Investigating the Significance of the Subiculum Through Neuronal Inhibition

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Abstract

From locating a food source to returning to a safe home, animals use navigation every day. As the spatial function of the hippocampus becomes more clear, specifically of the CA1, the role of the subiculum as the hippocampus' major output region is still unclear. In this study, we examine the significance of the subiculum during travel of scaled routes in rat models. The rats (N = 3) were trained to run either a small or large triangle based on different call sites and tested on various manipulations of the maze relative to the environment. The spatial navigation task demonstrated that the rats relied on the orientation of the call site relative to the room and the distal cues in the room to perform the correct runs. To test the extent of the significance of the subiculum for this task, an optogenetic method was applied to inactivate the subiculum region. The inactivation of these subicular neurons resulted in minimal change in task performance. However, additional information is still needed to come to a definitive conclusion on the significance of this major hippocampal output region.

Introduction

Both humans and animals are able to efficiently navigate through a complex environment to achieve a goal, be it to locate food or return home. The cognitive map, or mental representation of the environment, was first coined by Edward Tolman, whose research demonstrated how rats utilize trial and error learning to build a representation of their environment in their brain (Tolman, 1948). The mental representation allowed them to make less errors to obtain a reward more quickly when compared to the initial introduction to the task.

The neurophysiological basis of the cognitive map was supported through discoveries identifying specialized neurons in the hippocampus. The first discovery to support the neurophysiological basis of the cognitive map were place cells in the CA1 of the hippocampus (O'Keefe and Dostrovsky, 1971). Place cells were found to specifically fire when an animal was in a specific location of the environment. Further work identified head direction cells in postsubiculum, which have increased firing when the animal's head is pointed in a specific direction (Taube, Muller, and Ranck, 1990), grid cells in the entorhinal cortex, which encode the environment through equilateral triangles gridded-patterned firing (Hafting, Fyhn, and Molden, et al., 2005), and boundary vector cells, which are responsible for encoding the borders of an environment. Notably, boundary cells were found both in the subiculum (Lever, et al., 2000) and later in the entorhinal cortex (Solstad et al., 2008). The extensive findings of these navigation-specialized neurons strongly support the physiological basis of cognitive maps. Our study focuses on the role of subicular neurons in the neural basis of cognitive mapping.

The subiculum was identified as the major output region of the hippocampal formation (Witter & Groenewegen, 1990). Various regions including the CA1 (Witter et al., 1989), anterior thalamus (Shibata, 1993; Frost, 2025), and entorhinal cortex (Steward, 1976; Wyss, 1981) project directly into the subiculum. The information is then sent to numerous sites including the retrosplenial cortex (van Groen and Wyss, 1990) and back to both the CA1 and entorhinal cortex (Tamamaki and Nojyo, 1995). There are additional subicular connections (O'Mara et al., 2001) beyond the scope of this study that further suggest that subiculum may serve as an integration point of information.

The type of neural information derived from the subiculum would provide insight into the region's function. Sharp and Green (1994) revealed distinctive neural firing as the subicular neurons tended to fire throughout the environment at varying levels and with multiple activity peaks. Relatively more recently, a subpopulation of subicular neurons were discovered to bidirectionally encode the axis of travel along a route specified by a track. This firing characteristic was independent from the actual environment location and utilized an allocentric spatial representation (Olson, Tongprasearth, and Nitz 2017). Furthermore, these neurons demonstrated poor yet multi-directional tuning, indicating another potential significant feature of the subiculum (Place and Nitz, unpublished).

Despite these multiple studies on the connectivity and firing patterns of the subiculum, the field's understanding into the significance of the subiculum is limited. The first study to investigate the subiculum through lesion was performed by Schenk and Morris (1985). Results suggested the region's implication in spatial navigation, but the exact function was undetermined. A following subiculum lesion study by Morris et al. (1990) revealed this lesion may primarily affect long-term memory rather than short-term memory and spatial understanding. Recent studies have taken advantage of genetic engineering methods, such as chemogenetics and optogenetics. Through the chemogenetic use of designer receptor exclusively activated by designer drugs, DREADDs, Frost et al. (2025) found the inhibition of the dorsal subiculum projection to the anterior thalamus was critical to processing of allocentric information. The allocentric perception emphasizes the significance of understanding the environment for navigational tasks. These studies on isolated neural inhibition to the subiculum have been limited thus far.

In this study, we extend on the findings from Ryan Place through optogenetic inhibition strictly in the subiculum. Previous work by Place suggested an importance of orientation and alignment of the maze in relation to the boundaries of the environment and found multi-directional tuning characteristics in the subiculum. The experiment began with replicating the manipulations on a spatial navigation task on a hexagon-shaped maze. The task allowed for the isolation of the specific features of the environment or maze that may have been used to perform successful runs. The addition of optogenetics studied the significance of the subiculum on the task, as the multi-directional tuning neurons were discovered on the same maze.

