ROLE OF AGE, SPATIAL PROCESSING, AND NMDA RECEPTORS IN THE ONTOGENY OF CONTEXTUAL RECOGNITION MEMORY

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Bachelor of Science in Neuroscience with Distinction

Spring 2015

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ACKNOWLEDGMENTS

First, I would like to offer thanks to Dr. Mark Stanton for his mentorship over the last 2.5 years. His support and guidance over the course of my undergraduate career has contributed significantly to my development as a researcher and scholar. I am very grateful to the extraordinary group of graduate and undergraduate students of the Stanton Laboratory—Dr. Sarah Jablonski, Dr. William Schreiber, Patrese Robinson-Drummer, Sara Westbrook, Lauren Brennan, Jacob Wilmot, Hollie Sanders, Katelyn Buban, and Nicholas Heroux—for their training in experimental procedures, assistance in data collection and analysis, frequent scientific discourse, and friendship. Additionally, I would like to thank the members of my thesis committee, including Drs. Amy Griffin and Jaclyn Schwarz, for their constructive comments on this work. Finally, I would like to acknowledge my mother and father, Bebe and Prakash Ramsaran, and siblings, Amanda, Andrew, and Amelia Ramsaran, for their unwavering love and support.

This work was supported by Academic Year Scholar Program Awards (2013, 2014), a Supply and Expense Grant (2013), and a Senior Thesis Winter Session Scholars Award (2015); all from the University of Delaware Undergraduate Research Program. Summer research was funded by the Ronald E. McNair Postbaccalarueate Achievement Program (2013, 2014) and the Delaware IDeA Network of Biomedical Research Excellence (INBRE) Summer Scholars Program (2014).

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ABSTRACT

Determining the ontogenetic emergence of contextual learning and memory remains a challenge for developmental behavioral neuroscientists (for review, see Revillo et al., 2015). Research in this area suggests that the ability to acquire context representations emerges around the time of weaning in the rat (e.g., Jablonski et al., 2012), similar to hippocampus-dependent spatial cognition. However, this work has primarily utilized contextual fear conditioning paradigms that involve long delays and require interactions between cognitive and emotional brain memory systems, making it difficult to attribute preweanling rats' failure to express fear to contextual stimuli to the underdevelopment of memory systems supporting contextual learning per se. In addition, a key difficulty surrounds the spatial versus nonspatial nature of the contextual cues processed by preweanling rats (e.g., Pugh & Rudy, 1996), which may recruit different neural systems.

The current study examined contextual learning and memory in developing rats using an incidental learning paradigm known as the object-in-context recognition (OiC) task. We demonstrate that rats as young as 17-days-old can exhibit short-term contextual memory using this paradigm (Experiment 1A), and that learning in this task is associative (Experiment 1B). To address the spatial versus nonspatial context problem, we next manipulated the contextual cues or object placements in our OiC preparation and observed delayed development of contextual recognition memory when contexts in the OiC task were distinguishable only by the distal spatial environment (Experiment 2A) or when spatial information about the objects needed to be associated with contextual stimuli (object-place-context recognition; OPC task; Experiment 2B). Finally, we explored the role of NMDA receptors in contextual

recognition memory by administering an NMDA receptor antagonist prior to the aforementioned tasks. Systemic injections of .06 mg/kg MK-801 prior to training did not impair performance in our standard OiC task (Experiment 3A), but did in the latter two spatial task variants (Experiments 3B and C). These data provide strong support for contextual learning and memory in the preweanling rat and further support the notion that the ontogeny of contextual memory is influenced by the degree of spatial processing necessary for task performance. Furthermore, spatial cognition may contribute to the neurobiology of contextual recognition memory by rendering it dependent on NMDA-receptor-related plasticity.

Chapter 1

INTRODUCTION

Historically, contextual learning and memory has been attributed to the hippocampus (Kim & Fanselow, 1992; Kim, Rison, & Fanselow, 1993), and accordingly, its development to the ontogeny of hippocampal function (Rudy, 1993). It was previously thought that the hippocampus was involved in processing polymodal stimuli associated with a context (Phillips & Ledoux, 1992); however, it is now known that context learning can be supported by extrahippocampal substrates in scenarios where the hippocampus has been compromised (Wiltgen, Sanders, Anagnostaras, Sage, & Fanselow, 2006), or when contextual learning is mediated by an elemental associative system that obviates hippocampal function (Fanselow, 2000; Rudy, 2009). The development of contextual learning and memory processes has been well-defined primarily using fear conditioning paradigms (Rudy, 1993; Pugh & Rudy, 1996; Schiffino, Murawski, & Stanton, 2011; Jablonski, Schiffino, & Stanton, 2012). Yet, it is unclear whether the previously reported ontogenetic, behavioral, and neural determinants of contextual learning are applicable to other context-dependent learning tasks. Importantly, the use of contextual fear conditioning to study the ontogeny of contextual learning can be problematic, as discussed below.

First, under normal circumstances, contextual fear learning requires coordinated activity of emotional (amygdalar) and cognitive (hippocampal) brain memory systems, which make distinct contributions to contextual fear learning (Phillips & Ledoux, 1992; Zelikowsky, Hersman, Chawla, Barnes, & Fanselow,

2014). This issue can be addressed by employing a variant of contextual fear conditioning known as the context preexposure facilitation effect (CPFE), which temporally dissociates encoding of the context representation and context-shock association (Fanselow, 1990). However, the CPFE cannot overcome the fact that long delays are necessary for consolidation of contextual fear memories (Rudy & Morledge, 1994; Rudy & Wright-Hardesty, 2005). This is less than ideal as developing animals, particularly infants, show rapid rates of forgetting (for review, see Josselyn & Frankland, 2012). Thus, the ontogenetic emergence of contextual fear conditioning cannot be unambiguously interpreted as the development of contextual learning. Rather, it could represent the development of an interaction between brain memory systems involved in context and emotional learning, or the development of long-term memory systems. Lastly, an issue that has plagued this area of research is the spatial versus nonspatial nature of contextual cues processed by developing animals. This can be addressed with systematic experiments that use similar tasks to probe developing animals' memory for spatial and nonspatial contexts (e.g., Pugh & Rudy, 1996). Developments in behavioral techniques, such as the novelty-preference paradigm (Ennaceur & Delacour, 1988), now allow researchers to address these issues.

The novelty-preference paradigm (also known as novel object recognition), may be more suitable for studying the ontogeny of contextual learning and memory. In recent decades, the novelty-preference paradigm (Ennaceur & Delacour, 1988; for review, see Dere, Huston, & De Souza Silva, 2007) 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 (Berlyne, 1950), 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 (e.g., Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; Barker & Warburton, 2011). Novelty recognition paradigms are advantageous for studying the neurobiology of memory because they typically involve a one-trial training phase, memory can be probed within minutes of training or after longer delays, and recognition memory has been shown to emerge during early development (Reger, Hovda, & Giza, 2009; Ainge & Langston, 2012; Jablonski, Schreiber, Westbrook, Brennan, & Stanton, 2013; Westbrook, Brennan, & Stanton, 2014). In adult rats, variants of this paradigm rely on different neural systems (e.g., Barker & Warburton, 2011; Langston, Stevenson, Wilson, Saunders, & Wood, 2010b), which makes these tasks particularly useful for investigating neurocognitive development.

Object-in-context recognition (OiC) (Dix & Aggleton, 1999) 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) (Schiffino et al., 2011; Jablonski et al., 2012), which are also used to study memory functions of medial

temporal lobe structures including the hippocampus and associated neocortex (Rudy, 2009).

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 or other context-dependent recognition tasks. Performance of the OR task emerges before postnatal day (PD) 17 in the rat (Westbrook, Brennan, & Stanton, 2014; Krüger, Brockmann, Salamon, Ittrich, & Hanganu-Opatz, 2012), whereas our lab demonstrated that the OL task, which relies on hippocampal function (Mumby et al., 2002; Barker & Warburton, 2011; Oliveira, Hawk, Abel, & Havekes, 2010; Assini, Duzzioni, & Takahashi, 2009), emerges between PD17 and 21 (Westbrook et al., 2014). Likewise, the CPFE, a form of contextual fear conditioning that also requires incidental context learning and the hippocampus (Schiffino et al., 2011; Rudy, Barrientos, & O'Reilly, 2002; Matus-Amat, Higgins, Barrientos, & Rudy, 2004; Barrientos, O'Reilly, & Rudy, 2002), ontogenetically emerges around the same time (Schiffino et al., 2011; Jablonski et al., 2012; but see Pisano, Ferreras, Krapacher, Paglini, & Arias, 2012). The convergence of these findings and other reports on the development of spatial cognition (Rudy, Stadler-Morris, & Albert, 1987; Green & Stanton, 1989) suggest that behavioral performance in contextual recognition tasks may have a similar ontogenetic profile, given that similar underlying mechanisms are responsible for OiC memory.

The purpose of this thesis was to evaluate the determinants of contextual learning and memory during development using contextual recognition tasks. The present study aimed 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 1A). We also tested whether associative processing of object-context associations is necessary for OiC task performance in developing rats (Experiment 1B). To address the nonspatial versus spatial context problem, we examined the ontogenetic profiles of two spatial variants of the OiC task, the distal cue OiC task (Experiment 2A) and the object-place-context recognition (OPC) task (Eacott & Norman, 2004) (Experiment 2B). Based on previous work from our lab on the development of object versus spatial recognition (Westbrook et al., 2014), we predicted that these spatial task variants would emerge later in ontogeny compared to the standard OiC task. Developmental delays of contextual recognition memory associated with spatial task demands may reflect altered neurobiology of task performance in the distal cue OiC and OPC tasks compared to the standard OiC task. To this end, we evaluated the involvement of NMDA receptors (NMDAR), which are implicated in learning-related plasticity, in all three contextual recognition task variants (Experiments 3A, B, and C). Task dissociations with regard to NMDAR function would further support the notion that NMDARs are involved in spatial, but not nonspatial forms of short-term recognition memory.

Chapter 2

EXPERIMENT 1: ONTOGENY OF CONTEXTUAL RECOGNITION MEMORY

Experiment 1A: Ontogeny of Object-in-Context Recognition

Introduction

Recent studies conducted by our lab on the development of the CPFE suggest that the ability to acquire context representations may ontogenetically emerge around the time of weaning (approximately PD21) in the rat (Schiffino et al., 2011; Jablonski et al., 2012; Robinson-Drummer & Stanton, 2014), similar to forms of hippocampus-dependent spatial cognition, like the CPFE. However, the CPFE paradigm involves long delays and requires interactions between cognitive and emotional brain memory systems (supporting contextual and fear learning, respectively), making it difficult to attribute preweanling rats' failure to express fear to contextual stimuli to the underdevelopment of memory systems supporting contextual learning per se. To address this, Experiment 1 examined the ontogenetic profile of the object-in-context recognition (OiC) task, a behavioral paradigm that be used to assess short-term memory for context representations and that does not seem to require amygdala activity (Balderas et al., 2008). OiC task performance was observed in PD17, 21, 26, and 31 rats. These ages were chosen in order to extend our recent findings on the development of the OR and OL tasks (Westbrook et al., 2014). This experiment's data (as well as data described in Experiment 1B and a subset of data reported in Experiment 2A) have been reproduced from our recent report (Ramsaran, Westbrook, & Stanton, 2015).

