Research

Recovery of memory from infantile amnesia is developmentally constrained

Reto Bisaz,[1] Benjamin Bessières,[1] Janelle M. Miranda, Alessio Travaglia, and Cristina M. Alberini

Center for Neural Science, New York University, New York, New York 10003, USA

Episodic memories formed during infancy are rapidly forgotten, a phenomenon associated with infantile amnesia, the inability of adults to recall early-life memories. In both rats and mice, infantile memories, although not expressed, are actually stored long term in a latent form. These latent memories can be reinstated later in life by certain behavioral reminders or by artificial reactivations of neuronal ensembles activated at training. Whether the recovery of infantile memories is limited by developmental age, maternal presence, or contingency of stimuli presentation remains to be determined. Here, we show that the return of inhibitory avoidance memory in rats following a behavioral reactivation consisting of an exposure to the context (conditioned stimuli [CS]) and footshock (unconditioned stimuli [US]) given in a temporally unpaired fashion, is evident immediately after US and is limited by the developmental age at which the reactivations are presented; however, it is not influenced by maternal presence or the time interval between training and reactivation. We conclude that one limiting factor for infantile memory reinstatement is developmental age, suggesting that a brain maturation process is necessary to allow the recovery of a "lost" infantile memory.

Hippocampus-dependent episodic memories formed early in life are rapidly forgotten. This process of forgetting is evolutionarily conserved and is associated with infantile amnesia, the inability of adults to recall early-life memories (Campbell and Spear 1972; Rovee-Collier 1999; Hayne 2004; Josselyn and Frankland 2012; Callaghan et al. 2014; Madsen and Kim 2016; Alberini and Travaglia 2017).

Although they are rapidly forgotten, early-life experiences influence brain functions throughout life (Jacobs and Nadel 1985; Meaney et al. 1988; Sroufe et al. 1990; Brunson et al. 2005; Pryce et al. 2005; Mineka and Zinbarg 2006; Bale et al. 2010; Poulos et al. 2014; Perry and Sullivan 2014) and produce long-lasting biological changes in the brain. For example, aversive early-life experiences regulate the expression of hippocampal glucocorticoid receptor and hypothalamic–pituitary–adrenal (HPA) functions in a persistent fashion (Champagne and Curley 2009). Moreover, threatening experiences in early life can predispose individuals to psychopathologies such as post-traumatic stress disorder (PTSD) and mood and anxiety disorders (Heim and Nemeroff 2001). Consistent with this long-lasting influence on behavior, studies in rodent models have shown that, in contrast to what was previously believed, memories formed in infancy (at postnatal days 16–18 [PN16–PN18]) although not expressed, are not lost. Rather, they are stored over the longer term in a latent form and can reemerge at later times, up to adulthood, following behavioral reactivations or artificial stimulation of the neuronal ensembles activated at learning (Travaglia et al. 2016; Guskjolen et al. 2018; Bessières et al. 2020).

In our previous studies based on rat inhibitory avoidance (IA), a paradigm in which the animal learns to avoid a context previously paired with a footshock, we confirmed that an infantile episodic learning event given at PN17 resulted in the typical rapid forgetting. We then found that, however, this memory was stored long term in a latent form, as demonstrated by the observation that it could reemerge following a behavioral reactivation consisting of exposure to the training context (conditioned stimulus [CS]) and a later time footshock of the same intensity that was used during training (unconditioned stimulus [US]) but now given in a distinct context. Reexperiencing either the context or the footshock alone failed to reinstate the infantile memory (Travaglia et al. 2016), suggesting that the return of infantile memory is limited by certain boundaries.

Several questions about the conditions for the recovery of latent, infantile episodic memories remain to be addressed. First, is infantile memory reinstatement following the unpaired US presentation temporally regulated? Second, is there an age limit for memory reinstatement? Third, given that other types of learning, such as non-hippocampus-dependent cued conditioning, are regulated by the maternal presence during infancy (Moriceau and Sullivan 2006), is infantile episodic memory reinstatement limited or regulated by the presence of the mother? In this study, we set out to address these questions using IA in infant rats.

Results

Latent infantile memory returns immediately after unpaired CS and US presentation

In previous studies, we showed that forgotten infantile IA memory in rats was significantly reinstated following the presentation of the CS (exposure to the training context during a retention test, indicated as T) and the US (a reminder footshock, indicated as RS) of the same intensity as that experienced during training but given in a different context hours or even days later. T + RS, but not T or RS

[1]**These authors contributed equally to this work.**
Corresponding author: ca60@nyu.edu
Article is online at http://www.learnmem.org/cgi/doi/10.1101/lm.052621.120.

alone, was able to reinstate a strong, long-lasting, and context-specific IA memory (Travaglia et al. 2016).