METHODS

Subjects. Adult male Sprague-Dawley rats (N = 3) were studied. Rats were separately housed under a 12 hour light-dark cycle. Behavioral motivation was maintained through food-restriction, in which their weight was kept at 85-95% of their free-fed weight. Water was freely available. The experiment involving rat models complied with the Institutional Animal Care and Use Committee guidelines.

Apparatus. The behavioral tasks used the “Hex” maze, a black hexagon shaped track with six large triangles composed of four smaller triangles inside. Each corner has three axis of travel and each edge has four. The track was built 7.62 cm wide and 1 cm walls to allow for the use of distal cues and environment. The baseline position of the maze was positioned in the center of the room (Figure 1). Animals were called to the call sites of either an edge or a corner through an auditory stimulus of tapping on the track and were rewarded with half of a cheerio following a correct run.

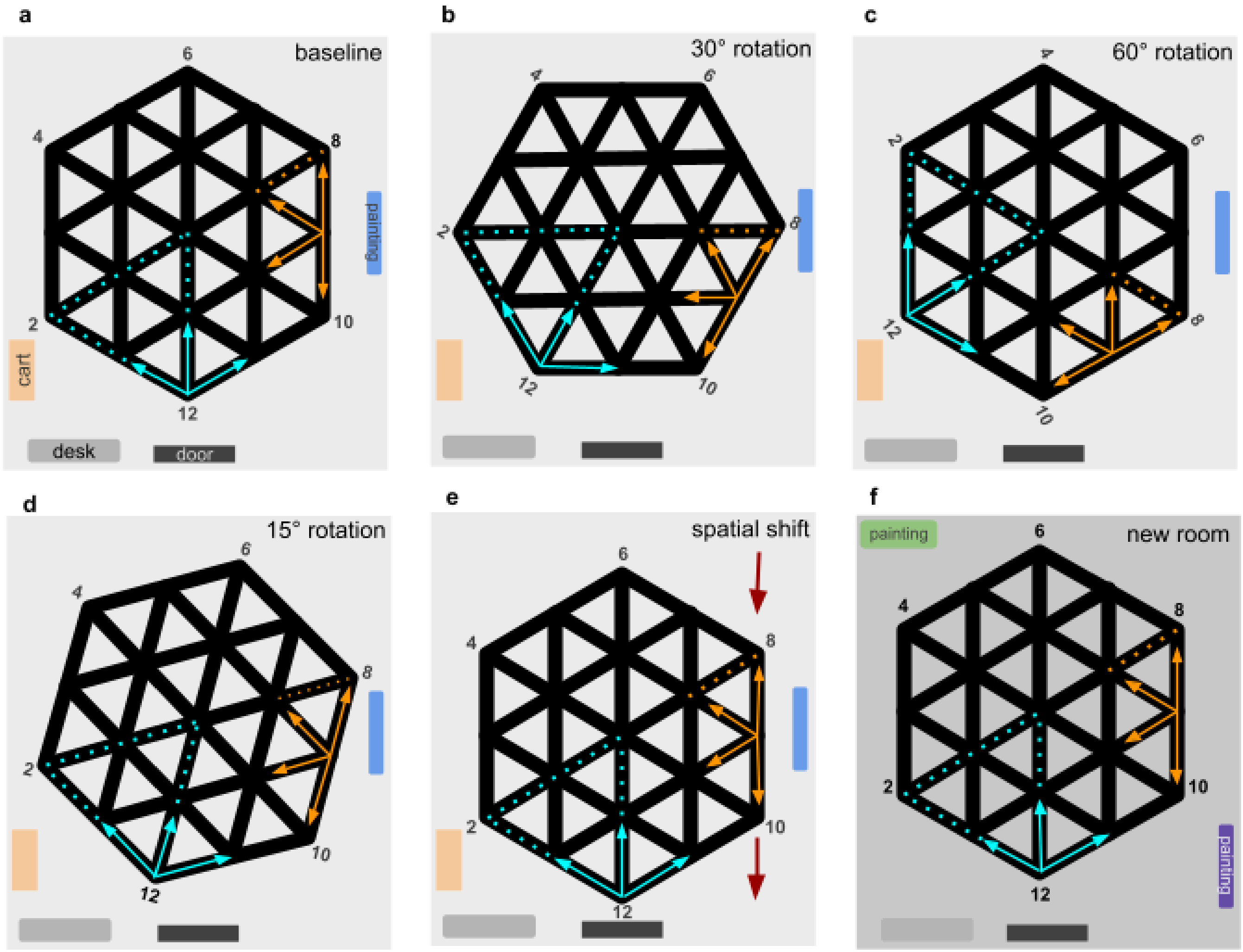

Figure 1. Hexagon-shaped maze, referred to as the “Hex” maze. (**a**) The baseline position has 12 call sites. Cyan arrows indicate possible paths that could be taken at a corner call site, while the cyan dotted lines indicate a correct large triangle run. Orange arrows indicate the possible pathways at an edge call site, while the orange dotted lines indicate the correct small triangle run. Colored objects represent the distal cues in the room visible to the rats. (**b-f**) The maze manipulations used to examine different features used for spatial navigation.

