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Effects of Hippocampal Impairment on Rodent Spatial and Non-Spatial Memory

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The human hippocampus is a critical brain region for the encoding and retrieval of both episodic and spatial memory. Although it has been established that the rodent hippocampus is involved in spatial memory, its role in non-spatial memory has been largely debated. The purpose of the current study is to establish the role of the rodent hippocampus in non-spatial memory.

To determine the necessity of the hippocampus during memory retrieval, muscimol is infused through previously surgically implanted bilateral dorsal CA1 cannulae, thereby temporarily deactivating the hippocampus. The Morris Water Maze, a well established hippocampal-dependent spatial navigation task, was initially utilized in order to confirm that the experimental procedures effectively impair proper hippocampal function. Next, Novel Object Recognition (NOR) experiments began. In the NOR experiments, mice are given three arena habituation/sample sessions to become familiar with the arena and with two identical objects. During a test session 24 h after the last habituation session, the mice are presented with one of the familiar objects one novel object. Successful retention of object memory is inferred if the mice exhibit a preference for exploring the novel object over the familiar one during the test session.

The current study is still in progress, but the findings are sure to address the long-standing debate regarding the function of the rodent hippocampus in non-spatial memory. Understanding the similarities between rodent and human hippocampal function could enable future animal studies to effectively answer questions about diseases and disorders affecting human learning and memory.

Effects of Hippocampal Impairment on Rodent Spatial and Object Memory Retrieval

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Introduction

In humans, both spatial and non-spatial components of declarative memory rely on the integrity of the hippocampus. Aging and Alzheimer's disease both compromise declarative memory. Developing effective treatments for memory impairment requires animal models of declarative memory, but first the similarities between the animal model and human memory systems must be established. The rodent hippocampus is known to be essential for spatial memory, but its role in non-spatial memory continues to be debated. It is also unclear whether declarative memories eventually become hippocampal independent, being supported by neocortical structures, instead.

The Novel Object Recognition (NOR) task was used here to examine the role of the rodent hippocampus in non-spatial object memory. Additionally, one cohort of mice was exposed to the NOR arena and familiar objects 21 days prior to testing, making it possible to elucidate whether object memory can be supported by neocortical structures after a long delay. The muscimol microinfusion temporary hippocampal inactivation technique was validated with a Morris water maze (MWM) experiment, before testing its effects in the NOR paradigm.

Questions

- Does direct bilateral intrahippocampal muscimol microinfusion effectively block hippocampal activity?
- Is the rodent hippocampus an essential brain structure for object recognition memory?
- Is object memory eventually supported by extrahippocampal structures?

Methods & Materials

Surgery and Cannulae Implantation

8 - 16 week old male C57BL/6J mice were surgically implanted with chronic bilateral intracranial guide cannulae directed above the CA1 region of the dorsal hippocampus, and then given 8 - 12 days to recover before beginning behavioral testing.

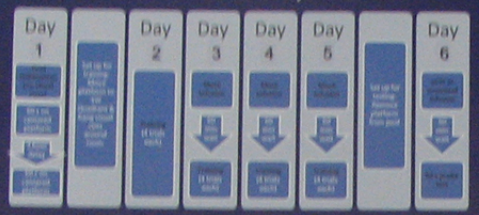
Intrahippocampal Microinfusions

Bilateral intracranial microinfusions were given 60 min prior to the probe test (MWM) or the test session (NOR). Infusion cannulae were inserted through the guide cannulae to permit microinfusion into the dorsal CA1 subfield of the hippocampus. Subjects received a total volume of 0.5 µL of artificial cerebrospinal fluid (aCSF) or muscimol (1 µg/µL), a $GABA_A$ agonist that effectively temporarily deactivates the hippocampus by increasing inhibition.

Methods & Materials

Morris Water Maze

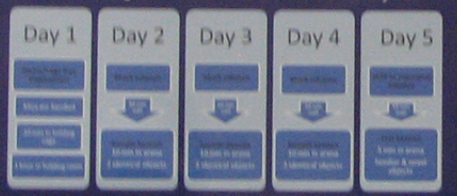
Mice were trained in a pool of water to use extramaze visual cues to successfully navigate to a submerged escape platform; this spatial navigation is known to be hippocampal dependent. The following figure presents the experimental design used in the current MWM study.



control (n = 7) muscimol (n = 8)

Novel Object Recognition

Mice have a natural tendency to explore novel objects in a familiar environment. The following figure presents the experimental design used in the current NOR study.