Here, we sought to determine how rapidly after the CS + US presentation the infantile memory encoded at PN17 is recovered. In agreement with our previous studies (Travaglia et al. 2016; Bessières et al. 2020), rats trained at PN17 had no memory when tested 7 d after training (T1) (Fig. 1). However, immediately after the presentation of an RS given 2 d after T1, the rats exhibited significant memory retention (T2) (two-way repeated-measures [RM] ANOVA followed by Bonferroni's multiple comparisons test; condition $F_{(2,104)} = 54.45$; $P < 0.0001$; testing $F_{(3,104)} = 18.61$; $P < 0.0001$; interaction $F_{(6,104)} = 13.75$; $P < 0.0001$) (Fig. 1). PN17 littermates, which were either left in their home cage (naive group) or received a shock of the same intensity immediately after being placed on a grid without being exposed to the context (shock-only group), exhibited no memory after T1 + RS, confirming that the reinstatement protocol alone did not evoke any IA response in these control groups (T2) (Fig. 1). The memory reinstated in the trained group was long-lasting, and persisted when retested 7 d later (T3, $P < 0.001$). Moreover, this memory did not generalize to a new context (Ctx B, trained vs. naive and shock-only, $P > 0.05$) (Fig. 1), indicating that the returned memory was selective for the context learned during the infantile experience. Collectively, these data revealed that the memory encoded in infancy emerges immediately after reexperiencing a US presentation subsequent to a CS given in a temporally unpaired fashion.

Reinstatement of infantile memory is developmentally regulated

We previously showed that the forgotten infantile IA memory can be recovered for a long period of time; in fact, T + RS effectively reinstates a memory even when presented up to 4 wk after training at PN17 (Travaglia et al. 2016). Similar results were obtained following artificial stimulations of the neuronal ensemble tagged by induction of immediate early genes (i.e., *c-Fos* or *Arc*) at contextual fear conditioning training in mice, leading to memory reinstatement up to 90 d after training (Guskjolen et al. 2018; Bessières et al. 2020). Hence, the recovery of the latent infantile memory can be evoked for a long time after the memory is formed. However, it remains to be determined whether there is an early age limit for the ability to reinstate infantile memory. To address

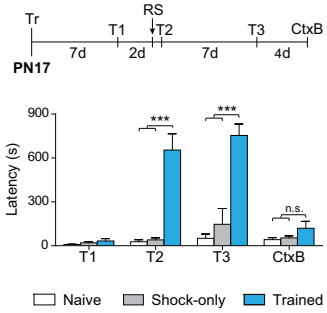

Figure 1. Memory acquired at PN17 is rapidly forgotten but reemerges immediately after reactivation. PN17 rats were left in their home cage (naive), exposed to a shock-only protocol, or subjected to a single IA training trial (Tr). Rats were tested 7 d after IA training (T1), or at matched time points for the other two groups. Two days later, all animals were given a reminder footshock (RS, black arrow) in a different context and then retested immediately after (T2) and 7 d later (T3) in the original IA training context. Four days after T3, the rats were tested in a new IA context (context B [CtxB]). Memory retention is expressed as mean latency ± SEM (in seconds [s]). Two-way repeated measures (RM) ANOVA followed by Bonferroni's multiple comparisons test: (***) $P < 0.001$. $n = 7$–8 rats per group.

this question, we presented the reinstatement protocol (T + RS) 1, 3, or 5 d after IA training of rats at PN17 (Fig. 2). Control littermates either remained in the home cage or received an immediate shock at PN17.

As expected, none of the trained rats exhibited memory retention at the first test (T1), given 1 d (Fig. 2A), 3 d (Fig. 2C), or 5 d (Fig. 2E) after training, at which they performed similarly to the naive and shock-only control groups (Fig. 2A,C,E). T + RS given 3 d (Fig. 2C) or 5 d (Fig. 2E) after training led to significant memory reinstatement (3 d: two-way RM ANOVA followed by Bonferroni's multiple comparisons test: condition $F_{(2,80)} = 36.10$, $P < 0.0001$; testing $F_{(3,80)} = 10.88$, $P < 0.0001$; interaction $F_{(6,80)} = 10.14$, $P < 0.0001$; 5 d: condition $F_{(2,76)} = 17.22$, $P < 0.0001$; testing $F_{(3,76)} = 5.136$, $P = 0.0027$; interaction $F_{(6,76)} = 4.655$, $P = 0.0004$), whereas T + RS presented 1 d after training completely failed to reinstate memory (two-way RM ANOVA followed by Bonferroni's multiple comparisons test: $F_{(4,66)} = 1.01$, $P = 0.4080$) (Fig. 2A). The memories reinstated by T + RS were long-lasting and context-specific (Fig. 2C,E).

