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Ontogeny of Object-in-Context Recognition in the Rat

Adam I. Ramsaran, Sara R. Westbrook, and Mark E. Stanton

University of Delaware

Abstract

The object-in-context recognition (OiC) task [19] is a spontaneous exploration task that serves as an index of incidental contextual learning and memory. During the test phase, rats prefer to explore the object mismatched to the testing context based on previous object-context pairings experienced during training. The mechanisms of OiC memory have been explored in adult rats [12, 35]; however, little is known about its determinants during development. Thus, the present study examined the ontogeny of the OiC task in preweanling through adolescent rats. We demonstrate that postnatal day (PD) 17, 21, 26, and 31 rats can perform the OiC Task (Experiment 1) and that preference for the novel target is eliminated when rats are tested in an alternate context not encountered during training (Experiment 2). Lastly, we show that PD26 but not PD17 rats can perform the OiC task when the training contexts only differed by distal spatial cues (Experiment 3). These data demonstrate for the first time that PD17 rats can acquire and retain short-term OiC memory, which involves conjunctive learning of object and context information. However, we also provide evidence that preweanling rats' ability to utilize certain aspects of a context (i.e., distal spatial cues) in the OiC task is not equivalent to that of their older counterparts. Implications for the development of contextual memory and its related neural substrates are discussed.

Keywords

context; hippocampus; ontogeny; short-term memory; incidental learning; Long-Evans rat

1. Introduction

The development of contextual learning and memory has been attributed to the ontogeny of hippocampal function [1]. It was previously thought that the hippocampus was involved in processing polymodal stimuli associated with a context [2]; however, it is now known that contextual learning can be supported by neural systems separate from the hippocampus in scenarios where the hippocampus has been compromised [3], or when contextual learning is mediated by an elemental associative system that obviates hippocampal function [4, 5]. The development of contextual learning and memory processes has been well-defined primarily using fear conditioning paradigms [1, 6-8]; yet, it is unclear whether the previously reported

Corresponding author: Mark E. Stanton, Ph.D., Department of Psychological and Brain Sciences, University of Delaware, Newark, DE 19716, 302-831-0175, stanton@udel.edu.

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ontogenetic, behavioral, and neural determinants of contextual learning are applicable to other context-dependent learning tasks. Developments in behavioral techniques, such as the novelty-preference paradigm, now allows for the examination of these questions.

In recent decades, the novelty-preference paradigm [9, 10] has become increasingly popular in behavioral neuroscience research due to its versatility in examining multiple forms of memory and different brain memory systems. The paradigm is based on rodents' innate preference for novel stimuli in their environments [11], and the many task variants within the paradigm can be used to assess different processes of incidental object, spatial, contextual, and temporal learning and memory [12, 13]. Novelty recognition paradigms are advantageous for studying the neurobiology of memory because they typically involve a one-trial training phase, and recognition memory has been shown to emerge during early development [14-17]. In adult rats, variants of this paradigm rely on different neural systems [13, 18], which makes these tasks particularly useful for investigating neurocognitive development.

Object-in-context recognition (OiC) [19] is a variant of the standard object recognition (OR) task that relies on contextual processing. In this task, rats are consecutively exposed to two pairs of identical objects within two distinct contexts. After a delay, rats are replaced into one of the contexts with both object types present. Rats preferentially explore the object mismatched to the testing context (novel target) based on the previous object-context pairings. The learning of object-context associations in the OiC task is incidental (without reinforcement); thus, research utilizing this task is relevant to other context-dependent incidental learning paradigms like the context preexposure facilitation effect (CPFE) [7, 8], which is also used to study memory functions of medial temporal lobe structures including the hippocampus and associated cortices [5].

While other forms of recognition memory such as object recognition (OR) and object location recognition (OL) have been studied ontogenetically, to our knowledge there are no studies of the ontogeny of object-in-context recognition. Performance of the OR task emerges before postnatal day (PD) 17 in the rat [16, 17], whereas our lab demonstrated that the OL task, which relies on hippocampal function [12, 13, 16, 20, 21], emerges between PD17 and 21 [17]. Likewise, the CPFE, a form of contextual fear conditioning that also requires incidental context learning and the hippocampus [7, 22-24], ontogenetically emerges around the same time [7, 8, but see 25]. The convergence of these findings and other reports on the development of spatial cognition [26, 27] suggest that behavioral performance in the OiC task may have a similar ontogenetic profile, given that similar underlying mechanisms are responsible for OiC memory.

The present study aims to expand the developmental literature on contextual learning and novelty recognition tasks by examining OiC task performance after a short delay in PD17, PD21, PD26, and PD31 rats (Experiment 1). In addition, the present study begins to explore the determinants of object-in-context recognition during development by testing whether conjunctive processing of object-context associations is necessary for OiC task performance (Experiment 2) and whether distal spatial cues are sufficient to support object-in-context learning (Experiment 3). If the OiC task recruits conjunctive (hippocampal) systems for the

processing of contexts, then the ontogenetic profile of the OiC task should resemble those of the OL task [17] and the CPFE [8].

2. Experiment 1: Ontogenetic Profile of Object-in-Context Recognition

In Experiment 1, we examined the ontogenetic profile of the OiC task by observing task performance in rats aged PD17, 21, 26, and 31. These ages were chosen in order to extend our recent findings on the development of the OR and OL tasks [17, 28].

