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**Automated analysis of rat exploratory behavior in an episodic-like memory  
task**

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October/2025

## **Automated analysis of rat exploratory behavior in an episodic-like memory task**

Master of science's dissertation project, to be defended at the Center for Humanities, Letters and  
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## Abbreviations and acronyms

**A1:** Old stationary object

**A2:** Old displaced object

**B1:** Recent stationary object

**B2:** Recent displaced object

**CA1:** Cornu Ammonis 1 hippocampal subregion

**CA2:** Cornu Ammonis 2 hippocampal subregion

**CA3:** Cornu Ammonis 3 hippocampal subregion

**DLPFC:** Dorsolateral Prefrontal Cortex

**ELM:** Episodic-Like Memory

**LEC:** Lateral Entorhinal Cortex

**mPFC:** Medial Prefrontal Cortex

**NOL:** Novel Object Location

**NOR:** Novel Object Recognition

**ROI:** Region of Interest

**TOM:** Temporal Order Memory

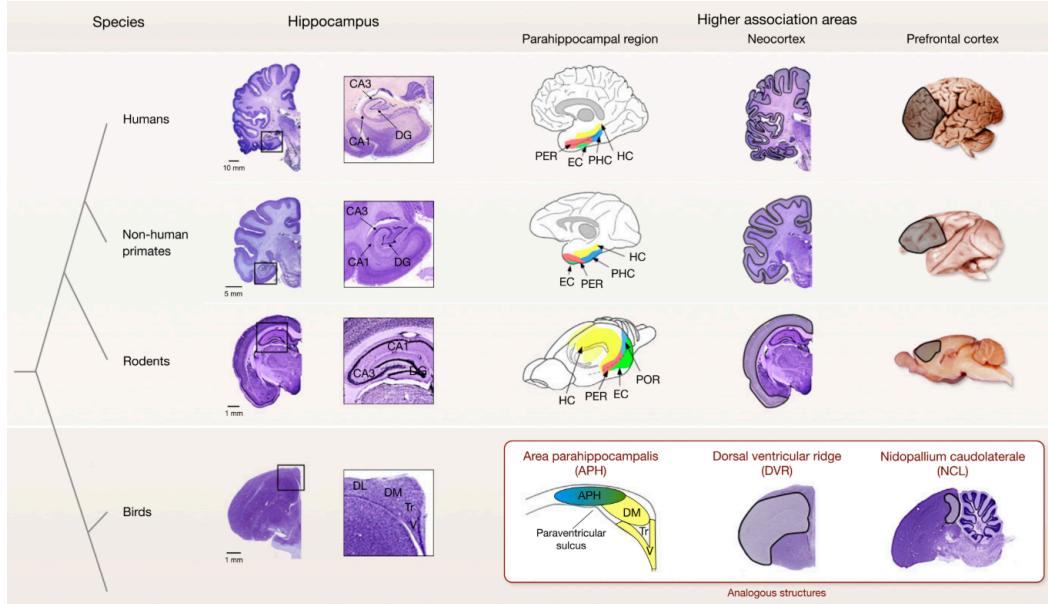
## Abstract

Episodic memory, i.e., the ability to recall specific spatiotemporal events, was initially thought to be an exclusively human capacity. Nonetheless, behavioral studies demonstrated that many animal species, including rats (*Rattus norvegicus*), are capable of expressing what is called episodic-like memory (Allen & Fortin, 2013). One of the protocols used to evaluate episodic-like memory expression uses object exploration behavior, along with the innate preference for novelty shown by the rodents, a way to assess memory for identity, location, and temporal order of objects, prerequisites for episodic-like memory. Some of the patterns shown by the animals in the task are not well explained. Also, recent evidence has shown that evaluating object exploration behavior alone is not enough to account for the ways animals read their environment during the course of the task. This dissertation investigated spatial and object processing in Wistar rats in all sessions of an episodic-like memory task, using automated analysis of exploratory behaviors, including object exploration, rearing, and zone occupancy. Results indicated a difference in exploratory behavior patterns between control and CA1-inactivated groups, with the latter showing a less organized spatial behavior. The results presented here highlight the importance of assessing exploratory behavior beyond object sniffing to understand memory-guided navigation and episodic-like memory processing.

## Introduction

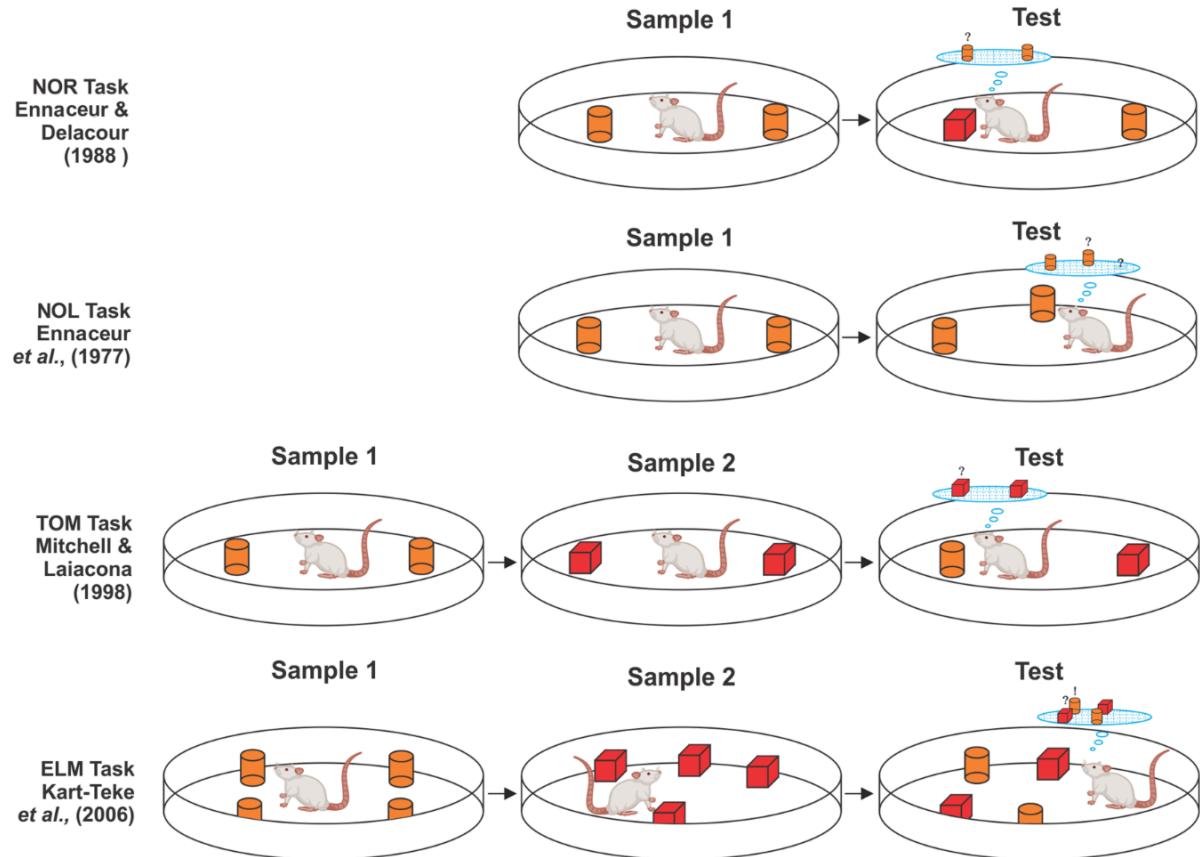
Episodic memory, i.e., the ability to recall specific events, is an essential part of the human experience. When proposed as a separate mental process, this cognitive ability was conceptualized as an integrated representation of the memorized item (or set of items), its spatial location and the time where it was encountered. Initially, it was assumed that episodic memory is dependent on an ability to mentally ‘time travel’ that would require a conscious representation of self. This led to the conclusion that episodic memory is a uniquely human ability (Tulving, 2002). Nonetheless, in the last decades, multiple lines of evidence showed the existence of a behavioral expression by various species, including rats (*Rattus norvegicus*), of the aforesaid integrated representation, called episodic-like memory (ELM; (Allen & Fortin, 2013; Barbosa & Castelo-Branco, 2022; Clayton & Dickinson, 1999; Kart-Teke et al., 2006).

These conclusions are further reinforced by studies showing that the neural correlates implicated in the episodic-like memory have a homologous structure that is present in many vertebrate species and relatively preserved in mammals (Fig. 1; Allen & Fortin, 2013). Furthermore, the three phases of memory formation: encoding, (the absorption of the information to be memorized); consolidation (the storage of this information for long-term use); and retrieval (the reactivation of the neural substrate associated with the behavioral expression of memory by a specific context or internal state) affect the behavioral expression of ELM when impaired (Barbosa & Castelo-Branco, 2022; Binder et al., 2015; Chao et al., 2020).



**Figure 1.** Brain areas implicated in episodic-like memory across species. For mammals, the regions involved in the process of ELM are highly homologous. Adapted from Allen & Fortin (2013).

Episodic-like memory expression is measured through protocols based on the assessment of behavioral criteria for the three components - item (what), location (where) and time (when) - that make up the integrated representation cited above. These protocols fall into two classes: training-based and training-free (Barbosa & Castelo-Branco, 2022). The former are based on the use of reinforcement schemes to generate learning of rules that involve the 3 components. These protocols have two important limitations. One is the time consuming nature of the training process. The other is the prospective nature of the tasks, since the animals learn to express the memory as a way to obtain a reward. This generates involvement of reward-system areas that can influence hippocampal activity and consequently, episodic-like memory processing. Training-free protocols are presented as more ecologically valid alternatives, by having an incidental nature more similar to how episodic-like memory formation and retrieval occur in the natural environment (Barbosa & Castelo-Branco, 2022; Huston & Chao, 2023). These are based on measuring spontaneous behaviors of the species as a way to assess expression of memory. A subset of these protocols, called spontaneous object recognition tasks (Fig. 2), use the innate preference of rodents for novel stimuli (Ennaceur & Delacour, 1988; Stretch, 1960) to assess memory, through the use of exploration behaviors in relation to objects. These tasks are going to be reviewed next.



**Figure 2:** Spontaneous object recognition tasks. Associative: tasks that evaluate one of the components of episodic-like memory (NOR, NOL, TOM); Non-associative: tasks that evaluate two or more components simultaneously. The ELM task for rodents by Kart-Teke et al. (2006) was designed to assess all three components. Adapted from Barbosa & Castelo-Branco (2022).

The first of these, called Novel Object Recognition (NOR) (Ennaceur & Delacour, 1988) was designed to evaluate memory for item (What). In the first phase, called sample session, the animals are exposed to two identical objects. After a predefined time interval, they are placed again on the same arena for a test session, with a copy of the object presented in the sample session (now a familiar object), and an exemplar of an object never shown to the animal before (called novel). Animals that memorize the familiar object show preference to the novel one. The same rationale was used later to design the Novel Object Location (NOL) task (Ennaceur et al., 1997) in which instead of a new object being presented in the test, one object is dislocated in

relation to the position it occupied in the sample. Animals show memory for spatial location (Where) by exhibiting preference for the displaced object in the test session.