Consistent with our previous studies (Travaglia et al. 2016), RS alone was unable to reinstate the latent infantile memory (Fig. 2B,D,F) as, indeed, rats trained at PN17 and exposed to RS 3 d (Fig. 2B), 5 d (Fig. 2D), or 7 d (Fig. 2F) later, did not exhibit any significant memory reinstatement (two-way RM ANOVA followed by Bonferroni's multiple comparisons test: for Fig. 2B, $F_{(2,36)} = 0.359$, $P = 0.7011$; for Fig. 2D, $F_{(2,36)} = 0.4188$, $P = 0.661$; for Fig. 2F, $F_{(2,36)} = 0.9411$, $P = 0.3996$). These results confirmed that exposure to the original training context followed by RS is necessary to reinstate the memory.

Collectively, these data indicate that a developmental or temporal boundary limits the ability to reinstate the infantile memory.

The age of the animal, but not the interval between training and reactivation, limits the ability to reinstate a latent infantile memory

Next, we asked whether the developmental boundary that limits the reinstatement of the memory encoded at PN17 is due to the interval between the training and reactivation (T + RS) or to the age of the animal at which the reactivation is presented. As shown above, for the T + RS reinstatement protocol to be effective, it needs to be given at least 3 d after training at PN17. Because training evokes biological and functional maturation (Travaglia et al. 2016; Bessières et al. 2020), we hypothesized that the time interval of 3 d between training and reactivation is necessary in order to allow the maturation process after learning to take place. An alternative explanation is that infantile memories can be reinstated only when the animal reaches a certain age. To distinguish between these possibilities, we designed two experiments. The first experiment tested whether training the rats a day earlier (at PN16) and allowing 3 d before presenting T + RS could reinstate the memory. As shown in Figure 3A, no memory reinstatement was detected 1 d (T2) or 7 d (T3) after the reactivation (T + RS, two-way RM ANOVA followed by Bonferroni's multiple comparisons test: $F_{(4,63)} = 0.4895$, $P = 0.7434$), indicating that the time interval between training and reactivation is not the factor that limits the reinstatement.

In the second experiment, we asked whether the developmental boundary that limits the reinstatement of the infantile memory is the age of the animal. Rats were trained at a later age (PN19), and the reinstatement protocol was started 1 d later. As shown in Figure 3B, when IA training was given at PN19, followed by T + RS 1 d later, the animals were able to reinstate a robust, long-lasting, and context-specific memory (two-way RM ANOVA followed by Bonferroni's multiple comparisons test: condition $F_{(2,80)} = 59.80$, $P < 0.0001$; testing $F_{(3,80)} = 21.38$, $P < 0.001$; interaction $F_{(6,80)} = 17.95$, $P < 0.0001$). Thus, the age of the animal at the time of experiencing reactivation, and not the time interval