2.1 Materials and methods

2.1.1 Subjects—Animal colony and maintenance have been described in our previous reports [17, 28]. Subjects were Long-Evans rats bred and housed in accordance with NIH guidelines at the University of Delaware, Office of Laboratory Animal Medicine (OLAM). Time-bred females were housed in clear polypropylene cages (45 cm × 24 cm × 21 cm) containing standard bedding and *ad libitum* access to food and water. Cages were checked for births during the light cycle (12:12), and the day on which newborn litters were found was designated PD0. On PD2, litters were transported from the breeding facility to the laboratory colony rooms, and on the following day (PD3), litters were culled to 8 pups (generally 4 males and 4 females) and paw-marked by a subcutaneous injection of nontoxic black ink for identification purposes.

A total of 90 (44 M; 46 F) Long-Evans rats derived from 21 litters were the subjects in Experiment 1. Subjects were assigned to one of four age groups: PD17, PD21, PD26, or PD31. These age designations were based on the day of testing, which varied by a day in the youngest and oldest groups (PD17: PD17 or 18, PD31: PD31 or 32). If same sex littermates were assigned to the same age group, they were placed in different context order groups (see section 2.1.4 below) as a counterbalancing measure so that no more than one same sex littermate was assigned to the same Age × Context Order combination. Rats in PD26 and PD31 age groups were weaned and housed by sex with littermates in clear polypropylene cages (45 cm × 24 cm × 17 cm) with *ad lib* access to food and water on PD21. On PD23 and PD28 (+1 d), rats in age groups PD26 and PD31 were housed individually in smaller, white polypropylene cages (24 cm × 18 cm × 13 cm). Alternatively, rats in PD17 and PD21 groups remained with their dam throughout the study except during habituation and testing sessions when they were placed in the same individual cages for transport to the behavioral testing rooms as PD26 and PD31 rats. These housing procedures are similar to our previous studies which have addressed the (lack of) effect of group vs. individual housing or age of weaning on age differences in novelty recognition task performance [17].

2.1.2 Apparatus—The apparatus was adapted from Jablonski et al. [28] and Westbrook et al. [17]. Two circular chambers made of white polyester resin panels and measuring 78.7 cm in diameter, 48.9 cm walls, and elevated 26.7 cm off the floor were configured as two contexts that could be easily distinguished by the rats during testing (Figure 1A-B). The first arena (Context A) was left unaltered and two local spatial cues—a black “X” and a striped circle—were respectively placed on the north and west walls of the arena out of reach of the rats. In the second arena (Context B), a laminated black-and-white striped poster insert was placed around the walls and a laminated black poster, overlaid by a clear acrylic sheet (76.2

cm diameter) and a circular mesh insert, was placed over the arena's floor. Additionally, a black "+" and bull's-eye pattern attached to the walls served as local spatial cues in Context B. Both contexts were located in separate rooms with ample lighting allowing rats to utilize the different distal spatial cues within the rooms. Thus, Context A and Context B were composed of contrasting visual, tactile, and spatial (proximal and distal) features. A camera was mounted on a tripod behind the south wall of both arenas which allowed for digital recording of all experimental sessions (see section 2.1.5 below). The stimulus objects (Figure 2A-B)—a fake green apple and glass jar filled with blue stones—were affixed to the arena floor with reusable Velcro in one of two object configurations (see Figure 1).

2.1.3 Habituation—Rats were habituated to both contexts during three sessions [17, 28]. Sessions 1 and 2 occurred the day prior to the testing session for each age group. The first session began between 0800-1200 hr and the second session began 5 ($\pm$ 1) hr later. Session 3 occurred the following morning, 5 ($\pm$ 1) hr before the testing session. Prior to each habituation session, rats were handled in the animal housing room for 3 min, weighed, and then carted to the behavioral testing rooms. For all habituation (and testing) sessions, rats were placed in the center of the arena facing the north wall. Rats were allowed to freely explore Context A or Context B devoid of objects for 10 min, with the order of context exposures counterbalanced across rats. Following the first 10 min context exposure, rats were removed for a delay of 3-5 min while the arenas were cleaned with 70% ethanol solution. Immediately following the cleaning period, the rats were placed into the opposite context for 10 min. As part of a context discrimination protocol (data not reported), a subset of animals received the two 10-min context exposures broken down into 9- and 1-min increments separated by additional experimenter handling and cleaning periods (this subset performed similarly to other animals).

2.1.4 Object-in-Context (OiC) Recognition task—Testing in the object-in-context (OiC; Figure 3) task took place on the afternoon of PD17 (+1 d), PD21, PD26, or PD31 (+1 d). In the testing session, rats were placed in either Context A or Context B where they encountered and explored an identical pair of objects (fake apple or glass jar; glass jar handle always pointed to the east wall) for 5 min (Sample 1). They were then removed for a delay of 3-5 min while the arenas and objects were cleaned with 70% ethanol solution and placed into the opposite context where they explored a different pair of identical objects for 5 min (Sample 2). After Sample 2, rats were again removed for a retention interval of 5 min. During the retention test (Test), which lasted 3 min, rats encountered one copy of each object within either Context A or Context B, yielding four possible context orders (Context Orders: ABA, BAB, ABB, BAA). The novel target was the object that was mismatched with the testing context relative to the previous experience in the sample phases. Object configuration (Figure 1), context order, and object order were counterbalanced across sex and age group variables.

