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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.

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3. Results

3.1. Retention of spatial memory increases with age

We first characterized age-dependent changes in spatial memory retention. To do this, different aged mice (P15–P150) were trained in the water maze (six trials per day over three days) and then given a probe test either 1 d or 30 d later to assess their spatial memory (Fig. 1A). During training the youngest cohort of mice (P15) swam more slowly than the older cohorts (ANOVA, main effect of Age: $F_{5,440} = 18.32$, $P < 0.01$; Tukey post hoc tests, all Ps < 0.001) (Fig. 1B). Therefore, to assess performance during training, we examined distance traveled (rather than escape latency) in order to remove swim speed as a confound. Across training days,

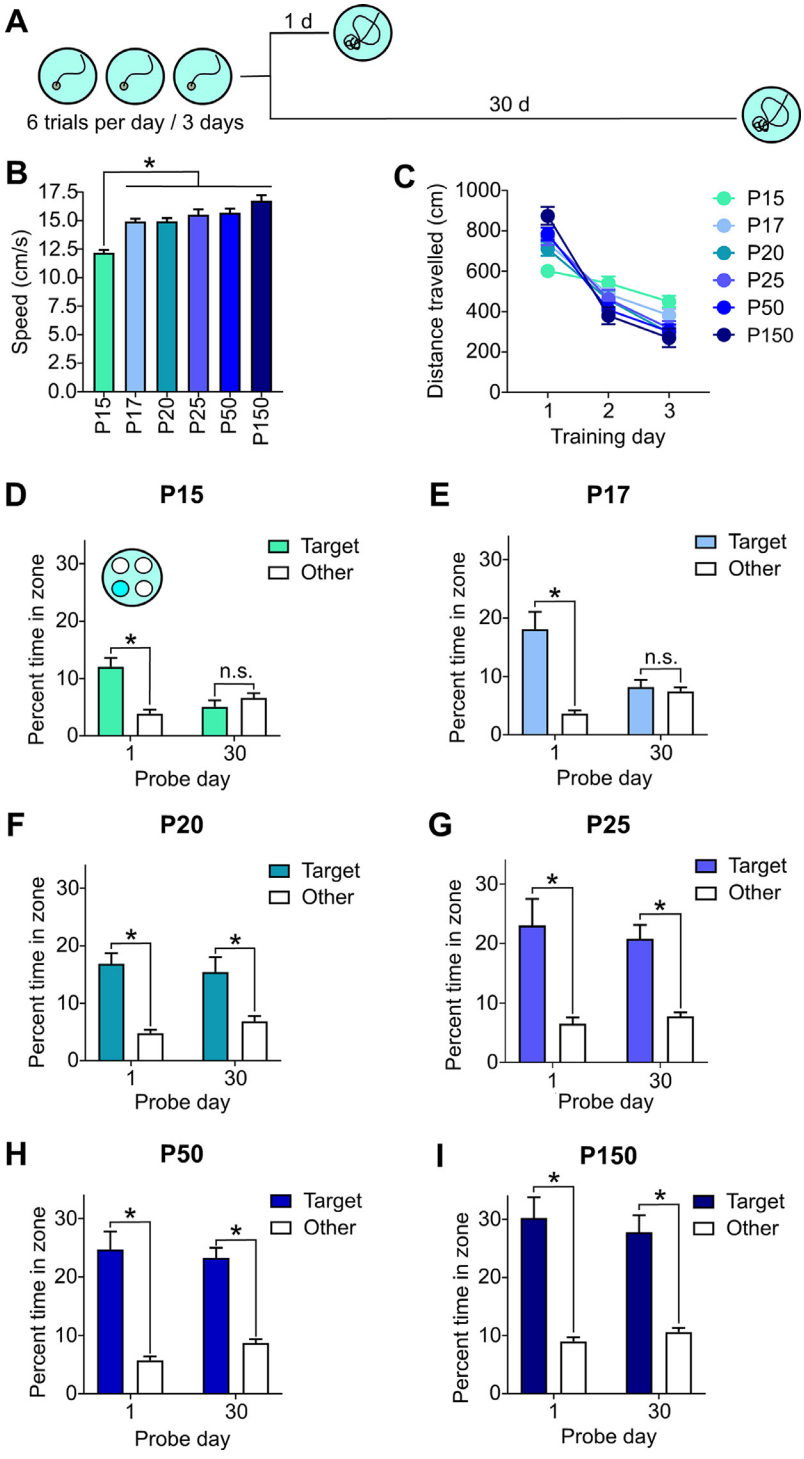

Fig. 1. *Age-dependent changes in spatial memory retention.* (A) Mice (P15–P150) were trained in the water maze and given a probe test either 1 d or 30 d later. (B) During training, average swim speed was lower in the youngest mice (P15) compared to other groups. (C) Average distance to platform declined across days during training. (D–I) Probe test performance in P15–P150 mice tested either 1 d or 30 d following the completion of training. Percent time spent in the target (closed bars) zone formerly containing the platform vs. the average of the three other (open bars) zones is shown. Graphs show means ± standard error of the mean. $^{*}P < 0.05$, n.s. = non-significant (Tukey's post hoc test).