Materials and Methods

Subjects

Animal colony and maintenance have been described in our previous reports (Jablonski et al., 2013; Westbrook et al., 2014). 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 Object-in-Context Recognition Task section 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 libitum* 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 (Westbrook et al., 2014).

Apparatus

The apparatus was adapted from Jablonski et al. (2013) and Westbrook et al. (2014). 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 Data Collection and Analysis

section 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).

Figure 1 Testing apparatuses. Contexts A (A) and B (B) were used in Experiments 1A, 1B, 2A, 2B, 3A, and 3C. Context C (C) was used only in Experiments 1B and 2B. In Experiment 2A, Context A′ (Context A located in a separate room) was implemented in addition to Contexts A and B. Experiment 3B used only Contexts A and A′. The two different object configurations (Configurations 1 and 2) for object placements during the tasks are shown in red.

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

Procedure

Habituation

Rats were habituated to both contexts during three sessions (Jablonski et al., 2013; Westbrook et al., 2014). 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 (±1) hr later. Session 3 occurred the following morning, 5 (±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).

Object-in-Context 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.

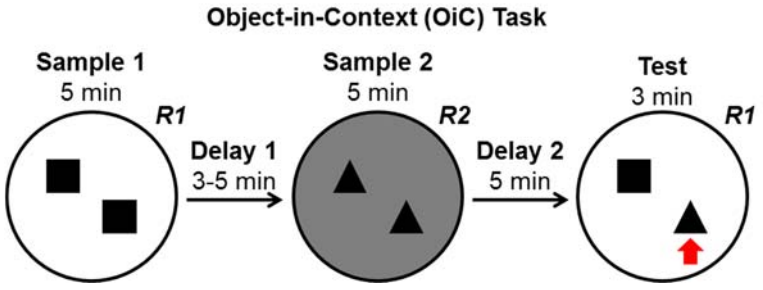

Figure 3 A schematic diagram of the object-in-context (OiC) task. Objects are shown in configuration 1. During the training phase, rats are exposed to two different pairs of objects within distinct contexts (Sample 1 and Sample 2) located in different rooms (R1 and R2), separated by a short delay (Delay 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. Memory for the training experience is indicated by preferential exploration of the object mismatched to the context (red arrow) during the test phase (Test).

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 (Jablonski et al., 2013; Westbrook et al., 2014). 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 sex, 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, $[t_{novel} / (t_{novel} + t_{familiar})]$ (Mumby et al., 2002). 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 (Dix & Aggleton, 1999). 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 in this experiment (and all others) was not influenced by sex (all ps>.11). These factors were consequently collapsed so exploration ratio data could be analyzed by the variable(s)-of-interest (in this case, age group).

Consistent with our previous report, ANOVAs were not used in analyzing exploration ratios (see Westbrook et al., 2014 for further explanation).

Results

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).

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) × 4 (Age Group) × 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 × Sample Phase interaction [$F(3, 73)=3.43$, $p<.02$]. No other main effects or interactions were observed (all Fs<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 (*p*s<.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.

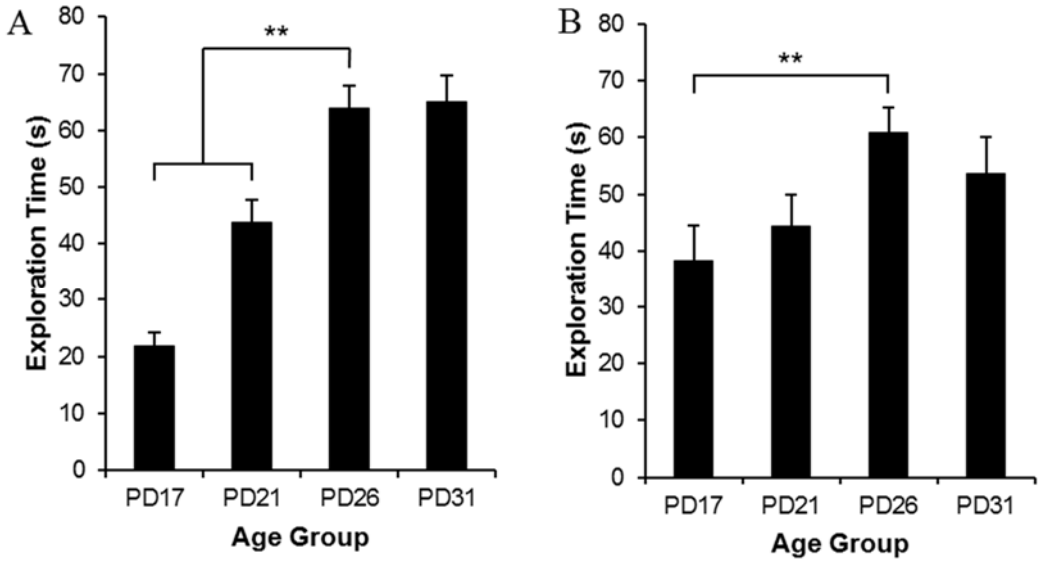

Figure 4 Exploration times for each age group during Sample 1 (A) and Sample 2 (B) of Experiment 1A. Mean exploration times (±SEM) are given. In general, mean exploration times increased with age until PD26. Mean exploration times only differed between sample phases in the PD17 age group. Significant differences between age groups for congruent sample phases are indicated. (**p<.01)

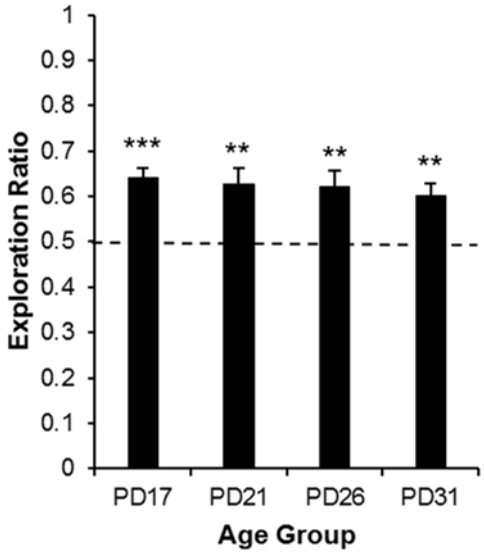

Figure 5 Mean exploration ratios (±SEM) during the OiC test phase for Experiment 1A. Exploration ratios were calculated as $t_{novel} / (t_{novel} + t_{familiar})$. Dashed line represents chance performance (0.5). All age groups performed significantly above chance levels. (**p<.01, ***p<.001)

Test Phase

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).

Discussion

Exploration times increased somewhat across age but, consistent with our previous report, this did not influence novelty preference performance (Westbrook et al., 2014). 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 contextual information incidentally and retrieve these memory traces after a short retention interval.

Experiment 1B: Object-in-Context Recognition and Associative Learning

Introduction

Experiment 1A 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 (Pugh & Rudy, 1996; Schiffino et al., 2011; Jablonski et al., 2012), Experiment 1B sought to determine whether developing rats were indeed associating the objects encountered during the sample phases with the contexts in which they were experienced. Following training, PD17 and PD26 rats were either tested in a context experienced during training (Same condition) similarly to Experiment 1A 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.

Materials and Methods

Subjects

Subjects were 60 (29 M; 31 F) Long-Evans rats from 16 litters. Animal colony and maintenance were identical to Experiment 1A. Subjects were assigned to one of two age groups (PD17 or PD26), corresponding to the rats' age on the day of testing, and one of the two testing context conditions (Same or Different) described above.

Apparatus

The apparatus was the same as Experiment 1A with the following exceptions. A third context (Context C; Figure 1C) was developed for Experiment 1B. Context C was a 50-gallon rectangular storage tub (Rubbermaid) measuring 108.5 × 54.4 × 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 "I" and checkered square—were placed respectively on the north and west walls. A fake apple (same as Experiment 1A), a glass jar filled with pink stones, a white plastic hook with a flat bottom, and an 8 oz. Pepsi can served as the stimulus objects (Figures 2A, C-E).

Procedure

The number and timing of habituation sessions, handling, and cleaning procedures were the same as described in Experiment 1A. 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 1A. 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 (Mitchell & Laiacona, 1998) and recognition memory based on context are not confounded as they are in Context Orders ABB and BAA. Additionally, consistent with our findings from Experiment 1A, it has been demonstrated that context order is not a significant factor in tests of short-term OiC memory (Martinez, Villar, Ballarini, & Viola, 2014). 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.

Data Collection and Analysis

Data collection and analysis for the OiC task were the same as Experiment 1A. 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. As mentioned above, Sex was also found to not influence the data, so exploration ratio data was analyzed by Age Group and Testing Context conditions.

Results

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).

Sample Phase

Sample phase exploration increased with age (Figure 6). Sample phase exploration times were analyzed using a 2 (Sex) × 2 (Age Group) × 2 (Testing Context) × 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 showed that exploration times were significantly lower in the PD17 groups relative to PD26 groups (p<.001).

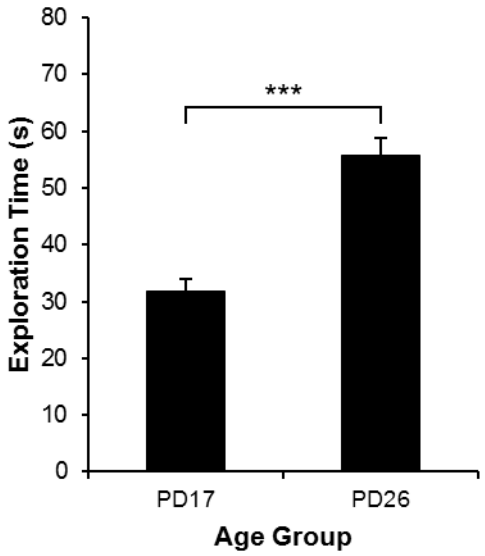

Figure 6 Mean exploration times (±SEM) by age group of Experiment 1B. Data are displayed collapsed across sex, testing condition, and sample phase. Exploration times were significantly lower in PD17 groups compared to PD26 groups. (***p<.001)

Test Phase

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 to chance 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 alternate context (p<.02).