Subsequently, a more complex variant, called Temporal Order Memory (TOM) task (Mitchell & Laiacona, 1998) was designed to measure memory for temporal order (When) of presentation of objects. In this version, there are 2 sample sessions, each with a different set of 2 objects (Fig. 2). In the test session, one object from the first set (old familiar object), and one from the second (recent familiar), is presented. Rats consistently show a pattern of preference for the old familiar object. There is still not an established explanation for this specific pattern. One proposal, the Trace Decay Hypothesis (Barker et al., 2019), states that time after the event (encounter with the object) causes a progressive decay (weakening) of the memory trace. Therefore, the old familiar object would have a weaker trace that would make it seem newer to the animal, eliciting novelty-based preference.

The tasks presented before are classified as non-associative, because they evaluate the components of ELM in a separate fashion. Based on them, many associative tasks, that evaluate the components in conjunction, were designed in the following years (Barbosa & Castelo-Branco, 2022; Chao et al., 2020).

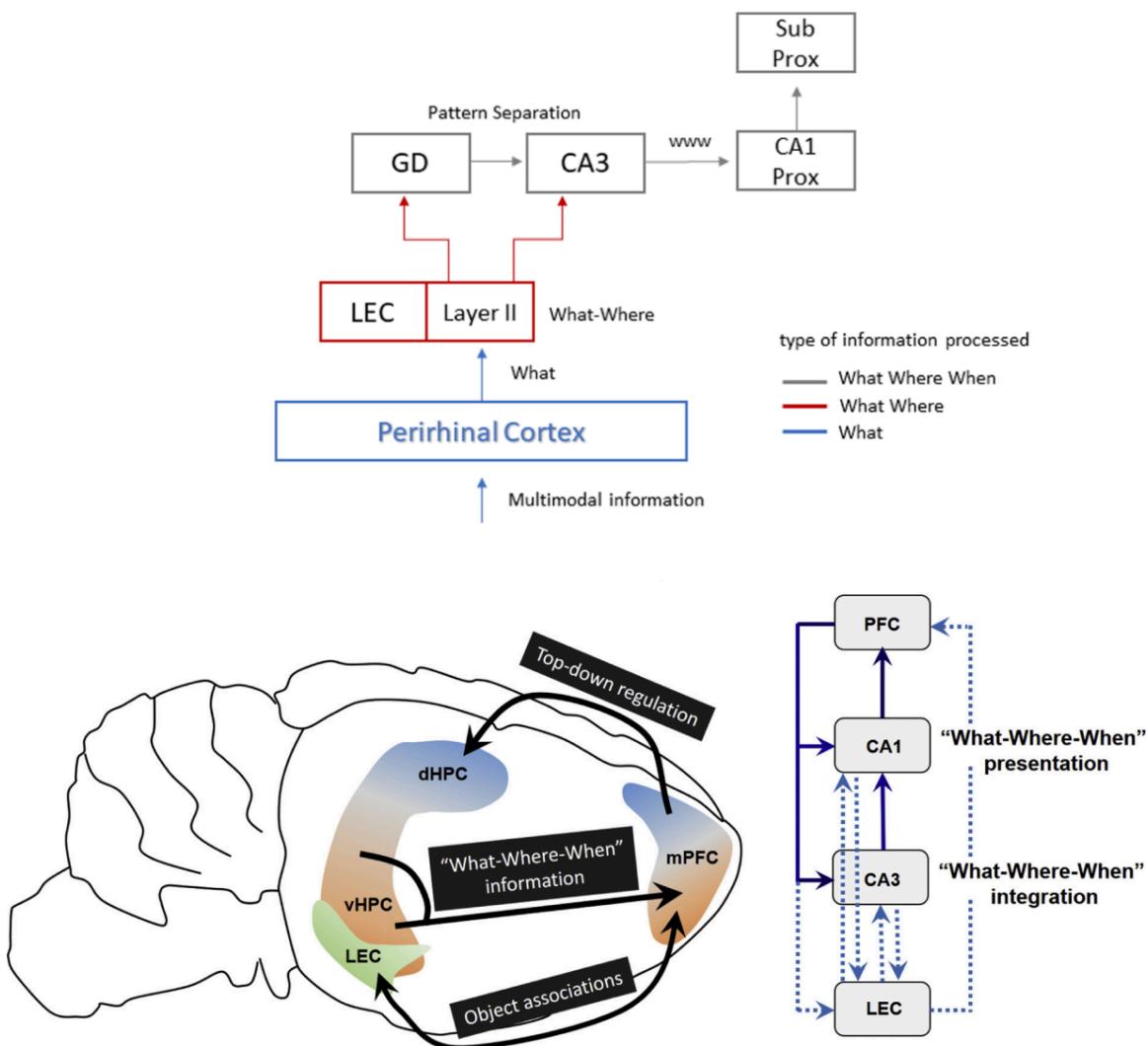
The one used in the present project was designed by Kart-Teke et al. (2006) to evaluate episodic-like memory through expression of all three components cited above (What, Where and When, Fig. 2). This task consists of 2 samples and 1 test session. In the first sample, the animal is exposed to 4 identical objects (A) placed in 4 predetermined positions in the arena. In the second sample session, the animal is exposed again to 4 objects (B), identical to each other, but different from the ones in sample 1. Two of these are placed in positions that were occupied by objects in the first sample session, and two in positions where there were none. In the test session, the animals are presented with 4 objects again: two A and two B objects. One of each set (A1, or old stationary; B1, or recent stationary) in the same position as presented before; Another (A2, or old displaced; B2 or recent displaced) in a novel one. Rats are able to consistently show preference for: B2 over B1, that corresponds to an expression of item + spatial memory, like in the NOL task; A1 over B1, that corresponds to an expression of item + temporal memory, like the TOM task; and A1 over A2, a pattern considered counterintuitive, since the authors expected the animals to prefer the A2 over the A1, as it happens with the B objects. Nevertheless, it is

interpreted as indicative of existence of an integrated representation, since the preference for old stationary in relation to old dislocated objects would still require memory for identity, location and time of presentation for the same item. Nonetheless, an explanation for this specific pattern of preference was not established yet.

A proposal, elaborated by (Chao et al., 2020) is based on the idea that ‘what’, ‘where’ and ‘when’ information is not being processed independently in the task. It states that the spatial novelty introduced in the second sample session influences the relation between the two ‘A’ objects in the test session. This would happen because the two ‘B’ objects put in novel positions in sample 2 introduce a novelty that creates a memory trace. In the test session, the old displaced object (A2) is put in one of the positions occupied by the ‘B’ objects. Therefore, an interaction would happen between the A2 object and the still active trace created in sample 2, making it appear ‘less new’. Because of that, the temporal difference between the old stationary object (A1) and the old displaced object (A2) would predominate, eliciting preference based on temporal order like what happens in the TOM task.

Associative and non-associative tasks have been used widely to investigate the neural substrate of ELM. These studies show different temporal and prefrontal areas implicated in different components (Fig. 3). The ‘what’ (item) component is mainly supported by the perirhinal cortex. The ‘where’ (spatial) is more dependent on the hippocampus, specially the CA1 and CA3 subregions (Fig. 3, a). The ‘when’ (temporal) component involves a circuit between the medial prefrontal cortex and the hippocampus (Fig. 3, b), with involvement of a subset of cells in CA1 that were termed ‘time cells’ (Eichenbaum, 2017).

Importantly, a direct circuit between mPFC and the hippocampus is necessary for the integration of the components for ELM (Fig. 3, b). Within this circuit, the lateral entorhinal cortex (LEC) provides integrated object and temporal information, CA3 binds what, where and when information and CA1 processes and relays these representations to the mPFC. The different areas engaged in ELM are extensively reviewed in Chao et al. (2020). The differential functioning of the hippocampus and its related areas on different components of ELM make the understanding of the relationship between a pattern of exploration to the components essential to uncover the mechanisms involved in the functioning (and dysfunction) of episodic-like memory.



**Figure 3. A:** Schematic representation of the anatomical areas and information flow of episodic-like memory representations in temporal and hippocampal regions. **B:** The medial prefrontal cortex - Hippocampus circuit that mediates episodic-like memory formation and expression. This circuit is also responsible for the relationship between CA1 spatial representation and mPFC mediated decision making. a: Adapted from Barbosa & Castelo-Branco (2022); b: Adapted from Chao et. al (2020).

Specially important for the current project is the function of the CA1 subregion. In the context of ELM processing, CA1 is seen as the selective gateway that takes the integrated representation and uses it to influence mPFC mediated decision-making and memory guided behavior (Fig. 3, b). The current evidence on the CA1 function for ELM portrays the region as a comparator between the integrated memory signal from CA3 with the current sensory input (Chao et al., 2020). Therefore, CA1 would be essential for the detection of and acting on novelty, which can be defined exactly as a mismatch between mnemonic representation and sensory input. This attributed function is in line with the extensive current literature on the function of CA1 for spatial representation/recognition (Eichenbaum, 2017; Griffin et al., 2007), novelty detection (Larkin et al., 2014; Park, 2023; Park et al., 2021; Tamboli et al., 2024) and memory guided decision making (Symanski et al., 2022; Tang et al., 2021).

Traditionally, object recognition is assessed through the manual measuring of object exploration behavior annotated when the animal goes near the objects and sniffs it. While object exploration is established as a strong measure of memory expression, there is evidence of other behaviors that are strongly elicited by novelty. One important example is rearing, i.e. the behavior by which the animals stand on its hind legs to inspect the environment. Rearing is a way for the animals to explore distal cues of the environment (Thompson et al., 2018), and consequently, is strongly associated with spatial encoding (Anderson et al., 2006; Lever et al., 2006). There is evidence showing that rearing is closely related to hippocampal activity and essential for memory encoding. Studies show that: inactivation of rats' dorsal hippocampus during rearing episodes impairs spatial memory formation (Layfield et al., 2023) and there are changes in theta and gamma hippocampal activity during rearing episodes (Barth et al., 2018). Importantly, a study by (Shan et al., 2025) shows that animals tend to rear more in zones related to object novelty, bringing important evidence to show that not only general presentation, but the locations where rearing happens are directed by previous experience. These data argue for the possibility that rearing is an active sampling behavior, i.e., a way for the animal to obtain new information for encoding and remapping based on detected novelty. In fact, studies show that attentive behaviors, like head-scanning, which is part of the rearing posture, optimize place-cell plasticity (Monaco et al., 2014).

Also importantly, beyond rearing and object exploration, rodent exploratory behavior is a structured sequence that involves excursions from a home-base in order to inspect the environment and create a spatial map (Thompson et al., 2018). This involves the use of rearing and scanning behaviors, and navigation through the environment, a behavior that is also novelty-sensitive (Anderson et al., 2006). Considering this evidence, we propose that analyzing other behaviors besides simple object exploration can bring a more complete picture of how the animals are memorizing the environment and its relations to the objects in ELM novel object based protocols, such as the one used in this project.

In summary, the evidence presented above indicates that analysis of object exploration in the test session by itself is insufficient to understand the processing of episodic-like memory in the course of the task aforementioned. Analyzing the spatial behavior of the animal and how it responds to the change in configurations between the sessions is then necessary for a complete picture. This involves not only analyzing rearing behavior, but where it happens, and more generally, where the animal is directing its navigation throughout the sample and test sessions.