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swim path lengths declined as expected (ANOVA, main effect of Training Day $F_{2,298} = 294.49$, $P < 0.01$). There was no effect of Age ($F_{5,149} = 0.64$, $P > 0.05$), indicating that different aged mice were equivalently efficient in locating the platform. However, we note that there was an Age × Training interaction ($F_{10,298} = 8.03$, $P < 0.01$), likely reflecting initially shorter swim paths in the P15 cohort on the first training day, and an overall flatter learning curve (Fig. 1C). Nonetheless, post hoc analyses revealed that swim paths were shorter on day 3 compared to day 1 in the P15 cohort ($P < 0.05$).

Following training mice were given a probe test either 1 d or 30 d later. For the P15 cohort, mice searched selectively when tested 1 d, but not 30 d, following training (Fig. 1D). Supporting this conclusion, an ANOVA with Retention as a between-subjects variable and Zone as a within-subjects variable revealed a significant Retention × Zone interaction ($F_{1,24} = 37.72$, $P < 0.01$), indicating that probe test performance varied with retention delay. Tukey post hoc tests confirmed that mice searched selectively at the 1 d (time spent in Target zone > time spent in Other zone; $P < 0.05$) but not 30 d ($P > 0.05$) test.

For the P17 cohort, a similar pattern emerged (Fig. 1E). Probe test performance varied with retention delay (Retention × Zone interaction, $F_{1,27} = 23.57$, $P < 0.01$). Tukey post hoc tests confirmed that mice searched selectively at the 1 d (Target zone > Other zone; $P < 0.05$) but not 30 d ($P > 0.05$) test.

For cohorts P20–P150 (Fig. 1F–I), probe test performance was similar regardless of retention delay. In each case, there was a Zone main effect (P20: $F_{1,28} = 37.37$, $P < 0.01$; P25: $F_{1,20} = 35.88$, $P < 0.01$; P50: $F_{1,25} = 82.49$, $P < 0.01$; P150: $F_{1,15} = 44.01$, $P < 0.01$) but no main effect of Retention (all Fs < 1) or Retention × Zone interaction (all Fs < 1). For each cohort, post hocs confirmed that mice searched selectively, spending more time in the Target zone compared to Other zones (all Ps < 0.05).