2.1.5 Data collection and analysis—Exploration during all habituation and testing sessions was recorded using a video camcorder (Panasonic USA, Model #SDR-H85P), and scored for exploration behavior as previously described [17, 28]. Digital recordings of sample and test phases were subsequently scored for exploration behavior by independent

observers using a dual-button timing program (Arun Asok, University of Delaware) with which scorers could track the time the rat explored each object present during a given phase of the task. Exploration was defined as active sniffing, whisking, and pawing directed toward the objects. A subset of data was reanalyzed by another observer in order to calculate inter-observer reliability. Correlation analyses revealed high agreement between observers (mean $r = .791$, SEM = ± 0.019 , all $ps < .02$).

STATISTICA 12 software was used for statistical analyses. Sample phase exploration times were analyzed by age and by sample phase (Sample 1 vs. Sample 2) using repeated measures ANOVA. When appropriate, post-hoc Student Newman-Keuls paired analyses were used. Object preference during the test phase was quantified by converting object exploration times to an exploration ratio, using the equation, $[t_{\text{novel}} / (t_{\text{novel}} + t_{\text{familiar}})]$ [12]. One-sample t -tests were used to compare the exploration ratios of each age group to a ratio representing chance performance (0.5), which is a convention in the object recognition literature [19]. Preliminary analyses showed that the outcomes of the experiment were not influenced by the different habituation protocols or context orders during the OiC task. Exploration ratio data were also not influenced by sex (see section 2.2.3 below). These factors were consequently collapsed so exploration ratio data could be analyzed by Age Group. Consistent with our previous report, ANOVAs were not used in analyzing exploration ratios [17].

2.2 Results and discussion

2.2.1 Subjects: Nine of the 90 subjects in Experiment 1 were excluded from the analyses. Three animals were removed due to technical errors (PD26, F, $n=1$; PD31, F, $n=1$; PD31, M, $n=1$) and 6 animals were excluded from the analyses for meeting the criteria of a statistical outlier (PD17, F, $n=2$; PD21, M, $n=1$; PD26, M, $n=1$; PD31, F, $n=1$; PD31, M, $n=1$). Outliers were defined as total exploration ratios (for the entire 3-min test phase) that exceeded ± 2 standard deviations from the mean of the age group. Data from the remaining 81 subjects were used in the analyses (PD17, $n=16$; PD21, $n=21$; PD26, $n=24$; PD31, $n=20$).

2.2.2 Sample phase: Sample phase analyses revealed an increase in sample phase exploration between preweanling (PD17) and juvenile (PD26) rats (Figure 4). The total amount of exploration time in each sample phase (Sample 1 vs. Sample 2) was analyzed between Age Group and Sex using a 2 (Sex) $\times$ 4 (Age Group) $\times$ 2 (Sample Phase) repeated measures ANOVA. The ANOVA showed a main effect of Age [$F(3, 73)=14.38$, $p<.000001$] and a significant Age Group $\times$ Sample Phase interaction [$F(3, 73)=3.43$, $p<.02$]. No other main effects or interactions were observed (all $F_s<3.2$). Post-hoc Student Newman-Keuls paired analysis using Age Group and Sample Phase as factors revealed that PD17 total exploration time during Sample 1 was less than PD17 exploration time during Sample 2 ($p<.007$), but exploration between Sample 1 and Sample 2 did not differ at any other age (all $ps>.21$). Additionally, exploration during Sample 1 increased with age until PD26. PD17 and PD21 Sample 1 exploration significantly differed from all other age groups ($ps<.004$), but PD26 Sample 1 exploration times did not differ from PD31 Sample 1 exploration. Exploration during Sample 2 only differed between ages PD17 and PD26 ($p<.01$) and PD21

and PD26 ($p < .04$). Overall, these data show an increase in sample phase exploration between PD17 and PD26, but not PD31.

2.2.3 Test phase: Two-sample t -tests comparing male versus female rats in each age group revealed no significant differences in OiC task performance (all $ps > .148$).

Figure 5 displays the results of the OiC retention test by Age Group. Rats in all age groups performed the OiC task, by preferentially exploring the novel target based on the testing context. One-sample t -tests of exploration ratios compared to chance performance (0.5) revealed high preference for the novel target, regardless of age (PD17: $p < .00002$; PD21: $p < .0012$; PD26: $p < .0017$; PD31: $p < .002$).

2.2.4 Discussion: Exploration times increased somewhat across age but, consistent with our previous report [17], this did not influence novelty preference performance. Rats in all age groups (PD17, PD21, PD26, and PD31) displayed significant novelty preference in the OiC task. These results indicate that performance in the OiC task emerges before PD17 and that rats as young as 17-days-old can learn incidental contextual information and retrieve these memory traces after a short retention interval.

3. Experiment 2: Role of Context Associations in Object-in-Context Recognition

Experiment 1 described the ontogenetic profile of the OiC task and found that preweanling rats could remember the contexts in which they previously encountered objects. Due to the OiC task's early emergence compared to other contextual learning paradigms [6, 7], Experiment 2 sought to determine whether developing rats were indeed associating the objects encountered during the sample phases with their respective contexts. Following training, PD17 and PD26 rats were either tested in a context experienced during training (Same condition) similarly to Experiment 1 or in an alternate (but familiar) context (Different condition). If preweanling and/or juvenile rats were performing the OiC task without using context associations, both groups would show similar novel target preference during the test. If object-context associations are required to perform the task, the Same group would show novel target preference whereas the Different group would not.

3.1 Materials and methods

3.1.1 Subjects: Subjects were 60 (29 M; 31 F) Long-Evans rats from 16 litters. Animal colony and maintenance were identical to Experiment 1.