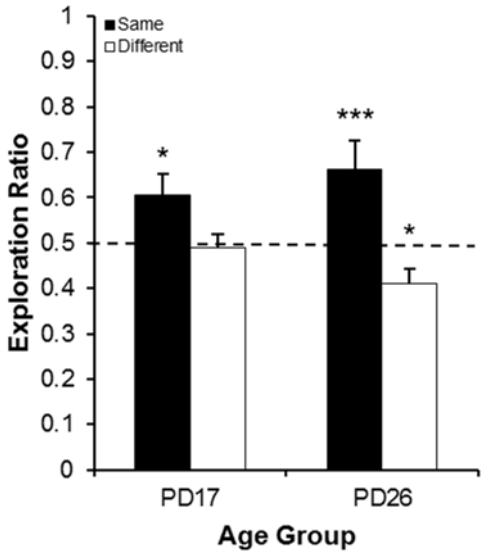

Figure 7 Mean exploration ratios (±SEM) during the OiC test phase by age group and test condition for Experiment 1B. Exploration ratios were calculated as $t_{novel} / (t_{novel} + t_{familiar})$. Dashed line represents chance performance (0.5). Both Same groups performed significantly above chance levels. The Different group at age PD26 showed a significant familiarity preference. (*p<.05, ***p<.001)

Discussion

Consistent with Experiment 1A, 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.

Chapter 3

EXPERIMENT 2: EFFECT OF SPATIAL MANIPULATIONS ON THE ONTOGENY OF CONTEXTUAL RECOGNITION MEMORY

Experiment 2A: Ontogeny of Object-in-Context Recognition Based on Distal Spatial Cues

Introduction

Results from Experiment 1 show that rats as young as 17-days-old can learn associations of object and context information. In Experiment 2A, we asked whether distal spatial cues were sufficient to support OiC learning in developing rats. Adult lesion and developmental literature on the contextual/spatial learning suggests that the hippocampus may be specifically involved in processing the distal, but not proximal spatial environment (Piterkin, Cole, Cossette, Gaskin, & Mumby, 2008; Dees & Kesner, 2013), and that distal spatial processing develops around or after the third postnatal week in the rat (Rudy et al., 1987; Pugh & Rudy, 1996). To examine this, PD17, 21, and 26 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 (proximal and distal) 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.

Materials and Methods

Subjects

Subjects were 81 (40 M; 41 F) Long-Evans rats from 23 litters. Animal colony and maintenance were the same as described for Experiment 1. Subjects were assigned to one of three age groups (PD17, PD21, or PD26), representing the rats' age on the test day, and one of two context conditions (Global or Distal), which determined whether they were tested in the standard (Global) or distal cue version (Distal) of the OiC task.

Apparatus

An identical set of chambers and context inserts as used in Experiment 1A were constructed for a total of 4 testing arenas. Subjects in the Global groups encountered Contexts A and B as is previous experiments, but subjects in the Distal groups experienced the same Context A 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 (Figure 8). A fake green apple and glass jar filled with pink stones (Figures 2A and C) served as the stimulus objects.

Procedure

The number and timing of habituation sessions, handling, and cleaning procedures were the same as described previously in Experiment 1B, except no

savings of habituation tests were given. 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 (Figure 9). Like Experiment 1B, only Context Orders ABA and BAB (for Global groups) and AA′A and A′AA′ (for Distal groups) were utilized so that novelty preference based on context associations versus relative recency could be dissociated.

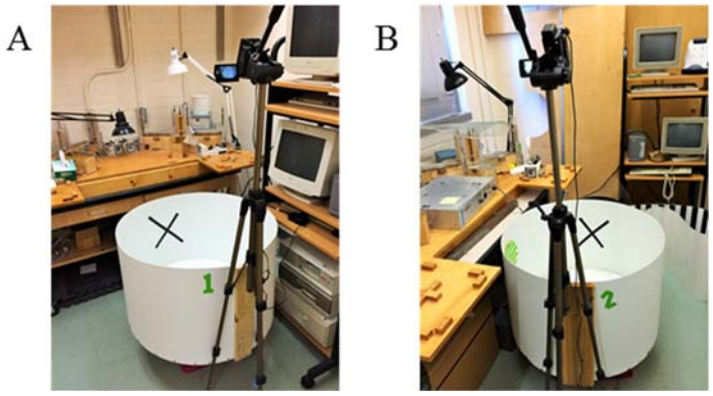

Figure 8 Example of the differences in distal room cues between contexts. Partial views of the rooms containing chambers 1 and 2 are shown. In the distal cue OiC task, identical chambers were surrounded by different stimuli (or stimuli in different locations relative to the open field chambers) that could be used as distal spatial cues. The most prominent distal spatial cues were workbenches, computer monitors, shelves, a whiteboard (not shown), conditioning chambers (not shown), and walls (not shown)

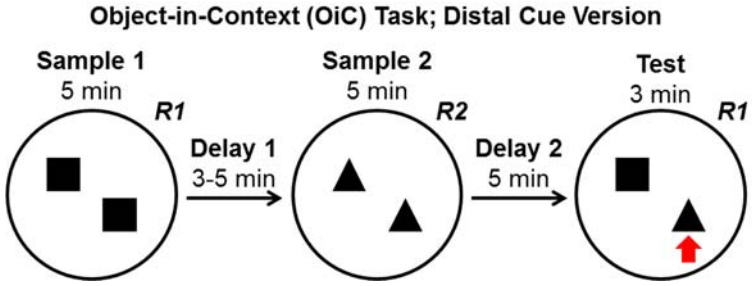

Figure 9 A schematic diagram of the distal cue version of the object-in-context (OiC) task. Objects are shown in configuration 1. During the training phase, rats are exposed to two different pairs of objects within identical chambers (Sample 1 and Sample 2) located in different rooms (R1 and R2), separated by a short delay (Delay 1). After a retention interval of 5 min (Delay 2), rats are replaced into the first context with a copy of both previously-encountered objects present. Memory for the training experience is indicated by preferential exploration of the object mismatched to the context (red arrow) during the test phase (Test).

Data Collection and Analysis

Data were collected and analyzed in the same manner as previous experiments. Sub-factors including context order, object configuration, and object order were counterbalanced across the variables of sex, age group, and context condition, and this collapsed before analyses.

Results

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). Four animals met the criteria of a statistical outlier and were additionally excluded from analyses (PD17, M, Global, n=1; PD21, F, Distal, n=1;

PD26, M, Global, n=1; PD26, F, Distal, n=1). Data from the remaining 71 subjects were used in the statistical analyses (PD17, Global, n=13; PD17, Distal, n=13; PD21, Global, n=13; PD21, Distal, n=11; PD26, Global, n=11; PD26, Distal, n=10).

Sample Phase

Sample phase exploration was influenced by Age Group (Figure 10). Sample phase exploration times were analyzed using a 2 (Sex) × 3 (Age Group) × 2 (Context Condition) × 2 (Sample Phase) repeated measures ANOVA which yielded a significant main effect of Age Group [$F(2, 59)=18.4$, $p<.000002$] and a marginally significant main effect of Context Condition [$F(1, 39)=5.07$, $p=.05$]. No other significant main effects or interactions were observed (all Fs<2.36). A post-hoc Newman-Keuls paired analyses showed that PD17 rats explored significantly less than PD21 and 26 rats (ps<.0002), but exploration times did not differ between PD21 and 26 groups ($p>.842$).

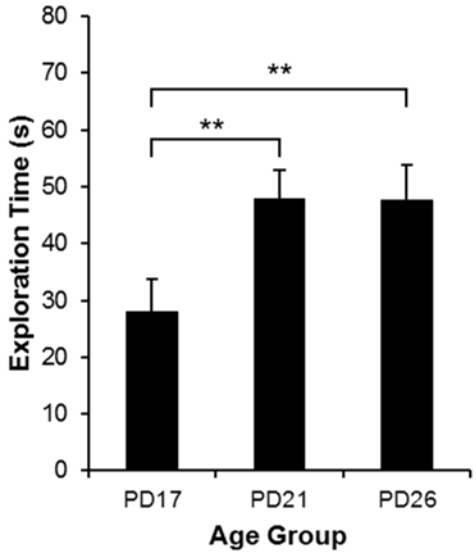

Figure 10 Mean exploration times (±SEM) by age for Experiment 2A. Data are collapsed across sex, context condition, and sample phase variables. Weanling and juvenile rats explored more overall than preweanling rats. (**p<.01)

Test Phase

Figure 11 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 and weanling 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), PD21-Global (p<.03), PD26-Global (p<.001), and PD26-Distal (p<.03) groups. In contrast, rats in the PD17-Distal and PD21-Distal groups did not show a preference for the novel target (ps>.18 and .08, respectively).

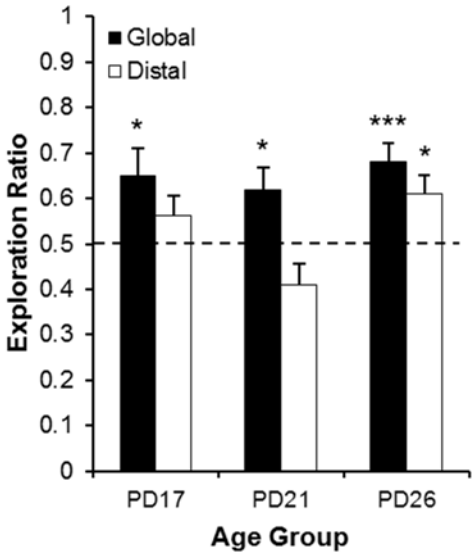

Figure 11 Mean exploration ratios (±SEM) during the OiC test phase by age group and context condition for Experiment 2A. Exploration ratios were calculated as $t_{novel} / (t_{novel} + t_{familiar})$. Dashed line represents chance performance (0.5). Both Global groups performed significantly above chance levels. Only the Distal group at age PD26 showed a significant novelty preference. (*p<.05, ***p<.001)

Discussion

Data from the Global groups are consistent with the preceding experiments, demonstrating that preweanling, weanling, and juvenile rats can perform the standard OiC task. However, rats in the PD26-Distal group, but not the PD17-Distal and PD21-Distal groups, could perform the OiC task when contexts were distinguished by the distal spatial environment. The key difference between the PD26-Distal, and PD17-Distal and PD21-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 younger ages depends on the presence of salient local chamber cues that can support associative context learning during early ontogeny. It is possible that

underdevelopment of the rat visual system may be responsible for the lack of OiC task performance in PD17-Distal and PD21-Distal groups (i.e., PD17 and 21 rats cannot see the distal spatial environment from within the testing chambers). Alternatively, ontogenetic differences between the two OiC task variants may reflect the differential involvement of neural substrates responsible for processing proximal versus distal spatial cues (see General Discussion).

Experiment 2B: Ontogeny of Object-Place-Context Recognition

Introduction

In the preceding experiment we observed protracted development of the OiC task after manipulating contextual cues in a manner that made spatial processing necessary for task performance. Therefore, it is possible that spatial task manipulations alter the ontogeny of contextual learning and memory. In the current experiment, we examined the ontogeny of the object-place-context recognition (OPC) task, which requires rats to associate an object's identity and spatial location with the context in which it is encountered. PD26 and 31 rats were tested in the OPC task, and like Experiment 1B, rats were tested in either a context experienced during training (Same condition) or an alternate context not experienced during training (Different condition). Because of its explicit spatial component, we expected the OPC task to emerge later in ontogeny relative to the OiC task (as observed in the Same groups). In addition, we predicted that task performance would be eliminated when rats were tested in an alternate context (Different groups), similar to Experiment 1B.

