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Using the context preexposure facilitation effect to study long-term context memory in preweanling, juvenile, adolescent, and adult rats

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HIGHLIGHTS

- Context memory persists in the context preexposure facilitation effect (CPFE).
- Context memory persists for 22 days in adult but for only 15 days in juvenile rats.
- The CPFE may be useful for studying the neural basis of infantile amnesia.

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ABSTRACT

The present study used the context preexposure facilitation effect (CPFE) to examine long-term retention of incidental context learning in periweanling, adolescent and adult rats. The CPFE is a variant of contextual fear conditioning in which encoding the context representation, associating this representation with shock, and expressing the context–shock association each occur on separate occasions. Experiment 1 manipulated the retention interval—1 d, 8 d, 15 d, or 22 d—between context preexposure and training with immediate shock to determine how long the encoded context could be remembered (testing always occurred 24 h following training). The other factors were age—postnatal day (PND) 24 vs 31—and training group—Preexposed to the training context (Pre) vs. an alternate context (Alt-Pre). At both ages, significantly more freezing was evident in the Pre vs. Alt Pre Groups at the 24 h, 8 d and 15 d retention intervals but not at the 22 d interval, indicating that juvenile–adolescent rats remember the context for up to 15 d. In contrast, context memory persists for 22 days in adult rats (Experiment 2); and is not evident after 24 h, 8 d, or 15 d retention intervals in PND 17 rats (Experiment 3). The present study illustrates the value of the CPFE paradigm for investigations of long-term context memory in developing rats. Implications for the neurobiology of infantile amnesia are discussed.

1. Introduction

Infantile amnesia, the inability of adults to recall events learned in early childhood, has been studied for many years in both animal models [10] and humans [28–31]. The idea that infantile amnesia has a biological basis is also longstanding [10]. However, understanding of the neural mechanisms of the ontogeny of long-term memory remains poorly developed [2,8,21].

Recent proposals attribute poor long-term memory in infancy to hippocampal neurogenesis [2] altered neocortical storage [21] and/or retrieval [18]. Recent work on this problem has used hippocampus-dependent tasks, such as contextual fear conditioning [1,2] although standard contextual fear conditioning (sCFC), in which context exposure is directly paired with foot shock, only depends on the hippocampus under certain conditions [32,40]. In contrast, a variant of sCFC known as the context preexposure facilitation effect (CPFE) cannot be learned without a conjunctive representation of the context [20,34] and therefore always requires a functional hippocampus [27,32,33,36]. The CPFE procedure consists of the three phases that typically occur at 24-h intervals—context exposure, training, and testing. Context learning occurs on the first day, a retrieved representation of the context is associated with immediate-shock on the second day, and this results in enhanced freezing (relative to non-preexposed controls) on the test day [13]. Thus, in the CPFE, acquisition of the context representation and the association of the context with shock occur on different occasions, making it easier to manipulate and analyze these two components of context fear learning independently.

The CPFE has been used to probe the different components of context learning in rats during adulthood [13,35] and over the course of development [20,36]. Recently, data featuring the retention of context memory have shown that PND 17 mice retain sCFC for one day, with partial forgetting at 7 or 14 days, and no memory evident at 28 days [2]. In contrast, adult mice trained at PND 60 show full retention

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of sCFC at 28 days. A similar effect was observed in this same study using the CPFE where infant mice showed increased levels of forgetting relative to the adults at the 7, 14 and 28 day intervals. Currently, there is a lack of data concerning the ontogeny of long-term context memory in rats.

The CPFE emerges between PND 17 and PND 24 in rats in parallel with standard contextual fear conditioning, depends on hippocampal NMDA-receptor function [36] and requires preexposure to the combined elements of the context and not just its elemental features [20]. The present study used the CPFE to examine the ontogeny of long-term retention of context learning in Long Evans (LE) rats. The interval between preexposure and training was manipulated whereas the interval between training and testing was always 24 h. Rats were preexposed on either PND 24 or PND 31 with a 24 h, 8 day, 15 day, or 22 day retention interval between preexposure and training (Experiment 1). These ages were chosen because they show comparable levels of context fear with a 24-h retention interval [36] but long-term retention of context learning is likely to increase over this period [9]. In order to confirm the ability of adult rats to retain the context memory under the specific parameters of our CPFE paradigm, and to replicate previous reports of adult context memory retention [35], rats were also preexposed on PND 52 with only a 24-h retention interval or the longest interval (22 days) at which PND 24 and PND 31 failed to show the CPFE (Experiment 2). Finally, we preexposed pre-weanling rats on PND 17 and tested them with a 24-h, 8 day and 15 day retention interval (Experiment 3) to explore age differences in retention in pre-weanlings that might be masked by their inability to express the CPFE after 24-h [14]. Taken together these studies sought to determine whether there are ontogenetic differences in context memory retention that would result in differential expression of conditioned fear at testing after extended retention intervals between preexposure and training.