Behavioral task. The rats were initially trained on a linear track to pair the auditory stimulus of tapping on the track to a reward. Upon successful reward pairing in this subtask, the rats moved onto training on the Hex maze .The animals are called to the call sites to perform the corresponding run and rewarded with half a cheerio upon completion. The rats began with running a large triangle when called to a corner of the track and repeated the behavior across the six corner call sites. Once the rats demonstrated proficiency in the large triangle runs, the rats were trained to run a small triangle when called to the edge of the maze in the same manner. While there are two possible triangular routes for each corner and four possible triangular routes for each edge, the rats chose the correct triangle every time regardless of the endpoint of the previous run.

The position of the maze was altered from the baseline position in four different ways: 30 degree rotation (Figure 1b), 60 degree rotation (Figure 1c), 15 degree rotation (Figure 1d), spatial shift 0.91 m towards the door to match the length of 30 degree rotation (Figure 1e), and a relocation to a new room (Figure 1f). Every recorded session would consist of 144 randomized trials to test all 12 call sites 12 times each with a 5 minute break after the first 72 trials. As the rat finishes the reward, the next call site would be called. The rats were initially trained and mastered the task of 144 trial sessions in 8 weeks. Recorded test trials also utilized 144 trials.

Viral injection. One of the three rats received an adeno-associated virus of pAAV-CKIIa-stGtACR2-FusionRed (Figure 2a). The animal was anesthetized with isoflurane and then placed into a stereotaxic frame. The viral injection targeting the subiculum was performed at the coordinates: -1.85, ±2.4, -4.5. The optic fiber was placed above the injection site (Figure 2b), allowing for manual remote control. Once the procedure was completed, the rat recovered in his home cage for two weeks before reintroduction onto the maze.

The virus introduced channelrhodopsin binded to a fluorescent protein, FusionRed, into the subiculum, to allow for real-time optogenetic manipulation. This virus was designed to induce neural inhibition upon light stimulation.The blue light was transmitted through the optic fiber at 5mV to saturate the region activated channelrhodopsin. The region was transiently inhibited for a 4 second period.

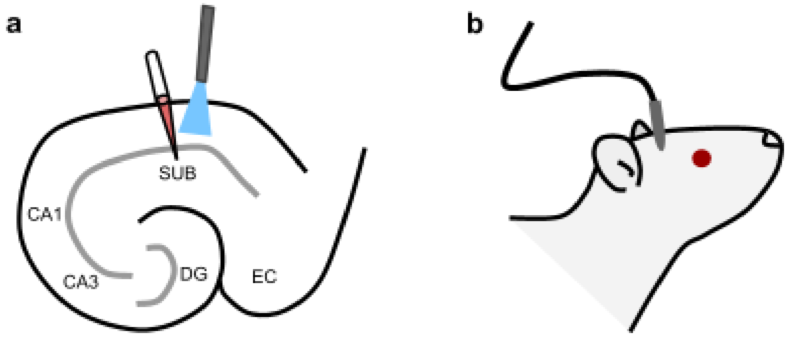

Figure 2. Optogenetic schematic. (**a-b**) The viral genetic injection of pAAV-CKIIa-stGtACR2-FusionRed in the subiculum (see Methods for details).

Data Analysis. This study used R for data visualization and interpretation. Correct and incorrect runs at the specific call site were recorded during the test sessions. Overall and triangle size performance accuracy was determined by percent of correct trials out of all trials.

RESULTS

Spatial orientation of call sites relative to boundaries are critical to navigation and not spatial location. To test what spatial features of the environment the animals use, the rats first learned and mastered the baseline position of the hex maze (Figure 1a). The animals were trained until they achieved an average accuracy of 96.9% in total, 5 sessions before the first manipulation day (Figure 3). Upon mastery of the baseline position, we rotated the maze by 30 degrees following the baseline half of the test session (Figure 1b). The location of the corner and edge call sites switched relative to the room. The orientation of the animal also was altered relative to the environment. The rats have the potential to utilize the geometry of the maze track to determine the correct run. However, the accuracy of the 30 degree runs dropped on average by 55.6% overall (Figure 4a). Notably, the large triangle runs had a dramatic decrease of 61% compared to the 27% in small triangles. These results suggest the use of either the location of the call sites or orientation of the maze relative to room.