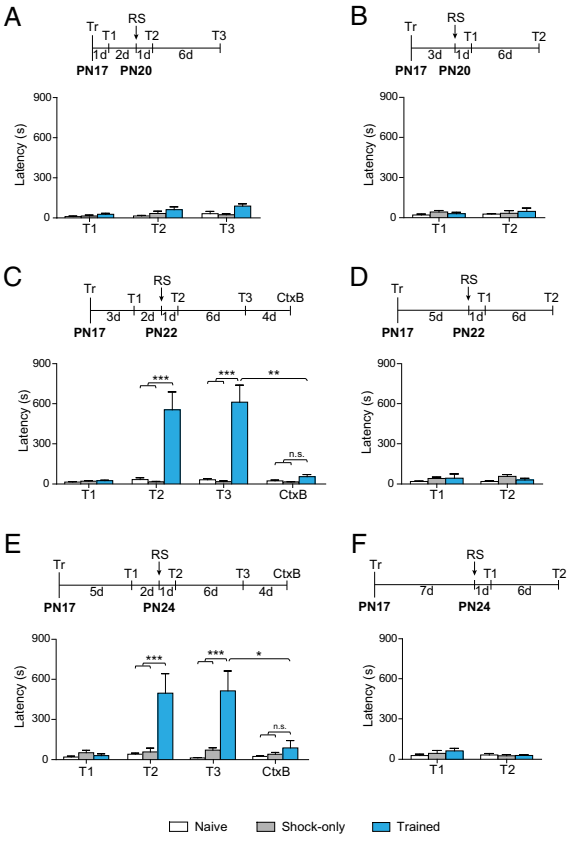

Figure 2. Reinstatement of infantile memory is developmentally limited. (*A*) PN17 rats were either left in their home cage (naive), exposed to a shock-only protocol, or subjected to a single IA training trial (Tr). Rats were tested 1 d (T1) after IA training or at matched time points for the other two groups. Two days later (at PN20), all animals were given a reminder footshock (RS, black arrow) in a different context and then retested 1 d (T2) and 7 d later (T3). (*B*) PN17 naive, shock-only, and trained rats received a RS (black arrow) at PN20 and then were tested 1 d (T1) and again 6 d later (T2). (C) PN17 rats were left in their home cage (naive), exposed to a shock-only protocol, or subjected to a single IA training trial. Rats were tested 3 d (T1) after IA training (Tr), or at matched time points for the other two groups. Two days later (at PN22), all animals were given a reminder footshock (RS, black arrow) in a different context, and then retested 1 d (T2) and 7 d later (T3). Four days after T3, the rats were tested in a new IA context (context B [CtxB]). (*D*) PN17 naive, shock-only, and trained rats received a RS (black arrow) at PN22 and were then tested 1 d (T1) and again 6 d later (T2). (*E*) PN17 rats were left in their home cage (naive), exposed to a shock-only protocol, or subjected to a single IA training trial. Rats were tested 5 d (T1) after IA training (Tr) or at matched time points for the other two groups. Two days later (at PN24), all animals were given a reminder footshock (RS, black arrow) in a different context and then retested 1 d (T2) and 7 d later (T3). Four days after T3, the rats were tested in a new IA context (context B [CtxB]). (*F*) PN17 naive, shock-only, and trained rats received a RS (black arrow) at PN24 and were then tested 1 d (T1) and again 6 d later (T2). Memory retention is expressed as mean latency ± SEM (in seconds [s]). Two-way RM ANOVA followed by Bonferroni's multiple comparisons test: (*) $P<0.05$, (**) $P<0.01$, (***) $P<0.001$. $n=6$–9 rats per group.

between training and reactivation, is the limiting factor that restricts the ability to recover the infantile memory.

Maternal presence does not affect the ability to reinstate the latent infantile memory

Given that the expression and behavioral modality of some early memories, such as cued learning, are regulated by maternal presence during infancy (Moriceau and Sullivan 2006), we considered that one factor that might affect the expression of episodic infantile memories is the presence of the dam. Hence, we investigated whether reinstatement of the latent infantile memory is influenced by the maternal presence.

Rats were trained at PN17 and weaned either at PN20 or PN23 —that is, before or after the reinstatement procedure (T + RS), which was given 4 d after training. As shown in Figure 4, A and B, exposure to T + RS 4 d after training at PN17 effectively reinstated the memory in both conditions, regardless of the presence of the dam in the home cage (two-way RM ANOVA followed by Bonferroni's multiple comparisons test: for Fig. 4A, $F_{(6,60)}=18.73$, $P<0.0001$; for Fig. 4B, $F_{(6,62)}=9.352$, $P<0.0001$). In fact, all the PN17-trained animals exhibited significant reinstatement of the latent IA memory when tested 1 d (T2) or 7 d (T3) after the RS (trained vs. naive and shock-only, $P<0.001$). We concluded that the presence of the dam does not influence the ability to reinstate infantile memory.

Discussion

Understanding the behavioral responses and underlying mechanisms of infantile memories is key to unraveling how memory systems develop and function in adulthood. Until recently, it was believed that the infant hippocampus and related memory system, because immature, are not functionally involved in episodic learning, and that they remain "offline" until relatively late in development when memories are formed and expressed long term (Kagan 1984; Rudy et al. 1987; Kraemer and Randall 1995; Pugh and Rudy 1996; Stanton 2000; Bauer 2006; Newcombe et al. 2007; Akers and Hamilton 2007). Our studies in rats demonstrated that this view was not correct: instead, the infant hippocampus, for example, at PN17, is necessary for forming long-lasting memories of contexts, objects, and places, even though these memories appear to be rapidly forgotten. In fact, these apparently forgotten memories can be recovered later in life by certain behavioral reminders or reactivations (Travaglia et al. 2016, Alberini and Travaglia 2017). Subsequent studies confirmed and extended these findings in multiple species, using different types of reminders as well as artificial reactivation of neuronal ensembles activated at training (Travaglia et al. 2018, Guskjolen et al. 2018; Bessières et al. 2020).

