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Memory capacity and prioritization in mice

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Abstract

Our brain’s capacity for memory storage may be vast but is still finite. Given that we cannot remember the entirety of our experiences, how does our brain choose what to remember and what to forget? Much like the triage of a hospital's emergency room, where urgent cases are prioritized and less critical patients receive delayed or even no care, the brain is believed to go through a similar process of memory triage while we sleep. Recent salient memories are prioritized for consolidation during sleep, which helps create stable, long-term representations in the brain; less salient memories receive a lower priority, and are eventually forgotten if not sufficiently consolidated. While rodents are a primary model for studying memory consolidation, common behavioral tests typically rely on a limited number of items or contexts, well within the memory capacity of the subject. A memory test exceeding an animal’s memory capacity is key to investigating how memories are selectively strengthened or forgotten. Here we report a new serial novel object recognition task designed to investigate the phenomenon of memory triage in mice, allowing us to directly test a rodent’s memory capacity and investigate factors influencing memory prioritization.

Keywords: Memory triage; Hippocampus; Rodent; Novel Object Recognition Task; Mice
Introduction
What determines whether a memory will be retained long-term or eventually forgotten? When items are presented in a series, memory retention is generally better for the first and last items, a phenomenon commonly referred to as the primacy and recency effect respectively (Ebbinghaus 1885, Murdock and Bennet, 1962, Greene et al, 2000). This phenomenon has been widely observed and studied in both humans (Murdock and Bennet 1962, Korsnes et al., 1996; Miles and Hodder, 2005; Daniel and Katz, 2018) and other animals, including monkeys (Gaffan, 1983), dolphins (Thompson and Herman, 1977), pigeons (Macphail 1980; Shimp, 1976) and rats (Reed et al. 1991, Kesner et al, 1984; Bolhuis and van Kampen, 1988; Harper et al, 1992 ), although usually over short delay periods and within a single context or task. A second factor affecting memory retention is repetition, where additional repetitions or training duration promotes stronger and longer-lasting memories (Zhan et al., 2018). Finally, a third major factor influencing whether a memory will be remembered or triaged is salience, the increased importance of the experience (or perceived future relevance) resulting from novelty, reward and/or emotion (Takeuchi et al., 2016; Foley et al., 2014; Madan 2017; Skavronska et al., 2020, Crowley et al. 2019).

Here, we describe a new behavioral task in mice, serial novel object recognition (sNOR), which provides us with a new tool for investigating the consolidation of multiple memories and how serial order, repetition, and salience may influence the triage of a memory. Importantly, this approach uses novel object recognition, allowing for the quick acquisition of new memories, with learning requiring only a single behavioral session. However, differing from a typically novel object recognition task, this behavioral paradigm uses multiple small behavioral arenas connected in series, each with a different set of novel objects for the mouse to learn. This approach can be scaled such that the memory capacity of the subject is exceeded, requiring the triage of one of more memories.

Results
We designed a serial object recognition task (Fig. 1), where mice (n=12) were trained to remember four distinct novel objects, each associated with a different behavioral arena. The experiment involved three distinct phases (habituation, familiarization, and testing) similar to the standard version of a novel object discrimination task involving a single arena (Leger et al 2013). In the habituation phase, mice received a 5 min exposure to each behavioral arena without any objects present. Twenty-four hours later, during the familiarization phase, a pair of identical novel objects were placed in each behavioral arena, and mice were allowed 5 min of exploration for each pair of objects. Novel objects were always different between arenas,
but identical within the same arena during the familiarization phase. At the end of the 5 min familiarization period in one arena, moveable doors were opened allowing the mouse to transition unaided to the next behavioral arena. After a familiarization period in each arena, mice were returned to their home cage to rest for 80 min. Following this, mice were reintroduced to the four behavioral arenas (in series) for the testing phase, which was identical to the familiarization phase except one object from each pair was swapped with a new object, now novel to the animal, and different from the objects in the remaining arenas. The behavior of the mice was monitored by an overhead camera, and exploration was analyzed offline (see Methods).

Figure 1. Serial object recognition task design.

To measure memory retention in our serial recognition task, we computed the Discrimination Index (DI) [see Methods], which ranged from -1 (only exploration of the familiar object) to 1 (only exploration of the novel object). We observed that the DIs in arena 1 and 3 tended to be the highest and lowest respectively (mean DI [arenas 1,2,3,4]= [0.41, 0.3, 0.012, 0.21], Fig. 2A), with a statistically significant difference in the DI between arenas 1 and 4 (P<0.0205, Signed rank, Bonferroni corrected) and between arena 3 and each of the other three arenas (all P<0.01, Signed rank test, Bonferroni corrected).