3.2. Forgetting occurs in younger mice even following over-training

In the first experiment mice searched selectively in the probe test 1 d following training, regardless of their age at the time of training. However, the degree of selectivity appeared to differ across groups, with the older (e.g., P50) mice spending more time in the target zone in the 1 d probe test than younger (e.g., P17) mice ($F_{1,27} = 8.16$, $P < 0.01$). Therefore, differences in performance at the 30 d retention delay might reflect either age-dependent differences in forgetting or, alternatively, age-dependent differences in initial memory strength. We addressed this second possibility by over-training young mice (P17; 12 trials per day for three days) and comparing their retention to older mice (P50) trained using a weak protocol (three trials per day for 3 days) (Fig. 2A). We reasoned that if differences in retention were due to differences in initial memory strength, then over-training might mitigate the observed forgetting in young mice. During training, swim path lengths declined in both groups of mice (main effect of Training Day: $F_{2,40} = 61.64$, $P < 0.01$) and, overall, path lengths were shorter in younger mice (main effect of Age: $F_{1,20} = 23.37$, $P < 0.01$) (Fig. 2B), reflecting the benefits of additional training. Post hoc analyses confirmed that path lengths were shorter in the younger mice on days 1 and 2 of training (Ps < 0.01).

In the probe test 1 d following training, young and adult mice both searched selectively. However, young mice spent more time in the target zone (Fig. 2D). Supporting this conclusion, an ANOVA with Age as a between-subjects variable and Zone as a within-subjects variable revealed a significant Age × Zone interaction ($F_{1,22} = 8.13$, $P < 0.01$), indicating that probe test performance varied with Age. Tukey post hoc tests confirmed that young and adult mice both searched selectively (Target > Other; Ps < 0.05), and young mice spent more time in the target zone compared to adult mice ($P < 0.05$). In the probe test 30 d following training, the adult, but not young, mice searched selectively (Fig. 2E). This was supported by a significant Age × Zone interaction ($F_{1,21} = 12.41$, $P < 0.01$), indicating that probe test performance varied with Age. Tukey post hoc tests confirmed that adult (Target > Other; $P < 0.05$) but not young ($P > 0.05$) mice searched selectively, and that adult mice spent more time in the target zone compared to young mice ($P < 0.05$).

Young mice initially expressed a stronger spatial bias compared to old mice (Fig. 2D). Yet, at the remote delay the young, but not old, mice exhibited forgetting (Fig. 2E). Supporting this dissociation, an ANOVA on the target zone data with Age and Retention as between-subjects variables revealed an Age × Retention interaction (Time spent in Target zone: $F_{1,43} = 16.69$, $P < 0.01$). Post hoc tests confirmed that young mice spent more time searching the target zone in the probe test 1 d following training, whereas old mice spent more time searching the target zone in the probe test 30 d following training (Ps < 0.05) (Fig. 2C).

3.3. Forgotten memories may be recovered following appropriate reminding

Next, we characterized the temporal profile of accelerated forgetting in infant mice. To do this, infant mice (P17) were trained as before (six trials per day for three days), and then separate groups were given a probe test to assess their spatial memory either 1, 15 or 30 d later (Fig. 3A). In this probe test, mice searched selectively when tested 1 or 15 d, but not 30 d, following training (Fig. 3B). An ANOVA with Retention as a between-subjects variable and Zone as a within-subjects variable revealed a Zone × Retention interaction ($F_{2,34} = 9.21$, $P < 0.01$). Post hoc tests confirmed mice spent more time searching the Target vs. Other zone in the 1 d and 15 d probe tests (Ps < 0.05). In contrast, mice spent equivalent time in the Target vs. Other zone in the probe test 30 d following training ($P > 0.05$).

This forgetting might be because the spatial memory no longer exists (i.e., a storage failure) or because it is no longer accessible (i.e., a retrieval failure). We next designed an experiment to distinguish between these two possibilities (Fig. 3C). Young mice (P17) were trained in the water maze as before (six trials per day for three days). Thirty days following training, mice received a reminder. This involved placing the mouse on the platform (for 30 s) in its original training location. Then either 1 or 24 h later the mice were given a probe test (with the platform removed from the pool). When tested 1 h after the reminder, mice searched selectively ($t_8 = 3.80$, $P < 0.05$) (Fig. 3D), indicating that the reminder was sufficient to recover the forgotten spatial memory. However, when a separate group was tested 24 h after the reminder, mice no longer searched selectively ($t_{13} = 1.95$, $P > 0.05$) (Fig. 3E), suggesting that this recovery is transient. An additional group received a misleading reminder (i.e., they were placed on a platform that was located in the opposite quadrant to training). In this case, the reminder was ineffective, and mice searched non-selectively ($t_{14} = 1.95$, $P > 0.05$) (Fig. 3F).

