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# Age-dependent changes in spatial memory retention and flexibility in mice

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ABSTRACT

In humans, memories for events happening early in life are forgotten more rapidly than those for events later in life. This form of accelerated forgetting in infancy is also observed in non-human species, and has been most extensively characterized in rats. Here we expand the characterization of infantile forgetting to mice, a species where a broader range of genetic tools can be used to understand the neurobiological mechanisms underlying this form of forgetting. Using a hidden platform version of the water maze task, we first assessed retention in mice that ranged in age from 15 to 150 days-old at the beginning of training. All groups exhibited spatial memory when tested one day after training. However, only mice that were 20 days or older at the time of training could remember one month later. Second, forgetting in younger cohorts of mice was not due to weaker encoding, since when younger mice were over-trained, such that their performance exceeded that of adult mice, they still exhibited forgetting. Third, in young mice, presentation of a reminder one month following training led to memory recovery, indicating that forgetting was due to a retrieval, rather than storage, deficit. Fourth, younger mice exhibited superior reversal learning compared to older mice, raising the possibility that a by-product of infantile forgetting might be greater flexibility.



## 1. Introduction

Infantile amnesia refers to the loss of episodic memories from our earliest childhood years. For example, as adults we have virtually no memories for events in the first three years of our lives, and then only inconsistent memory for events occurring between the ages of 3–7 (Rubin & Schulkind, 1997). Loss of these memories is due to accelerated rates of forgetting in infancy compared to adulthood (Wetzler & Sweeney, 1986). Psychological accounts of infantile amnesia have emphasized the co-emergence of a sense of self (Howe & Courage, 1993), theory of mind (Perner & Ruffman, 1995), and/or language (Nelson, 1993) with the emerging ability to form persistent memories of important events. However, similar accelerated forgetting is observed in non-human species, suggesting that human faculties such as self-identity and language are unlikely to provide a complete understanding of this phenomenon.

In non-human species, the majority of studies of infantile forgetting have been conducted in rats. Starting with the influential work of Byron Campbell and his colleagues, infantile amnesia has been demonstrated across a wide range of behavioral paradigms. These include conditioned suppression (Campbell & Campbell, 1962), passive avoidance (Campbell, Misanin, White, & Lytle, 1974; Feigley & Spear, 1970; Schulenburg, Riccio, & Stikes, 1971; Travaglia, Bisaz, Sweet, Blitzer, & Alberini, 2016), active avoidance (Campbell et al., 1974; Kirby, 1963; Klein & Spear, 1969), appetitive discrimination (Campbell, Jaynes, & Misanin, 1968), contextual fear conditioning (Rudy & Morledge, 1994; Weber, McNally, & Richardson, 2006), incidental context learning (Robinson-Drummer & Stanton, 2015), eyeblink conditioning (Brown & Freeman, 2014) and water maze (Brown & Kraemer, 1997).

Similar accelerated forgetting is observed in mice following contextual fear conditioning (Akers, Arruda-Carvalho, Josselyn, & Frankland, 2012; Akers et al., 2014). For example, adult mice exhibit robust contextual fear memories for up to one month following training. In contrast, infant mice (postnatal day 17; P17) exhibit robust contextual fear memory when tested 24 h following training, but these memories are forgotten at longer retention delays (Akers et al., 2014).

Genetic manipulations in mice provide additional opportunities to understand the neurobiological mechanisms of infantile

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amnesia. Therefore, the primary goal of the current study was to characterize forgetting in infant mice in another hippocampus-dependent learning paradigm. We elected to do this using the water maze, as young mice can be trained in the water maze (Chapillon & Roullet, 1996; Paylor, Baskall-Baldini, Yuva, & Wehner, 1996). Our results support the hypothesis that young mice show poorer retention compared to older mice. We find that over-training does not mitigate the accelerated forgetting observed in young mice, suggesting that forgetting is not simply due to inferior encoding. Moreover, we find that appropriate reminders lead to recovery of otherwise 'lost' memories, suggesting that neurodevelopmental changes do not erase spatial memories, but render these memories harder to access. Finally, we find that young mice exhibit superior reversal learning, and suggest that this, in part, is due to higher rates of forgetting at this developmental stage.

## 2. Methods

### 2.1. Mice

Mice were a cross between C57BL/6 (paternal) and 129Svev (maternal) strains (Taconic), which were bred in the Hospital for Sick Children animal facility. Mice were maintained on a 12 h light/dark cycle (lights on at 0700 h) with food and water available ad libitum. The day of birth was designated P0, and litter sizes ranged from 4 to 9 pups. After weaning (P21), mice were group-housed according to sex (2–5 per cage). To control for potential litter-dependent effects on memory, each litter was split across experiments such that no more than 3 mice per litter was included in a single experimental condition (Abbey & Howard, 1973). Females and males were assigned evenly across experimental conditions. All procedures were approved by the Animal Care Committee at The Hospital for Sick Children and Use Committee policies and conformed to both the Canadian Council on Animal Care (CCAC) and National Institutes of Health (NIH) Guidelines on the Care and Use of Laboratory Animals.

