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Research Article

Object Recognition in P14 mice

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Abstract

There are a lack of validated learning and memory paradigms in neonatal mice, which hampers translational modeling of pediatric learning and memory. In this study, we demonstrate that neonatal mouse pups at P13-P14 are able to perform the object recognition task soon after eye opening. We first utilized a control group, with which we tested whether the mice could perform and meet the criteria for object recognition, including nasal directed object exploration. Once that portion was confirmed, we utilized a larger object recognition group to validate the paradigm.

Keywords: *Object Recognition; P14 Mice*

Introduction

There are many behavioral learning tests for adult mice, however, to date, there is a lack of validated learning and memory paradigms for neonatal mice. The object recognition test is typically performed after mice are weaned from their mother and in the stage of pre-adolescence with fully developed visual ability at P30 or later. In this study, we sought to establish that P13-14 pups are able to perform this task.

Mice have unique visual development and neonatal mice generally do not open their eyes until their 12th post gestational day (P12) [1]. The timing and sequence of visual inputs and cortical processing remain to be defined. It is known that the rodent visual apparatus has high-level visual processing acumen with invariant object recognition [2,3]. However, it is unknown whether postnatal P14 mice have the capacity to perform a task which involve multisystem cuing, including visual recognition, of different laboratory objects very soon after eyelid opening (EO). The period around EO is characterized by a flood of synaptic activity involving numerous regions of the brain [4,5]. There are several variations of the object recognition test [6]. We used a one-trial learning task, wherein the animal displays a preference for a novel object over a familiar one on day 2 [7,8] after a single session of exposure to 2 similar objects on day 1. This activity relies on several areas of the brain, most notably: 1) the visual processing system, 2) the hippocampus, where new memories and experiences are encoded [9,10] and 3) the medial temporal lobe [11,12]. The additional knowledge provided by this study includes behavioral characterization and confirmation of this learning paradigm in P13-14 mice, which allows for earlier postnatal testing of mammalian learning and memory.

Materials and Methods

The institutional animal care and use committee (IACUC) at Baylor College of Medicine has approved the use of animals in this study.

Animals: All animals were from the C57BL/6 wild type strain (Jackson Laboratories). Animals were cared for in the vivarium with access to food and water and checked daily. The day light cycle of the vivarium was 12h/12h with lights out at 8 PM and on at 8 AM. Animals used for the study were handled per protocol described below. At the conclusion of the analysis, animals were euthanized with CO2 as approved on the IACUC protocol at Baylor College of Medicine and consistent with the recommendation of the Panel on Euthanasia of the American Veterinary Medical Association. All investigators have completed all necessary pre-requisites and have been certified to work with rodents.

Setup: The arena in which the test is performed is a small 22 cm x 44 cm plexiglass chamber, surrounded on three sides by a white screen to limit spatial information and prevent spatial biases that may influence object exploration. The total exploration time per session is 5 minutes with an intersession interval of 24 hours. An angled mirror is placed behind the cage to enable the observer to see the back side of the arena. Mice are placed into the arena and the time spent interacting with the objects is recorded from above utilizing pre-defined zones (ANY-maze video tracking system (Stoelting Co., USA). On day 1, two similar objects are placed in the arena and the time spent interacting with each object is recorded. On day 2, one of the objects is changed for a novel object and the time spent interacting with each object is recorded. Mirrors were used on either side of the setup, as well as in the back, to account for the small mouse size and to enhance scoring rigor.

Protocol: This study had two parts: 1) the control portion to establish task feasibility and 2) the Object Recognition (OR) task. For part 1, P13-14 mice were introduced to 2 identical objects on both sides of the arena over 2 contiguous days. For part 2, the OR task also occurred over 2 contiguous days with an intersession interval (ISI) of 24 hours between testing sessions. Familiarization of the arena and objects occurred on day 1 and testing on day 2 when the novel object is introduced. The first day of testing occurred on day P13 (both sides with identical objects), and the second day with the novel object on P14, as calculated from P0, the day of birth. The testing design, as well as scoring criteria for a positive OR recognition test are presented below.

Group	Task	Discrimination Ratio for a positive test
Control Group	2 identical objects on both sides over 2 contiguous days, ISI = 24 hours	Discrimination Ratio < 0.5 on both days
OR task groups	2 identical objects on day 1, 1 novel and 1 same object from previous day, ISI = 24 hours	Discrimination Ratio < 0.5 on day 1, Discrimination Ratio > = 0.5 towards novel object on day 2

Table

The time of testing was identical on both days, and the postnatal pups were all transferred to the holding cage (which is identical to the OR cage in figure 1) in the same order as the prior day for 5 minutes. The mice were habituated with the handler to avoid stress response. Specifically, a sleeved and gloved hand was placed in their cages for 1 minute each day for one week prior to the test being performed. Testing was done during the day. After the test, they were returned to their home cage with their parents. The objects were cleaned with alcohol and dried between animals to prevent olfactory cuing. The total test period was 5 minutes for each day and for each mouse, and the metrics were recorded as the number of interactions with each object, mean time spent interacting with each object, and the latency to the first-time object exploration. These are provided in the supplementary data tables as recorded by ANY-Maze software. The novel object was changed between batches, and the side of placement was changed as well.