Analyses of this type have only become possible recently, due to the emergence of computational programs that allow for the automated detection and classification of animal behavior and movement (Luxem et al., 2023). These tools overcome barriers related to human perceptual and time limitations, making it possible to investigate the relationship between complex variables (like rearing and navigation) during different behavioral protocols. The collection of data on these relationships allows for a continuous and broad view of the progression of animal behavior during these tasks, in contrast to the discrete and manual annotation of behaviors that was the rule previously. This progress is especially important for research in tasks where there is freedom of movement on the part of the animal, as is the case in the previously mentioned tasks (Datta et al., 2019).

The project presented here used these tools to analyze how the patterns of rearing presentation and zone occupancy change in response to muscimol mediated CA1 inactivation in the context of an episodic-like memory task.

## **Justification**

The investigation of the cognitive and behavioral processes subjacent to episodic memory is essential for the characterization of the functioning of this process. In this sense, the application of episodic-like memory tasks for rodents is an important tool. The previously explained protocol (Kart-Teke et al., 2006), widely used to study the process, has explanatory gaps related to the behavioral expression of memory by the animals. Furthermore, memory has traditionally been assessed with the exclusive annotation of object exploration behavior in the test session, a practice that overlooks the complexity of spatial exploration known to be presented by rodents in tasks like the one used here. The measuring of behaviors like rearing of the way the animals navigate the environment during the whole course of the task can bring valuable insights into the way animals process episodic-like memory and how it is affected by hippocampal function impairment.

## **Objectives**

### **General**

To investigate Wistar rats' spatial and object processing in an episodic-like memory task using automated behavioral analyses.

### **Specifics**

1. To quantify object exploration, rearing behavior, and zone occupancy across all phases of the episodic-like memory task.
2. To analyze behavioral responses to the changes in spatial and object novelty introduced throughout the task.
3. To evaluate the influence of muscimol-induced CA1 inactivation on altering these behavioral patterns and the expression of episodic-like memory.

## Hypotheses

### **Sample Session 1 (S1)**

Animals, for both groups, will not show any object or zone preference.

### **Sample Session 2 (S2)**

H1: During S2, animals will show a preference for exploring the two objects placed in novel locations (previously unoccupied in S1) over the two objects placed in familiar locations.

H2: Animals will rear more frequently and spend more time in the zones where there were objects in S1, but there are none in S2.

### **Test Session**

H3: Animals will show the expected episodic-like memory expression by spending more time exploring the novel displaced (B2) and old stationary (A1) objects.

H4: This integrated memory will be reflected in their spatial navigation: animals will rear more frequently and spend more time in the zones containing the novelty-related objects (B2 and A1).

H5: As a marker of latent spatial memory, animals will spend more time in zones that were occupied by objects in previous sessions but are now empty.

### **Effect of CA1 Inactivation**

H6: Animals with CA1 inactivation (muscimol group) will fail to express episodic-like memory, showing no significant preference for the critical objects (B2, A1) in the test session.

H7: The muscimol group will not show the patterns of increased rearing and zone occupancy in novelty-related zones (H2, H4, and H5) observed in control animals.

## Materials and Methods

### Dataset

The dataset used in this project originated from experiments made at the Memory and Cognition Studies Laboratory at the Federal University of Paraíba (LEMCOG-UFPB) (Drieskens, 2016; Drieskens et al., 2017). In this set of experiments, the hippocampal area CA1 was pharmacologically inactivated with muscimol, a GABAa agonist, before the first sample session of an episodic-like memory task (Kart-Teke et al., 2006, Experimental protocol). In these experiments, the experimental group (muscimol) was compared against a (saline) control group. For the purpose of our analysis, data from both groups were used.

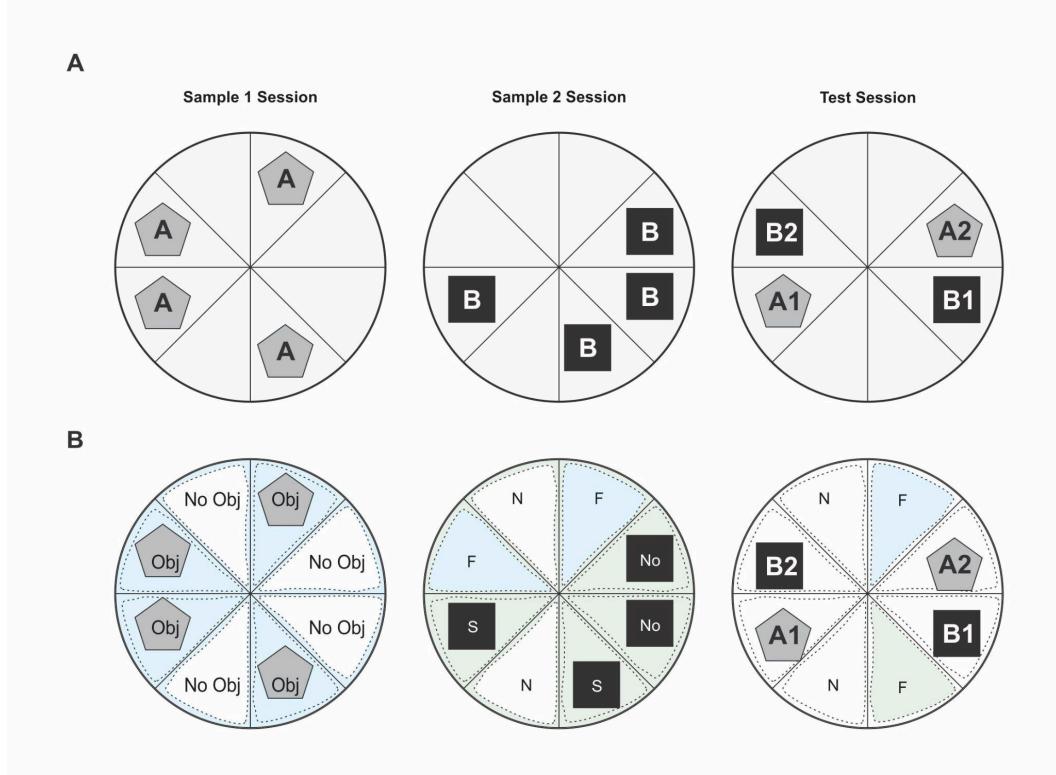
### Animals

24 male rats (*Rattus norvegicus*) were used in this set of experiments. Animals were housed in plastic-made rectangular boxes (30x37x16 cm), with water and food available *ad libitum*, in a 12/12 light-dark cycle. All experimental procedures were carried out in the light phase of the cycle, with controlled lighting. All experiments and procedures were approved by the local Animal Use Ethics Committee (CEUA UFPB Nº 050/2015).

### Experimental protocol

The experimental protocol used by Drieskens et al. (2017) consisted of a modified version of the episodic-like memory task for rodents designed by Kart-Teke et al. (2006) . In our lab, object positions were adapted specifically for a circular open field (Fig. 4, A). In this version, as in the original task, the arena is divided into 8 equal sub-areas; objects are positioned in these areas according to task specifications, and their position is counterbalanced between animals (Introduction). This adaptation was validated and successfully implemented by our

group in other studies (De França Malheiros et al., 2021; De Souza et al., 2019; Dias et al., 2022; Drieskens et al., 2017).



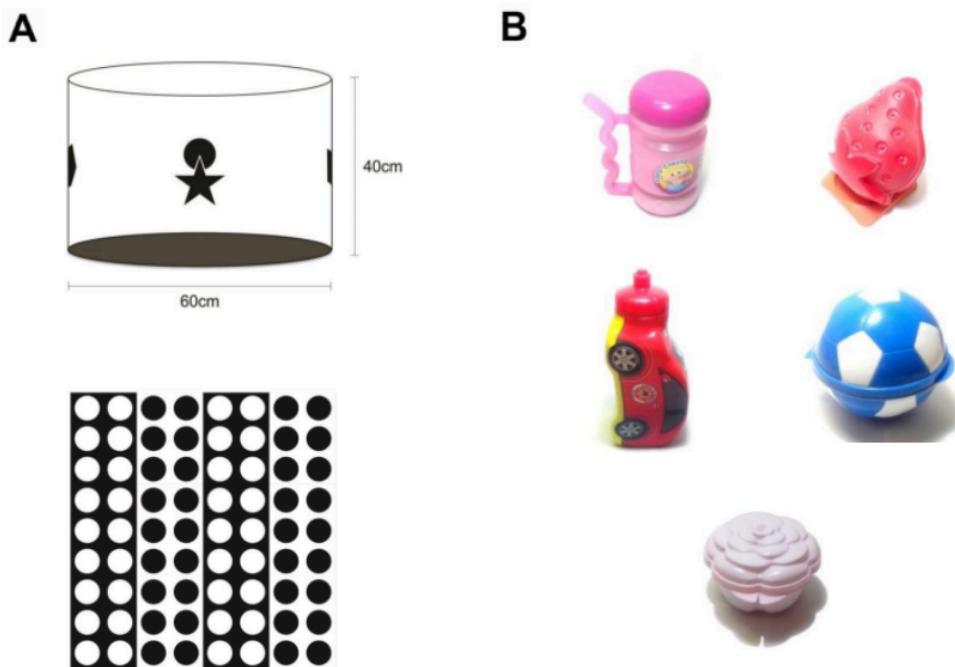
**Figure 4.** Object and zone classification for analysis. **A:** Adapted version of the Kart-Teke et al. (2006) ELM task for rodents. Sample 1: 4 novel objects are presented to the animals; Sample 2: Another set of 4 novel objects is presented - 2 in positions already occupied in sample 1, 2 in novel positions; Test: 4 Objects (2 from S1, 2 from S2) are presented. A1, B1: Objects from each sample session in the same position as in the first time they were presented; A2, B2: Objects presented in novel positions. **B:** Zone classification used for rearing and occupation measures. Sample 1: Zones that had objects x zones that did not; Sample 2: Former zones (F): There were objects in Sample 1, but no longer; Novel zones (No): There were not objects in Sample 1, but now there are; Same zones: There were objects in both sessions; Never (N): There were no objects for both sessions.

The protocol consisted of 5 handling sessions (24-hour interval), where the animals were kept in contact with the experimenter for 15 minutes (Fig. 5). After that, the subjects were exposed to 3 habituation sessions (10 minutes, 24-hour interval) where the animals were put in the open field without any objects. A day after the habituation sessions, animals performed the episodic-like memory task. The interval between sample and test sessions was 1 hour, similar to the original task (Kart-Teke et al., 2006)



**Figure 5:** Schematic representation of the timeline of the experimental protocol done in Drieskens et al. 2015. Interval between the phases of the task was 1 hour.

The open-field arena consisted of a plexiglass circular arena of 60 cm in diameter and 40 cm in height, with a black floor and a transparent wall. The field contained 4 proximal cues (black stickers of different shapes) (Fig. 6, A). The 4 curtains that isolate the field from the rest of the experimental room contained distal cues (posters with different patterns). 9 types of objects were used in the task. They differed in color, height, texture, and shape (Fig. 6, B).