Materials and Methods

Subjects

Animal colony and maintenance were the same as described in previous experiments. Subjects were 55 Long-Evans rats (24 M, 31 F) derived from 12 litters. Like Experiment 1B, subjects were assigned to one of two age groups (PD26 or PD31) and one of two testing context conditions (Same or Different).

Apparatus

The testing arenas and objects were identical to those used in Experiment 1B. Contexts A, B, and C were employed (Figure 1) and the stimulus objects were a fake green apple, a glass jar filled with pink stones, a white plastic hook with a flat bottom, and an 8 oz. Pepsi can served as the stimulus objects (Figures 2A, C-E).

Procedure

All procedures (handling, habituation, cleaning, etc.) were the same as those described for Experiment 1B, except for the ages on which habituation and testing occurred, and the behavioral preparation. Habituation began on the morning of PD25 or 30 and testing took place on the afternoon of PD26 or 31 for PD26 and PD31 groups, respectively. For animals in the Different groups, Context B or C was assigned as the alternate context.

The OPC task operated similarly to the OiC task described in previous experiments, except for the manner in which rats encountered the objects. A schematic diagram of the standard OPC task (Same condition) is shown in Figure 12. The number and duration of each phase of the OPC task were the same as described for the OiC task. During Samples 1 and 2 (5-min each), rats encountered the same pair of

dissimilar objects in each context with the spatial locations of each object reversed in the Sample 2 context relative to the Sample 1 context. During the test phase, two copies of one of the object types was presented to the rats for 3 min. Rats in the Same condition were always tested in the temporally distant context from the training phase (see Experiment 1B for explanation). Because both objects were experienced in both contexts, preference during the test phase was derived from the novel configuration of the object identity, space, and context. In the Different condition, testing occurred in an alternate context not experienced during either sample phase, thus neither object during the test should be considered novel.

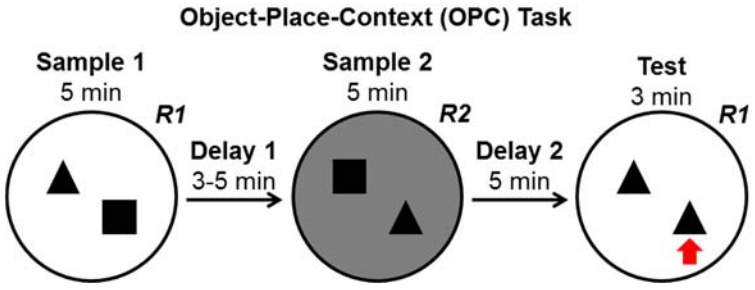

Figure 12 A schematic diagram of the object-place-context (OPC) task. Objects are shown in configuration 1. During the training phase, rats are exposed to the same object pair in two distinct contexts (Sample 1 and Sample 2) located in different rooms (R1 and R2), separated by a short delay (Delay 1). The locations of the objects are reversed in Sample 2 relative to Sample 1. After a retention interval of 5 min (Delay 2), rats are replaced into the first context with two copies of one of the previously-encountered objects. Memory for the training experience is indicated by preferential exploration of the object in a novel configuration of location and context (red arrow) during the test phase (Test).

Data Collection and Analysis

Procedures for data collection and analysis were the same as Experiments 1 and 2. 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, these data were collapsed across these subgroups before statistical analyses.

Results

Subjects

Two subjects were removed from the data set due to a technical error (PD26, F, Same, n=1; PD26, F, Different, n=1). Another four subjects' data were classified as statistical outliers and removed (PD26, M, Different, n=1; PD26, F, Different, n=1; PD31, F, Same, n=1; PD31, M, Different, n=1). Analyses proceeded using data from the remaining 49 subjects (PD26, Same, n=13; PD26, Different, n=11; PD31, Same, n=13; PD31, Different, n=12).

Sample Phase

Sample phase exploration times are shown by Age Group and Sample Phase (Figures 13A and B, respectively). Data were analyzed using a 2 (Sex) × 2 (Age Group) × 2 (Testing Context) × 2 (Sample Phase) repeated measures ANOVA, which revealed significant main effects of Age Group [$F(1, 41)=5.95$, $p<.020$] and Sample Phase [$F(1, 41)=5.95$, $p<.0002$]. There were no other significant main effects or interactions (all Fs<2.78). Post-hoc Newman Keuls paired comparisons determined that PD31 rats explored significantly more than PD26 rats ($p<.01$) and that rats explored more during Sample 1 compared to Sample 2 ($p<.0002$).

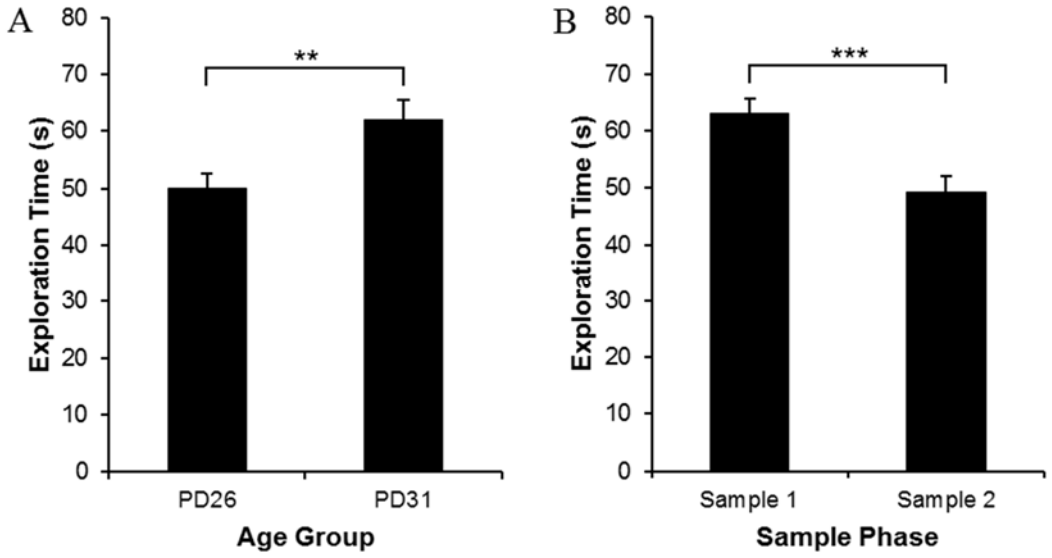

Figure 13 Sample phase exploration times (±SEM) by age group (A) and sample phase (B) for Experiment 2B. Data are shown collapsed across sex, testing context, and and age group (A) or sample phase (B) variables. Adolescent rats explored more than juvenile rats and rats explored more during Sample 1 compared to Sample 2. (**p<.01, ***p<.001)

Test Phase

Results from the OPC task test phase are displayed in Figure 14. Only PD31 rats tested in the training context (PD31-Same group) showed significant preference for the novel target relative to chance performance (p<.04, one-sample t-test). Rats in the PD26-Same group showed no preference for the novel target (p>.96). When tested in an alternate context, PD26 rats preferred to explore the object designated as "familiar" (p<.02). A similar trend was observed in the PD31-Different group (p<.08).

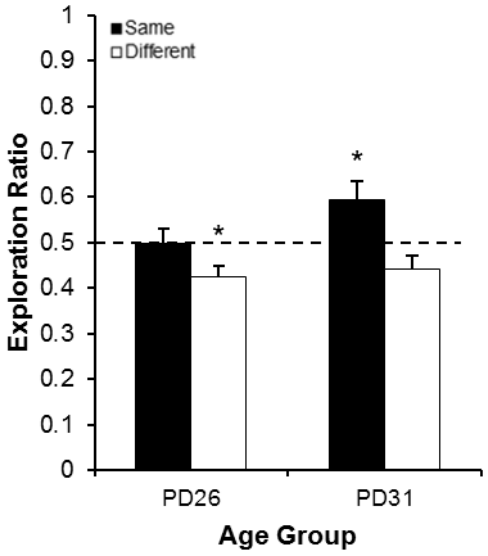

Figure 14 Mean exploration ratios (±SEM) during the OPC test phase by age group and testing context for Experiment 2B. Exploration ratios were calculated as $t_{novel} / (t_{novel} + t_{familiar})$. Dashed line represents chance performance (0.5). Rats were able to perform the OPC task on PD31, but not PD26 (Same groups). When rats were tested in an alternate context (Different groups), preference for the novel target was eliminated. (*p<.05)

Discussion

Object-place-context recognition appears to ontogenetically emerge between PD26 and 31 in the rat. To our knowledge, this is the first demonstration of OPC memory in the developing rat. Not surprisingly, preference for the novel target during the test phase was eliminated in both age groups when rats were tested in an alternate context, confirming the context-dependent nature of OPC memory. Compared to the OiC task, which does not necessarily require spatial processing, the OPC task is drastically delayed during development. The ontogeny of the OPC task is also protracted compared to the distal cue version of the OiC task (Experiment 2), which

may suggest that “episodic-like” memory (Norman & Eacott, 2004) develops even later than memory for a spatial context, which is a component of episodic memory.

Chapter 4

EXPERIMENT 3: ROLE OF NMDA RECEPTORS IN CONTEXTUAL RECOGNITION MEMORY

Experiment 3A: Effect of NMDA Receptor Antagonism on Object-in-Context Recognition

Introduction

In addition to the lack of literature on the ontogeny of object-in-context recognition and similar context-dependent recognition tasks (that we addressed in Experiments 1 and 2), the neural mechanisms involved in the OiC task are poorly understood. NMDAR-related plasticity has been implicated in numerous forms of recognition memory (for review, see Warburton, Barker, & Brown, 2013), yet its role in contextual recognition memory has not been examined. Therefore, in the current experiment we examined the role of NMDARs in the OiC task by administering intraperitoneal (i.p.) injections of MK-801, a non-competitive NMDAR antagonist, 30 min prior to training in the OiC task. Only short-term memory was assessed (5-min retention interval), thus impairments in OiC task performance would suggest that NMDARs are involved in encoding and/or retrieval of object-context associations.

Materials and Methods

Subjects

Subjects were 36 Long-Evans (19 M, 17 F) rats from 9 litters. Animal care and maintenance were the same as previous experiments. All subjects were PD25 at the beginning of the experiment and PD26 on the testing day. Animals were assigned

either receive i.p. injections of MK-801 solution (MK-801 group) or saline vehicle (SAL group).

Apparatus

Four chambers were configured as Contexts A and B (two each; Figures 1A and B) as in previous experiments. A fake apple and glass jar filled with pink stones (Figures 2A and C) were used as the stimulus objects.

Drugs

MK-801 (dizocilpine maleate) was purchased commercially from Tocris (Ellisville, Missouri). Prior to the experiment, MK-801 was dissolved in 0.9% sterile saline solution to achieve a concentration of .06 mg/ml MK-801 in saline vehicle. This dose was taken from our previous studies of weanling-adolescent rats and is effective in disrupting performance in the OL task but not the OR task (Chadman, Watson, & Stanton, 2006; Jablonski et al., 2013). MK-801 solution was administered via i.p. injections at volume of 1.0 ml/kg body weight. Control animals received i.p. injections of 1.0 ml/kg saline vehicle.