3.1.2 Apparatus: The apparatus was the same as Experiment 1 with the following exceptions. A third context (Context C; Figure 1C) was developed for Experiment 2. Context C was a 50-gallon rectangular storage tub (Rubbermaid) measuring $108.5 \times 54.4 \times 45.7$ cm in dimensions and raised 26.7 cm off the ground. Context C had similar surface area compared to Contexts A and B, but differed in all other attributes. The walls and floor of context C were dark purple, and the floor was uneven giving it a distinct texture. Context C was located in a separate room from the other arenas and different local spatial cues—a black “T” and checkered square—were placed respectively on the north and west walls. A

fake apple (same as Experiment 1), a glass jar filled with pink stones, a white plastic hook with a flat bottom, and a 8 oz. Pepsi can served as the stimulus objects (Figures 2A, C-E).

3.1.3 Procedure: The number and timing of habituation sessions, handling, and cleaning procedures were the same as described in Experiment 1. However, in this experiment, rats were habituated to all three contexts such that each rat explored two contexts per session, with all possible combinations of context pairs represented equally across habituation sessions and age groups. As a result, each context was encountered twice rather than three times as in Experiment 1. In addition, a savings of habituation procedure was added to the end of the third habituation session to confirm that rats in both age groups could discriminate the three contexts (data not reported). Following the second 10-min context exposure of the third habituation session, rats were removed for a delay of 3-5 min during which the arenas were cleaned and then rats were either placed into the context that they just explored or the context which they did not encounter during Session 3, for a 3-min savings of habituation test.

The OiC task procedure was the same as Experiment 1 for the Same group, except only Context Orders ABA and BAB were used for the comparison against the Different group. Exploration ratios for these are a more conservative measure in our short-term memory test because recognition memory based on recency [29] and recognition memory based on context are not confounded as in Context Orders ABB and BAA. Additionally, consistent with our findings from Experiment 1, it has been demonstrated in the literature that context order is not a significant factor in tests of short-term OiC memory [30]. In the Different group, where rats were tested in a familiar context not experienced during either sample phase, the novel target was designated as the object that would have been novel if the rat was tested in the Sample 1 context. In this way, groups Different and Same were treated identically except for the context where testing occurred. Object configuration, context order, alternate context (Context B or C), object identity (apple and jar or hook and can), and object order were counterbalanced across sex, age, and testing context conditions.

3.1.4 Data collection and analysis: Data collection and analysis for the OiC task were the same as Experiment 1. One-sample *t*-tests were used to compare exploration ratios to chance performance. Context order, alternate context (Context B or C), object identity (apple and jar or hook and can), object configuration, and object order were counterbalanced across sex, age, and testing context groups. Thus, data were collapsed across these sub-factors. Sex was also found to not influence exploration ratio data (see section 3.2.3 below), so exploration ratio data was analyzed by Age Group and Testing Context conditions.

3.2 Results and discussion

3.2.1 Subjects: Data from 5 animals were excluded due to technical error (PD17, F, Different, *n*=1; PD17, M, Same, *n*=1; PD26, M, Same, *n*=1; PD26, M, Different, *n*=1; PD26, F, Different, *n*=1) and 1 animal was removed because it did not meet the minimum criteria for exploration (1 s in each phase; PD26, F, Different, *n*=1). An additional 5 animals met the criteria of an outlier (PD17, F, Different, *n*=1; PD17, M, Same, *n*=1; PD26, F, Different, *n*=1; PD26, M, Same, *n*=2), and were excluded from analyses. Data from the remaining 49

subjects were used in the analyses (PD17, Same, $n=14$; PD17, Different, $n=12$; PD26, Same, $n=11$; PD26, Different, $n=12$).

3.2.2 Sample Phase: Sample phase exploration increased with age (Figure 6). Sample phase exploration times were analyzed using a 2 (Sex) $\times$ 2 (Age Group) $\times$ 2 (Testing Context) $\times$ 2 (Sample Phase) repeated measures ANOVA. The ANOVA revealed a main effect of Age [$F(1, 41)=43.7, p<.000001$], but no other significant main effects or interactions (all $F_s<2.66$). A post-hoc Student Newman-Keuls paired test using Age and Sample Phase as factors showed that there were no differences between Sample 1 and Sample 2 exploration times at both ages, but exploration was significantly lower in PD17 relative to PD26 groups ($p<.001$).

3.2.3 Test Phase: Test performance was not influenced by sex. Two-sample t -tests comparing male and female rats in each condition (Age Group $\times$ Testing Context) yielded no significant differences (all $p_s>.644$).

Results from the OiC task are displayed in Figure 7 by Age Group and Testing Context. Rats in the Same group displayed a preference for the novel target during the test phase at ages PD17 and PD26, whereas rats in the Different group did not. One-sample t -tests comparing total exploration ratios for the 3-min test revealed high novelty preference in the PD17-Same ($p<.05$) and PD26-Same ($p<.0003$) groups. In contrast, the PD17-Different group showed no preference for either object during the test ($p>.87$) and the PD26-Different group showed a significant preference for the object designated as “familiar” when tested in the familiar alternate context ($p<.02$).

3.2.4 Discussion: Consistent with Experiment 1, rats as young as 17-days-old could perform the OiC task, as demonstrated in the Same group. When rats were tested in a familiar context not encountered during the sample phases (Different group), preference for the designated novel target was abolished, and this effect was observed at both ages. This effect does not reflect differences in exploration times between the Same vs. Different groups. In summary, these results indicate that preweanling and juvenile rats associate the encountered objects with their respective contexts during the sample phases of the OiC task and later retrieve that information during the retention test.