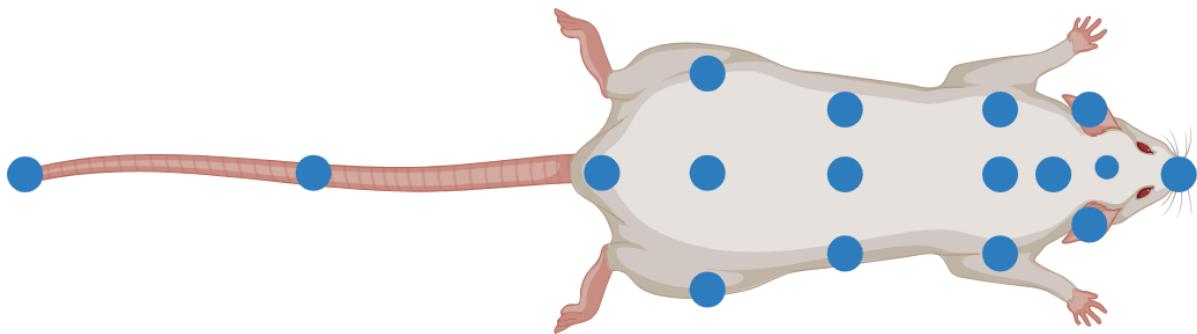
**Figure 6:** Apparatus used for implementation of the episodic-like memory tasks. **A:** Open field (upper) and distal cues (lower). **B:** Objects used. Those differed in size, shape and color, and their use was validated with object bias testing (De Souza, 2015; Drieskens, 2016).

The 24 animals used received either a muscimol (12) or a saline (12) injection bilaterally in the CA1 hippocampus subregion before the first sample session of the task (access Drieskens et al., 2017 for details). Distance traveled and total exploration time for all sessions were compared between groups in the original experiment, and no significant difference was found (Drieskens, 2016; Drieskens et al., 2017).

## Data Analysis

### Tracking

Tracking of the animals' movement was implemented with DeepLabCut (Mathis et al., 2018), a software written in Python. The software pipeline uses computer vision models based on deep learning for pose estimation, i.e., automatic detection of the position of previously specified points in the animal's body in video frames. For this project, a model was trained to track 17 points spanning the animal's head, neck, torso, and tail (Fig. 7). The video analysis function on DeepLabCut returns .csv files with the position of each body part in each frame for all the videos analysed. All the following automated analyses in the current project were based on these outputs.



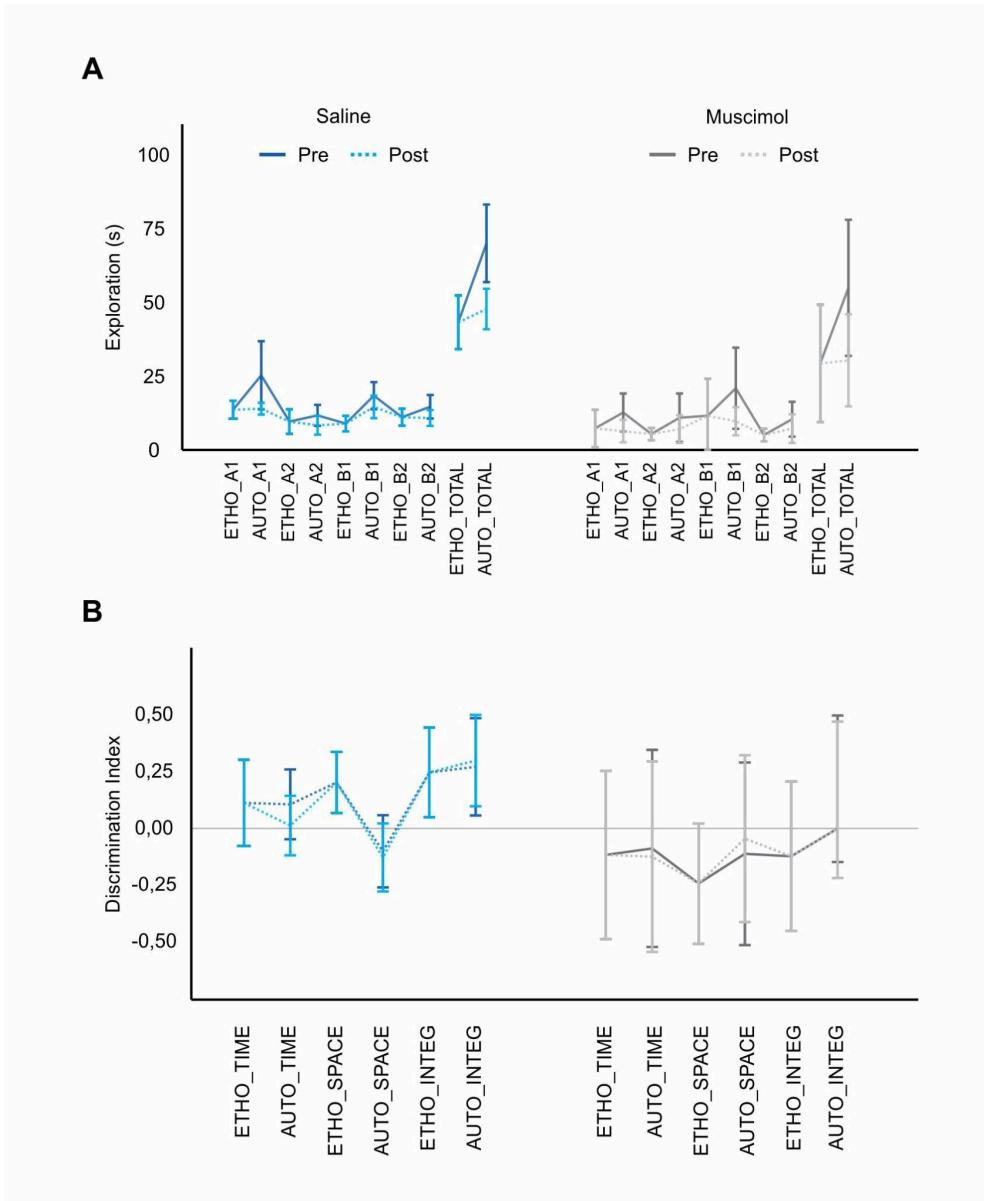
**Figure 7:** Keypoints used for tracking of the animals. For exploration, snout was used. For distance, the center point of the torso was used. Training of the rearing classified used features based on all points (see Rearing behavior section).

## Object exploration analysis

Extraction of object exploration episodes for the test session videos was attempted with the Region of Interest (ROI) function in the SimBA pipeline (Goodwin et al., 2024). This function uses pose-estimation data from DeepLabCut for a specific point in the body to count every occurrence of entry in a predefined region in the image, as well as the time spent in that region for that occurrence. With this data, total frequency and duration of occupancy for each region marked can be calculated. ROIs were drawn manually around the object, and the program counts total duration of time and frequency of occurrences where a selected keypoint (snout) enters each of the ROIs (objects).

For validation of the automated extraction, Spearman correlations were performed between automatically x manually extracted exploration times for each object, with 0.7 being considered as the minimum acceptable correlation value. The initial analysis revealed variable, and sometimes very low (~0.2) correlations, depending on the object. Visual inspection of videos generated with SimBA's ROIs showed three classes of artifacts: moments where the animals stood still near the objects with its snout inside the marked ROI; moments where the animal would pass with its snout near the object while not exploring the object itself; moments where the animals would climb the object to inspect the environment.

Python programs were then written to remove from the automated exploration time output moments where: the animal is still (body-center speed [cm/s] < 1.5) for more than 1.0 sec with its snout inside the object ROI; and/or is climbing the object (both snout and body center point inside the ROI). These artifact remotions significantly improved the correlation values (Fig . 8), but specially for the B1 object (Figure 10, Statistical Analyses), which is the less explored when the animals express memory, the differences were still enough to affect Discrimination Index values (see Statistical Analysis). For those reasons, manually extracted exploration time values for the test sessions were used for further analysis.



**Figure 8:** Comparisons between object exploration and discrimination annotated manually (ETHO) and automatically (AUTO) before (Pre) and after (Post) artifact removal separated by experimental groups (blue for saline, grey for muscimol). **A:** Exploration time, in seconds, for each object in the test session (Fig 4, A) and the sum of their exploration times (TOTAL). **B:** Temporal (TIME), Spatial (SPACE) and Integrative (INTEG) indexes for the test session. Slopes between ETHO and AUTO are inversely proportional to their correlations. Notice that artifact removal significantly reduced the slopes.

Exploration times for the sample sessions were manually annotated with ELAN software (Version 7.0, Max Planck Institute for Psycholinguistics, The Language Archive, [2025], Wittenburg et al., 2006). Exploration, as in the original experiment, was counted when the animal sniffed the object with its snout directed towards it, even in rearing episodes. Correlations between exploration times annotated in the time of the first experiment analysis and by the author of the current project were performed for validation, with satisfactory ( $>0.9$ ) values.

Objects were categorized depending on the dynamics of the specific session (Fig. 4, A).

## Rearing behavior

A random-forest classifier, which is a supervised machine learning algorithm, was trained with SimBA to automatically detect rearing behavior, defined as moments where the animal was standing on its hind legs, leaning on the object or not. Moments where the animal was exploring the object or a cue were not counted (Fig. 9).

To train the model, software's default features (mostly related to movement between frames of the body-part coordinates) and a custom feature set (Two-point body-part distances [mm]; Body-part angles [degrees]; Convex-hull perimeters and area [ $\text{mm}^2$ ]) were extracted. The extraction process returns .csv files with values for each of these features in each frame for all the videos. Then, examples of rearing episodes were manually annotated using SimBA's own interface. The random forest model was then trained to detect correlations between rearing positive frames and certain sets of values for the features. The model was tested with precision and recall measuring, as well as visual inspection of videos to see if rearing detection was satisfactory.

Total rearing duration and frequency for all sample and test session videos, both in the field as a whole, and for session-specific zones (Fig. 4, B), were extracted. Both sets of data were segmented for: the first and third minutes; for the session as a whole (cumulatively).

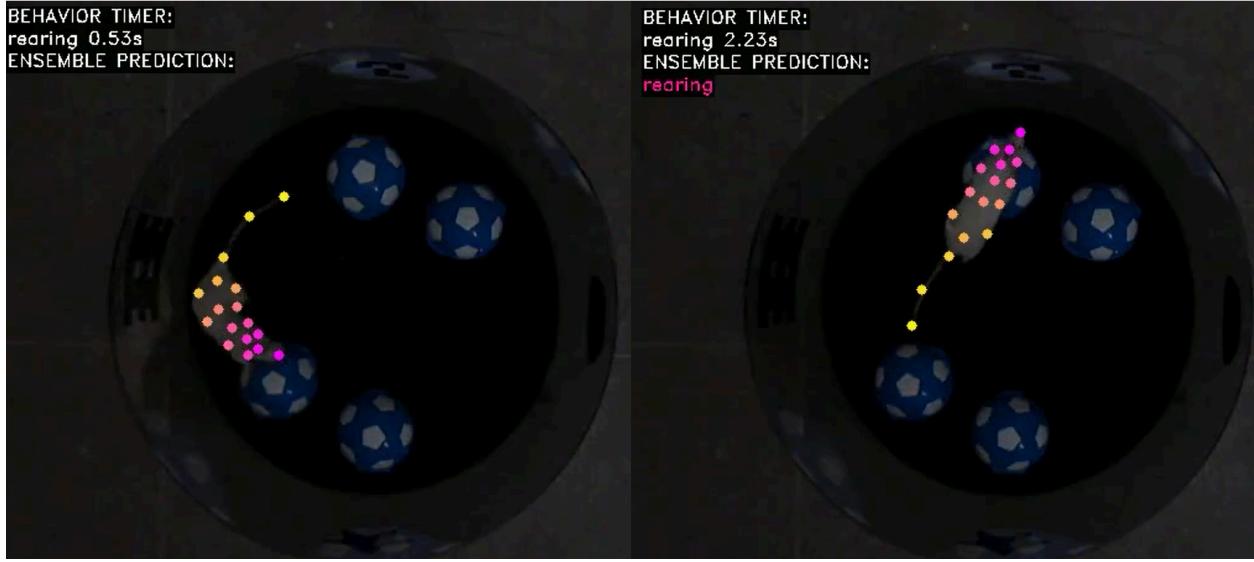


Figure 9: Example frames illustrating the criteria of the rearing classification model. Rearing was only assigned when the animal was: in its hind legs and looking at the environment (right). Moments where the animal was leaning on the object in order to sniff were not considered by the model.