Procedure

Handling, habituation, cleaning, and testing procedures were identical to those described for the PD26-Global group in Experiment 2A with the exception of the drug manipulation.

MK-801 (.06 mg/kg) or saline vehicle was administered to rats via i.p. injection 30 min before the start of the testing session. Rats were weighed in the animal colony room before receiving injections in an adjacent room. All rats were

returned to the colony room for the period between drug administration and behavioral testing. Following the delay, rats were trained and tested in the OiC task (Figure 3).

Data Collection and Analysis

Data collection and analysis were the same as the previous experiments. Context order, object order, and object configuration were counterbalanced across sex, and drug treatment conditions and therefore collapsed before analyses.

Results

Subjects

Four animals were excluded from analyses due to experimental errors before test phase (M, SAL, n=1; F, SAL, n=1; M, MK-801, n=1; F, MK-801, n=1). An additional four animals met the criteria of a statistical outlier and were also excluded (M, SAL, n=1; F, SAL, n=2; F, MK-801, n=1). Data from the remaining 28 subjects were used in the analyses (SAL, n=13; MK-801, n=15).

Sample Phase

Sample phase exploration times are shown in Figure 15. Data were analyzed using a 2 (Sex) × 2 (Treatment) × 2 (Sample Phase) repeated measures ANOVA, which yielded a significant main effect of Treatment [$F(1, 24)=6.86$, $p<.02$]. This was the only significant main effect or interaction (all Fs<1.2). Post-hoc Newman-Keuls paired analysis determined that MK-801 animals explored more during the sample phases than SAL animals ($p<.02$).

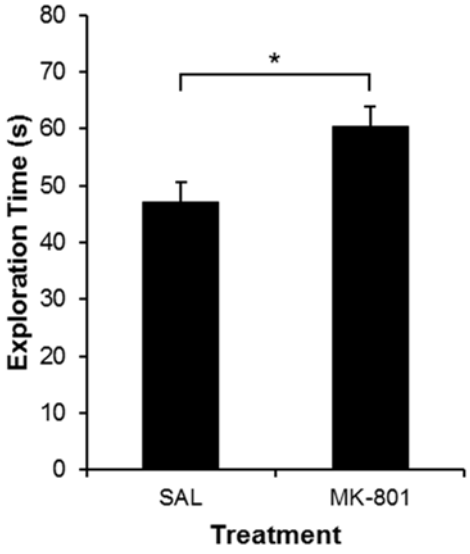

Figure 15 Mean exploration times (±SEM) by drug treatment for Experiment 3A. Data are shown collapsed across sex and sample phase variables. Sample phase exploration times were heightened in rats injected with MK-801 compared to controls. (*p<.05)

Test Phase

Administration of MK-801 prior to training had no effect on OiC task performance (Figure 16). Both SAL and MK-801 groups displayed a robust preference for the novel target during the test phase, as confirmed by one-sample *t*-tests comparing group exploration ratios to chance performance (p<.000001 and .01, respectively).

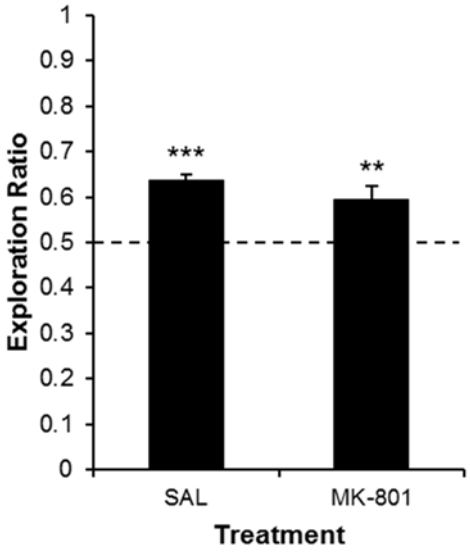

Figure 16 Mean exploration ratios (±SEM) during the OiC test phase for Experiment 3A. Both groups preferentially explored the novel target. (**p<.01, ***p<.001)

Discussion

Sample phase exploration was increased by the MK-801 drug manipulation, but as observed in these data and previous studies (e.g., Westbrook et al., 2014), this did not affect test performance (see General Discussion). Importantly, antagonizing NMDARs prior to training did not disrupt behavioral performance in the OiC task in juvenile rats. Thus, NMDAR plasticity does not seem to be involved in encoding or retrieval of short-term OiC memory. This lack of effect is similar to our previous study in developing rats which demonstrated that the OR task, but not the OL task, is not disrupted by systemic administration of MK-801 (Jablonski et al., 2013). Taken together, these results suggest that the neural mechanisms involved in OiC memory, especially the role of NMDARs, is more similar to OR memory than OL memory.

This hypothesis is also supported by the developmental data from the preceding experiments.

Experiment 3B: Effect of NMDA Receptor Antagonism on Object-in-Context Recognition Based on Distal Spatial Cues

Introduction

Experiment 3A determined that NMDAR function is not essential for OiC task performance when a short retention interval is used and when contexts are defined by salient proximal and distal cues. Numerous research groups, including our own, have shown that NMDARs are involved in some forms of short-term recognition memory, e.g., object location recognition, but not object recognition (Larkin et al., 2008; Assini et al., 2009; Jablonski et al., 2013; for review, see Warburton et al., 2013). In particular, NMDARs seem to be involved in recognition memory that includes some spatial aspect. To further examine the hypothesis that NMDARs are involved in spatial but not nonspatial recognition memory, we antagonized NMDARs in juvenile rats while performing the OiC task variant in which performance is dependent on processing of distal spatial cues (see Experiment 2A). Here, we expected to observe impairment in task performance because unlike the task used in the previous experiment, there is an explicit spatial component in the distal cue OiC task.

Materials and Methods

Subjects

Animal care and maintenance was the same as described in previous experiments. Subjects were 27 Long-Evans rats (14 M, 13 F) from 9 litters. Rats were

assigned to the MK-801 group, which received drug injections prior to training, or the SAL group, which received injections of saline. Subjects were 25-days-old at the start of the experiment (PD26 on the testing day).

Apparatus

The apparatus were the same as described for the Distal groups in Experiment 2A. Chambers were arranged as Contexts A and A′, which were identical except for the room in which they were placed (i.e., different distal spatial cues). The stimulus objects were a fake green apple and a glass jar filled with pink stones (Figures 2A and C).

Drugs

Drug preparation was the same as Experiment 3A. Rats in the MK-801 group received a dose of .06 mg/ml MK-801 delivered in a volume of 1.0 ml/kg body weight. Subjects in the SAL group received injections of saline vehicle delivered in the same volume.

Procedure

The procedure was identical to that of Experiment 3A, except that the distal cue version of the OiC task was employed. Subjects were habituated to the testing chambers on PD25 (two sessions, morning and afternoon) and PD26 (one session, morning). On the afternoon of PD26, rats were carted to a nearby room where they received MK-801 or saline injections and then were returned to the animal housing room. The testing session began 30 min later and context memory was probed using the OiC task based on processing of distal spatial cues (Figure 9).

Data Collection and Analysis

As described in the previous experiment, context order, object order, and object configuration were counterbalanced across sex, and drug treatment conditions. These variables were collapsed before analyses.

Results

Subjects

One rat was excluded from analyses for meeting the criteria of a statistical outliter (M, SAL, n=1). Data from the remaining 26 subjects were used in the analyses (SAL, n=13; MK-801, n=13).

Sample Phase

Sample phase exploration was not influenced by the drug treatment (Figure 17). Exploration times were analyzed using a 2 (Sex) × 2 (Treatment) × 2 (Sample Phase) repeated measures ANOVA. No significant main effects or interactions were observed (all Fs<3.91, ps>.06; the interaction of Sex × Sample Phase approached significance). Importantly, objects were explored for substantial amounts of time during the sample phases by all groups.

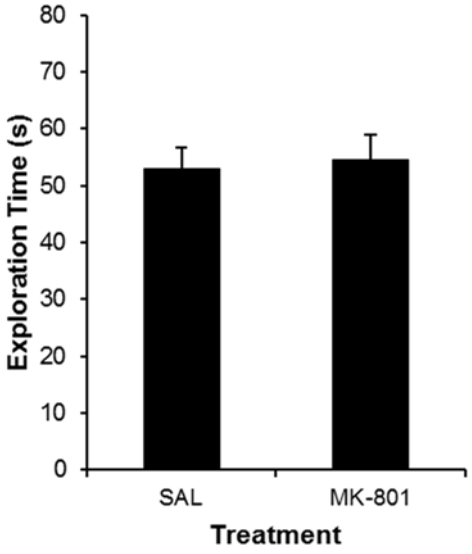

Figure 17 Mean exploration times (±SEM) by drug treatment for Experiment 3B. Data are shown collapsed across sex and sample phase variables. Drug treatment had no effect on object exploration during the sample phases.

Test Phase

Results from the test phase are shown in Figure 18. Saline injections had no effect on task performance, as PD26 rats showed robust performance of the distal cue version of the OiC task compared to chance levels ($p<.011$). In contrast, rats that received injections of MK-801 prior to training did not display a preference for the novel target ($p>.16$). Thus, blocking NMDARs impaired this spatial variant of the OiC task.

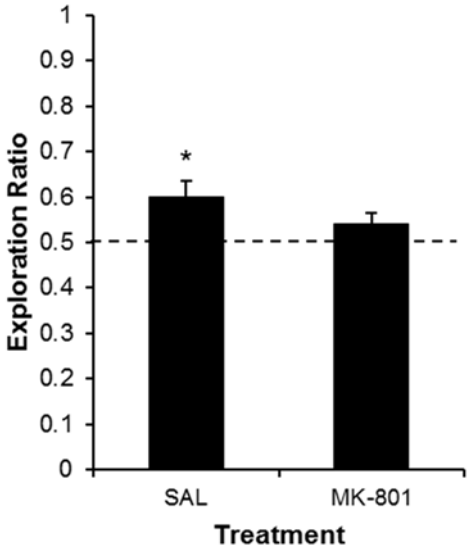

Figure 18 Mean exploration ratios (±SEM) during the OiC test phase for Experiment 3B. When contexts were defined only by the distal spatial environment, injections of MK-801 impaired task performance but control injections did not. (*p<.05)

Discussion

Unlike Experiment 3A, the drug treatment had no effect on sample phase exploration times. These effects (or lack of effects) are not likely to contribute to differential memory strength for the training experience between groups because as seen in all of the previous experiments, juvenile rats' level of object exploration is significantly higher than necessary to support recognition memory (see General Discussion). Notably, we observed an impairment in task performance in the MK-801 group in the distal cue version of the OiC task. This is in stark contrast to Experiment 3A which utilized the standard (global) version of the OiC task, which was not affected by NMDAR antagonism. These data might suggest that NMDARs are involved in spatial forms of recognition memory, which is consistent with our

previous report (Jablonski et al., 2013). If this is true, NMDAR plasticity should be essential for performance of the OPC task as well.