A spatial shift towards the door of the room was used to determine if the specific location of the call sites in relation to the room is responsible for the drop in performance. The distance of the shift matched the distance of the 30 degree rotation from the baseline position, which was 0.9 meters (Figure 1e). In this spatial shift manipulation, the location of the call sites are not in the same position as the baseline location as the sites were moved towards the door. The orientation of the corners and edges remained the same. The results revealed no significant changes to the animals' behavior following the spatial shift, indicating that the location of the call sites are not particularly important for successful runs (Figure 4b).

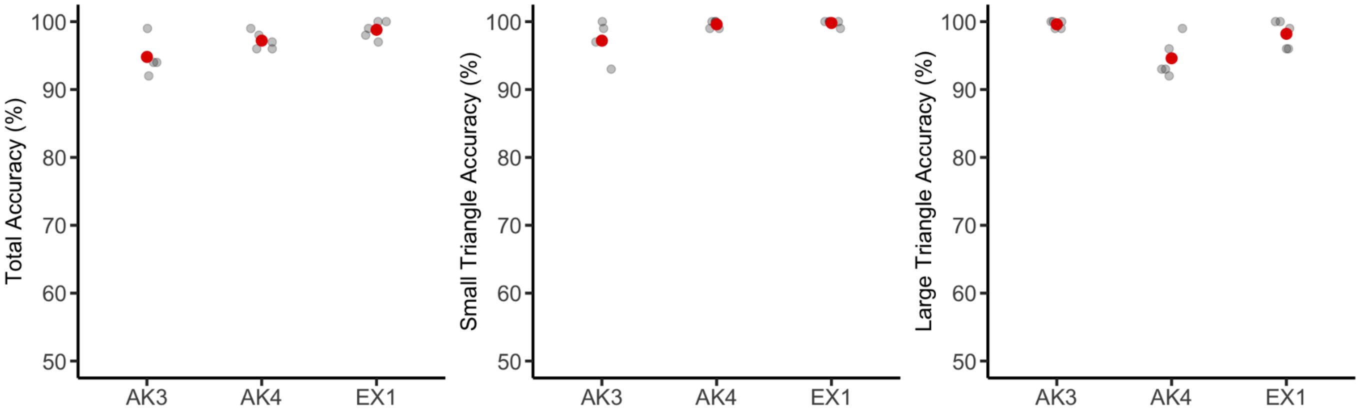

Figure 3. **All three rats (x-axis) mastered the Hex maze task**. Average accuracy (red dots) was calculated from the five training sessions (grey dots) prior to the first test session (manipulation day). Total task accuracy (left) was 94.8%, 97.2%, and 98.8%. Small triangle accuracy (middle) was 97.2%, 99.6%, and 99.8%. Large triangle accuracy (right) was 99.6%, 94.6%, and 98.2%.