## Zone preference analyses

Analysis of the time spent by the animals as well as rearing behavior in zones of the open field for sample and test sessions was implemented with the Region-of-Interest function in the Python-based pipeline Simple Behavioral Analysis (SimBA, Goodwin et al. 2024). Zones in the arena were drawn according to specific novelty dynamics for each session (Fig 4, B), based, when adequate, on the zone classification done in Shan et al. (2025).

For sample session 1, zones were classified as: Object (presence of objects) x No-object (absence). For sample session 2, there were: Former, i.e., zones that had objects in S1 but no longer have any in S2; Novel, zones where there are objects now but there were not any in S1; Same, zones where there were objects in both sessions; Never, zones where there were not any objects in both sessions. For the test session, zones were classified according to the objects in them (Fig. 4, A, B); Never and Former, classified in the same way as in S2.

Duration and frequency of rearing (see Rearing Behavior), as well as occupancy in the zones for the first and third minutes, and for the whole session (cumulatively) were used for comparisons: between experimental groups; and between zones within groups.

## Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp, 2020). Initially, discrimination indexes (Figure 10) were calculated for object exploration in the sample 2 and test sessions. Boxplots with the ID values for the test sessions were generated, and the cases marked as outliers (more than three interquartile ranges beyond the quartiles) were excluded from all the subsequent analyses for all the sessions. After outlier removal, 19 animals (12 control, 7 muscimol) remained. Shapiro-Wilk normality tests were performed for all variables, and if one of the variables to be used in a specific comparison showed significant differences from the gaussian, non-parametric statistics were used.

Rearing duration and frequency for the whole arena were compared between groups for sample and test sessions. Based on Shapiro-Wilk normality tests, differences between groups were assessed with independent samples t-tests or Mann-Whitney U tests.

Rearing duration, frequency and time-spent in the zone-relative ROIs drawn in the field were compared between, and within groups. All comparisons done for rearing in the session as a whole were also done for rearing in the first and three initial minutes of the session.

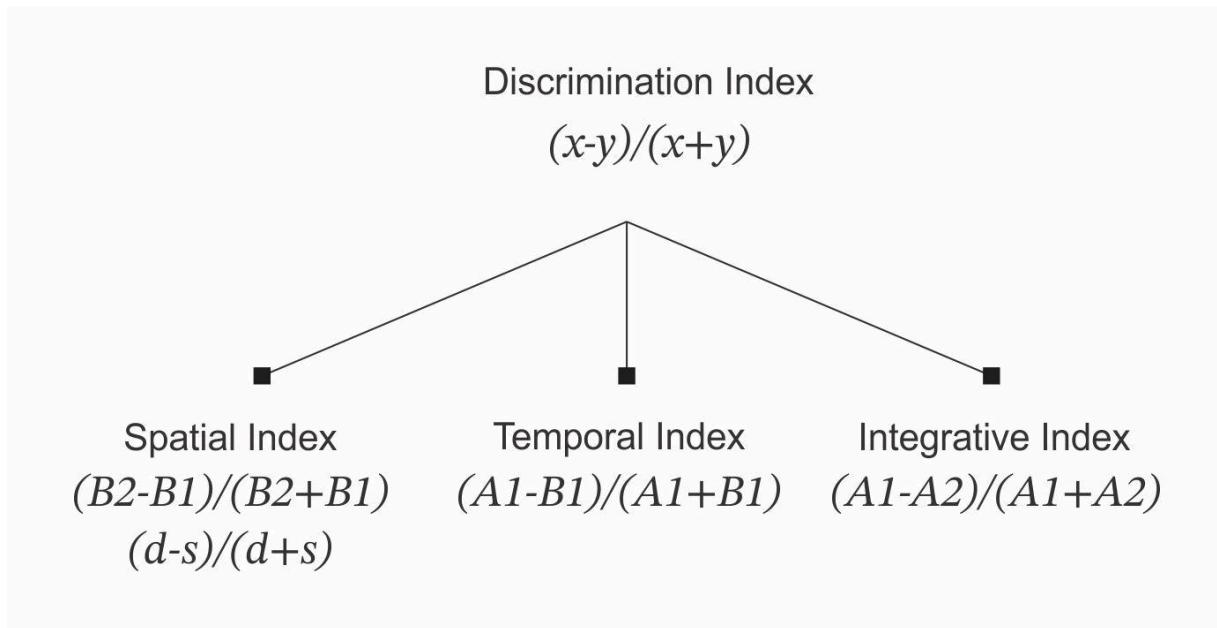
For the first sample session (S1), between object and between no-object zone comparisons within each group were made using repeated-measures ANOVA or Friedman's test. These analyses did not show significant differences. Therefore, new variables for the average between the two objects and between the two no-object zones were generated. All further comparisons used these variables. Repeated measures (or Friedman's) ANOVA were used to compare between zone averages for each group. Independent samples t-test (or Mann-Whitney's U) were used to compare between groups, for each of the zone averages.

In the second sample session (S2), zone-type comparison and averaging was done such as in sample 1. All zone averages were compared between groups. Within group comparisons were

performed between: former x never; and same x novel zones for each group separately (Fig. 4, B).

For the test session, zone-type averaging was used for the 3 never zones (Fig. 4, B). Then, between-group zone comparisons were done between all zones, like in the sample sessions. Within each group, comparisons were made between: Former and the averaged Never zones (Mann-Whitney's U); A1, A2, B1 and B2 zones (Friedman's ANOVA).

Object exploration was analyzed for the sample sessions. For sample 1, comparisons were made between the 4 objects. For sample 2, the 4 objects were classified as stationary or dislocated in relation to sample 1 (Fig. 4, A). Comparisons and averaging of objects of the same type were done in the same way as with field zones. A spatial index (Fig. 10) was then calculated using the stationary and dislocated object averages, and a one sample t-test x chance (0) was performed.



**Figure 10:** The discrimination index (upper) and the versions used to assess Spatial ( $B_2 \times B_1$ ), Temporal ( $A_1 \times B_1$ ) and Integrative ( $A_1 \times A_2$ ) discrimination.

## Results

### Object Preference

Object exploration was manually extracted for sample and test sessions of the episodic-like memory task recorded in the dataset. Total exploration data was already analyzed in Drieskens et al. (2017), as well as discrimination index results for the test session. The latter =are summarized in Fig. 11, C. In this section, object preference results will be presented for the sample sessions.

In the first sample session (S1) of the task, four identical objects were presented to the animals. A repeated-measures (rm) ANOVA using exploration times revealed no significant effect of object ( $F(3, 51) = 2.149, p = .105$ ) and no significant interaction between object and group ( $F(3, 51) = 0.947, p = .425$ ). These results show that the animals in both groups explored objects equally in this phase of the task.

In the second sample session (S2), four different objects were presented: two in the same position as objects in S1 (stationary) and two in new positions (displaced objects). To investigate whether animals display preference for the objects in novel positions, a spatial discrimination index (methods) was calculated between the averages of the exploration times for the stationary and displaced objects. One-sample t-tests comparing the index values with chance (0) showed no significant difference for neither the Saline ( $t(11) = -0.247, p = .810$ ) nor the Muscimol ( $t(7) = -1.843, p = .108$ ) group. These results indicate the animals also explored the objects equally in this session, showing no preference for objects in novel locations.

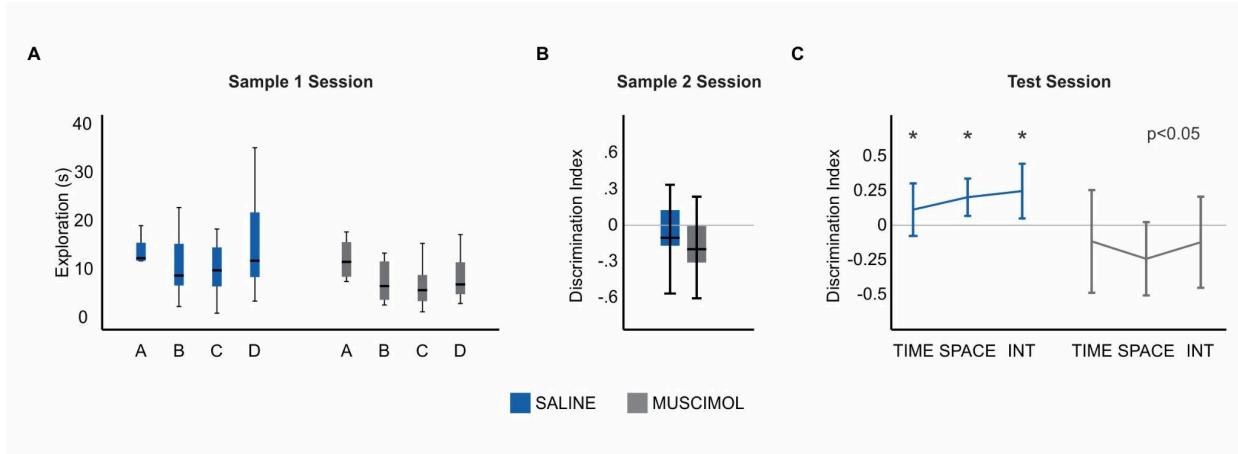


Fig 11: Results for object exploration analyses, separated by experimental group. Blue: saline; grey: muscimol. A) Sample 1 session: No significant differences were observed for any of the experimental groups in the exploration time of the objects. B) Sample 2: Spatial discrimination index between the stationary and dislocated objects. Animals in both groups did not show preference for the stationary object. C: Test session: t-test revealed a significant difference from chance (0) for all indexes in the saline group; No such difference was found for the muscimol group. More details in Drieskens et al. (2017).

## General rearing presentation

The total frequency and duration of rearing behavior was automatically quantified for all experimental sessions. Initially, general presentation of both measures in the field as a whole was compared between groups.

For S1, a Mann-Whitney test revealed a statistically significant difference ( $U = 12.000$ ,  $p = .016$ ) for rearing duration between groups with the Saline group presenting a higher average (Fig. 12). No such difference was found for rearing frequency ( $U = 25.500$ ,  $p = .246$ ).

For S2, an independent t-test showed only a non-significant trend ( $t(17) = 1.867$ ,  $p = .080$ ), with a medium to large size effect (Hedge's  $g = 0.7$ ), for a difference between groups in the total duration of rearing, with saline's average duration being higher. As in S1, no significant difference ( $t(17) = 1.317$ ,  $p = .205$ ) was observed for the total frequency.

For the test session, Mann-Whitney U revealed no significant differences in either total duration ( $U=33.000$ ,  $p=.482$ ) or frequency ( $U=33.500$ ,  $p=.482$ ) of rearing behavior.

No significant differences were found for either variable in any of the sessions when only the initial minute or the first three minutes were considered (Sup. Tables, Table 1).

