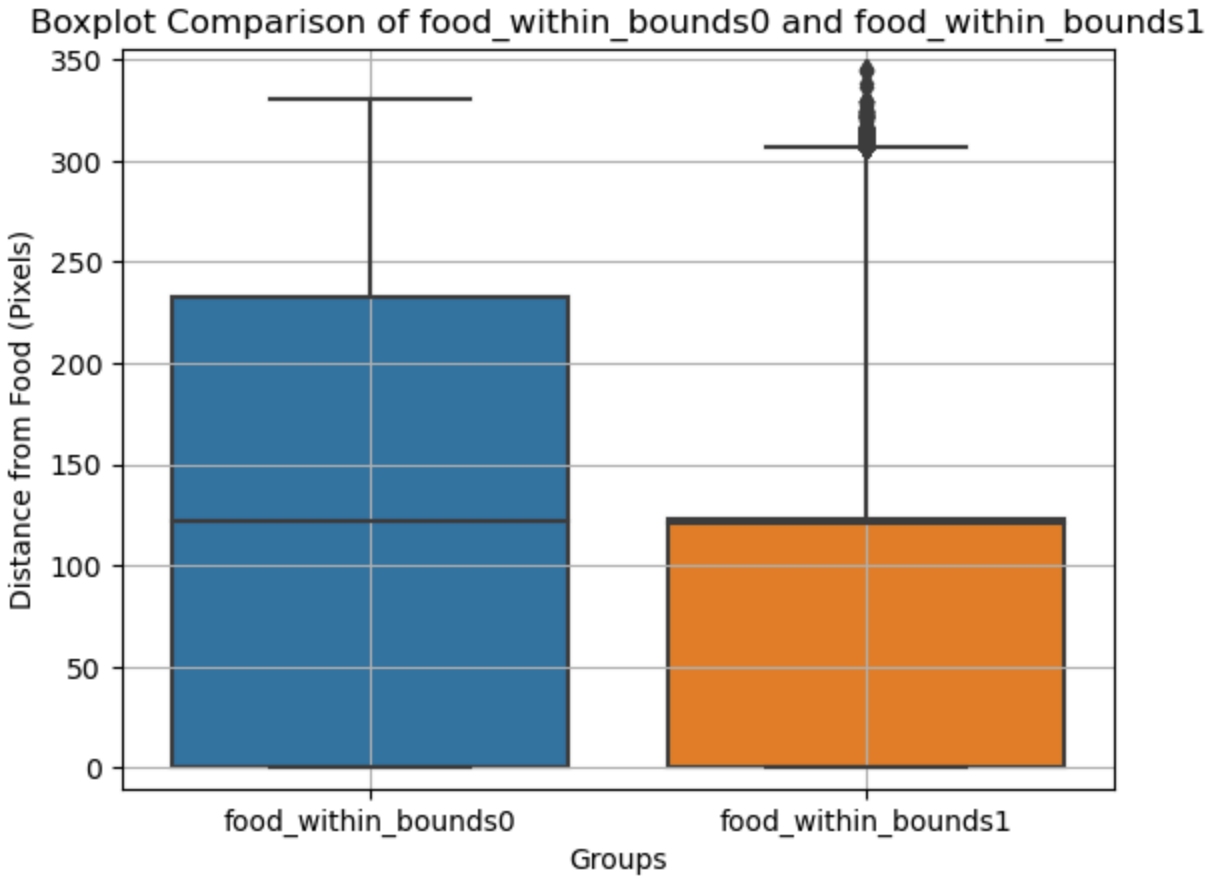
In [123…
```
food0 = (mouth0 - food_center).pow(2).sum(axis=1).pow(1/2)
food1 = (mouth1 - food_center).pow(2).sum(axis=1).pow(1/2)
```

In [124…
```
statistic, p_value = mannwhitneyu(food_within_bounds0,food_within_bounds1)

# Interpret the result
alpha = 0.05
if p_value < alpha:
    print("Reject null hypothesis: There is a significant difference between g
else:
    print("Fail to reject null hypothesis: There is no significant difference b

print("P-value:", p_value)
```

```
Reject null hypothesis: There is a significant difference between groups.
P-value: 0.0
```