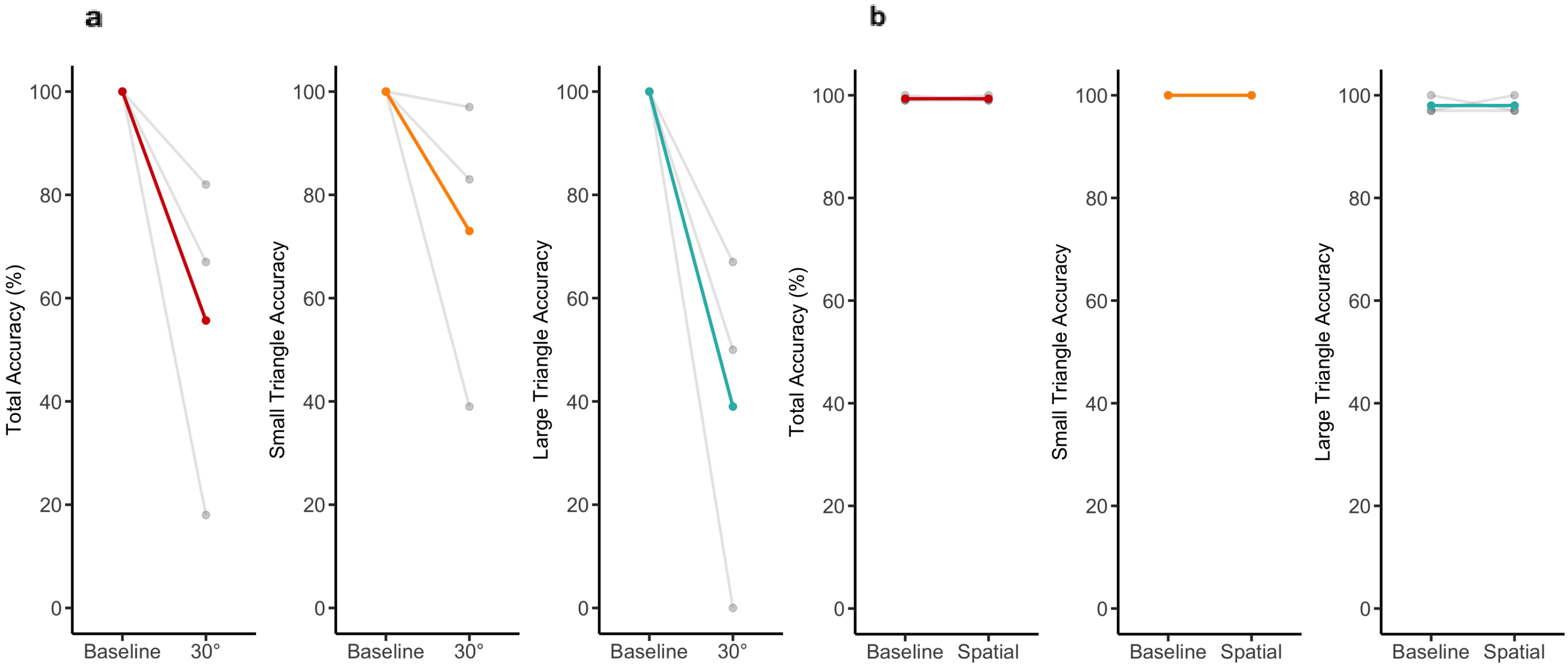

Figure 4. **30° rotation and spatial shift tested the significance of spatial orientation and location, respectively**. (**a**) Total task average accuracy (red line) began with 100% at baseline and dropped to 55.6% following the 30°. Small triangle accuracy (orange line) began with 100% at baseline and dropped to 73%. Large triangle accuracy (cyan line) began with 100% at baseline to 39% . Individual animal performance is indicated by the grey lines. (**b**) Total task average accuracy began with 99.3% at baseline and stayed at 99.3% following the spatial shift of 0.91 meters. Small triangle accuracy began with 100% at baseline and stayed at 100% after the shift. Large triangle accuracy began with 98% at baseline and stayed at 98% .

Distal cues decrease task accuracy while local cues do not. A 60 degree rotation and exposure to a new room was used to test whether the local and/or distal cues of the environment, respectively. Local cues of the Hex maze included odors, divots or any other physical irregularities on the maze tracks. It may be possible that the rats learned 12 different call sites to guide their navigation. The 60 degree rotation of the baseline position rotated the specific corner and edges clockwise (Figure 1c). The orientation of the animals at the call sites and location of the corner and edge shapes remained the same while the local cues of call sites were rotated by 60 degrees. This rotation did not significantly affect the animals' performance (Figure 5a).

Distal cues of the Hex maze refer to the distant cues of the environment, which include the markings on the four walls, furniture, and other visual stimuli in the room. To examine whether these animals are using the distal cues to successfully perform the task, the old distal cues were replaced by changing to a new room following the baseline condition (Figure 1f). The position and orientation of corner and edge call sites remained constant relative to the walls of the original room. The overall average accuracy did significantly decrease by 42.3% following the new room implementation (Figure 5b). The average accuracy for large triangles dropped by 52.7% while 30% for small. The initial drop and following increase in performance suggests the animals rely on distal cues and can learn new features to support their navigation on the Hex maze.