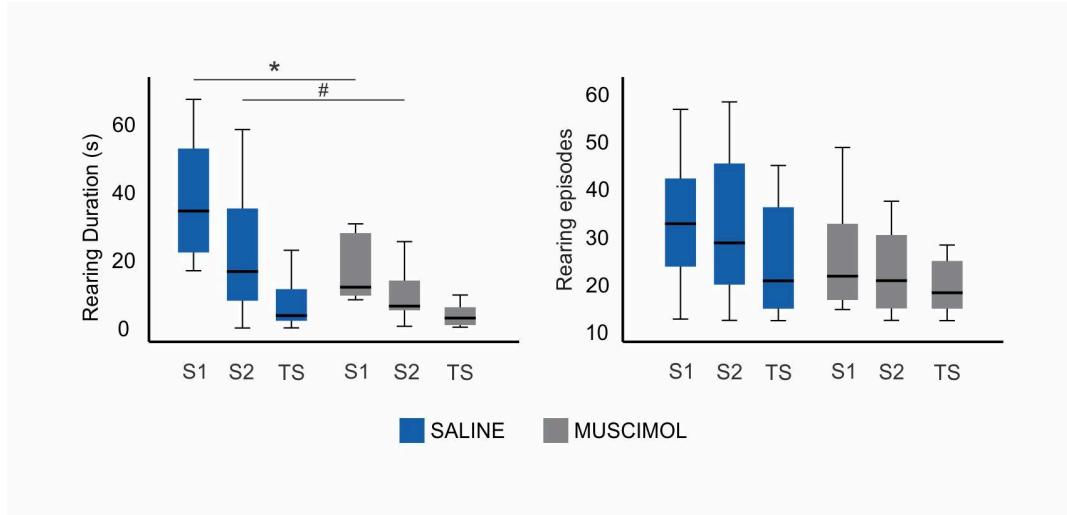


Fig 12: Rearing duration and total number of episodes, separated by experimental group and sessions (S1: Sample 1, S2: Sample 2, TS: Test session). Mann-Whitney revealed a significant difference ( $U = 12.000$ ,  $p = .016$ ) for the duration of rearing between groups in the first sample session. A trend ( $t(17) = 1.867$ ,  $p = .080$ ) was also observed for the duration in the second sample session. No difference was observed between groups for the number of episodes.

## Rearing behavior and zone preference

Region-of-interest algorithms were used to separate the field in zones and classify them, according to object configuration/history, for each session (Zone preference analyses; Fig 4, B). To investigate how these zones influence animal's environment exploration, rearing behavior and occupancy time were measured and compared (between and within-group) in relation to those zones for all sessions.

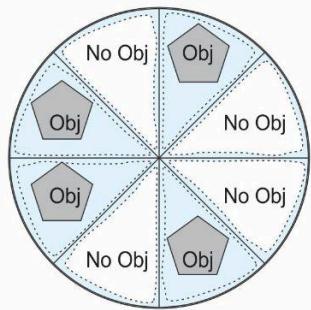
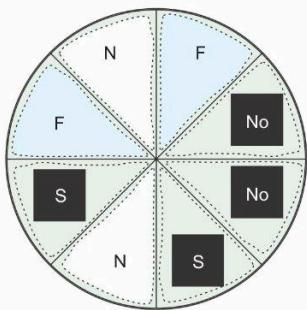
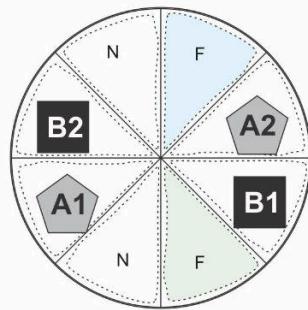
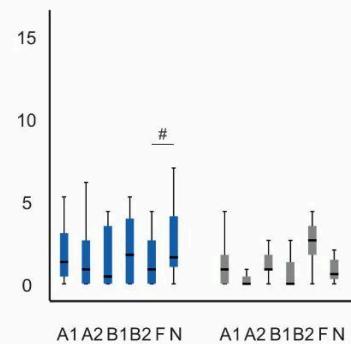
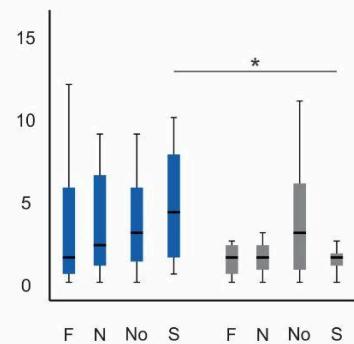
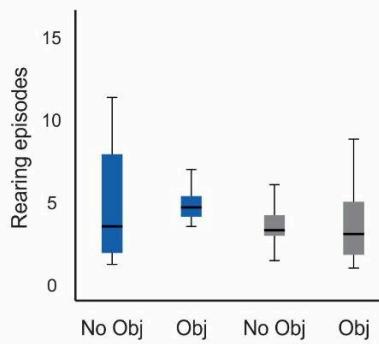
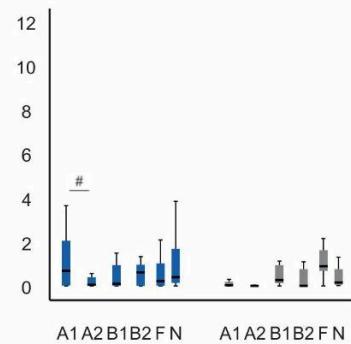
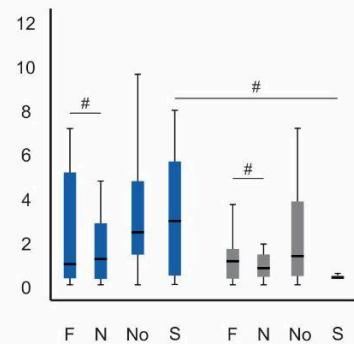
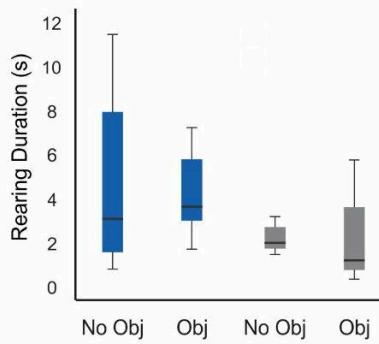
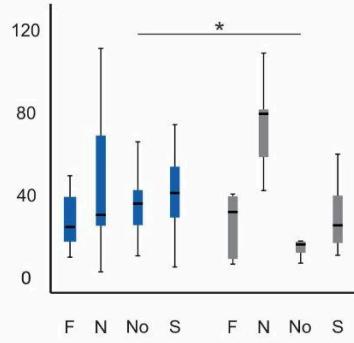
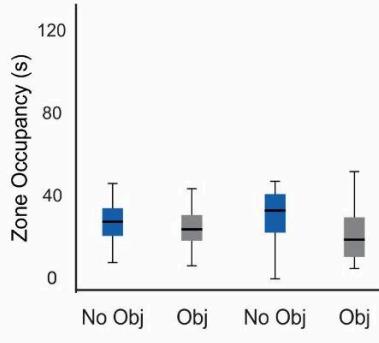
For S1, the 8 zones in the field were classified according to the presence or absence of objects (Fig 4, B). Frequency and duration of rearing in the zones in the same zone type (4 object and 4 no-object zones) were averaged. A rm-ANOVA on these variables revealed no significant effect of Zone type ( $F(1, 16) = 0.001$ ,  $p = .978$ ) and no significant Zone type x Group interaction ( $F(1, 16) = 0.015$ ,  $p = .906$ ) for duration of rearing, but a significant main effect of Group (replicating the general rearing analysis, see previous subsection) was found. No significant differences were found for the time spent by the animals in the zones for this session (Zone type:  $F(1, 16) = 0.728$ ,  $p = .406$ ; Zone type x Group:  $F(1, 16) = 0.069$ ,  $p = .796$ ).

For S2, zones were classified as: Former (two zones that had objects in S1, but no longer do); Same (the ones that had objects both in S1 and S2); Novel (zones that had no objects in S1 but had objects in S2); Never (zones that were empty in both sample sessions). Mann-Whitney's

tests were used to compare duration and frequency of rearing between groups for all zones. This analysis revealed a significant difference between frequency of rearing in the Same zones ( $U = 18.500$ ,  $p = .046$ ), as well as a trend for duration ( $U=21.000$ ,  $p = 0.083$ ), with the Saline group having more and longer episodes of the behavior. No other between-group comparisons were significant for that session (Sup. Tables, Table 2). Within-group comparisons were made between: Former x Never zones, where a significant trend was also found for muscimol group ( $Z = -1,787$ ,  $p=0.074$ ); Same x Novel zones, where no significant differences were found for either either frequency or duration in any of the experimental groups (Sup. Tables, Table 3). As for the time spent in zones, Mann-Whitney U between groups revealed that the Saline group spent significantly more time ( $U = 12.000$ ,  $p= .010$ ) in the Novel zones compared to the Muscimol group. No other between (Sup. Tables, Table 2), or within-group (Sup. Tables, Table 3). No differences were found for time spent in zones for that session.

For the test session, zones were divided according to the object configuration of this phase of the task (A1, A2, B1, B2) and in its relations to S2 object positions (Former and Never zones). Mann-Whitney's U used to compare the zones between groups showed no statistical differences, for frequency or duration of rearing behavior (Sup. Tables, Table 4). Wilcoxon's signed rank test was used to compare rearing duration and frequency within-groups between: A1 x A2; B1 x B2; B1 x A1; B2 x A1; Former x Never zones. An almost-significant trend ( $Z = 1.955$ ,  $p = 0.051$ ) was found in the Saline group for the duration of rearing between the A1 and A2 zones, and a trend for frequency of rearing in the former x never zones for the saline group ( $Z = -1,788$ ,  $p=0.074$ ). No significant differences were found for any of the other pairwise comparisons in any of the experimental groups (Sup. Tables, Table 9). For zone occupancy, a Friedman's ANOVA between zones indicated only a non-significant trend towards differential occupancy by the Saline group ( $\chi^2(5) = 9.952$ ,  $p = .077$ ; Muscimol:  $\chi^2(5) = 2.102$ ,  $p = .835$ ). Wilcoxon's signed rank tests between the zones showed a significant preference ( $Z = 2.589$ ,  $p = .010$ ; Muscimol:  $Z = 0.676$ ,  $p = .499$ ), by the Saline group, for the Never when compared to the Former zones. No between groups, and no other within-group differences were found for time spent in zones (Table 4, Table 9).

No significant effects were observed for any of the rearing variables compared above when considering only the first or the three initial minutes of any of the sessions (Sup. Tables, Tables 5, 6, 7, 8).