Numerous questions remain to be addressed about the processes underlying the storage of infantile memories in latent form and their recovery following reactivations. In this study, using rat IA, we showed that reexposure to CS (test, T) and US (reminder shock, RS) given in a temporally unpaired manner (i.e., two or more days apart) resulted in the recovery of memory, which was expressed immediately after the US. We also showed that the ability to recover infantile memory has a developmental age limit: The memory can be reinstated if the reactivation is given starting with the CS at PN20, but not at PN18, suggesting that some developmental maturation must occur in order to enable the system to reinstate the memory. Finally, we showed that memory recovery is not influenced by maternal presence.

The brain regions and systems necessary for memory reinstatement remain to be identified. Consistent with the well-known role of the hippocampus in associative learning (Maren et al. 2013; Weiss and Disterhoft 2015), the dorsal hippocampus is required for formation of infantile memory, but it is dispensable for memory reinstatement evoked by T followed by RS given in a different context 2 d later (Travaglia et al. 2016). Furthermore, memory reinstatement does not occur if the US is presented before CS, that is, if the RS is presented before testing (Fig. 2; Travaglia et al. 2016), implying that the return of memory requires the sequence

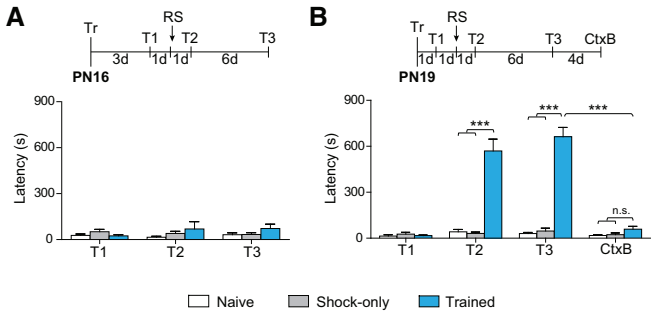

Figure 3. The age of the animal, but not the time elapsed between training and reinstatement, limits the ability to express infantile memory. (*A*) PN16 rats were left in their home cage (naive), exposed to a shock-only protocol, or subjected to a single IA training trial (Tr). Rats were tested 3 d after IA training (T1), or at matched time points for the other two groups. One day later, all animals were given a reminder footshock (RS, black arrow) in a different context and then retested 1 d (T2) and 7 d later (T3). (*B*) PN19 rats were left in their home cage (naive), exposed to a shock-only protocol, or subjected to a single IA training trial (Tr). Rats were tested 1 d after IA training (T1), or at matched time points for the other two groups. One day later, all animals were given a reminder footshock (RS, black arrow) in a different context and then retested 1 d (T2) and 7 d later (T3). Four days after T3, the rats were tested in a new IA context (context B [CtxB]). Memory retention is expressed as mean latency ± SEM (in seconds [s]). Two-way RM ANOVA followed by Bonferroni's multiple comparisons test: (***) $P < 0.001$. $n = 7$–8 rats per group.

of the conditioning experience to be reexperienced in some way, albeit in a temporally unpaired fashion.

Previous investigations in both humans and rodents have shown that the persistence of memory in infants is prolonged by the experience of reminders. Studies in rats showed that infantile amnesia could be alleviated by "reminders" given periodically throughout the retention interval, leading to the hypothesis that infantile forgetting is due to retrieval failure (Campbell and Jaynes 1966; Spear and Parsons 1976; Spear and Smith 1978). Similar conclusions were proposed more recently (Richardson et al. 1986; Kim and Richardson 2007), based on evidence in rodents that memory reacquisition (retraining) following infantile experience is accompanied by molecular changes typical of a memory previously experienced. These data led Li et al. (2014) to conclude that infantile memories are not lost but are actually stored as a persistent memory "trace" and to suggest that infantile forgetting must be due to a retrieval failure rather than an inability to store memories over the long term (i.e., true memory loss).

In humans, elegant and compelling studies conducted by Rovee-Collier and collaborators reported that prelinguistic infants retain information about events in which they participated for periods of weeks and even months (Rovee-Collier et al. 1980; Davis and Rovee-Collier 1983; Rovee-Collier and Hayne 1987; Perris et al. 1990), and that memory retention persists because of reactivations. They showed that repeated retrievals within a given time window prolong memory retention, whereas failure to retrieve a memory within a time window may result in a permanent retention deficit (Rovee-Collier 1990). In addition, Rovee-Collier and collaborators challenged the dogma that in infancy hippocampus-dependent memories cannot be formed and proposed that it is not necessary to invoke a different memory system to explain episodic memories in infants, an hypothesis that was extensively debated (Schacter and Moscovitch 1984; Rovee-Collier and Cuevas 2009).