3.4. Flexibility decreases with age

One possible benefit of accelerated rates of forgetting might be a reduction in proactive interference (Epp, Silva Mera, Kohler, Josselyn, & Frankland, 2016). That is, forgetting one platform location might, in turn, facilitate learning a new, conflicting platform location.

We tested this possibility in the final experiment. Mice (P17–P150) were trained in the water maze to find the platform in a fixed location (six trials per day for three days). Thirty days later they were then retrained (reversal learning; 10 trials for one

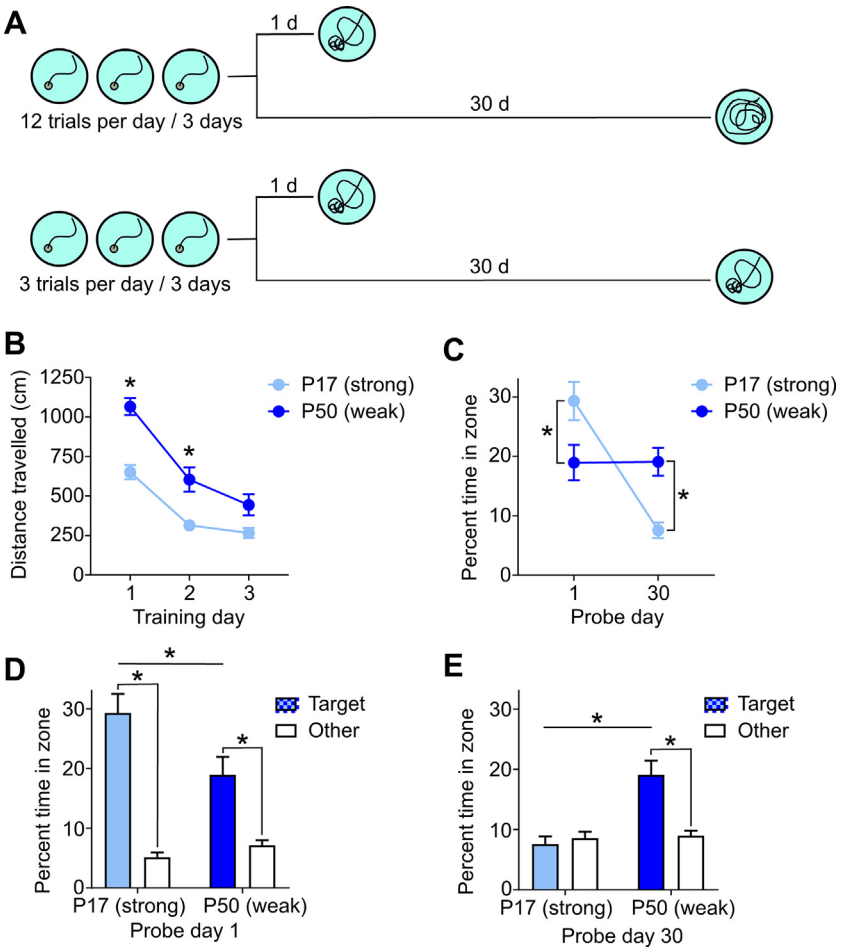

Fig. 2. *Over-training does not mitigate forgetting in infant mice.* (A) Infant (P17) mice were trained using a strong protocol (12 trials per day for three days) and adult mice (P50) were trained using a weak protocol, and then retention was tested either 1 d or 30 d later. (B) Across training, swim paths to the platform were shorter in infant mice. (C) In the probe test 1 d following training the infant mice outperformed the adult mice, spending more time in the target zone. In contrast, in the probe test 30 d following training the adult mice outperformed the infant mice, spending more time in the target zone. (D–E) Probe test performance in infant and adult mice tested either 1 d or 30 d following the completion of training. Percent time spent in the target (closed bars) zone formerly containing the platform vs. the average of the three other (open bars) zones is shown. Graphs show means ± standard error of the mean. $^{*}P < 0.05$, n.s. = non-significant (Tukey's post hoc test).