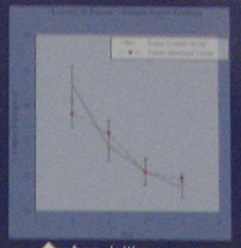
control (n = 10) muscimol (n = 7)

Exploration was measured using EthoVision XT tracking software (Noldus). Successful retention of object memory was inferred if the mice exhibited a preference for exploring the novel object over the familiar one during the test session.

Cannulae Placement

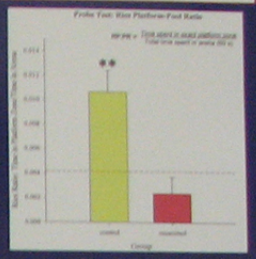
Fixed tissue sliced at 50 µm and stained with Neutral Red. The cannulae placements for all mice were verified and the data for any mice with incorrectly placed cannulae were removed from analyses, resulting in the group sizes indicated above.

Morris Water Maze Results



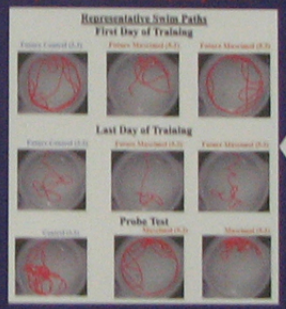
Acquisition

There was no significant difference between future treatment groups ($F_{3, 13}$ = 0.002, P = 0.962). The future groups acquired the task equally well.



Probe Test

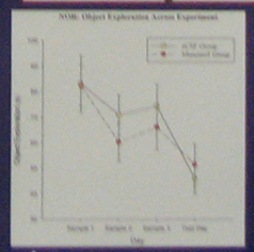
The muscimol group's RIOS platform:pool ratio (RP:PR) was not different from chance (t_7 = 1.691, P=0.135), but was different from the control group (t_{13} = 3.758, P = 0.002, **). These results indicate that the muscimol effectively impaired hippocampal function.



Representative Swim Paths

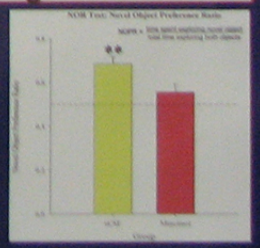
Mice that received muscimol microinfusion before the probe test failed to search accurately in the pool, and behaved similarly to the first training day, suggesting that they had no memory of the MWM task.

Novel Object Recognition Results



Object Exploration

There was no significant difference in object exploration between groups ($F_{1, 15}$ = 0.377, P = 0.548). The future groups acquired similar amounts of object exploration across the experiment and the muscimol infused prior to the test session did not affect the amount of total object exploration.



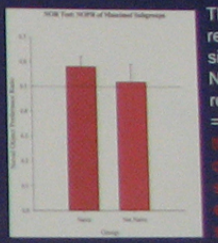
Test Session

The muscimol group's NOPR was not different from chance (t_5 = 1.532 P = 0.176, but it was significantly less than the aCSF group's NOPR (t_{15} = 2640, P = 0.019, **). The muscimol microinfusion impaired object recognition, indicating that the hippocampus is necessary for retrieval of non-spatial memory.

NOR with 21 day delay

Eight of the 17 mice used in the NOR study were run in an NOR study 21 days prior, and therefore had previous experience with the arena and the familiar objects. If the object memory is transferred to neocortical regions and is supported independently of the hippocampus over time, then intrahippocampal muscimol microinfusions should not impair object memory retrieval in this cohort of mice.

Test Session



The NOPR of naïve mice that received muscimol was not significantly different than the NOPR of non-naïve mice that received muscimol (t_5 = 0.814, P = 0.453). Having experienced the arena and familiar objects 21 days prior (in addition to the 3 sample sessions of the current experiment) did not result in savings, indicating that the memory had not become independent of the hippocampus.

Conclusion

- Microinfusion of muscimol effectively impaired hippocampal function, as indicated with the MWM study.
- Muscimol microinfusion prior to MWM probe test impaired more than just the retrieval of spatial memory, as illustrated by swim paths.
- Muscimol microinfusion prior to NOR test decreased NOPR to chance, indicating that the hippocampus is necessary for object memory retrieval.
- Object recognition does not become independent of the hippocampus even after a 21-day consolidation period.

Discussion

These findings clearly demonstrate a role for the rodent hippocampus in non-spatial object memory. These results help confirm similarities between mechanisms of rodent and human memory. To further define such mechanisms, future studies could, (i) address the argument that hippocampal impairment in the NOR might be due to a spatial component by conducting the test in a different contextual environment; (ii) aim to better visualize the diffusion of muscimol by using fluorescent-tagged muscimol; and (iii) address whether the rodent hippocampus is necessary for the encoding of object memory by administering hippocampal microinfusions prior to the NOR sample sessions.

Acknowledgements

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