Our data based on molecular manipulations of the dorsal hippocampus in rats (Travaglia et al. 2016, Bessières et al. 2020) is in agreement with Rovee-Collier's hypothesis, as they demonstrated that the hippocampus is necessary for the formation of infantile episodic memories (Travaglia et al. 2016; Bessières et al. 2020).

The apparent loss of memories formed early in life cannot be explained entirely by retrieval failure. Retrieval failure is not consistent with the lack of retrieval at ages when the animal has fully developed this function. Furthermore, recent data from our laboratory and others (Akers et al. 2014; Travaglia et al. 2016; Tsai et al. 2018; Farooq and Dragoi 2019; Bessières et al. 2020) lead us to suggest that the apparent forgetting of infantile memories is not due to simple inability to retrieve memories but rather to the fact that the infant hippocampus, although critically involved, operates in a distinctive manner relative to that of the adult system. In fact, it engages differential molecular and cellular mechanisms and circuitry that promote functional development and competence. Future studies should continue to identify which other brain regions are critical for infantile memory formation and storage as well as for the return of the memory following reactivations. For example, the potential functional contribution of cortical areas such as the prefrontal cortex, which plays a critical role in memory consolidation in the adult animal, remain to be determined. Some studies have reported that prelimbic cortex, entorhinal cortex, and piriform cortex are activated with infantile learning or retrieval (Kim et al. 2012; Guskjolen et al. 2018; Stanton et al. 2021); however, which area(s) functionally contribute to infantile memory storage remains to be

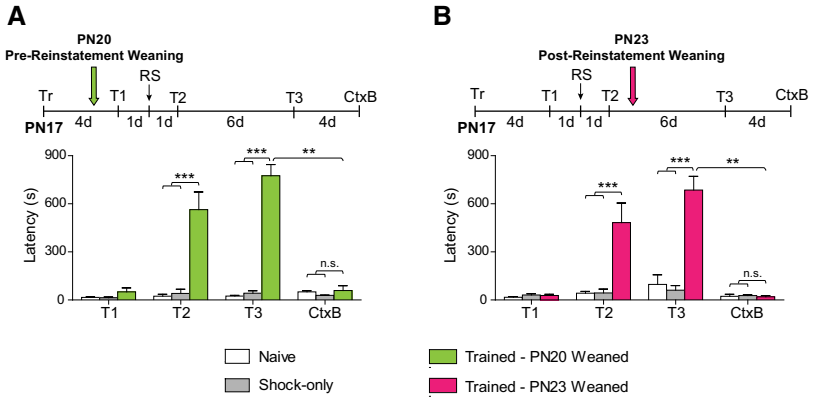

Figure 4. Maternal presence does not influence the reinstatement of the latent infantile memory. (*A*,*B*) PN17 rats were left in their home cage (naive), exposed to a shock-only protocol, or subjected to a single IA training trial (Tr). Rats were tested 4 d after IA training (T1) or at matched time points for the other two groups. One day after, all animals were given a reminder footshock (RS, black arrow) in a different context and then retested 1 d (T2) and 7 d later (T3). Four days after T3, the rats were tested in a new IA context (context B [CtxB]). The animals were weaned either at PN20 (*A*) or PN23 (*B*) (before or after the reinstatement protocol, respectively). Memory retention is expressed as mean latency ± SEM (in seconds [s]). Two-way RM ANOVA followed by Bonferroni's multiple comparisons test: (**) $P < 0.01$, (***) $P < 0.001$. $n = 6$–10 rats per group.

understood. Notably, the fact that infantile memory can be stored for a long time (Travaglia et al. 2016; Guskjolen et al. 2018) suggests that similarly to adult memories infantile memories may undergo a systems-level reorganization. However, given the immaturity of the infant hippocampal memory system, it cannot be excluded that this reorganization operates in a distinct manner compared to that taking place in the adult system.