day), but this time the platform was moved to the quadrant opposite the training quadrant. Twenty-four hours later, the mice were given a probe test, and we assessed the extent to which mice searched in the new platform location vs. the original platform location (Fig. 4A). We reasoned that mice with greater levels of flexibility might more readily switch to the new platform location, and hypothesized that this form of flexibility might be greater in younger mice due to faster rates of forgetting of the original platform location.

In the probe test following reversal training mice displayed divergent patterns of searching (Fig. 4B). Younger mice (P17–P20 at the beginning of training) spent more time searching the new platform location compared to the old platform location. In contrast, the oldest cohort of mice (P150 at the beginning of training) spent more time searching the old platform location compared to the new platform location. To examine this age-dependent shift in search patterns, we conducted an ANOVA with Age as a between-subjects variable and Zone as a within- subjects variable. A significant Age × Zone interaction ($F_{4,96} = 4.11$, $P < 0.01$) indicated that the extent to which mice searched the new vs. the old platform location depended on age. Post hoc analyses indicated that only P17 and P20 mice spent more time searching the new compared to old platform location (Ps < 0.01). We then computed an index (time spent searching new platform location – time spent searching old platform location) that allowed us to directly compare flexibility across ages. We found that this index declined with age ($F_{4,48} = 3.16$, $P < 0.05$) (Fig. 4C), suggesting that flexibility declines with age. Post hoc analyses indicated that P17 mice were more flexible than P50 and P150 mice (Ps < 0.05), and P20 mice were more flexible than P150 mice ($P < 0.05$).

4. Discussion

In this series of experiments we characterized spatial memory retention and flexibility in infant and adult mice using the Morris water maze. There were four main findings. First, mice that were 15 days-old or older were able to learn to find the submerged platform in the water maze, and searched selectively for it in a probe test 24 h after the completion of training. However, only mice that were 20 days or older at the time of training remembered its location when tested one month later. Second, over-training P17 mice did not mitigate this forgetting, indicating that the accelerated forgetting observed in younger mice is unlikely to be due to formation of an initially weaker spatial memory. Third, a reminder treatment (placing the mouse back on the training platform) transiently led to memory recovery in younger mice, suggesting that forgetting is due to retrieval, rather than storage, failure. Fourth, younger