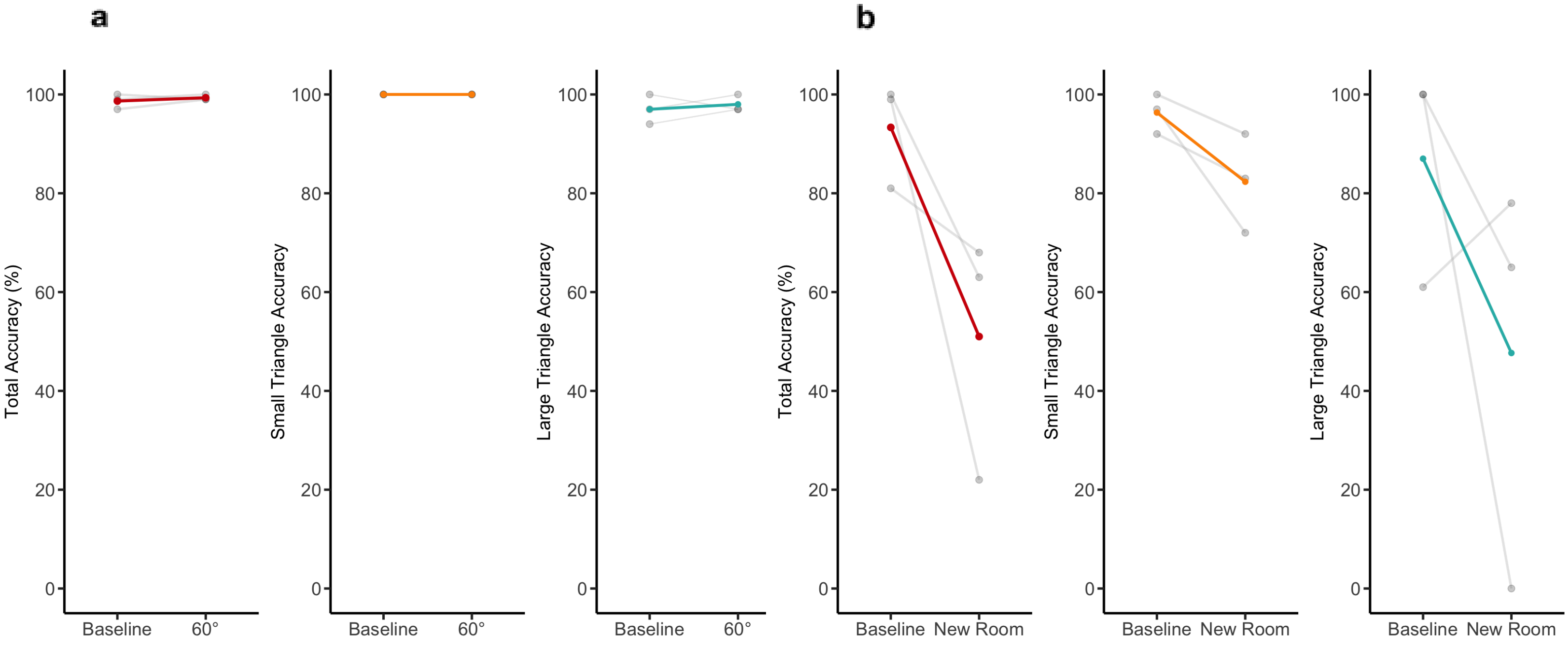

Figure 5. **60° rotation and new room tested the significance of spatial orientation and location, respectively**. (**a**) Total task average accuracy began with 98.7% at baseline and increased to 99.3% following the 60°. Small triangle accuracy began with 100% at baseline and stayed at 100%. Large triangle accuracy began with 97% at baseline and increased to 98% . (**b**) Total task average accuracy began with 93.3% at baseline and dropped to 51% following the new room introduction. Small triangle accuracy began with 96.3% at baseline and dropped to 66.3%. Large triangle accuracy began with 87% at baseline and dropped to 34.3% .

Change in alignment of the maze to room may influence task accuracy. The baseline position had a pair of sides that sat parallel to the walls of the room, or in other words sat in alignment with the environment (Figure 6a). The animals mastered this type of alignment to the room. Once the maze was rotated 30 degrees, the alignment shifted to create a 90 degree intersection between the sides of the baseline position and the initial walls (Figure 6c). The drop in performance in the 30 degree can also be considered as a result of the change in alignment, which is a component of the orientation of the animal during their navigation (Figure 4a). These results motivated a complete misalignment of the baseline position by a 15 degree rotation (Figure 6b). This rotation resulted in no pair of maze sides to align, or be in parallel, with the walls of the room. Interestingly, the large triangle average accuracy was more impacted compared to the small triangle, dropping by 15.7% compared to 1% respectively. The overall average accuracy of the 15 degree rotation decreased by 8.3% from baseline (Figure 6d). The selective drop in the large triangle accuracy offers additional consideration into the significance of the alignment of the environment in respect to the boundaries of the environment.