**A****Sample 1 Session****Sample 2 Session****Test Session****B****C****D**

█ SALINE   █ MUSCIMOL

Figure 13. A) Zone classification used for rearing and occupation measures. Sample 1: Zones that had objects x zones that didn't; Sample 2: Former zones (F): There were objects in Sample 1, but no longer; Novel zones (No): There weren't objects in Sample 1, but now there are; Same zones: There were objects in both sessions; Never (N): There were no objects for both sessions. B) Total number of rearing episodes for both experimental groups and for all classes of zones (methods, zone preference analyses). Significant differences between groups for the Same zones ( $U = 18.500$ ,  $p = .046$ ) for the S2. For the test, non-significant ( $Z = -1.788$ ,  $p=0.074$ ) trend for the Former x Never zones in the saline group. C) Total duration of rearing, in seconds. Non-significant trends for: Same zones between groups ( $U=21.000$ ,  $p = 0.083$ ); Former x Never zones, for both groups (Saline:  $Z = -1.689$ ,  $p = .091$ ; Muscimol:  $Z = -1.787$ ,  $p=0.074$ ); For the test session, an-almost significant trend between the A1 and A2 zones for the Saline group ( $Z = 1.955$ ,  $p = 0.051$ ). D) Zone occupancy, for both groups in all experimental sessions. Mann-Whitney U revealed significant differences ( $U = 12.000$ ,  $p=.010$ ) in the permanence in the novel zones by the saline group compared to the muscimol. Wilcoxon signed-rank tests also reveal a significant difference ( $Z = 2.589$ ,  $p = 0.010$ ) between the former and never zones for the Saline group in the test session

## Discussion

Episodic memory studies with Novel Object Recognition tasks traditionally relied on the measuring of object exploration in the test session as a way to assess memory expression (Barbosa & Castelo-Branco, 2022). Evidence shows that, specially for associative tasks, other behavioral measures, such as rearing, are an essential part of how the animal encodes and retrieves episodic-like memory (Shan et al., 2025). In the present study, we investigated the influence of hippocampal CA1 inactivation on measures of object exploration, zone occupancy and rearing behavior in a set of animals exposed to an episodic-like memory task.

The task consisted of 2 samples and a test session (Methods, Experimental Protocol, Fig. 4, A). In the samples, 2 sets of objects, different between but identical within sessions, were presented. A spatial novelty was introduced, since 2 of the objects presented in sample session 2 were in positions where no objects had been before. One of the goals of the study presented here was to analyze if and in what way animals responded to that novelty.

In regard to object exploration, we hypothesized that: animals (for both groups) would show no object preference for S1, but saline animals would show a preference for the object in novel positions in S2. Our analysis showed no preference by the animals to any of the objects in sample session 1, consistent with our hypothesis; But no pattern of preference was found for objects in novel position in S2, for any experimental group. This result indicates that the animals did not express object exploration differently based on the novelty introduced between sample sessions. It also shows that general expression of object exploration behavior is not affected by

muscimol based inactivation, in line with previous results (Drieskens et al., 2017; Stackman et al., 2016).

Rearing expression was measured and compared between groups for all sessions (Results, Fig. 12). We found a significant difference in rearing duration in the first sample session by the muscimol group relative to the saline controls, as well as a similar (non-significant) trend in the second. For the test session, the rearing presentation was not significantly different between groups, with very low frequency and duration for both.

A possible explanation for the results above is that CA1-inactivated animals, while still able to perform rearing, lose context-specific guidance for expression of the behavior. The novelty-sensitive nature of rearing is well-established in the literature (Lever et al., 2006). Furthermore, a study by Layfield et al. (2023) demonstrated that dorsal hippocampus inactivation during rearing episodes significantly impaired spatial memory performance, showing that the information acquired during rearing moments is essential for the encoding of spatial variables. The results aforementioned point to the possibility that the diminished rearing time observed in the muscimol group reflects a lack of spatial novelty detection, affecting the propensity of the animal to further investigate general spatial relationships in the environment. This idea is further reinforced by evidence showing that hippocampal activity codes not only spatial representation, but task demands and movement goals related to the environment (Qian et al., 2025; Smith & Bulkin, 2014; Zeng et al., 2024). Then, the diminished amount of rearing observed for the muscimol group would be expected in light of a lack of memory-based guidance for the presentation of the behavior.

We also analyzed rearing and occupancy relative to zones in the field, done accordingly to object position and history, in a similar way to Shan et al. (2025) (Methods, Zone Preference Analyses; Fig. 13). The results found indicate a disorganized and non-selective zone navigation by the CA1-inactivated group.

In sample session 2, zones were classified in: Former (a zone where there was an object in sample session 1, but there is none in sample 2); Never (there was never an object); Novel (there was no object in sample 1, but there is now); Same (there were objects in both session) (Methods, Zone preference analyses). Saline controls showed more rearing episodes in the Same zones. Also, the saline group spent significantly more time in the Novel zones compared to the CA1-inactivated animals. The results on rearing go against our hypothesis, since we expected the

animals to rear more in the Former zones, like what has been seen in Shan et al. (2025). Nonetheless, the saline animals showed indicatives of a pattern of zone occupancy consistent with a novelty preference.

In the test, zones were classified according to: object positions (A1, A2, B1, B2); object history (Former and Never zones, similarly to sample 2) (FIG 13, A). The saline group performed rearing for a longer period of time in the A1 compared to the A2 zones. Also, the saline animals spent more time in the Never zones compared to the Former ones. These results contradict our hypothesis, which stated a novelty related zone preference for both rearing and zone occupancy.

Notice that the A1 zone for the test is the only one in which there is an object for all phases of the task (Fig. 4). Importantly, no distinctive pattern of rearing or zone occupancy was observed in the Muscimol group. Also, no zone preference for either variable was observed for the first sample session, where there is still no memory related to object configuration. These findings strongly suggest that the zone preference patterns found here were related to processing of the changes (and the maintenance, in case of the Same/A1) in object configuration across sessions.

As said before, the patterns found in the present study are contrary to the ones observed in Shan et al. (2025), in the sense that the subjects in that study tended to rear more frequently in the Former when in comparison to the Never zone. A possible explanation for this result is the type/complexity of the task, since they used an Novel Object Location task (Introduction, Fig. 2), which is non-associative and only uses two objects and 1 sample session. Accounts of rodent exploratory behavior (Thompson et al., 2018) show that rodents explore the environment using their home-base as reference points. From these reference points, the animals use scanning behaviors (like rearing) to inspect distal cues in the environment, and then to excursions from there to the spatial location they want to investigate. In the context of object recognition tasks, a more salient novelty would lessen the need for the animals to use this reference point to assess the differences between current sensory input and mnemonic representations. This would translate into the analysis performed in their study as more rearing in the novelty-related zones in comparison with the familiarity-related ones. In our study, where multiple instances of novelty are introduced in a task involving multiple representations in conjunction, there would be a bigger need for the use of the reference point for exploration. An example in the same direction

is the study by Ross & Easton (2022), which observed a change in exploration pattern depending on the type of mismatch presented in an associative episodic-like memory task. This study concluded that the type of novelty (temporal x contextual) modulated the exploratory strategy used.

In studies done with space-related decision making in mazes (reviewed in Wijnen et al., 2024), rodents show what is called Vicarious trial and error (VTE) behavior, which is an exploratory-like sampling behavior where the animals look back and forth between the choice options. Evidence (summarized in Johnson et al., (2012)), shows that hippocampal place-cell activity during VTE expression simulates (act as if) the possible paths, being interpreted by the authors as evidence of the animal trying to predict the outcome of the decision of going through each one of these paths. One possible explanation for the patterns seen in this study is that, considering the aforementioned interconnectedness of spatial representations and decision-making processes in the hippocampus, there is a possibility that the presentation of rearing behavior observed in our study would be analogous to a VTE: the animal uses rearing in reference points to sample novelty, and consequently, path possibilities. And then, based on what it sees, moves to the novelty related part of the environment to remap its representations. A study on the hippocampal network activity concludes that rearing episodes are triggered by novelty relating to allocentric cues, and arise as a behavior for exploration of these distal cues to start remapping based on the novelties in the environment (Barth et al., 2018). Another study by Xu et al.(2019) showed that a spatial maze task triggers remapping exactly during the moment where the animal enters the new goal-related arm of the maze. The evidence presented above favours the possibility of existence of a sequential pattern for spatial recognition in these tasks: The animal uses a scanning behavior, like rearing, to look at the distal cues, and then move to places of interest to investigate the novelty related zones in the environment. In this direction, a study by Stackman et al. (2016) shows an example of how CA1-inactivation disorganizes spatial search behavior. Their results show, besides a loss in spatial memory for the inactivated group, a higher tendency for thigmotaxis (i.e., wall hugging behavior) and a lack of search in the maze as a whole, a typical result in hippocampal function impairment, further reinforcing the function of CA1 in spatial search strategies related to mnemonic representations. In addition, there is evidence linking hippocampus activity to object position novelty (or object absence) and intention to explore a specific object at a certain location (Zeng et al., 2024). These data

reinforce the idea of a relationship between inactivation of CA1 and a loss of spatial guidance in exploratory behaviors, as seen in the rearing and occupancy behaviors analyzed in the present project (Results, Fig. 13).

These conclusions point to the possibility that rearing, ambulation to a zone, and direct object exploration serve different cognitive functions in the course of the task. Based on our results, we hypothesize that the animals would modulate the use of this behavioral repertoire in varied moments and use them in a specific sequence. More analyses are necessary to investigate if these behaviors present a structured sequence in the context of the episodic-like memory task.

Interpretation of our results is limited due to the low sample size of the dataset, especially considering the high variability of the rearing behavior across sessions and animals, amplified even more when divided by zones. Possible solutions to this problem would be the use of General Linear Mixed Models, capable of accounting for non-parametric distributions and individual variability without the downside of loss of statistical power (Lazic, 2015).

Another limitation is the fact that we used average-like data for the analyses. While this is a common practice and it succeeds in showing group differences, one of the advantages of the automated measures used here is their capacity to treat behavior as a time-series data. This allows an episode-to-episode analysis of behavior, enhancing sensibility and making it possible to use other measures, such as Markov Models (Datta et al., 2019) to analyze structure in a more fine-grained way.

One important behavior not looked at in the present project was head-scanning. The literature shows that this behavior is an essential part of the space representation repertoire, including a function for place-cell plasticity (Lever et al., 2006; Monaco et al., 2014; Poulter et al., 2018). Further analyses are needed to account for where the animal is looking at, when it is scanning, and to analyze the structure of the trajectories it makes when looking at the environment.

## Conclusion

The work presented here investigated the object exploration, rearing and spatial occupation patterns of Wistar rats exposed to an episodic-like memory task. It also analyzed the impact of muscimol mediated CA1-inactivation in the presentation profile of these behaviors. A general reduction was observed in the muscimol group in comparison to the saline controls. Furthermore, the CA1-inactivated group showed a more disorganized spatial behavior in relation to controls. The analyses presented here highlight the importance of assessing behavior beyond simple object exploration and in all phases of the task in order to better understand mnemonic processing. They also open up possibilities and questions that can contribute to the understanding of how animals process space in object recognition based tasks.