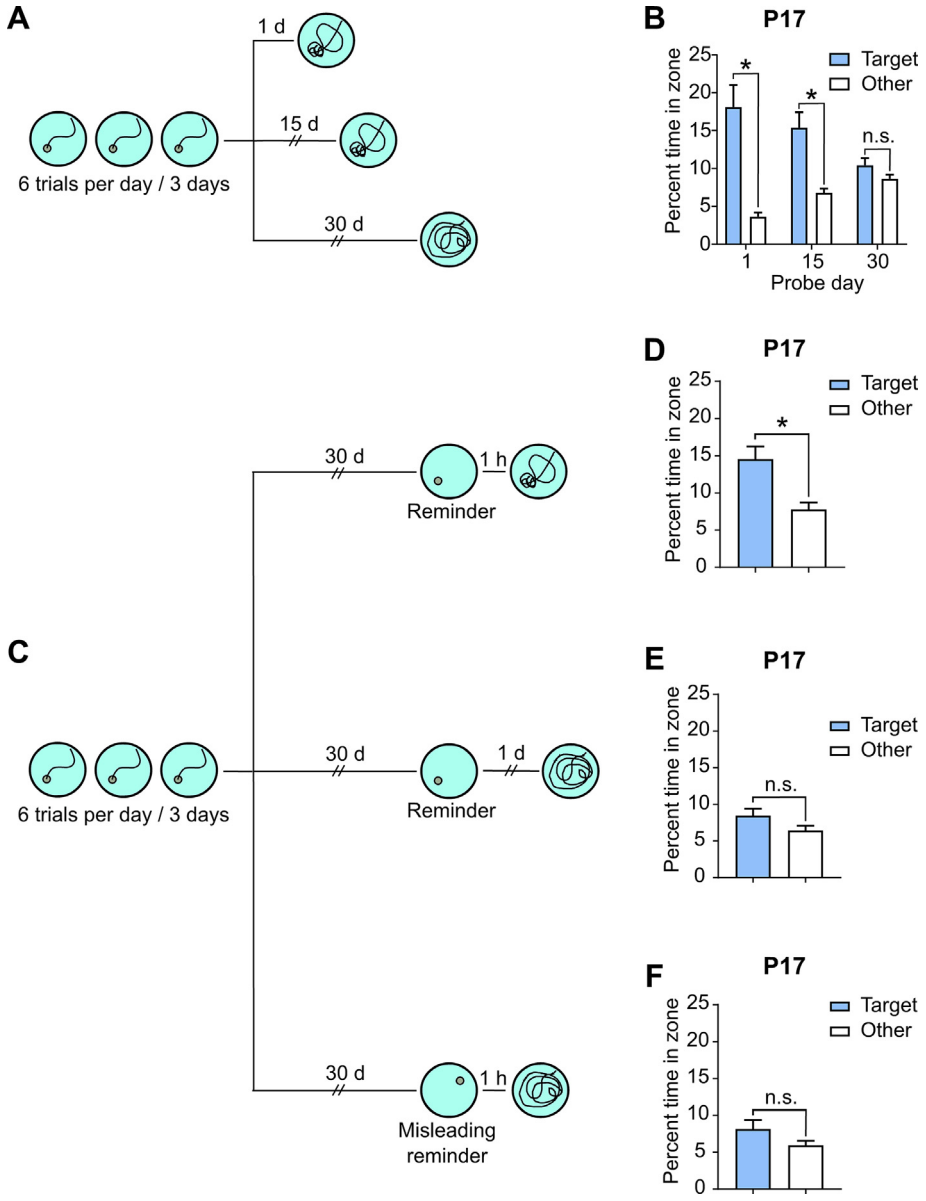

Fig. 3. *Reminders lead to transient recovery of lost memories in infant mice.* (A) Infant (P17) mice were trained tested either 1 d, 15 d or 30 d later. (B) In the probe tests, percent time searching the target (compared to Other) zones declined with increasing retention delay. (C) Infant (P17) mice were trained in the water maze and received a reminder (placement on training platform for 30 s) or misleading reminder (placement on platform in opposite zone) 30 days following training, and then were tested 1 h or 1 d later. (D–F) Probe test performance in mice receiving reminders. Percent time spent in the target (closed bars) zone formerly containing the platform vs. the average of the three other (open bars) zones is shown. Graphs show means ± standard error of the mean. $^{*}P < 0.05$, n.s. = non-significant (Tukey's post hoc test).

mice were more adept at reversal learning compared to older mice. Accelerated forgetting in younger mice likely contributes to this superior flexibility.