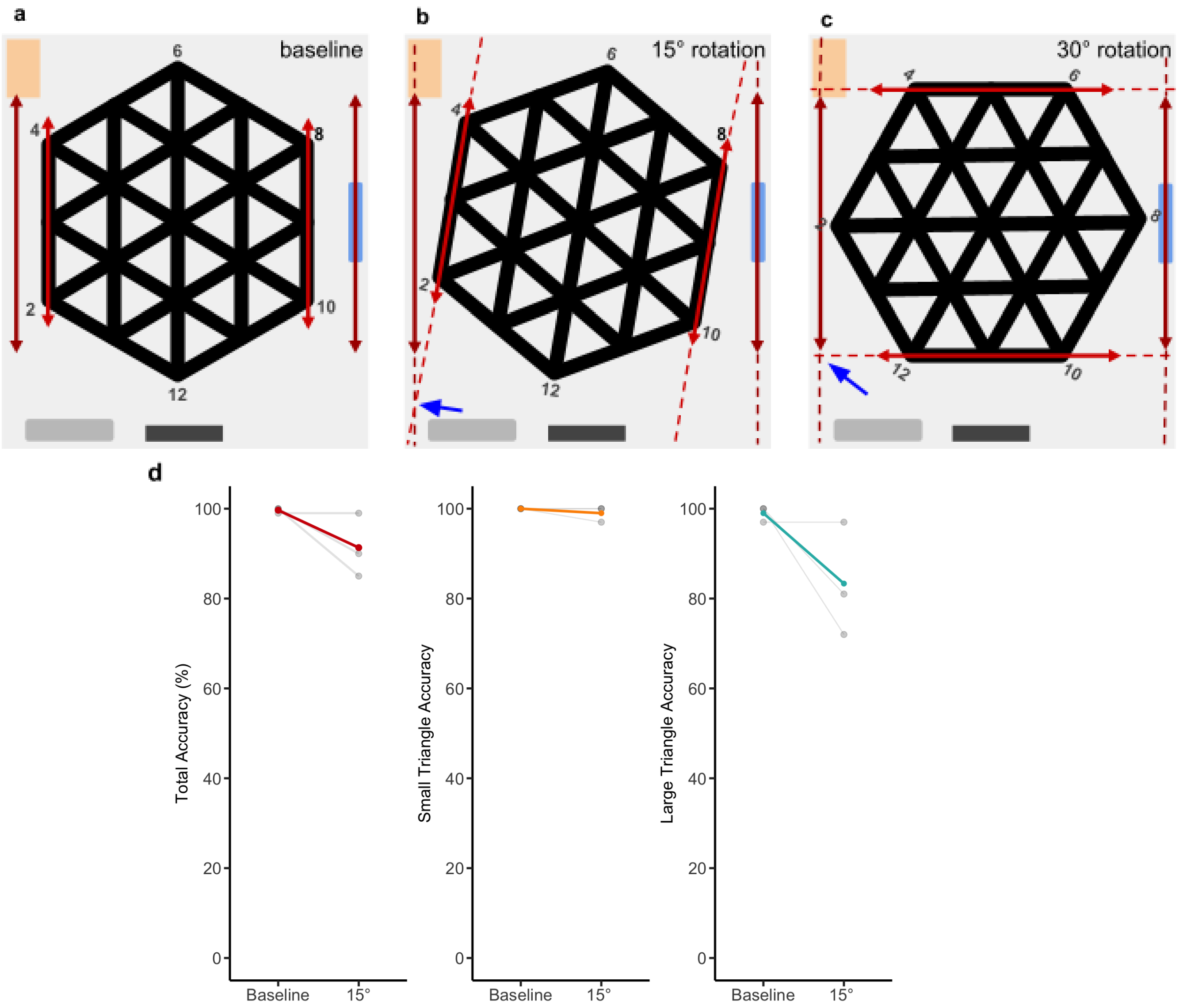

Figure 6. **The alignment of the maze relative to the walls may influence the animals' accuracy.** (**a**) The baseline position of the maze edges (#3 and #9 edges) in respect to the parallel walls of the room. (**b**) 15° rotation of the Hex maze, baseline edges intersect the alignment of the walls. No edges were in alignment with any of the walls. (**c**) 30° rotation of the Hex maze altered the alignment of the edges and created a perpendicular intersection with the walls. (**d**) Total task average accuracy began with 99.6% at baseline and dropped to 91.3% following the 15° rotation. Small triangle accuracy began with 100% at baseline and slightly dropped to 99%. Large triangle accuracy began with 99% at baseline and dropped to 83.3% .

Optogenetic inhibition has yet to demonstrate the significance of the subiculum. The animal with the adeno-associated virus was tasked to run the baseline position of the Hex maze with optogenetic stimulation as it approached the call site, lasting until the decision intersection (Figure 7a). At the intersection point, the animal has the option to either run a small or large triangle. The subiculum was inactivated at the critical point of making the correct run. The test session was split between two conditions: light off (subiculum under normal activity) or light on (subiculum inactivated). The test began with no light stimulation, in which the total accuracy was 92%. The lower baseline accuracy was not unusual to the rat's previous performance following his surgery. The following trials altered between light stimulation and no stimulation. The test session ended with no stimulation trials, bringing the subiculum to baseline conditions. Interestingly, the accuracy between altering trials and the last trials of no simulations revealed no significant difference in behavior. The total accuracy during the altering trials was 99% while the last section of trials had a 100% accuracy. These results suggest the altering optogenetic inhibition has no effect on the animal's performance on the Hex maze. However, given the sample size of one rat and one optogenetic test, these results are not a definitive conclusion to the significance, or lack of, for the function of the subiculum.