How and what types of reactivations are able to produce the return of memories? Artificial stimulation is a very useful tool to dissect circuitry possibly involved in the return of memory, but knowing which types of natural behavioral reactivations are capable of recalling infantile representations is key to understand behavioral responses. We previously reported that an unpaired CS+US presentation with an interval between CS and US that can be quite extended, up to 1 wk, successfully reinstate infantile memories (Travaglia et al. 2016). Although behavioral or artificial reinstatement can be effective for a long time after training (i.e., 1–3 mo) (Travaglia et al. 2016; Guskjolen et al. 2018; Bessières et al. 2020); here, using behavioral protocols, we showed that reinstatement is limited by developmental age. The latent infantile memory cannot be reinstated if T is provided 1 d after training and RS 2 d later; however, it becomes fully effective when the same T+RS is presented starting 3 d after training. We also provided evidence that the age of the animal at the time of the reinstatement, and not the time interval between training and reinstatement, limits the return of memory. Thus, these data led us to conclude that the expression/reinstatement of infantile episodic memories is limited by developmentally regulated mechanisms. Notably, the developmental age that limits memory reinstatement corresponds to the age at which hippocampus-dependent types of memories, including spatial and contextual memories, begins to be expressed long term (Rudy et al. 1987; Kraemer and Randall 1995; Pugh and Rudy 1996; Stanton 2000; Akers and Hamilton 2007; Langston et al. 2010; Tan et al. 2017). Implying that the recovery of memory requires the hippocampal system to have acquired the ability to express memories long term. Thus, we speculate that the ability to recover memory is limited by mechanisms of biological and circuitry maturation necessary for long-term memory expression. This idea is also supported by our previous work showing that infantile learning evokes slow and distinctive biological changes in the dorsal hippocampus, including (1) a mGluR5- and BDNF-dependent switch in the expression of *N*-methyl-D-aspartate (NMDA) receptor subunits GluN2A/GluN2B; (2) a slow and persistent increase in the immediate early genes (IEGs) c-Fos, Zif268, and activity-regulated cytoskeleton-associated protein (Arc/Arg3.1); (3) elevated expression of excitatory synapse markers synaptophysin and postsynaptic density 95 (PSD-95); and (4) the maturation of α-amino-3-hydroxy-5-methyl-4-isox-azoleproprionic acid (AMPA) receptor synaptic responses (Travaglia et al. 2016; Bessières et al. 2020). One additional intriguing finding is that memory reinstatement, which required both context re-exposure and a subsequent RS, emerges immediately after RS. The reasons for this rapid memory expression after RS are unclear, and future studies shall determine whether immediate reinstatement is a general prerogative of infantile memory recovery after reactivations or if it is due to the two factors reminder T+RS. It is possible, for example, that T reactivates a representation, which then rapidly associate with the RS presented at later times.

Finally, our results clearly indicated that the presence of the mother does not affect the ability of animal to reinstate a latent infantile memory. Previous work showed that the presence of the mother can regulate the maturation of the emotional learning system in pups (Liu et al. 1997; Champagne and Curley 2009; Callaghan and Richardson 2013). In fact, cued learning is regulated by the maternal presence during infancy (Moriceau and Sullivan 2006), and maternal separation from P2–P14 can promote the development of adult-like fear learning and memory (Callaghan and Richardson 2011, 2012). In contrast, the reinstatement of hippocampus-dependent memories is not influenced by circuitry or mechanisms regulated by the maternal presence.

To conclude, our results on the developmental age limit for memory reinstatement leads us to propose that the recovery of infantile memory requires that the brain has matured the ability to express memory long term.

Materials and Methods

Animals

Seventeen-day-old and 24-d-old male and female offspring were obtained from pregnant Long Evans female rats (Charles River Laboratories). Rats were housed in 30.80 cm × 40.60 cm × 22.23 cm plastic cages containing ALPHA-dri bedding under a 12 h light–dark cycle (lights on at 07.00 a.m.) with food and water ad libitum. All experiments were carried out during the light cycle. The birth date was considered PN0, and the litters were culled to 10–12. Only one male and one female per litter were used in any experimental condition. For all experiments, statistical analyses revealed no significant difference in males versus females (unpaired two-tailed Student's *t*-test, $P > 0.05$). Rats were weaned at PN21. All procedures complied with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the New York University Animals Care Committees.

Inhibitory avoidance

Inhibitory avoidance (IA) was carried out as previously described (Travaglia et al. 2016). The IA chamber (Med Associates Inc.) consisted of a rectangular Perspex box divided into a safe compartment and a shock compartment (each 20.3 cm × 15.9 cm × 21.3 cm). The safe compartment was white and illuminated, and the shock compartment was black and dark. The apparatus was located in a sound-attenuated room with dim red light illumination. During training sessions, each rat was placed in the safe compartment with its head facing away from the door. After 10 sec, the door separating the compartments was automatically opened, allowing the rat access to the shock compartment. The door closed automatically when the rat crossed with all four limbs the invisible infrared light photosensors located in the shock compartment. Two seconds later, a footshock (2 sec, 1 mA) was automatically delivered to the grid floor of the shock chamber via a constant current scrambler circuit. The animal remained in the dark compartment for additional 10 sec, returned to its home cage, and then tested for memory retention at designated time points. As controls, we used naive animals (handled and left in their home cage) and rats exposed to a footshock without the IA context experience (shock-only). Shock-only treatment consisted of placing the rat onto grid of the shock compartment and immediately delivering a footshock of the same duration and intensity used in IA training. The animal returned to its home cage immediately after the footshock delivery. This protocol does not induce any association between the context and the footshock. Retention tests were performed by placing the rat back in the safe compartment and measuring its latency to enter the dark compartment. Footshocks were not administered during the retention tests (unless otherwise specified), and testing was terminated at 900 sec. During retraining sessions, rats were tested for memory retention and received a footshock upon entering the dark compartment. Locomotor activity was measured during training and testing by automatically counting the number of times each rat crossed the invisible infrared light photosensors located in both the safe and the shock compartment. Reminder footshock (RS) was administered with duration and intensity identical to that of training, in a novel neutral chamber with transparent walls, located in a different white light-illuminated experimental room. Context generalization was tested in a modified IA box that had a smooth plastic floor and walls