Experiment 3C: Effect of NMDA Receptor Antagonism on Object-Place-Context Recognition

Introduction

Based on previous data from our lab, we hypothesized that NMDAR plasticity may be essential for spatial learning in short-term recognition memory tasks (Jablonski et al., 2013). This idea has gained support from the current study, which has thus far shown that NMDARs are essential for a spatial variant of the OiC task, but not the standard OiC task, which does not necessarily require spatial processing. In the current experiment, we examined the role of NMDARs in another spatial variant of contextual recognition memory, the OPC task. We predicted that NMDARs would be essential in this recognition memory task, similar to the previous experiment, as spatial learning is necessary to perform the target behavior.

Materials and Methods

Subjects

Animal maintenance and care were the same as previous experiments. Subjects were 40 Long-Evans rats (21 M, 19 F) derived from 12 litters. Like Experiments 3A and 3B, subjects were assigned to SAL control group or MK-801 drug treatment group. Unlike previous experiments, subjects were PD30 at the beginning of the experiment and PD31 on the testing day. This age was used because in Experiment 2B, we observed that OPC memory emerges between PD26 and 31.

Apparatus

The apparatuses were the same as those in Experiment 3A. Chambers were arranged as Contexts A and B (Figures 1A and B) and the stimulus objects were a fake green apple and a glass jar filled with pink stones (Figures 2A, C).

Drugs

Drugs were prepared and administered in the same doses as preceding experiments. MK-801 was dissolved in sterile saline to achieve a dose of .06 mg/ml. MK-801 and saline control injections were given i.p. in a volume of 1.0 ml/kg body weight.

Procedure

Handling, habituation, and cleaning procedures were the same as Experiment 3A. As in the preceding experiments, MK-801 or saline i.p. injections were given 30 min prior to the testing session. The OPC task (Figure 12) was conducted in the same manner as in Experiment 2B for animals in the Same condition (i.e., rats were always tested in the temporally distant context from the training phase).

Data Collection and Analysis

Data collection and analysis were conducted identically to previous experiments. Context order, object order, and object configuration were counterbalanced across sex, and drug treatment conditions and therefore collapsed before analyses.

Results

Subjects

One subject was excluded from analyses for not exploring either object during the second sample phase (M, SAL, n=1). Another two rats were excluded for meeting the criteria of a statistical outlier (F, SAL, n=1; F, MK-801, n=1). The remaining subjects' data were used in the analyses (SAL, n=18; MK-801, n=19).

Sample Phase

Sample phase exploration times are shown in Figure 19 by Treatment and Sample Phase. A 2 (Sex) × 2 (Treatment) × 2 (Sample Phase) repeated measures ANOVA revealed a significant Treatment × Sample Phase interaction [$F(1, 33)=11.1$, $p<.002$]. No other significant main effects or interactions were observed (all Fs<3.14). Post-hoc analysis (Newman-Keuls) of the Treatment × Sample Phase interaction showed that exploration SAL rats explored less during Sample 2 than during Sample 1 ($p<.02$) and less than MK-801 rats during Sample 2 ($p<.03$).

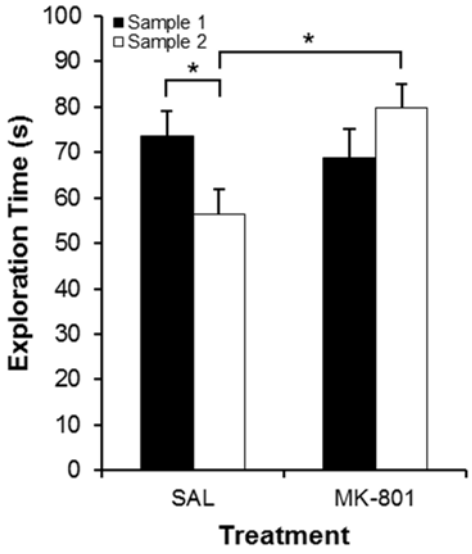

Figure 19 Mean exploration times (±SEM) by drug treatment and sample phase for Experiment 3C. Exploration time data are shown collapsed across sex. Exploration during Sample 2 in the SAL group was lower compared to Sample 1 in the same animals and Sample 2 in the MK-801 group. ($p<.05$)

Test Phase

Exploration ratio data from the OPC task test phase are presented in Figure 20. As supported by one-sample *t*-tests, the SAL group displayed a non-significant trend toward preferential exploration of the novel target ($p=.077$) and the MK-801 group preferred to explore the familiar target ($p<.03$).

While retroactively observing the data set, we discovered an aberrant cohort of rats that did not showed no preference for the novel target (mean=.500), which differed from the other cohorts in the current experiment and other saline control data from our lab on the OPC task (Ramsaran & Stanton, unpublished observations). This atypical cohort was removed from the data set (SAL, n=8; MK-801, n=8) and the exploration ratio data were reanalyzed using one-sample *t*-tests (Figure 20, inset).

After removal of these data, saline controls showed robust preference for the novel target (p<.012) and rats treated with MK-801 showed no preference for either object (p>.26).

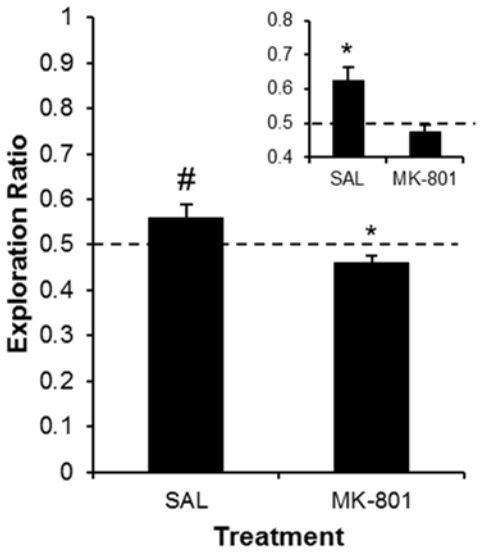

Figure 20 Mean exploration ratios (±SEM) during the OPC test phase for Experiment 3C. Inset shows exploration ratios with the anomalous cohort removed. Animals injected with saline performed the OPC task, whereas animals injected with MK-801 were impaired. (#p<.08, *p<.05)

Discussion

The current experiment examined the effects of NMDAR blockade on OPC memory. The drug treatment did contribute to differential exploration times during the sample phase, but as stated earlier, all sample exploration times were sufficient in supporting recognition memory (Akkerman et al., 2012). Importantly, we demonstrated here that short-term OPC memory requires NMDAR plasticity in adolescent rats. This is similar to our finding in juvenile rats using the distal cue

version of the OiC task—another contextual recognition memory task involving processing of space—but not the standard OiC task. Thus, our hypothesis that NMDARs are involved in recognition memory involving space was further supported. Alternatively, NMDARs may play different roles in these two spatial recognition tasks, e.g., learning multimodal associations in the OPC task versus encoding the distal spatial environment in the spatial OiC task. However, more research is needed to determine the exact function of NMDARs in these contextual recognition memory tasks.

Chapter 5

GENERAL DISCUSSION

The experiments in this thesis begin to characterize contextual recognition memory during development in the rat. Experiment 1A showed that object-in-context recognition is evident in preweanling (PD17) through adolescent (PD31) aged rats. Experiment 1B determined that object-in-context recognition involves associative learning of object and context information. Experiment 2 extended these findings by demonstrating that performance of two spatial OiC task variants (distal cue OiC task, Experiment 2A; OPC task, Experiment 2B) emerge during the postweanling-preadolescent period in the rat. Lastly, Experiment 3 found that performance of the standard OiC task does not require NMDAR function (Experiment 3A), but NMDAR function is necessary for performance of the distal cue OiC task (Experiment 3B) and OPC task (Experiment 3C).

Experiment 1A showed that PD17, 21, 26, and 31 rats 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 (Dix & Aggelton, 1999; Mumby et al., 2002; Langston et al., 2010b; Martinez et al., 2014; Bekinstein, Renner, Gonzalez, & Weisstaub, 2013; Balderas et al., 2013; Barsegyan, McGaugh, & Roozendaal, 2014; Langston & Wood, 2010; Norman & Eacott, 2005; Spanswick & Sutherland, 2010; Wilson et al., 2013a; Wilson, Wtanabe, Milner, & Ainge, 2013b); however, the youngest age at which OiC memory has been examined is approximately PD50 (Li et al., 2011). The emergence of OiC task performance by PD17 is similar to the emergence of object recognition (OR) (Reger et al., 2009; Ainge & Langston, 2012; Krüger et al., 2012; Westbrook et

al., 2014), but not other forms of spatial recognition memory like the object location recognition (OL) task, which emerges between PD17 and PD21 (Westbrook et al., 2014), or the 2-object variant of the object-in-place (OiP) task, which emerges between PD24 and 31 (Ainge & Langston, 2012). A study by Krüger and colleagues (2012) showed that not only is OR evident during the preweanling period of development, but also OL and temporal order recognition (TOR). It should be noted that this study 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 memory.

The early development of OiC task performance observed in Experiment 1A was unusual when compared to the ontogeny of spatial cognition. Therefore, in Experiment 1B a context manipulation was applied that confirmed that PD17 and PD26 rats displayed novelty preference during the OiC test phase based on previously learned object-context associations. This is the first study to test the assumption that novelty preference in the OiC task is driven by contextual stimuli. Previous studies employing similar context manipulations in the OR task highlight the differences between OR and OiC memory. In these object recognition 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 (O'Brien, Lehmann, Lecluse, & Mumby, 2006; Dellu, Fauchey, Le Moal, & Simon, 1997; Cohen et al., 2013; Piterkin et al., 2008). Conversely, developing rats in our experiment only exhibited novelty preference when tested in a context congruent with the sample phases, but not when tested in a different

familiar context. Thus, context plays a critical role in OiC memory but not OR memory, which is easy to reconcile when considering the differences between OR and OiC task procedures. In an OR test, novelty can be derived from the novel target's identity as the rat encounters the new object for the first time. In contrast, in the OiC task both objects present during the test are considered familiar based on their identities, so novelty is derived from the objects' relationship to the context. Indeed, OiC memory is distinct from OR memory despite their similar developmental profiles. By implementing the alternate context test, we demonstrated that contextual cues are salient to developing rats and these cues drive novelty preference during the OiC task.

Infant rats may be able to solve the OiC task by associating the stimulus objects with features of the immediate (proximal) environment and not the distal environment. Therefore, the OiC task may only assess spatial cognition under certain circumstances, i.e., when a spatial context is involved (Langston et al., 2010b). In Experiment 2A, we investigated whether distal spatial cues alone were sufficient to support OiC memory during ontogeny. Here, we found that PD26 rats, but not PD17 or 21 rats, were able to perform the OiC task by associating the stimulus objects with the spatial environment of the training rooms (proximal chamber cues were held constant). It is unlikely that low exploration times in the PD17 groups can account for their inability to detect the novel target, as PD21 rats did not perform the task even with exploration levels comparable to PD26 rats. It is important to note that 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 are not correlated with novelty preference during the test phase (Ozawa, Yamada, & Ichitani,

2011; Westbrook et al., 2014; for review, see Akkerman et al., 2012). It is possible that poor visual acuity in PD17 and 21 rats may have contributed to their inability to perform the distal cue version of the OiC task, as they would be unable to form distinct representations of the context. We note however that in our distal cue OiC task, room cues greatly differed between contexts so task performance did not require rats to make fine discriminations, but rather notice crude shifts in the distal environment (Figure 8). Still, more experiments are still needed to evaluate this view.