4. Experiment 3: Contribution of Distal Spatial Cues to Object-in-Context Recognition

Results from Experiments 1 and 2 show that rats as young as 17-days-old can learn conjunctions of object and context information. In Experiment 3, we asked whether distal spatial cues were sufficient to support OiC learning in preweanling and juvenile rats. Adult lesion and developmental literature on the contextual/spatial learning suggests that the hippocampus is specifically involved in processing the distal, but not proximal spatial environment [31], and that distal spatial processing develops around or after the third postnatal week in the rat [6]. To examine this, PD17 and PD26 rats were probed in the OiC task under the same context condition as previous experiments (chambers in separate rooms with distinct proximal cues) or under conditions that necessitate the processing of specifically distal spatial cues to perform the task (chambers in separate rooms with identical

proximal cues). Thus, rats were run in two Context Conditions, a Global group that experienced a global shift in contextual cues when moved between contexts versus a Distal group, for which only distal contextual cues distinguished the contexts. We expected to observe age-related differences in task performance when comparing the Global and Distal groups, which might reflect developmental differences in a more explicit form of spatial learning (Distal task) than our standard (Global) OiC task.

4.1 Materials and methods

4.1.1 Subjects—Subjects were 53 (26 M; 27 F) Long-Evans rats from 16 litters. Animal colony and maintenance were the same as described for Experiment 1.

4.1.2 Apparatus—An identical set of chambers and context inserts as used in Experiment 1 were constructed for a total of 4 testing arenas. Subjects in the Global groups encountered Contexts A and B as in previous experiments, and subjects in the Distal groups experienced the same Context A but in different rooms. We refer to Context A presented in the alternate room as Context A'. Thus, Contexts A and A' were identical in their proximal features but differed by their distal spatial environments. A fake green apple and glass jar filled with pink stones (Figures 2A, C) served as the stimulus objects.

4.1.3 Procedure—The number and timing of habituation sessions, handling, and cleaning procedures were the same as described previously. Rats in the Global groups were habituated and tested in Contexts A and B as in previous experiments, whereas rats in the Distal groups were habituated and tested in Contexts A and A'. During each habituation session, rats explored both contexts for 10 min each, without objects present.

The OiC task was conducted identically as described in Experiment 1, except for the context manipulations described above. Like Experiment 2, only Context Orders ABA and BAB (for Global groups) and AA'A and A'AA' (for Distal groups) were utilized so that novelty preference due to context associations versus relative recency could be dissociated.

4.1.4 Data collection and analysis—Data were collected and analyzed in the same manner as previous experiments. Exploration ratios were compared to chance performance using one-sample *t*-tests. Sub-factors including context order, object configuration, and object order were counterbalanced across the variables of sex, age group, and context condition.

4.2 Results and discussion

4.2.1 Subjects—Data from 2 subjects were excluded from analyses because of technical errors occurring during experimentation (PD17, F, Distal, *n*=1; PD26, F, Distal, *n*=1). One rat did not meet the minimum criteria for object exploration (1 s in each phase; PD17, M, Distal, *n*=1). Three animals met the criteria of a statistical outlier and were additionally excluded from analyses (PD17, M, Global, *n*=1; PD26, M, Global, *n*=1; PD26, F, Distal, *n*=1). Data from the remaining 47 subjects were used in the statistical analyses (PD17, Global, *n*=13; PD17, Distal, *n*=13; PD26, Global, *n*=11; PD26, Distal, *n*=10).

4.2.2 Sample Phase—Sample phase exploration was influenced by the factors of Age Group and Context Condition (Figure 8). Sample phase exploration times were analyzed using a 2 (Sex) $\times$ 2 (Age Group) $\times$ 2 (Context Condition) $\times$ 2 (Sample Phase) repeated measures ANOVA. The ANOVA yielded significant main effects of Age Group [$F(1, 39)=28.9, p<.000005$] and Context Condition [$F(1, 39)=5.07, p<.05$] as well as a significant interaction of Age Group $\times$ Sample Phase [$F(1, 39)=4.50, p<.05$]. No other significant main effects or interactions were observed (all F s < 4.05); however, there was a marginally significant interaction for Age Group $\times$ Context Condition ($F=4.04, p=.051$). Post-hoc Student Newman-Keuls paired analyses of the Age Group $\times$ Sample Phase interactions revealed that exploration times did not differ between sample phases at either age (all p s $> .09$), despite the significant Age Group $\times$ Sample Phase interaction term (Figure 8A). Post-hoc tests of the marginal interaction of Age Group $\times$ Context Condition revealed that rats in the PD17-Global group explored more during the sample phases than rats in the PD17-Distal group ($p<.004$), but this difference was not observed between the Global and Distal groups on PD26 ($p>.864$) (Figure 8B).

4.2.3 Test Phase—Two-sample t -tests comparing male versus female rats in each condition (Age Group $\times$ Context Condition) showed that sex did not influence OiC test performance (all p s $> .160$).

Figure 9 displays the results of the OiC task by Age Group and Context Condition. Juvenile rats were able to perform the OiC task under both Global and Distal conditions, but preweanling rats were only able to show preference for the novel target in the Global condition. One-sample t -tests comparing group exploration ratios to chance performance (0.5) showed significant novelty preference in the PD17-Global ($p<.03$), PD26-Global ($p<.001$), and PD26-Distal ($p<.03$) groups. In contrast, rats in the PD17-Distal group did not show a preference for the novel target ($p>.18$).