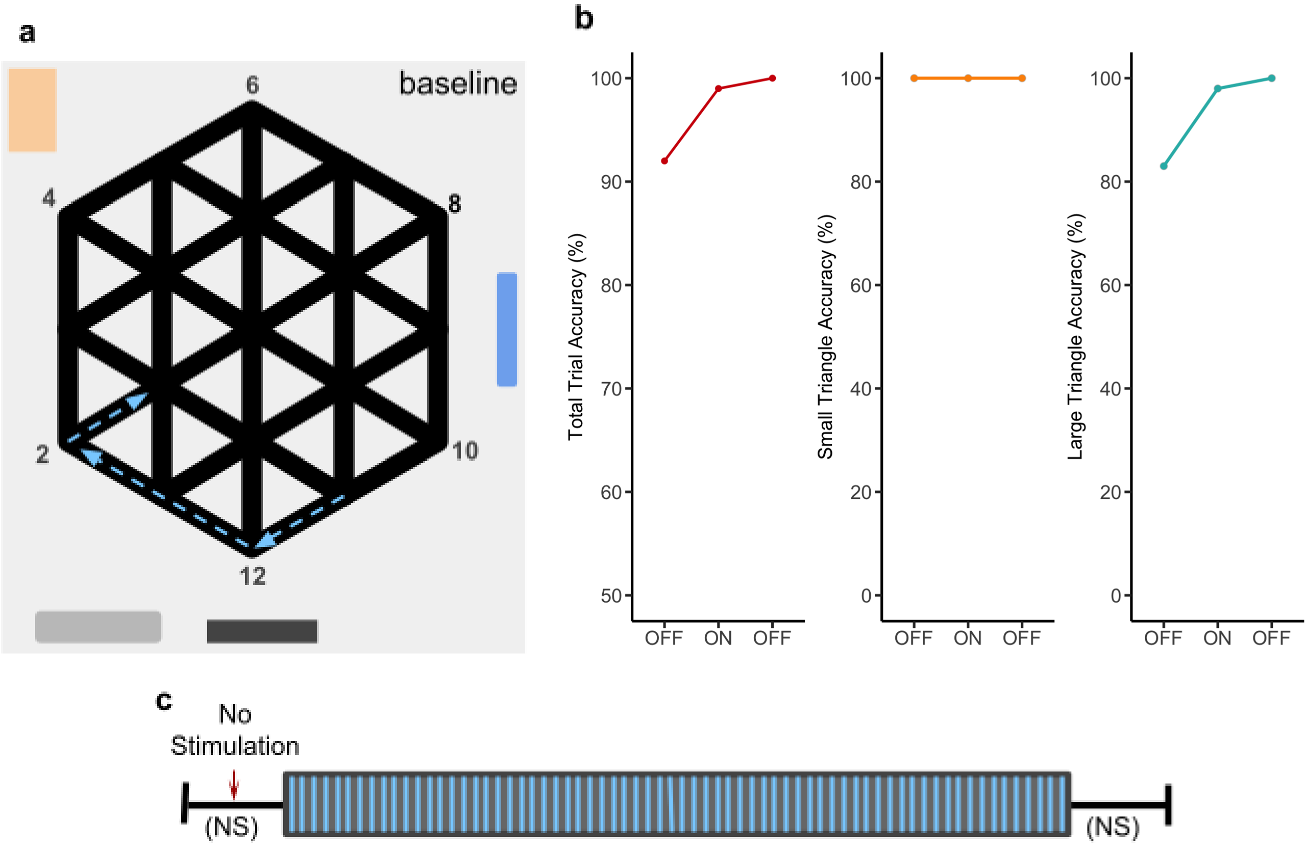

Figure 7. Optogenetic inhibition of the subiculum does not result in significant behavioral changes. (**a**) Blue dashed inhibited the distance the animal traveled when the blue light stimulation. (**b**) Under no stimulation, the total task average accuracy began with 92% with 100% accuracy for small triangles and 83% for large triangles. Under the altering stimulation, the total accuracy was 99%, with 100% for small and 98% for large triangles. The last trials (**c**) The schedule of optogenetic inactivation. First 24 trials were under normal conditions with no light stimulation.

The following 96 trials underwent altering light stimulation for every other trial. The last 24 trials under no light stimulation had a 100% total accuracy with 100% for small and 100% for large triangles.

Discussion

The results from the various behavioral manipulations identified specific aspects of the navigation that supports the successful runs on the Hex maze. The 30° rotation and spatial shift confirmed the significance of the orientation of the maze in respect to the boundaries of the room, as opposed to the particular location of the call sites. The new room and 60° rotation verified the importance of the distal cues of the environment and the lack of importance of the local cues of the maze. These behavioral findings were additionally consistent with Place's results. These results further highlight the role of the environment and the relative orientation in spatial navigation on the Hex maze.

While the findings from the optogenetic section of the study presented no significant behavior changes, it does not definitively identify the true significance of the subiculum. These results are limited to one animal and one approach thus far. Previous results from this study demonstrated the variability in behaviors across the animals (see example Figure 5b). Therefore, future expansion on this study would require additional animals to confirm the validity of the previous stimulation pattern (Figure 2c). The presence of the adeno-associated virus in the subiculum region currently needed to be confirmed through histology. Further work would utilize electrophysiology methods to verify the inhibition of the neurons when under optogenetic stimulation. Additionally, this current approach of altering light stimulation at the baseline position may not challenge the animal enough to produce meaningful results. Another recent study used a chemogenetic approach and introduced a challenging condition which resulted in positive significant behavioral changes (Yanakieva S, 2024). Future considerations for challenging the animals may include introducing the animals to complete darkness during the task and continuing to explore the 15° rotation among other spatial manipulation.

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