We next compared these results to a standard novel object discrimination task (single arena), during both the familiarization period (both objects were novel) and the testing phase (one object was familiar and one object was novel). Compared to the standard version of the novel object recognition task, involving a single arena and single pair of objects (Mean DI=0.33), we did not observe any statistically significant differences in the DI for arenas 1, 2 and 4 (P>0.05, Rank sum test, Bonferroni corrected). In contrast to this, arena 3 had a significantly lower DI compared to a single arena test (P<0.0017, Rank sum test, Bonferroni corrected).
We next determined how many objects each subject could remember, using a discrimination index criterion (0.153) based on the DI distribution obtained during the single arena familiarization period (Fig. 2A, see Methods). Most mice could remember 2-3 pairs of objects (Fig. 2B), with all mice successfully recalling the objects from arena 1, in contrast to none of the mice being able to remember the objects from arena 3 (Fig 2C). No statistically significant difference in the total exploration time was observed between arenas 2, 3 and 4, with the total exploration time slightly greater for objects in arena 1 compared to the remaining arenas, albeit only with a statistically significant difference when compared with arena 2 and 3 (Signed rank test, Bonferroni corrected, $P=0.047$ [vs. arena 2], $P=0.047$ [vs arena 3], Fig 2D).

It is important to note that even when the memory capacity of the mouse was exceeded, the newly added items did not disrupt previously encoded memories (objects from arenas 1 and 2). While the serial position effect would predict the best memory recall for arenas 1 and 4, this was only partially true in our data; we observed a strong primacy effect (best performance in arena 1), but a weaker recency effect (50% of mice performed above chance in arena 4). Additionally, memory recall in arena 2 was not significantly different from arena 1 or 4 ($P>0.05$, Sign ranked test, Bonferroni corrected).

**Figure 2. The memory capacity of mice.** (A) Discrimination index in each arena (blue), compared to a single arena version of the task with 2 novel objects during the familiarization period (orange) or during the test period with one familiar and one novel object (green). (B) The distribution of the total number of object pairs remembered. (C) The fraction of subjects with significant a Discrimination index in each arena. (D) Total exploration time in each subject.
To investigate how memory triage is affected by factors other than serial order, namely training duration and salience, we performed a modified novel object recognition task which included an additional high-salience arena with a longer exploration time (referred to here as “the playground”). Compared to the standard arena used, this high-salience arena was larger in size (>11 times the area), had a longer exploration time (>3 times the duration), and contained a greater number of novel objects (30 objects + 1 tube + 1 running wheel) which the animal was free to explore either before or after the familiarization phase- referred to here as P\text{interference} (n=8, proactive interference) and R\text{interference} (n=8, retroactive interference), respectively [see Methods]. For comparison, we also compared these two groups with a standard serial novel objective discrimination task, which we refer to as the control group (n=7, no interference) (Fig. 3B). Given that most mice could remember 2 pairs of objects [see above], we only used a simplified, two arena version of the serial NOR task (Fig. 3A).

We observed that in both arenas, the mean discrimination index of the R\text{interference} mice was significantly impaired compared with the control group, while no statistically significant difference was observed between the P\text{interference} and control group (Mean DI [arena 1,2]: (R\text{interference} [0.002,-0.09], P\text{interference} [0.37, 0.23], control [0.33, 0.43], Kruskal Wallis test with Bonferroni post hoc comparisons, R\text{interference} vs control- [P=0.0438; P=0.0079], P\text{interference} vs control- [ P>0.05, P>0.05], Fig. 4A).
We next observed that most mice remembered objects in both arenas for the control group (no interference), one arena from the proactive interference group, and neither arena from the retroactive interference group (Fig. 4B, see Methods). Interestingly, the task performance typically declined between the first and second arena for the pre- and post-interference groups, while opposite trend was observed for the control group (memory recall was better for the second arena compared with the first arena) (Fig. 4C). No statistical difference in total exploration time was observed between arena 1 and 2 under all three interference conditions (Fig 4D; arena 1: p=0.2174; arena 2: p=0.3817).