4.2.4 Discussion—Data from the PD17-Global and PD26-Global groups are consistent with the preceding experiments, demonstrating that preweanling and juvenile rats can perform the OiC task. However, rats in the PD26-Distal group, but not the PD17-Distal group, could perform the OiC task. It is unlikely that low levels of exploration behavior during the sample phases accounts for this age difference, as the observed amounts of exploration (>15 s per sample phase) are sufficient to support recognition memory [17, 32]. The key difference between the PD26-Distal and PD17-Distal groups suggests that, although our data support the notion that rats as young as 17-days-old can perform the OiC task, task performance at this age depends on the presence of salient local chamber cues that can support associative context learning during early ontogeny.

5. General Discussion

The present study defines the ontogenetic profile of object-in-context recognition and begins to explore the behavioral determinants of the OiC task in developing rats. Experiment 1 demonstrated that PD17, 21, 26, and 31 rats can all display preference for the novel target in the OiC task. Experiment 2 replicated these findings in PD17 and PD26 rats and additionally showed that when rats were tested in an alternate context, novelty preference was

eliminated. The inability of this group to perform the OiC task was not due to a neophobic response to the alternate context because the rats were preexposed to the alternate context during habituation sessions. Also, this effect (and the effects observed in Experiment 3) cannot be explained by object exploration times during training, as demonstrated in our data and in previous reports. Multiple research groups including our own have shown that recognition memory can be supported in adult and developing rats by a minimal level of object exploration during sample phases (~10 s), and that exploration levels during the sample phases is not correlated with performance during the test phase [17, 32, 33]. Thus, conjunctive processing of objects and contexts appears necessary for OiC task performance in developing rats. Finally, Experiment 3 found that the OiC task can be supported by distal spatial cues in PD26 but not PD17 rats. Together, the present study contributes to the literature by reporting that a form of short-term contextual memory, object-in-context recognition, develops by PD17 in the rat, OiC learning in developing rats is in fact associative in nature, and OiC learning can be supported by distal spatial cues in juvenile but not preweanling rats.

The results of Experiment 1 indicated that preweanling rats (PD17) can perform the OiC task. To our knowledge, this is the first study to report the ontogeny of object-in-context recognition. Numerous studies have demonstrated the ability of adult rats to perform the OiC task [12, 18, 19, 30, 34-40]; however, the youngest age reported is approximately PD50 [41]. Our observation that OiC task performance emerges by PD17 is similar to the emergence of standard object recognition (OR) [14-17], but not other forms of spatial recognition memory like the object location recognition (OL) task, which emerges between PD17 and PD21 [17], or the 2-object variant of the object-in-place (OiP) task, which emerges between PD24 and 31 [15]. A study by Krüger et al. showed that not only is OR apparent during the preweanling period of development, but also OL and temporal order recognition (TOR) [16]. It should be noted that Krüger and colleagues used a within-subjects design that may have facilitated the early ontogeny of the OL task on PD16 and the TOR task on PD17. In the current study, all rats were naïve prior to the OiC task; thus, we demonstrated the earliest instance of apparent OiC recognition, which appears to emerge before other forms of spatial recognition memory.

The early development of the OiC task observed in Experiment 1 was unusual when compared to the ontogeny of other forms of spatial cognition (see below). Therefore, a context manipulation was implemented in Experiment 2 that confirmed that PD17 and PD26 rats displayed novelty preference during the test phase on the bases of learning the object-context pairings during the sample phases. This is the first study to test the assumption that contextual stimuli drive novelty preference in the OiC task. In a recent study in which long-term OiC memory was examined, contexts were manipulated during the second sample phase [30]; however, this manipulation did not allow the experimenters to identify the strategy by which rats were performing their task. Context has also been manipulated in studies employing the OR task. In these studies, adult rodents were tested in either the same context as the sample phase, a different familiar context, or a different novel context and showed high preference for the novel object in the former two groups, but not the latter [42-45]. The results of the present study highlight the differences between the OiC and OR tasks by showing that standard object recognition is not a strategy employed by rats in our

alternate context test. In our task, both objects present during the test phase were previously encountered, thus object novelty is derived from the objects' relationship to the context. Interestingly, PD26 rats in the Different condition showed a modest preference for the "familiar" target during testing. It is possible that PD26, and not PD17 rats, are exhibiting preference for the temporally distant object when tested in the alternate context, as is found in the temporal order recognition task. Preliminary data from our laboratory support this notion as juvenile rats, but not preweanling rats, can exhibit temporal order recognition memory (Wilmot, Ramsaran, & Stanton, unpublished observations). Nonetheless, we demonstrated here that contextual cues are salient to developing rats in a test of spontaneous exploration, and, unlike versions of the OR task implemented in other studies, processing of object-context associations drives novelty preference during the OiC task.