Another interpretation of this finding is that younger rats' capacity for spatial learning is inadequate. Earlier reports on the ontogeny of spatial cognition support this. For example, distal cue utilization in the water maze develops after PD20 (Rudy et al., 1987) and contextual fear conditioning in a clear plexiglass chamber (allowing sensory input from the distal environment) significantly increases between PD20 and 23 (Rudy, 1993; Pugh & Rudy, 1996). Not surprisingly, both of these tasks can be performed by preweanling rats when spatial cues are moved to the immediate environment. These findings are in alignment with the present study. Although PD17, 21, and 26 rats showed robust novelty preference when distinct proximal cues were available, only PD26 rats showed preference for the novel target when these cues were removed. It is likely that the distal cue version of the OiC task employed here involves spatial learning whereas the version of the task used in the preceding experiments may not. Importantly, we showed here that the ontogeny of the OiC task is delayed when this spatial manipulation is applied.

In Experiment 2B we further examined the impact of spatial learning on the development of contextual recognition by examining the ontogeny of OPC memory. The results indicated that PD31 but not 26 rats could perform the OPC task, and like in

Experiment 1B, preference for the novel target was eliminated when rats were tested in an alternate context. Literature on the OPC task is scarce and restricted to adult rats (Eacott & Norman, 2004; Langston et al., 2010b; Langston & Wood, 2010; Easton, Fitchett, Eacott, & Baxter, 2011; Wilson et al., 2013b). This is the first study to examine OPC task performance in during development, therefore we cannot compare these data to the adult literature. Interestingly, the ontogenetic emergence of the OPC task is even later than the distal cue OiC task. This may reflect the different cognitive demands of these two tasks. The OPC task requires association of information from three dimensions; the rat must associate not only the objects' identities with the context, but also the objects' locations within each context. Thus, this task does not involve a spatial context as in the last experiment, but rather additional spatial information that must be associated with the objects and contexts.

We suggest here that the ability to associate space with object or context information, and not spatial learning alone, is likely responsible for the late emergence of the OPC task. OPC can be viewed as a composite of OiC and the 2-object variant of OiP (Eacott & Norman, 2004; Ainge & Langston, 2012), therefore these forms of recognition memory can be likened to component processes of OPC. This thesis establishes that the associative learning for objects and contexts (OiC) emerges before PD17, but Ainge & Langston (2012) show that associative learning of objects and locations in the 2-object OiP task emerges between PD24 and 30. This is later than the development of spatial recognition (OL) between PD17 and 21. Future experiments should examine the ontogeny of place-context recognition (Easton et al., 2011; Wilson et al., 2013b) to provide converging evidence for this hypothesis. Alternatively, this interpretation of OPC as a composite of OiC and 2-object OiP may be inappropriate as

the mechanisms of OPC memory are currently unknown. OPC memory mechanisms may be distinct from, and not simply the sum of, those involved in OiC and OiP memory. Nonetheless, it is clear that spatial cognition influences the ontogeny of performance in contextual recognition tasks.

In addition to affecting the ontogeny of contextual memory, spatial cognition may also impact the neurobiology of contextual memory. NMDARs have been widely implicated in synaptic plasticity and various forms of spatial/contextual learning and memory (for review, see Riedel, Platt, & Micheau, 2003), so we investigated the role of NMDARs in contextual recognition. Antagonizing NMDARs before training with MK-801 did not impair our standard OiC task (Experiment 3A), but did impair the distal cue version OiC task (Experiment 3B) and the OPC task (Experiment 3C) in developing rats. These findings are consistent with the developmental data presented earlier in that the OiC task shares characteristics with the OR task and the distal cue OiC task and OPC task are similar to higher-order recognition memory tasks (e.g., OL task), even during development.

Previous studies have shown that NMDAR function is essential for spatial memory in the OL task (Assini et al., 2009; Hunsaker, Mooy, Swift, & Kesner, 2007; Larkin et al., 2008; Jablonski et al., 2013; for review, see Warburton et al., 2013). In these studies, pretraining administration of NMDAR antagonists (e.g., MK-801, APV, CPP) into the peritoneum or hippocampal subfields CA1, CA3, or dentate gyrus (DG) of adult rodents abolished preference for the displaced object in the OL task. Pretraining administration of the NMDAR agonist D-cycloserine also improved OL task performance, further implicating NMDAR plasticity in spatial recognition (Assini et al., 2009).

On the other hand, the NMDAR-dependency of nonspatial (object) memory seems to be delay-dependent. OR memory has been shown to be disrupted by pretraining injections or microinfusions (into perirhinal cortex or hippocampus) of NMDAR antagonists (Baker & Kim, 2002; Winters & Bussey, 2005; de Lima, Laranja, Bromberg, Roesler, & Schröder, 2005; Nilsson, Hansson, Carlsson, & Carlsson, 2007; van der Staay, Rutten, Erb, & Blokland, 2011), but in all of these studies the delay between training and testing was ≥1 hr. In contrast, NMDAR antagonists have no effect on nonspatial memory in OR tasks using a shorter (5 min) retention interval (Winters & Bussey, 2005; Jablonski et al., 2013). Because the length of the retention interval has a significant influence on the neurobiology of various forms of recognition memory (e.g., Hammond, Tull, & Stackman, 2004; Barker & Warburton, 2011), for the moment we will only consider short retention intervals (~5 min) that assess short-term memory/working, as results from these studies are most applicable to the experiments in this thesis.

Combined, the aforementioned data suggest that NMDAR plasticity is involved in spatial but not nonspatial forms of short-term recognition memory, both in adulthood and during ontogeny. This hypothesis is supported by previous data from our lab showing that performance of the OL task but not OR task depends on NMDAR function (Jablonski et al., 2013), and the current thesis. Using a short retention interval, we observed no memory impairment in the OiC task following administration of MK-801. It is possible that a configural representation of the spatial context is not a requisite for OiC memory. In other words, OiC memory may not involve encoding of the spatial features of the context in relation to the objects. However, the distal cue OiC task and the OPC task likely involve conjunctive spatial processing. Like the OL

task, these tasks cannot be performed without encoding the spatial features of the context. Also like the OL task, we showed here that these tasks are impaired by NMDAR antagonism. Because MK-801 has a brain half-life of approximately 2 hr (Vezzani et al., 1988), we were unable to discern whether NMDAR disrupted encoding or retrieval of memory in the distal cue OiC and OPC tasks as training and testing occurred consecutively (session time < 35 min). Future studies should address what process of disrupted by NMDAR blockade in contextual recognition by manipulating the timing of drug administration (e.g., pretraining vs. pretesting), however this will likely require a longer retention interval between training and testing. In addition, further research using intracranial drug infusions should attempt to localize the brain region(s) where NMDAR plasticity is necessary to support memory in the distal cue OiC and OPC tasks. This research will provide key insight into the poorly understood neural substrates of contextual recognition memory during ontogeny.

Although this thesis did not employ direct brain manipulations, it is instructive to consider the present findings in relation to the neuroanatomical systems of contextual recognition memory and related tasks during early ontogeny. In particular, the hippocampal system (including the hippocampal formation and parahippocampal cortices) is recruited in tests of spatial recognition memory (Mumby et al., 2002; Barker & Warburton, 2011) and contextual fear memory (Phillips & Ledoux, 1992; Kim & Fanselow, 1992; Matus-Amat et al., 2004; Schiffino et al., 2011). Therefore, the hippocampal system is likely to be involved in contextual recognition memory, as some studies using adult rodents have found (see below).

Our findings suggest that OiC ontogenetically emerges earlier than other forms of spatial cognition mediated by the hippocampal system. For example, two incidental learning tasks that share behavioral mechanisms of contextual recognition 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 (Dix & Aggleton, 1999). 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 (Fanselow, 1990). Our lab has demonstrated that rats display preference for the displaced object in the OL task on PD21, but not PD17 (Westbrook et al., 2014), and that moderate levels of freezing are observed in a CPFE paradigm when rats are preexposed to the conditioning context on PD21, but not PD17 (Schiffino et al., 2011; Jablonski et al., 2012; Robinson-Drummer & Stanton, 2014). The OL task and CPFE rely on conjunctive spatial processing (Jablonski et al., 2012; Jablonski et al., 2013) subserved by the hippocampal system, which has been proposed for the OiC task (in Rudy, 2009). These claims are supported by research showing that reversibly inactivating, lesioning, or preventing plasticity in the hippocampus disrupts performance in the OL task (Mumby et al., 2002; Barker & Warburton, 2011; Krüger et al., 2012; Assini et al., 2009; Warburton et al., 2013) and the CPFE (Schiffino et al., 2011; Rudy et al., 2002; Barrientos et al., 2002; Matus-Amat et al., 2004).

Likewise, the current literature suggests that the OiC task relies on function of the hippocampal system in adult rodents (Mumby et al., 2002; Martinez et al., 2014; Balderas et al., 2008; Bekinstein et al., 2013; Norman & Eacott, 2005; Spanswick &

Sutherland, 2010), with a few exceptions (Langston & Wood, 2010; Langston et al., 2010b). The disparate findings regarding the role of the hippocampus in OiC memory may reflect variations in task parameters across studies. For example, the variable delays employed differentially recruit short-term vs. long-term memory processes. As discussed before, the length of the retention interval between training and testing is determinant of the neurobiology of the OR task. Not only does the length of the retention interval influence whether NMDAR mechanisms are necessary for OR memory (Winters & Bussey, 2005), but also whether the hippocampus plays a role in this type of learning (Baker & Kim, 2002; Barker & Warburton, 2011; Hammond et al., 2004). Thus, it is possible that the hippocampus is differentially recruited for learning processes in the OiC depending on the retention interval. Despite this, some studies report impaired OiC memory using a short delay (2-5 min) following lesions of the fornix (Norman & Eacott, 2005) or hippocampus (Mumby et al., 2002). Clearly, variables other than retention length contribute to the role of the hippocampus in OiC memory. Our lab is currently investigating the ontogeny of long-term memory in the OiC task. This work will further elucidate the different processes involved in short-term versus long-term contextual memory.