Previous studies have examined the ontogeny of memory in rodents, focusing in particular on the age at which hippocampus-dependent learning emerges. In rats, for example, the ability to form contextual fear memories (Brasser & Spear, 2004; Esmoris-Arranz, Mendez, & Spear, 2008; Kim & Richardson, 2010; Pisano, Ferreras, Krapacher, Paglini, & Arias, 2012; Raineki et al., 2010; Rudy, 1993; Rudy & Morledge, 1994; Schiffino, Murawski, Rosen, & Stanton, 2011) or allocentric spatial memories in the water maze (Akers & Hamilton, 2007; Brown & Kraemer, 1997; Carman, Booze, & Mactutus, 2002; Carman & Mactutus, 2001; Chapillon & Roullet, 1996; Paylor et al., 1996; Rudy, Stadler-Morris, & Albert, 1987) emerges between P17–P24. These forms of learning require that rats form representations of space, and spatial representations depend on head direction, place, and grid cells in the hippocampal formation. Interestingly, the emergence of these forms of spatial learning coincides with the emergence of head direction (P15–16), place (P16–17) and grid (P20–21) cell firing in rats (Langston et al., 2010; Wills, Barry, & Cacucci, 2012; Wills, Cacucci, Burgess, & O'Keefe, 2010).

A previous study reported that water maze learning was possible in P24 but not P17 mice (Paylor et al., 1996). In contrast, in the current study we found that water maze learning was possible at P15. There are two major methodological differences between the two studies. First, in our study mice received fewer training trials (18 trials over three days vs. 36 trials over three days). Second, in our study we used F1 C57B6/129 hybrid mice, whereas the previous study used C57B6 mice. It is most likely this latter difference is most important since C57B6/129 mice show superior performance in the water maze, requiring less training and achieving superior asymptotic performance compared to C57B6 mice (Kee, Teixeira, Wang, & Frankland, 2007). Similar to the current study,

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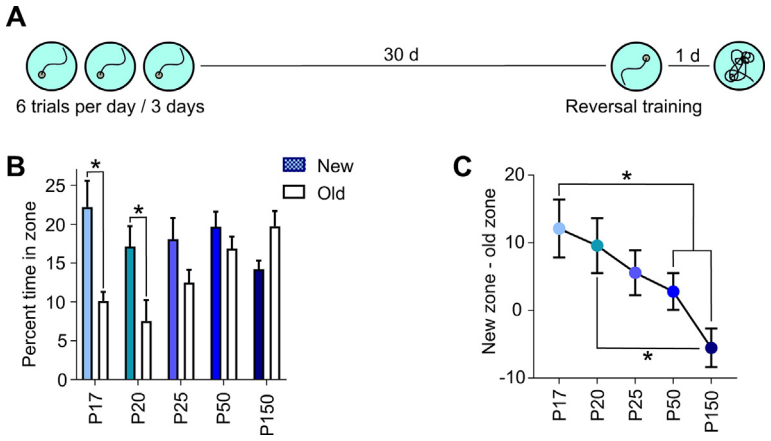

Fig. 4. *Superior reversal learning in young vs. old mice.* (A) Mice (P17–P150) were trained in the water maze and 30 d later retrained with the platform moved to the opposite zone. One day later, they were given a probe test and the amount of time searching the new vs. old platform location assessed. (B) In the probe tests, percent time searching the new platform zone (closed bars) compared to old platform zone (open bars) in P17–P150 mice. (C) Preference for new platform location (time spent in new platform zone - time spent in old platform zone) declined with age. Graphs show means ± standard error of the mean.

we previously found that contextual fear conditioning was possible at P13–14 in mice. This suggests that head direction, place and grid cell firing may emerge earlier in mice, although this has not yet been assessed. More generally, these data support the idea that brain and behavioral development is faster in mice compared to rats (Pellis & Iwaniuk, 2000; Whishaw, Metz, Kolb, & Pellis, 2001).

A major focus in our study was on the retention of spatial memories. We found that mice that were P20 or older at the beginning of training could remember the location of the submerged platform for at least one month. In contrast, mice that were P15 or P17 at the beginning of training exhibited pronounced forgetting when tested one month (but not one day) following the completion of training. Rates of forgetting might be modulated by a variety of factors (e.g., amount of initial training, type of learning). Nonetheless, these forms of accelerated forgetting in young mice have been consistently observed in many rodents (and other species) and correspond to infantile amnesia observed in humans. In rats, memories acquired in infancy or juvenility are rapidly forgotten (Josselyn & Frankland, 2012). In these rat studies, memories formed as late as P24–31 were forgotten (e.g., (Robinson-Drummer & Stanton, 2015)). In contrast, at this same age mice appear to be capable of forming stable, long-lasting, hippocampus-dependent memories. For example, in the current study, P20 mice can form spatial memories that last at least a month. This suggests that the transition from an infantile state, where memories are rapidly forgotten, to a more adult-like state, where memories persist, occurs earlier in mice compared to rats.