### 2.2. Water maze

*Basic training and test probes*: Mice were trained in the hidden platform version of the water maze. A circular pool (120 cm diameter, 50 cm height) was filled with water (28 °C) to a depth of 40 cm. Water was made opaque by the addition of nontoxic paint. A circular escape platform (10 cm diameter) was submerged approximately 0.5 cm below the surface of the water in the centre of one of the pool quadrants (N, S, E, W). The pool was surrounded by a curtain painted with five large, distinct geometric shapes located 1–1.5 m from the pool wall. In most experiments, mice received six training trials per day (in blocks of three trials separated by approximately 1 h) for three consecutive days. Each trial began by placing the mouse into the pool, facing the wall, from one of four possible start positions. The order of the release points varied pseudorandomly across days. The trial ended when the mouse reached the hidden escape platform or after 60 s had elapsed. If the mouse failed to locate the hidden platform, the experimenter's hand was placed over the platform (to serve as a visual cue) and the mouse was given an additional 15 s to find the platform. If the mouse failed to do so, it was gently guided to the platform. The mouse stayed on the platform for 15 s after which it was placed on a heated blanket for an additional 15 s (total inter-trial interval of approximately 30 s).

Memory was tested using a probe test. During the probe test, the escape platform was removed from the water and the mouse was allowed to swim freely for 60 s. The mouse's behavior in the pool was recorded by an overhead video camera and tracked using automated software (Watermaze 3.0, Actimetrics). During training, we analyzed escape latency, distance travelled, and swim speed. In the probe test, we quantified spatial memory by measuring amount of time mice spent searching in the target zone (20 cm radius, centered on location of platform during training, corresponding to 11% of pool surface) versus average time spent in three other equivalent zones in other areas of pool (Moser, Krobert, Moser, & Morris, 1998).

#### 2.2.1. Spatial memory retention

Different aged mice were trained and tested either one day (P15, $N = 12$; P17, $N = 14$; P20, $N = 15$; P25, $N = 10$; P50, $N = 14$; P150, $N = 10$) or 30 d (P15, $N = 14$; P17, $N = 15$; P20, $N = 15$; P25, $N = 12$; P50, $N = 13$; P150, $N = 17$) following training. In these experiments, we found that P15 mice (a) had slower swimming speeds and (b) weaker performance in the probe test 1 d after training compared to older mice. Therefore, in subsequent studies we used P17 infant mice to avoid these potentially confounding factors (slower swimming, weaker encoding).

#### 2.2.2. Overtraining and undertraining

In a subset of the experiments, P17 mice were extensively trained (12 trials a day for three days; 'overtraining' condition) and P50 mice were weakly trained (three trials a day for three days; 'weak training' condition). As before, separate cohorts of mice were tested at either 1 d (P17, $N = 10$; P50, $N = 14$) or 30 d (P17, $N = 10$; P50, $N = 13$) following training.

#### 2.2.3. Time course of forgetting in P17 mice

P17 mice were trained and tested either one day ($N = 14$), 15 d ($N = 11$) or 30 d ($N = 10$) following training.

#### 2.2.4. Reminders

In some experiments, P17 mice were given a 'reminder' of the platform location 30 days following the completion of training. The reminder consisted of placing a mouse on the platform (positioned in the training location) where they remained for 30 s. One ($N = 14$) or 24 h ($N = 14$) later, the mice were given a probe test. Some mice were presented with a 'misleading' reminder ($N = 14$). In this case, they were placed on the platform for 30 s. However, the platform was located in a position opposite to the training location. Memory was probed 1 h later.

#### 2.2.5. Reversal training

P17 ($N = 14$), P20 ($N = 7$), P25 ($N = 11$), P50 ($N = 13$) and P150 ($N = 8$) mice were trained for six trials a day over three days (as above). On day 30, reversal training took place. Mice received 10 training trials (in blocks of five, separated by 1 h) during which the hidden platform was located in the position opposite to that of initial training. A probe test was performed 24 h later. Amount of time spent in a 20 cm zone around where the platform was located during initial training (old zone) was compared to a similarly-sized zone centered on the new (reversal training) zone location.

### 2.3. Statistical analysis

We analyzed training data and probe test data using analysis of variance (ANOVA) or *t* tests, where appropriate. Following ANOVA, significant effects were further analyzed with Tukey's or Fisher's LSD post hoc tests.