**Figure 4. Effect of salience on the serial novel object discrimination task**

(A) Discrimination index of no interference group (blue), proactive interference group (red) and retroactive interference group (yellow) in each arena. (B) The distribution of the total number of object pairs remembered. (C) Fraction of subjects with significant discrimination index in each arena. (D) Total exploration time in each subject for three groups in each arena.
Discussed

Here we describe a new behavioral paradigm that can test both the memory capacity and prioritization in rodents, using multiple novel object recognition tests performed serially. Using this approach, we examined the main factors thought to influence memory prioritization—serial order, repetition, and salience.

Using a four-arena version of the task (Fig 1), we observed that the memory capacity of mice is approximately 2-3 object pairs, with a strong primacy effect (highest DI occurred in the first arena). Importantly, the introduction of additional arenas (each with a novel object pair) did not affect overall performance (measured as the discrimination index) compared to the standard version of the task performed using a single arena and object pair. This suggests that as memory demands increase beyond the brain's capacity to consolidate, the brain's strategy is to triage specific memories rather than try to remember everything at a lower fidelity. We do not know the true upper limit of memory in mice, as increasing the salience or exploration time could potentially increase the number of objects remembered beyond our observations here. Furthermore, our observations are based on a serialized version of a novel object recognition task; a similar approach could be applied to other recognition tasks, including novel location and novel context, where a different set of brain regions are required for the task, potentially leading to a different memory capacity (Dix and Aggleton 1999, Langston and Wood 2010).

Introducing a new arena (“the playground”) with a longer exploration time and a higher degree of salience (many novel objects) negatively affected the memory retention of objects from the 2-arena version of the serial novel object task. However, retroactive interference (exploration of the playground after familiarization in the 2-arena task) was more detrimental to memory retention than if the playground was explored before familiarization (proactive). This contrasts with our 4-arena task, where the first two arenas were generally remembered the best, with a similar memory performance when compared to the single arena task, despite any possible retroactive interference from arenas 3+4. However, as we did not vary salience, exploration time and the delay period either independently or parametrically, the relative role and influence of these factors remain an open question.

What neural mechanisms are responsible for the prioritization and triage of memories. Memory consolidation is postulated to require hippocampal replay, the spontaneous reactivation of neural sequences that are experience-dependent (Silva et al. 2015, Takigawa et al 2022), and reinstate brain activity patterns representing recent behavioral episodes (Wilson and McNaughton 1994, Lee and Wilson 2002, Girardeau et al. 2009, Tirole, Huelin Gorriz, et
During sleep, more hippocampal replay is postulated to lead to memory strengthening while an insufficient amount of replay would result in memory triage (and eventual forgetting) (Lewis and Bendor 2019). Replay also occurs in the awake animal (Foster and Wilson 2006, Diba and Buzsaki 2007, Carr et al. 2011) and this form of replay has recently been shown as a candidate mechanism for “tagging” salient memories for later sleep replay (Huelin Gorriz et al. 2023). Because awake replay can occur remotely (while the animal is awake but not in the same context as the replayed behavioral episode), this may also help increase the likelihood of the memory later replaying during sleep. Retroactive interference potentially disrupts such remote awake replay, especially in the case of our highly salient “playground”, biasing subsequent awake replay events towards this new experience and away from the 2-arena novel object recognition task, in turn leading to a lower priority for the 2-arena behavioral episode to later replay during sleep.
Methods

Animals
C57BL/6J female mice (3 months of age) were used in all experiments. Mice were allocated to their home, four per cage, where were subject to a reverse light cycle (12h/12h dark/light cycle). All the mice were housed in their home cage for 10 days before the experiment with ad libitum access to food and water. No food or water restriction was performed before the behavioral tasks. All experimental procedures performed were first approved by a local ethical review committee at University College London. Procedures were carried out under license from the UK Home Office in accordance with the Animals (Scientific Procedures) Act 1986 under Project license- PPL P61EA6A72. All methods were also carried out in accordance with ARRIVE guidelines.

Apparatus and materials
The four arena behavioral apparatus was 55 cm L × 55 cm W × 80 cm H, with an open-top field made of corrugated fluted board. This was used for all stages of the object recognition task. Barriers further subdivided the open field into four equal sub-areas (A, B, C, D) of approximately 27 cm x 27 cm (L x W) to create individual arenas. These barriers could be lifted individually, allowing the animal to walk to, and explore all relevant sub-area within a single session. Using paper clips, large white shapes were hung down one wall of each sub-area to create four different contexts that could be easily discriminated by the mice.