In [125…
```
data = pd.DataFrame({'food_within_bounds0': food0,
                     'food_within_bounds1': food1})

# Create boxplots using Seaborn
sns.boxplot(data=data)
plt.ylim(min(min(food0), min(food1)) - 10, max(max(food0), max(food1)) + 10)
```

```
plt.xlabel("Groups")
plt.ylabel("Distance from Food (Pixels)")
plt.title("Boxplot Comparison of food_within_bounds0 and food_within_bounds1")
plt.grid(True)
#mean0 = sum(food0) / len(food0)
#mean1 = sum(food1) / len(food1)
#plt.axhline(y=mean0, color='blue', linestyle='--', label=f'Mean 0: {mean0:.2f}
#plt.axhline(y=mean1, color='red', linestyle='--', label=f'Mean 1: {mean1:.2f}
plt.show()
```



In [126…

```
# Create DataFrames with the values of exercise0 and exercise1
df_food0 = pd.DataFrame({'Food0_Values': food0})
df_food1 = pd.DataFrame({'Food1_Values': food1})

# Merge the two DataFrames into one
df_combined = pd.concat([df_food0, df_food1], axis=1)

# Specify the Excel file path
excel_file = 'food2_values.xlsx'

# Export the combined DataFrame to Excel
df_combined.to_excel(excel_file, index=False)
```

In [ ]:

In [1]:
```
import pandas as pd
import h5py
import numpy as np
import os
import matplotlib.pyplot as plt
from scipy.stats import ttest_ind
from scipy.stats import shapiro
from scipy.stats import mannwhitneyu
import seaborn as sns
```

In [2]:
```
pip install openpyxl
```

```
Requirement already satisfied: openpyxl in c:\users\shared_grosslab\anaconda3
\lib\site-packages (3.1.2)
Requirement already satisfied: et-xmlfile in c:\users\shared_grosslab\anaconda
3\lib\site-packages (from openpyxl) (1.1.0)
Note: you may need to restart the kernel to use updated packages.
```

In [3]:
```
# Get the path to the user's home directory
home_directory = os.path.expanduser('~')

# Set the working directory to the desktop directory
desktop_directory = os.path.join(home_directory, 'Desktop')
os.chdir(desktop_directory)

# Print the current working directory to verify
print("Current working directory:", os.getcwd())
```

```
Current working directory: C:\Users\shared_grosslab\Desktop
```

In [302…
```
file_paths = [

r"\\170.140.232.175\aquamice_working\painted20240314\box2_y2023m09d16_h00m14s5
r"\\170.140.232.175\aquamice_working\painted20240314\box2_y2023m09d16_h01m14s5
r"\\170.140.232.175\aquamice_working\painted20240314\box2_y2023m09d15_h23m14s5
```