In Experiment 3, we investigated whether distal spatial cues alone were sufficient to support OiC learning. Here, we found that PD26 rats, but not PD17 rats, were able to perform the OiC task by associating the objects encountered during the sample phases with the spatial environment of the training rooms (proximal chamber cues were held constant). Unlike Experiments 1 and 2, there is a clear dissociation in task performance when comparing PD17 and 26 rats under Distal conditions. One interpretation of this effect is that because of their inadequate sensory-perceptual capacity, preweanlings cannot see the distal spatial cues and therefore cannot use them to form distinct representations of the contexts. More experiments are needed to evaluate this view. Alternatively, it is possible that preweanling rats in the Distal group cannot perform the OiC task because of their poor spatial learning skills, which have been documented in the literature using other behavioral paradigms. An early report on the ontogeny of spatial cognition show that PD17 rats can use proximal but not distal spatial cues to locate the goal platform in the Morris watermaze [26]. Another developmental study found that standard contextual fear conditioning in PD18 rats is significantly weaker when training occurs in a clear plexiglass chamber (allowing sensory input from the distal spatial environment) compared to when training occurs in a solid black chamber [27]. However, behavioral performance in both experiments of groups utilizing distal spatial cues significantly increases by PD20 and 23, respectively. These findings are in alignment with our results—whereas PD17 rats in our experiment could perform the OiC task when distinct proximal cues were available, robust object novelty preference was not present when contexts were only distinguished by distal cues. Furthermore, our results suggest that PD17 rats are unlikely to integrate distal spatial cues into their acquired representations of the contexts. Together, these data suggest that PD17 rats may use a different contextual learning strategy to perform the OiC task compared to postweanling rats, as is observed in other behavioral paradigms such as conditional discrimination [46]. Importantly, proximal- versus distal-cue utilization is likely to influence the neurobiology of task performance and thereby affect the ontogeny of behavior in spatial learning paradigms, including the OiC task (see below).

It is instructive to consider the present findings in relation to the neurobiology of performance on OiC and related tasks during early ontogeny. In particular, the hippocampal system is recruited in both tests of spatial recognition memory [12, 13] and contextual fear memory [7, 23]. Our findings suggest that OiC recognition ontogenetically emerges earlier than other forms of spatial cognition mediated by the hippocampal system. For example, two

incidental learning tasks that share behavioral and neurobiological mechanisms of the OiC task emerge later in development compared to OiC recognition. First, the object location recognition (OL) task is a variant of the novelty-preference paradigm in which rats preferentially explore a displaced object (i.e., a familiar object in a novel location) during the test phase [19]. Second, the context preexposure facilitation effect (CPFE) is a form of contextual fear conditioning in which encoding of the conjunctive context representation and context-shock association occur on separate days [47]. Our lab has demonstrated that rats can display preference for the object in the novel location in the OL task on PD21, but not PD17 [17], and that moderate levels of freezing are observed in a CPFE paradigm when rats are preexposed to the conditioning context on PD21 [7, 8]. The OL task and CPFE rely on conjunctive spatial processing [5, 8, 28] subserved by the hippocampal system, which has been proposed for the OiC task [5]. These claims are supported by research showing that reversibly inactivating, lesioning, or blocking plasticity in the hippocampus disrupts performance in the OL task [12, 13, 16, 20, 21, 48] and the CPFE [7, 22-24].

Likewise, the current literature suggests that the OiC task relies on function of the hippocampal system in adult rats [12, 30, 34, 36, 38, 39], with a few exceptions [18, 37]. The disparate findings regarding the role of the hippocampus in OiC memory may reflect variable delays employed by different studies which differentially recruit short-term vs. long-term memory processes, or variation in the degree of spatial processing required for discrimination of the contexts during the task. In studies that manipulated proximal (chamber) spatial cues but not distal (room) spatial cues, the hippocampus was not critical for OiC task performance [18, 37]. In contrast, a study by Mumby and colleagues in which there was a shift in both proximal and distal spatial cues of the contexts, OiC performance was hippocampus-dependent [12]. Thus, discrepancies in the OiC literature regarding the role of the hippocampus may be attributed to differences in cue utilization among these studies, as is mentioned by Langston and Wood [37]. We addressed this issue in Experiment 3 and observed object novelty preference in PD17 and PD26 rats when contexts differed in both proximal and distal cues, but only at PD26 when contexts differed only in distal spatial cues. Evidence from object recognition (OR) studies support the view that the hippocampus is involved in processing the distal but not proximal spatial environment. In one study, complete lesions of the hippocampus did not disrupt OR when subjects were trained and tested in contexts that differed only by proximal cues [45]. Moreover, dentate gyrus lesions disrupted OR when the task was performed in a clear chamber that allowed for processing of the distal spatial context, but not in an opaque black chamber [31]. Our developmental data are consistent with these data and their implications for hippocampal development; performance in the OiC task may not require the hippocampus when salient proximal cues are involved, but when task performance requires utilizing the distal spatial environment, the hippocampus is likely to be involved. Accordingly, performance in the OiC task emerges by PD17 in the former case and after PD17 in the latter case. With this perspective, our data are consistent with the ontogeny of conjunctive spatial learning as described above.