The forgetting in mice younger than 20 days-old may be mediated by multiple mechanisms acting both in the short- and long-term. For example, following learning, the rapid internalization of AMPA receptors (and associated depotentiation of learning-dependent increases in synaptic strength) contributes to memory weakening in the short-term (i.e., hours-days; Migues et al., 2016). In the long term (i.e., days-weeks), the continuous integration of new neurons into hippocampal circuits also weakens memories and leads to forgetting (Akers et al., 2014; Epp et al., 2016). In this case, the integration of new neurons is associated with both the addition of new synaptic connections, as well as the elimination of existing synaptic connections (Toni et al., 2007; Toni et al., 2008), and this remodeling likely renders information stored within hippocampal circuits harder to access (i.e., a retrieval deficit) (Frankland & Josselyn, 2016; Frankland, Kohler, & Josselyn, 2013). In our experiments we did not manipulate AMPA trafficking or neurogenesis levels, and it is plausible that both these factors contribute to the observed forgetting. Certainly, we have previously linked heightened levels of hippocampal neurogenesis in young mice to accelerated forgetting observed at this stage of development (Akers et al., 2014). Whether there are age-dependent changes in AMPA trafficking that could account for accelerated forgetting in infancy has not been assessed.

When P15 or P17 mice were tested one month after training, they spent an equivalent amount of time searching the target zone (that formerly contained the platform) vs. other equivalent zones in the pool, suggesting the memory loss was profound. However, we found that reminders led to recovery of the seemingly 'lost' memories in infant P17 mice, suggesting that memories are inaccessible (i.e., retrieval failure) rather than erased (i.e., storage failure) at this developmental stage. This conclusion is consistent with several other studies showing that pharmacological (Kim, McNally, & Richardson, 2006) or behavioral (Travaglia et al., 2016) interventions can serve to recover otherwise 'lost' memories in young rodents. We note that the memory recovery was transient. Perhaps stronger (or multiple) reminders would be sufficient to produce more sustained memory recovery.

In our final experiment, we found that younger mice exhibited better reversal learning compared to older mice. One month following training, young mice were able to quickly locate the platform when it was moved to the opposite part of the pool. In contrast, older mice tended to search more at the original platform location more than the new platform location. P17 mice exhibit pronounced forgetting one month following training, and therefore in many respects enhanced flexibility is unsurprising given that there should be little or no interference from memory for the original platform location. However, the P20 group is more interesting. These mice did not forget over the course of a month (e.g., Fig. 1F), yet still exhibit enhanced flexibility compared to older mice (Fig. 4B and C). Our interpretation is that there is degradation of the memory trace for the original platform position over the course of a month in the P20 mice. However, this deterioration does not manifest at the behavioral level during the probe test. Nonetheless, a weaker memory trace for the original platform location interferes to a lesser extent with learning the new platform location, and so these mice exhibit enhanced flexibility. These observations echo several previous studies that have examined age-dependent changes in reversal learning in a number of different species. For example, age-dependent reversal learning impairments have been observed in mice (Johnson & Wilbrecht, 2011), rats (Schoenbaum, Nugent, Saddoris, & Gallagher, 2002), dogs (Milgram et al., 2004), and monkeys (Bartus, Dean, & Fleming, 1979) in a variety of behavioral paradigms. While these deficits are usually attributed to fron-

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tal cortex dysfunction (Izquierdo, Brigman, Radke, Rudebeck, & Holmes, 2016), our data suggest that reduced rates of forgetting (or engram deterioration) in older animals might also contribute to reduced flexibility.

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