A more likely determinant of the role of the hippocampus in contextual recognition memory is the spatial nature of the behavioral task (Nadel, 1991). The hippocampus has long been the focus of research on spatial memory systems (O'Keefe & Dostrovsky, 1971), and decades of research have supported the theory that hippocampal place cells support a neural representation system for the spatial environment, i.e., a "cognitive map" (for review, see Moser, Kropff, & Moser 2008; Moser, Rowland, & Moser, 2015). In alignment with this cognitive map theory, the

hippocampus may only be involved in contextual memory when spatial information about the environment must be encoded. Interestingly, data from studies examining the neural substrates of OiC memory support this idea. In studies that manipulated proximal (chamber) spatial cues but not distal (room) spatial cues, the hippocampus was not critical for OiC task performance (Langston et al., 2010b; Langston & Wood, 2010; Norman & Eacott, 2005). In the study by Norman & Eacott (2005), fornix lesions did lead to impaired performance in the OiC task relative to rats that underwent sham surgeries, but fornix lesioned rats still explored the novel target significantly above chance levels. In contrast, a study by Mumby and colleagues (2002) in which the distal spatial environment was the most salient difference between the contexts, OiC performance was eliminated by hippocampal lesions. 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 (2010). If the spatial nature of a given behavioral task determines its hippocampal-dependency (Nadel, 1991), the hippocampus may become engaged in the recognition memory tasks when spatial features of the contexts or objects must learned to perform the task.

Evidence from object recognition studies support the view that the hippocampus is involved in processing the spatial environment in recognition memory tasks. The hippocampus is normally not involved in short-term memory for objects (Mumby et al., 2002; Barker & Warburton, 2011; Langston et al., 2010b; Langston & Wood, 2010); however, the hippocampus becomes critical for OR memory when the spatial environment is made a prominent within the task. In two studies, lesions of the hippocampus did not disrupt OR when subjects were trained and tested in contexts that

differed only by proximal cues (Piterkin et al., 2008) but did disrupt OR memory when the change in context included the distal spatial environment (i.e., room cues), which could be perceived by animals (Piterkin et al., 2008; O'Brien et al., 2006). Moreover, dentate gyrus lesions impaired 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 (Dees & Kesner, 2013). These results suggest that the hippocampus is engaged in memory for the distal spatial environment. In Experiment 2A of this thesis we observed preference for the novel target in PD17, 21, and 26 rats when contexts differed in both proximal and distal cues, but only at PD26 when contexts differed only in distal spatial cues. It is possible that the OiC task does not require hippocampal function when salient proximal cues are provided, but the hippocampus becomes necessary when task performance requires utilizing the distal spatial environment. 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 development of hippocampus-mediated conjunctive spatial learning, as described previously.

Spatial learning in contextual recognition tasks can also involve learning spatial information about the objects, as in the OPC task. The hippocampus is known to be involved in forms of spatial recognition memory including the OL task (Mumby et al., 2002; Barker & Warburton, 2011; Langston et al., 2010b; Langston & Wood, 2010), the 4-object OiP task (Barker & Warburton, 2011), and the OPC task (Eacott & Norman, 2004). The ontogeny of these tasks are all similar in that performance emerges around or after the time of weaning in the rat, similar to hippocampus-dependent conjunctive spatial learning (Jablonski et al., 2012). Notably, associative

spatial recognition (OiP and OPC memory) develops between PD26 and 31 (Ainge & Langston, 2012; see Experiment 2B), which is much earlier than the emergence of OL memory between PD17 and 21 (Westbrook et al., 2014). The later development in these tasks may represent the development of a larger neural circuit mediating OiP and OPC memory. Associative spatial recognition in the 4-object OiP task relies on a functional circuit between the hippocampus, perirhinal cortex, and medial prefrontal cortex (mPFC) (Barker & Warburton, 2011). To make sense of these ontogenetic differences, future research should determine whether the perirhinal cortex and/or mPFC are necessary for OPC memory and other forms of contextual recognition memory during development.

The cognitive map theory of the hippocampus is amenable with, but not perfect in explaining, accounts of hippocampal involvement in spatial but not nonspatial recognition memory. In disagreement with this idea is the finding that performance of the 2-object OiP task is not disrupted by hippocampal lesions (Norman & Eacott, 2004; Langston et al., 2010b; Langston & Wood, 2010). This task is impaired by lesions of the lateral entorhinal cortex (LEC), which is thought to be part of the 'what' pathway conveying nonspatial (object) information to the hippocampus (the medial entorhinal cortex, or MEC, is thought to convey spatial information to the hippocampus via firing properties of grid cells) (Wilson et al., 2013b). The same study found that the LEC is not necessary for object recognition, but is necessary for place-context recognition, which does not involve object memory. These results and others such as the discovery of spatial selectivity of LEC neurons firing patterns following the placement and removal of an object in an environment (Deshmukh & Knierem, 2011). As discussed by Knierem, Neunuebel, and Deshmukh (2013), the dichotomous

view of nonspatial and spatial streams of information to the hippocampus needs to be reassessed. This growing body of work suggests that substrates upstream of the hippocampus may be able to process spatial features of a context during learning tasks, including those used in the current thesis.

Finally, we will consider the development of contextual recognition memory in terms of configural association theory (Sutherland & Rudy, 1989) which like the cognitive map theory, describes one of the most plausible models of hippocampal function. The configural association theory postulates that the hippocampal formation uniquely contributes to memory through its ability to form configural/conjunctive representations of stimuli (Figure 21A). This conjunctive representation is special in that it is distinct from the mere summation of the bound stimuli (i.e., the representation is greater than the sum of its parts). The phenomenon of conjunctive learning can be observed in negative patterning experiments, in which animals can learn to respond to rewarded individual stimuli (e.g., A+ and B+) but withhold responding to the unrewarded compound stimulus (AB-) (for review, see Sutherland & Rudy, 1989; Fanselow, 1999). Damage to the hippocampus prevents learning of the conjunction of AB- and animals respond twice as much to the unrewarded compound stimulus in expectation of a reward twice in magnitude compared to A+ or B+. This behavior represents the antithesis of conjunctive learning, simple associative learning, in which the compound stimulus is merely equal to the individual stimuli that compose it (i.e., AB = A + B). As noted by Nadel (1991), the theoretical frameworks for the configural association and cognitive map theories are not mutually exclusive. This idea is best exemplified by the CPFE, which requires conjunctive encoding of a spatial context

before that context can be associated with an aversive footshock (Jablonski et al., 2012).

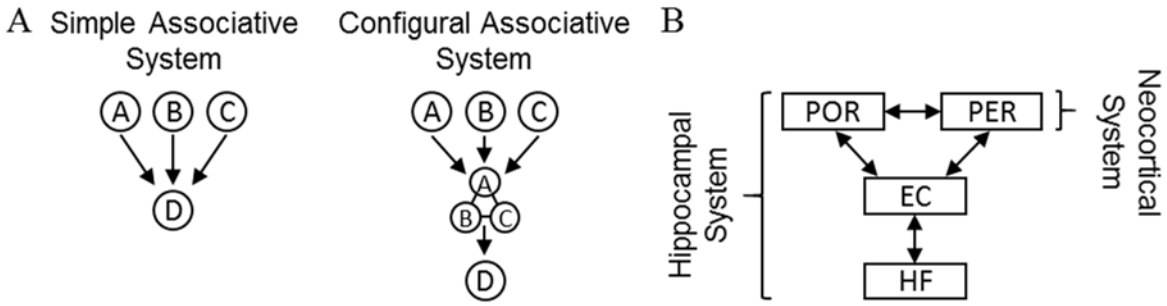

Figure 21 Illustration of the dual systems theory of stimulus associations (Panel A) and neural correlates (Panel B). In the simple associative system, stimuli/features/elements (A, B, and C) are individually associated with event D. In contrast, in the configural associative system, elements A, B, and C are bound into a unified, conjunctive representation that then is associated with event D. The simple associative system is thought to be mediated by neocortical areas including the POR and PER. The hippocampal system, including HF (hippocampus proper, dentate gyrus, and subiculum), is necessary to support configural associations. (POR) Postrhinal cortex; (PER) perirhinal cortex; (EC) entorhinal cortex; (HF) hippocampal formation. Adapted from Rudy, 2009.

At a neural systems level, the configural and simple associative systems are both supported by medial temporal lobe structures, especially in the case of learning and memory of conjunctive context representations (for review, see Rudy, 2009). The significant difference between these systems is that configural learning is dependent on the hippocampus (hippocampal system) whereas simple associative learning can be supported by extrahippocampal structures such as the perirhinal and postrhinal cortices (neocortical system; Figure 21B). In terms of development, the configural

associative system can support the CPFE on PD21 in the rat, but it is not known whether the conjunctive learning is possible before this age.

It is currently unknown whether contextual recognition memory is dependent on the hippocampal system to process conjunctive context representations. As stated above, it is currently unknown if PD17 rats, like those in our OiC task, can process conjunctions of contextual stimuli. It is possible that preweanling rats can perform the OiC task by associating the encountered objects with elemental, or features-based, context representations using the simple associative system. This strategy is independent of the hippocampal system, but instead relies on a neocortical system composed of primarily the rhinal cortices (Rudy, 2009), of which, the perirhinal region shows early ontogeny during the first two weeks of postnatal life (Furtak, Moyer, & Brown, 2007). The role of the perirhinal cortex in the OiC task is of particular interest as human fMRI studies have shown elicited activity in this region, and not the hippocampus, while viewing objects in incongruent contexts (Rémy, Vayssiére, Pins, Boucart, & Fabre-Thorpe, 2014) and during successful encoding of object-context associations in a visual task (Watson, Wilding, & Graham, 2012). In the rat, *c-fos* expression in the LEC (which is heavily innervated by the perirhinal cortex; Knierem et al., 2013), and not MEC or hippocampus, was elevated following the OiC task (Wilson et al., 2013a), and lesions of the LEC impaired OiC memory (Wilson et al., 2013a; Wilson et al., 2013b). Furthermore, perirhinal cortex lesions disrupted memory for object-object associations (Norman & Eacott, 2005), which may be the method preweanling rats perform our OiC task if they are using a simple associative (elemental) strategy.

Alternatively, preweanling rats may be able to learn conjunctive representations of the contexts in the OiC task. 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 (Experiment 2A), 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 (Wiltgen et al., 2006). 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. Ongoing studies in our lab aim to determine what contextual cues are used by PD17 rats during the OiC task. These experiments will further elucidate whether preweanling rats can learn conjunctive context representations.

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, place, grid, and border cells are detected in PD16-18 rats during environment exploration, with head direction and border cells exhibiting adult-like firing patterns at this very young age (Ainge & Langston, 2012; Langston et al., 2010a; Bjerknes, Moser, & Moser, 2014). Despite place and grid cells showing protracted development, head direction and border cells, and perhaps even developing grid cells, may provide rudimentary input to the

hippocampal place cells during this period. 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 (for review, see Stanton, 2000). For example, spatial delayed alternation (SDA) in a T-maze emerges as early as PD18 depending on task parameters (Green & Stanton, 1989; Freeman & Stanton, 1991; Jablonski, Watson, & Stanton, 2010; Stanton, Jensen, & Pickens, 1991). 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 (Stanton, 2000). Future research on the ontogeny of the OiC task will aim to better define the cognitive and neural processes that determine contextual recognition memory during early development, which will also inform our understanding of the ontogeny and neural substrates of contextual learning.

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