In the present experiment, we did not examine the neural basis of the OiC task; therefore, it is currently unknown whether our version of the task is dependent on the hippocampal system to process conjunctive context representations. This ability exists in PD21 rats [8], but it is not known whether PD17 rats can encode conjunctive context representations. It is

possible that preweanling rats can perform the OiC task by associating the encountered objects with elemental, or features-based, context representations. This strategy is independent of the hippocampal system, but instead relies on a neocortical system composed of primarily the rhinal cortices [5], of which, the perirhinal region shows early ontogeny during the first two weeks of postnatal life [49]. This view is supported by studies showing (1) that PD17 rats can solve the Morris watermaze task by processing proximal, but not distal, spatial cues [26], which may not require the hippocampus; (2) that the hippocampus is necessary for processing distal or global, rather than proximal, spatial cues in a modified OR task [45]; and (3) that *c-fos* expression is increased in the lateral entorhinal cortex (nonspatial pathway), and not the medial entorhinal cortex (spatial pathway), following an OiC task in which distal spatial cues were not manipulated [40]. We showed that PD17 rats likely associate the encountered objects with the salient proximal cues of the context based on their inability to associate object identities with distal spatial cues, but this does not preclude the possibility that the proximal spatial cues are learned conjunctively. There is also evidence that in the absence of the hippocampal system (i.e., after hippocampal ablation), the neocortical system can support context processing through a slower, less efficient mechanism that either requires a longer duration of context exposure, or multiple context exposures [3]. If a similar compensatory mechanism is active during early development, the extra context exposure requirement is satisfied in our OiC task protocol during habituation when rats are exposed to the contexts for extended periods on multiple occasions. More studies are needed to determine if PD17 rats are using a strategy based on elemental (neocortical) or conjunctive (hippocampal) context learning.

It is important to note that the ontogenetic emergence of a preponderance of spatial learning tasks around the time of weaning (~PD21) does not necessarily mean that functional development of the hippocampal system itself occurs at this age. Electrophysiological recordings confirm that head direction cells, place cells, and grid cells are detected in PD16-18 rats during environment exploration, and despite place and grid cells showing protracted development, these three cell types may provide rudimentary input to the hippocampus during this period [15, 50]. This raises the possibility that the hippocampus is functioning to support conjunctive context representations in our OiC task at PD17. Behavioral evidence also supports the view that hippocampus-dependent tasks can be learned by preweanling rats [51]. For example, spatial delayed alternation (SDA) in a T-maze emerges as early as PD18 depending on task parameters [27, 52-54]. In accordance with this, the development of neural substrates like the hippocampus should be considered in the context of what neurobehavioral systems they are interacting with during a specific learning task, rather than how these substrates function alone [51]. Future research on the ontogeny of the OiC task will aim to better define the cognitive and neural processes that determine performance on the OiC task during early development, which will also inform our understanding of the ontogeny and neural substrates of contextual learning.

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Highlights

- Conjunctive learning of objects and contexts develop prior to weaning in the rat.
- Control tasks confirm that task performance requires conjunctive learning.
- Object-in-context learning in preweanling rats depends on salient proximal cues. When distal cues define the contexts, object-in-context learning is present in juvenile but not preweanling rats.

Figure 1.

Testing apparatuses. Contexts A (A) and B (B) were used in Experiments 1, 2, and 3, whereas Context C (C) was used only in Experiment 2. Two different object configurations (Configurations 1 and 2) for object placements during the OiC task are shown in red.

Figure 2.
Stimulus Objects. Objects displayed in A and B were used in Experiments 1 and 3. Objects displayed in A and C-E were used in Experiment 2.

Figure 3.

A schematic diagram of the object-in-context (OiC) task. Objects are shown in configuration 1. During the training phase, rats are exposed to two different pairs of objects within distinct contexts (Sample 1 and Sample 2), separated by a short delay (Delay 1). After a retention interval of 5 min (Delay 2), rats are replaced into one of the sample contexts with a copy of both previously-encountered objects present.

Figure 4.

Exploration times for each age group during Sample 1 (A) and Sample 2 (B) of Experiment 1. Mean exploration times ($\pm$ SEM) are given. In general, mean exploration times increased with age until PD26. Mean exploration times only differed between sample phases in the PD17 age group. Significant differences between age groups for congruent sample phases are indicated (** $p < .01$).

Figure 5. Mean exploration ratios ($\pm$ SEM) during the OiC test phase for Experiment 1. Exploration ratios were calculated as $t_{\text{novel}} / (t_{\text{novel}} + t_{\text{familiar}})$. Dashed line represents chance performance (0.5). All age groups performed significantly above chance levels. (** $p < .01$, *** $p < .001$)

Figure 6.

Mean exploration times ($\pm$ SEM) for each testing condition (Same vs. Different) by age group during Sample 1 (A) and Sample 2 (B) of Experiment 2. Testing condition did not affect object exploration times during the sample phases. Exploration times were significantly lower in PD17 groups compared to PD26 groups. (***) $p < .001$

Figure 7.

Mean exploration ratios ($\pm$ SEM) during the OiC test phase by age group and test condition for Experiment 2. Exploration ratios were calculated as $t_{\text{novel}} / (t_{\text{novel}} + t_{\text{familiar}})$. Dashed line represents chance performance (0.5). Both Same groups performed significantly above chance levels. The Different group at age PD26 showed a significant familiarity preference. (* $p < .05$, *** $p < .001$)

Figure 8.

Mean exploration times ($\pm$ SEM) by age and sample phase (A) or context condition (B) for Experiment 3. Juvenile rats explored more overall than preweanling rats and PD17 rats in the Global group explored more than PD17 rats in the Distal group. (** $p < .01$, *** $p < .001$)

Figure 9.

Mean exploration ratios ($\pm$ SEM) during the OiC test phase by age group and context condition for Experiment 3. Exploration ratios were calculated as $t_{\text{novel}} / (t_{\text{novel}} + t_{\text{familiar}})$. Dashed line represents chance performance (0.5). Both Global groups performed significantly above chance levels. Only the Distal group at age PD26 showed a significant novelty preference. (* $p < .05$, *** $p < .001$)