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## Supplementary tables

**Table 1: segmented (1 and 3 minutes, cumulative) results for rearing number of episodes (FREQ) and total duration (DUR) between groups.**

Variable	S1	S2	T
<b>FREQ_MIN_1</b>	U = 30.000, p = .479	t = 0.296, p = .771	U = 38.000, p = 1.000
<b>DUR_MIN_1</b>	U = 37.000, p = .930	t = 0.121, p = .905	U = 32.000, p = .596
<b>FREQ_MIN_3</b>	U = 31.500, p = .536	t = 0.858, p = .403	U = 31.500, p = .536
<b>DUR_MIN_3</b>	U = 21.000, p = .126	t = 0.771, p = .451	U = 36.000, p = .860

**Table 2: Results for all comparisons of rearing number of episodes (FREQ), and total duration (DUR), as well as zone occupancy (OCC) between groups for the sample session 2.**

Zone	Freq	Dur	Occ
<b>FORMER_AVG</b>	U = 35.500, p = .592	U = 34.000, p = .536	U = 39.000, p = .837
<b>NOVEL_AVG</b>	U = 28.500, p = .261	U = 33.500, p = .482	U = 12.000, p = .010
<b>NEVER_AVG</b>	U = 40.000, p = .902	U = 32.000, p = .432	U = 27.000, p = .227
<b>SAME_AVG</b>	U = 18.500, p = .045	U = 21.000, p = .083	U = 27.000, p = .227

**Table 3: Results for within-group comparisons for rearing number of episodes (FREQ) and total duration (DUR), as well as object occupancy for the sample session 2.**

Variable	Salina	Muscimol
NEVER_AVG_FREQ - FORMER_AVG_FREQ	Z = -0.625, p = .532	Z = -1.787, p = .074
SAME_AVG_FREQ - NOVEL_AVG_FREQ	Z = -0.982, p = .326	Z = -0.171, p = .864
NEVER_AVG_DUR - FORMER_AVG_DUR	Z = -1.689, p = .091	Z = -1.363, p = .173
SAME_AVG_DUR - NOVEL_AVG_DUR	Z = -1.490, p = .136	Z = -0.676, p = .499
NEVER_AVG_OCC - FORMER_AVG_OCC	Z = -1.177, p = .239	Z = -1.183, p = .237
SAME_AVG_OCC - NOVEL_AVG_OCC	Z = -1.334, p = .182	Z = -1.183, p = .237

**Table 4: Results for the comparisons of rearing number of episodes (Freq), total duration (Dur) and zone occupation (Occ) between groups for all zone types.**

Zone	Freq	Dur	Occ
A1	U = 32.500, p = .432	U = 23.000, p = .120	U = 34.000, p = .536
A2	U = 29.500, p = .299	U = 29.000, p = .299	U = 34.000, p = .536
B1	U = 40.500, p = .902	U = 34.000, p = .536	U = 35.000, p = .592
B2	U = 26.000, p = .196	U = 33.000, p = .482	U = 40.000, p = .902

Zone	Freq	Dur	Occ
<b>FORMER</b>	U = 26.500, p = .196	U = 22.500, p = .100	U = 41.000, p = .967
<b>NEVER_AVG</b>	U = 24.000, p = .142	U = 27.000, p = .227	U = 36.000, p = .650

**Table 5:** Segmented results (MIN\_1, and MIN\_3, cumulative) for number of episodes and total duration (DUR) for rearing comparisons between groups in the second sample session.

Zone	DUR_MIN_1	FREQ_MIN_1	DUR_MIN_3	FREQ_MIN_3
<b>FORMER_AVG</b>	U = 24.000, p = .239	U = 22.500, p = .213	U = 36.000, p = 1.000	U = 35.000, p = 1.000
<b>NEVER_AVG</b>	U = 29.000, p = .797	U = 33.000, p = .530	U = 31.500, p = .213	U = 55.000, p = .537
<b>NOVEL_AVG</b>	U = 20.000, p = .126	U = 50.000, p = .772	U = 109.500, p = .008	U = 100.000, p = .014
<b>SAME_AVG</b>	U = 36.000, p = .008	U = 54.000, p = .518	U = 43.500, p = .219	U = 46.000, p = .324

**Table 6:** Segmented results (MIN\_1, and MIN\_3, cumulative) for number of episodes (FREQ) and total duration (DUR) for rearing comparisons within groups in the second sample session.

Variable	Salina	Muscimol
<b>NEVER_AVG_DUR_MIN_1</b>	-	Z = -0.866, p = .386
<b>FORMER_AVG_DUR_MIN_1</b>	-	Z = -1.472, p = .141
<b>NEVER_AVG_FREQ_MIN_1</b>	-	Z = -0.898, p = .369
<b>FORMER_AVG_FREQ_MIN_1</b>	-	Z = -0.541, p = .589

<b>NEVER_AVG_DUR_MIN_3</b>	-	Z = -0.816, p = .415	Z = -0.734, p = .463
<b>FORMER_AVG_DUR_MIN_3</b>			
<b>NEVER_AVG_FREQ_MIN_3</b>	-	Z = -0.154, p = .878	Z = -1.461, p = .144
<b>FORMER_AVG_FREQ_MIN_3</b>			
<b>SAME_AVG_DUR_MIN_1</b>	-	Z = -0.854, p = .393	Z = -1.485, p = .138
<b>NOVEL_AVG_DUR_MIN_1</b>			
<b>SAME_AVG_FREQ_MIN_1</b>	-	Z = -1.515, p = .130	Z = -1.028, p = .302
<b>NOVEL_AVG_FREQ_MIN_1</b>			
<b>SAME_AVG_DUR_MIN_3</b>	-	Z = -0.153, p = .879	Z = -1.377, p = .168
<b>NOVEL_AVG_DUR_MIN_3</b>			
<b>SAME_AVG_FREQ_MIN_3</b>	-	Z = -0.990, p = .322	Z = -0.997, p = .318
<b>NOVEL_AVG_FREQ_MIN_3</b>			

**Table 7: Segmented results (MIN\_1, and MIN\_3, cumulative) for number of episodes (FREQ) and total duration (DUR) for rearing comparisons between groups in the test session.**

Zone	DUR_MIN_1	FREQ_MIN_1	DUR_MIN_3	FREQ_MIN_3
<b>A1</b>	U = 33.5, p = .659	U = 32.0, p = .596	U = 60.0, p = .211	U = 38.5, p = .536
<b>A2</b>	U = 24.5, p = .211	U = 24.5, p = .211	U = 52.5, p = .328	U = 49.0, p = .328
<b>B1</b>	U = 36.5, p = .860	U = 61.5, p = .860	U = 52.5, p = .724	U = 55.0, p = .724

<b>B2</b>	U = 23.0, p = .740	U = 102.5, p = .659	U = 93.0, p = .328	U = 97.5, p = .724
<b>FORMER_AVG</b>	U = 37.0, p = .179	U = 52.5, p = .328	U = 100.0, p = .669	U = 93.0, p = .724
<b>NEVER_AVG</b>	U = 34.0, p = .930	U = 52.5, p = .724	U = 64.0, p = .724	U = 102.0, p = .724

**Table 8: Segmented results (MIN\_1, and MIN\_3, cumulative) for number of episodes (FREQ) and total duration (DUR) for rearing comparisons within groups in the test session.**

Variable	Salina	Muscimol
<b>A2_FREQ_MIN_1 - A1_FREQ_MIN_1</b>	Z = -1.190, p = .234	Z = -1.000, p = .317
<b>A2_DUR_MIN_1 - A1_DUR_MIN_1</b>	Z = -0.631, p = .528	Z = -1.000, p = .317
<b>B2_FREQ_MIN_1 - B1_FREQ_MIN_1</b>	Z = 0.000, p = 1.000	Z = -1.000, p = .317
<b>B2_DUR_MIN_1 - B1_DUR_MIN_1</b>	Z = 0.000, p = 1.000	Z = -1.000, p = .317
<b>B1_FREQ_MIN_1 - A1_FREQ_MIN_1</b>	Z = 0.000, p = 1.000	Z = -1.000, p = .317
<b>B1_DUR_MIN_1 - A1_DUR_MIN_1</b>	Z = 0.000, p = 1.000	Z = -1.000, p = .317

<b>B2_FREQ_MIN_1 - A1_FREQ_MIN_1</b>	Z = -1.000, p = .317	Z = -1.000, p = .317
<b>B2_DUR_MIN_1 - A1_DUR_MIN_1</b>	Z = -1.000, p = .317	Z = -1.000, p = .317
<b>FORMER_FREQ_MIN_1 NEVER_AVG_FREQ_MIN_1</b>	- Z = -0.531, p = .595	Z = -0.595, p = .552
<b>FORMER_DUR_MIN_1 NEVER_AVG_DUR_MIN_1</b>	- Z = -0.734, p = .463	Z = -0.463, p = .643
<b>A2_FREQ_MIN_3 - A1_FREQ_MIN_3</b>	Z = -0.768, p = .443	Z = -0.102, p = .919
<b>A2_DUR_MIN_3 - A1_DUR_MIN_3</b>	Z = -1.000, p = .317	Z = -0.102, p = .919
<b>B2_FREQ_MIN_3 - B1_FREQ_MIN_3</b>	Z = -0.638, p = .523	Z = -0.322, p = .747
<b>B2_DUR_MIN_3 - B1_DUR_MIN_3</b>	Z = 0.000, p = 1.000	Z = -0.322, p = .747
<b>B1_FREQ_MIN_3 - A1_FREQ_MIN_3</b>	Z = -0.108, p = .914	Z = -0.362, p = .717

<b>B1_DUR_MIN_3 - A1_DUR_MIN_3</b>	Z = -0.314, p = .753	Z = -0.362, p = .717
<b>B2_FREQ_MIN_3 - A1_FREQ_MIN_3</b>	Z = -1.156, p = .248	Z = -0.330, p = .741
<b>B2_DUR_MIN_3 - A1_DUR_MIN_3</b>	Z = -0.840, p = .401	Z = -0.330, p = .741
<b>FORMER_FREQ_MIN_3 NEVER_AVG_FREQ_MIN_3</b>	- Z = -1.289, p = .197	Z = -0.619, p = .537
<b>FORMER_DUR_MIN_3 NEVER_AVG_DUR_MIN_3</b>	- Z = -0.524, p = .600	Z = -0.619, p = .537

**Table 9: Within-group comparison results for rearing number of episodes (FREQ), total duration (DUR) and occupancy (OCC) in test.**

Variable	Salina	Muscimol
<b>A2_FREQ - A1_FREQ</b>	Z = -0.705, p = .481	Z = -1.633, p = .102
<b>A2_DUR - A1_DUR</b>	Z = -1.955, p = .051	Z = -1.414, p = .157
<b>B2_FREQ - B1_FREQ</b>	Z = -0.862, p = .389	Z = -0.507, p = .612
<b>B2_DUR - B1_DUR</b>	Z = -0.420, p = .674	Z = -1.521, p = .128
<b>B1_DUR - A1_DUR</b>	Z = -1.244, p = .214	Z = -1.521, p = .128

<b>B1_FREQ - A1_FREQ</b>	Z = -1.244, p = .214	Z = -0.552, p = .581
<b>B2_FREQ - A1_FREQ</b>	Z = -0.520, p = .603	Z = -0.944, p = .345
<b>B2_DUR - A1_DUR</b>	Z = -1.172, p = .241	Z = -1.788, p = .074
<b>NEVER_AVG_FREQ FORMER_FREQ</b>	- Z = -1.788, p = .074	Z = -1.609, p = .108
<b>NEVER_AVG_DUR FORMER_DUR</b>	- Z = -1.245, p = .213	Z = -1.183, p = .237
<b>A2_OCC - A1_OCC</b>	Z = -0.784, p = .433	Z = -0.507, p = .612
<b>B2_OCC - B1_OCC</b>	Z = -1.412, p = .158	Z = -0.507, p = .612
<b>B1_OCC - A1_OCC</b>	Z = -0.314, p = .754	Z = -0.507, p = .612
<b>B2_OCC - A1_OCC</b>	Z = -0.628, p = .530	Z = -0.169, p = .866
<b>NEVER_AVG_OCC FORMER_OCC</b>	- Z = -2.589, p = .010	Z = -0.676, p = .499