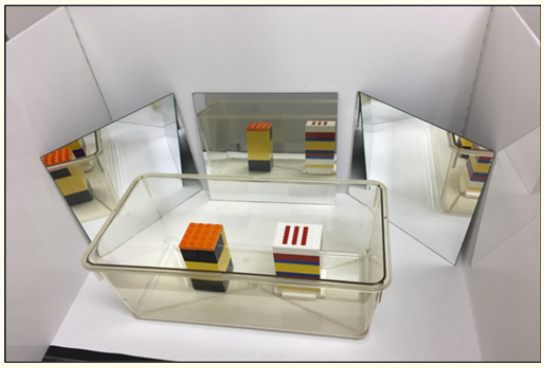

Figure 1: *Shows the object recognition (OR) setting, with mirrors and the cage with a familiar and novel object used on day 2.*

Exploratory behavior was defined as nose-directed behavior towards the object [13]. Non-nose directed behaviors including climbing and pawing of the objects without nasal cuing were excluded from the analysis. All analysis was done post-hoc in a blinded fashion. The scoring algorithm followed the protocol from Leger., *et al*. which includes both behaviors and the 2 mm nose distance that qualify as exploration [6]. Discrimination ratio (DR) was calculated utilizing the formula from Sivakumaran., *et al*. [14]:

DR= (T(new)-T(old))/(T(total) where T(total)=T(new)+T(old). T=time in seconds as measured by the recorder for qualified exploration activities. T(new) is the time spent with the new object on day 2. T(old) is the time with the old object. DR of 0.5 or greater is considered significant.

Results

In the control group, there were a total of 4 postnatal mice used (n = 2 female, n = 2 male). The DR < 0.5 in all mice over both days except for a single mouse on Day 1 (P13-14 control spreadsheet). This data is presented in figure 2 left panel.

OR task: There were a total of 16 males and 15 females over the entire group over 5 batches. The discrimination ratio, which measures recognition memory sensitivity [14], was found to be greater than 0.5 over all of the mice, with a mean day 1 DR of 0.1 (Figure 2 right panel). There was no difference in weight between batches or between male and female mice, p = 0.54 (0.59, 0.32). The supplemental video file demonstrates mouse exploration.

Discussion

Mice have poor vision at young ages where there is a high incidence of myopia [15]. The majority of postnatal exploration occurs through direct contact, including climbing, pawing, sniffing and other behaviors [8], which involve direct object interaction utilizing nasal direction for the OR test. Object recognition has been used as one of the primary learning and memory tasks to assess the effect of environmental stimuli on hippocampal function [16]. Object recognition interrogates several brain areas including the CA-1 region of the hippocampus [17]. This is evidenced by delayed and disrupted object memory especially with a delayed testing interval in mice with induced hippocampal deficits [18]. To ensure consistent interpretation of the OR and to decrease variability in the task, the object type must be consistent for a robust interaction between the mice and the objects [19,20]. Therefore, context, placing, and accurate postnatal timing are all important in executing the OR test correctly for neonatal mice.

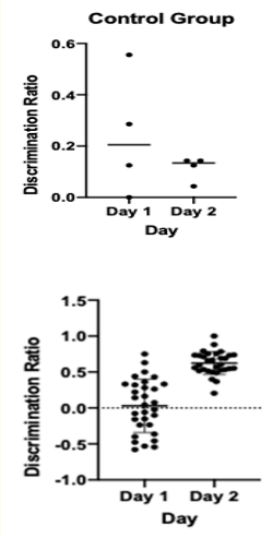

Figure 2: *Discrimination Ratio (DR) calculations as described for the control group (left panel) and Object Recognition (OR) group (right panel). As noted in the text, except for a single mouse on day 1, the mice in the control group did not discriminate between objects. In the OR group (right panel) 29/31 mice spent more time with the novel object on day 2 compared to day 1 and 25/31 spent more time with one object than another on day 1. However, both test by means showed non-significant batch DR (< 0.5) on day 1 and 2 of control group, and on day 1 of the OR group, with a significant DR on day 2 of OR group.*

Importantly, if the postnatal mice were unable to recognize the novelty, then the interaction would be random between objects, and the discrimination ratio would be < 0.5, which was seen in the control group. Notably, 29/31 of the mice within the OR group displayed an overt preference for the novel object, both in time spent and number of interactions with a DR of >= 0.5 over the cohort. This study demonstrates that the OR test is reproducible when used with neonatal mice as early as P14 and represents a non-stressful, non-aversive means of interrogating encoded memories in young mice.

Conclusion

The Object Recognition test can be successfully performed in P14 neonatal mice, demonstrating that this is non-stressful, non-aversive, enriched learning paradigm can be used soon after eyelid opening (EO). This OR test is easily administered, and quickly tests function, as long as basic tenets of the test are adhered to [6]. Use of the OR test will inform future studies utilizing neonatal mice in interrogating early learning and memory.

Supplemental Files

P13-14 control mice spreadsheet.

P13-4 Excel spreadsheet with gender, weight, and raw data of mice.

Videos of mouse motion indicating the mice are exploring the objects.

Funding Disclosures

None.

Disclosure of Interests

None.

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