Two objects were placed diagonally (∼5 cm from the wall) in each context. Objects differed in height (2–10 cm), base diameter (2–6 cm), color, and shape. During the familiarization phase, two identical objects were presented in each arena, while during the testing phase, one of the familiar objects was replaced by a novel object. There were three copies of each object so that different copies of the same object could be presented during the familiarization and testing phases, aiming to avoid the possibility of mice being attracted by their own or other mice’s odor left on objects that were presented. For all the experiments, objects and their relative positions were placed randomly and counterbalanced to avoid potential position effects in the arena. Pilot experiments were conducted to ensure mice could discriminate the different objects and did not show object preference.

For the high-salience arena, a 90 cm L × 90 cm W × 80 cm H open-top field made of corrugated fluted board was used to as “the playground” for the mice. Twenty individual or combined Lego blocks in five different colors, one wheel, one red tube and ten small colorful blocks were placed in the playground in a random arrangement. The layout of the playground remained
the same for every mouse and was constant throughout the whole experiment. Every mouse was allowed to actively explore for no less than 15 minutes.

**Novel Object Recognition Task**

All NOR tasks were based on the following stages: 1) Habituation phase: 24 hours before the familiarization, the mice were put into the apparatus for 5 minutes in each arena without the objects present. 2) Familiarization phase: mice were moved from the colony arena to the experimental arena 30 mins prior to each familiarization phase to minimize stress related to transportation. After this, animals were allowed to explore each arena (in the order of arenas 1–4) and its objects for 5 minutes. The interval period took place immediately after the familiarization session. Once the interval time elapsed the final testing phase of the NOR was conducted. 3) Testing phase: this phase was identical to the familiarization phase with the exception that one object in each context was replaced with a novel one. The mice were allowed to explore freely for 5 minutes in each arena. Any form of exploration beyond 5 minutes in each arena was not scored. The behavior of the mice was monitored by an overhead camera, and exploration was analyzed offline using the ANY-maze tracking system. To eliminate olfactory cues, after each phase, all the apparatus and objects were wiped and cleaned with water containing 50% ethanol.

**Interference conditions.** 36 female mice were divided into three groups – two of them with a playground as memory interference before and after the familiarization phase, respectively. For the no interference group, mice were kept in their home cage during the entire interval time. For the groups with interference – mice were placed in the playground for 20 minutes, and in their home cage for the remainder of the interval time. The total interval time for all three groups was 100 minutes (**Fig. 3A**).

**Automated tracking of object exploration.** Automated tracking of exploratory activity was conducted with ANYmaze software. For each video file, the 27.5 cm × 27.5 cm floor area of each arena and each object were outlined in ANYmaze as the main field. The head, body, and base of the tail of the mouse were automatically tracked by ANYmaze. Once a mouse satisfied the criteria for investigating an object, that period was accumulated to determine the total exploration time for a certain object. All files were coded to allow the experimenter to analyze the data blinded.

**Object investigating criteria.**

The criteria for active exploration was when the mouse’s head was less than 20 mm from the object and oriented towards it (**Dix & Aggleton, 1999; Langston & Wood, 2010**). Climbing
over or leaning on an object was not considered to be an investigating behavior, unless that action was accompanied by a nose-directing behavior toward the object (Besheer & Bevins, 2000; Stickgold & Walker, 2013). Our criteria for exploration also excluded time spent standing on the top of objects (Leger et al., 2013).

Data analysis

Memory performance on each recognition trial is expressed as the Discrimination Index (DI)

\[ DI = \frac{t_{\text{novel}} - t_{\text{familiar}}}{t_{\text{novel}} + t_{\text{familiar}}} \]

where \( t_{\text{novel}} \) and \( t_{\text{familiar}} \) represent the total amount of time spent exploring novel and familiar object during the tasks respectively (Leger et al., 2013; Tam et al., 2017). For the DI, the higher a ratio score is above zero (which indicates the mouse spent more time on the novel objects), the better the recognition memory performance. A significance level for the Discrimination index (0.153) was based on the DI distribution obtained during the familiarization period (2 SD above the mean DI) in the single arena version of the task. Custom scripts in MATLAB were used for data analysis.

Author Contributions:

Q.Q., C.M., and D.B. designed the experiment and analyzed the data, Q.Q. and C.M. collected the data. Q.Q and D.B. wrote and revised the manuscript.

Data Availability statement:

All data is available on Figshare- https://doi.org/10.6084/m9.figshare.22820450.v1

All code will be available via GITHUB (https://github.com/bendor-lab/serial_novel_object) upon publication.

Declaration of interests:

The authors declare that they have no competing interests.

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Reference