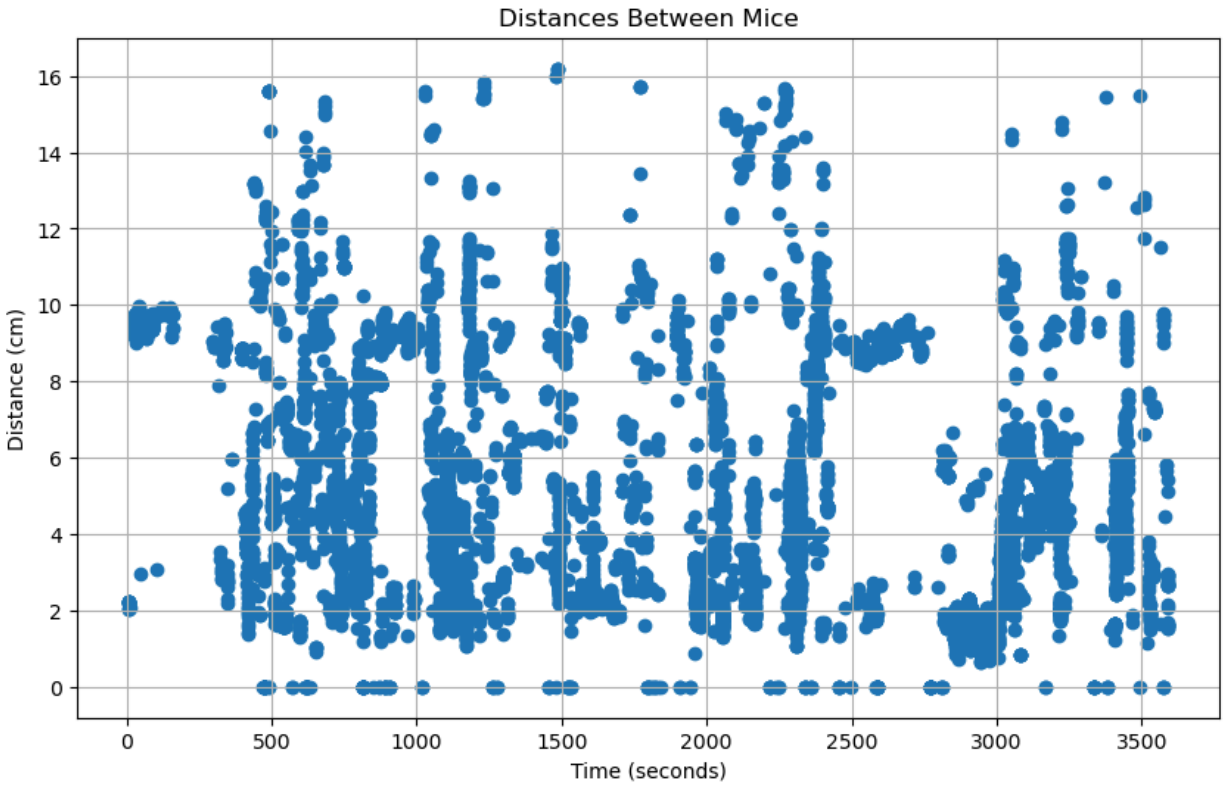
In [303…
```
combined_data = pd.DataFrame()
```

In [304…
```
confidence_threshold = 0.80

for file_path in file_paths:
    # Read data from each file into a DataFrame
    df = pd.read_hdf(file_path).droplevel(0, axis=1)

    # Calculate the mean of the likelihood values across all levels
    mean_likelihood = (df.loc[:, (slice(None), slice(None), 'likelihood')] > c

    # Filter rows based on the mean confidence (likelihood) values
    if mean_likelihood > confidence_threshold:
        combined_data = pd.concat([combined_data, df], ignore_index=True)

# Drop rows containing NaN values
combined_data = combined_data.dropna()
```

In [305…
```
body0 = df['mouse0']['body_center'][['y','x']]
body1 = df['mouse1']['body_center'][['y','x']]
```

In [306…
```
distances_between_mice = np.linalg.norm(body0 - body1, axis=1)
```

In [307…
```
# Conversion factor from pixels to centimeters
pixel_to_cm = 0.0264583333

num_frames = len(distances_between_mice)
time_seconds = np.arange(num_frames) / 30  # Assuming 30 frames per second

# Convert distances from pixels to centimeters
distances_in_cm = distances_between_mice * pixel_to_cm

# Plot the distances as a scatter plot
plt.figure(figsize=(10, 6))
plt.scatter(time_seconds, distances_in_cm, marker='o')
plt.xlabel('Time (seconds)')
plt.ylabel('Distance (cm)')
plt.title('Distances Between Mice')
plt.grid(True)
plt.show()
```



In [308…
```
# Drop NaN values from the distances
distances_between_mice = distances_between_mice[~np.isnan(distances_between_mi

# Create a DataFrame with the distances
df_distances = pd.DataFrame(distances_between_mice, columns=['Distance (cm)'])

# Define the file path for the Excel file
excel_file_path = 'distances_between_mice.xlsx'

# Save the DataFrame to an Excel file
df_distances.to_excel(excel_file_path, index=False)
```