2. General methods

2.1. Subjects

Subjects were Long–Evans rats born in the animal colony at the University of Delaware and moved to in-lab colony rooms on PND 2. On PND 3, litters were culled to 8 pups (typically 4 males and 4 females) and weaned on PND 21, except where noted. Dams and their litters were housed in polypropylene cages measuring 8 in. high × 18 in. long × ×9 in. wide in an animal colony maintained on a 12:12 h light/dark schedule and in accordance with the NIH guidelines. Following weaning, pups were housed with 2–4 same-sex littermates and provided ad libitum food and water throughout the entire course of the experiment. No more than 1 same-sex littermate was assigned to a given experimental condition.

2.2. Apparatus

The apparatus has been previously described [7,36]. Conditioning occurred in 1 of 4 identical Plexiglas conditioning chambers connected to a grid-floor shock generator [36] situated under a fume hood, which provided the only source of overhead lighting and low-level background noise. There was also a white opaque sheet covering any adjacent walls between two chambers so that the animals could not see each other. The alternate context consisted of wire mesh cages housed within BRS-LVE sound-attenuating shells used for eyeblink conditioning [6,36]. Preexposure sessions occurred in one of these two sets of chambers which were situated in two different rooms. Training and testing always occurred in the Plexiglas chambers (see below).

2.3. Data analysis

Conditioned fear was assessed by measuring freezing during the contextual fear tests. Freezing was defined as the cessation of all visible movement except for respiration. The data were analyzed using FreezeFrame software (Actimetrics, Wilmette, IL) as previously described [7]. Data were analyzed via ANOVA and post hoc tests (Newman–Keuls) using Statistica software [36]. As in our previous reports, data points within each group that were outliers (scores exceeding +/− 2 standard deviations from other data points in their group) were removed from the statistical analyses. In total, about 9% of animals (25 of 271) were removed with the average z-score for all outliers being 3.26. Details concerning experimental factors and designs appear separately for each experiment below.

2.4. Experiment 1

The multiple-exposure CPFE procedure has been previously described by our lab [12]. The purpose of Experiment 1 was to determine how long juvenile rats could retain a context memory and whether the duration of retention differed from adolescent rats that show comparable levels of initial acquisition. Because the CPFE is fully developed by PND 24 and doesn't develop further at PND 31 [36], Experiment 1 also sought to examine possible ontogenetic differences in retention of the context memory between PND 24 and PND 31.

2.4.1. Method

Subjects were from 25 litters with 79 pups (41 males, 38 females) preexposed on PND 24 and 74 pups (35 males, 39 females) preexposed on PND 31. The design was a 2 (Sex: male vs. female) × 2 (Age: PND 24 vs. 31) × 2 (Preexposure group: Pre vs. Alt-Pre) × 4 (Retention interval: 1, 8, 15, and 22 days) between-groups factorial design, in which the retention interval refers to the period between preexposure and training (see below). The pups were assigned to these groups as follows: PND24-Pre-1 day (7 males, 6 females), PND24-Alt-Pre-1 day (6 males, 6 females), PND24-Pre-8 days (3 males, 4 females), PND24-Alt-Pre-8 days (4 males, 2 females), PND24-Pre-15 days (7 males, 5 females), PND24-Alt-Pre-15 days (5 males, 5 females), PND24-Pre-22 days (5 males, 6 females), PND24-Alt-Pre-22 days (4 males, 4 females), PND 31-Pre-1 day (4 males, 5 females), PND31-Alt-Pre-1 day (4 males, 4 females), PND31-Pre-8 days (4 males, 4 females), PND31-Alt-Pre-8 days (4 males, 5 females), PND31-Pre-15 days (5 males, 5 females), PND31-Alt-Pre-15 days (4 males, 5 females), PND31-Pre-22 days (5 males, 5 females), and PND31-Alt-Pre-22 days (5 males, 6 females).

The CPFE procedure took place in three phases: preexposure, training and testing with the preexposure-to-training interval varying across groups but with the training and testing sessions always occurring 24 h apart. On the preexposure day (PND 24 or PND 31), pups were preexposed using a multiple preexposure procedure [12,27] to either the training context (Pre Group) or the alternate context (Alt-Pre Group). The Pre Group pups were taken from their home cages, weighed, placed into opaque transport boxes and wheeled to a waiting area outside of the conditioning room while the chambers were cleaned using a 5% ammonium hydroxide solution. The Alt-Pre Group pups experienced the same procedure but were taken to the alternate context on the preexposure day for a total time approximately equal to that of the Pre Group. Pups were taken from their opaque transport boxes and placed inside the chambers for 5 min then removed and placed back in the chamber 5 times for 1 min at approximately 1 minute intervals.

There were 4 retention intervals between preexposure and training: 1 day (replicating the conventional CPFE), 8 days, 15 days and 22 days. After the designated retention interval, all pups (Pre or Alt-Pre) were trained in the Plexiglas conditioning context in which the Pre Group had previously been preexposed. Training consisted of immediate delivery of a 2 s, 1.5 mA scrambled foot shock [36]. In order to ensure immediate delivery of the shocks, pups were placed in their conditioning chamber one at a time. The placement-to-shock interval was less than 5 s. Following the immediate shock, pups were removed as quickly as possible and returned to their transport box and, after all pups were trained (~5 min) they were returned to their home cages.