decorated with distinct geometric patterns and colors and was located in a separate white-illuminated experimental room.

Statistical analyses

Data analysis from Figures 1–4 was performed in Prism 6 (GraphPad Software). The data were analyzed by two-way RM analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons tests. Differences were considered significant at $P < 0.05$. The intent of this study was not to investigate sex differences; therefore, we included both female and male rats after preliminary statistical analyses of separate sex groups ($n = 3–4$ each sex) yielded no significant difference (unpaired two-tailed Student's *t*-test, $P > 0.05$) and the range of individual values was similarly distributed.

Acknowledgments

This work was supported by the DANA Foundation and the National Institutes of Health (NIH) grant MH065635 to C.M.A.

References

Akers KG, Hamilton DA. 2007. Comparison of developmental trajectories for place and cued navigation in the Morris water task. *Dev Psychobiol* **49:** 553–564. doi:10.1002/dev.20227

Akers KG, Martinez-Canabal A, Restivo L, Yiu AP, De Cristofaro A, Hsiang HL, Wheeler AL, Guskjolen A, Niibori Y, Shoji H, et al. 2014. Hippocampal neurogenesis regulates forgetting during adulthood and infancy. *Science* **344:** 598–602. doi:10.1126/science.1248903

Alberini CM, Travaglia A. 2017. Infantile amnesia: a critical period of learning to learn and remember. *J Neurosci* **37:** 5783–5795. doi:10.1523/JNEUROSCI.0324-17.2017

Bale TL, Baram TZ, Brown AS, Goldstein JM, Insel TR, McCarthy MM, Nemeroff CB, Reyes TM, Simerly RB, Susser ES, et al. 2010. Early life programming and neurodevelopmental disorders. *Biol Psychiatry* **68:** 314–319. doi:10.1016/j.biopsych.2010.05.028

Bauer PJ. 2006. Constructing a past in infancy: a neuro-developmental account. *Trends Cogn Sci* **10:** 175–181. doi:10.1016/j.tics.2006.02.009

Bessières B, Travaglia A, Mowery TM, Zhang X, Alberini CM. 2020. Early life experiences selectively mature learning and memory abilities. *Nat Commun* **11:** 628. doi:10.1038/s41467-020-14461-3

Brunson KL, Kramár E, Lin B, Chen Y, Colgin LL, Yanagihara TK, Lynch G, Baram T. 2005. Mechanisms of late-onset cognitive decline after early-life stress. *J Neurosci* **25:** 9328–9338. doi:10.1523/JNEUROSCI.2281-05.2005

Callaghan BL, Richardson R. 2011. Maternal separation results in early emergence of adult-like fear and extinction learning in infant rats. *Behav Neurosci* **1:** 20–28. doi:10.1037/a0022008

Callaghan BL, Richardson R. 2012. The effect of adverse rearing environments on persistent memories in young rats: removing the brakes on infant fear memories. *Transl Psychiatry* **2:** e138. doi:10.1038/tp.2012.65

Callaghan BL, Richardson R. 2013. Early experiences and the development of emotional learning systems in rats. *Biol Mood Anxiety Disord* **3:** 8. doi:10.1186/2045-5380-3-8

Callaghan BL, Li S, Richardson R. 2014. The elusive engram: what can infantile amnesia tell us about memory? *Trends Neurosci* **37:** 47–53. doi:10.1016/j.tins.2013.10.007

Campbell BA, Jaynes J. 1966. Reinstatement. *Psychol Rev* **73:** 478–480. doi:10.1037/h0023679

Campbell BA, Spear NE. 1972. Ontogeny of memory. *Psychol Rev* **79:** 215–236. doi:10.1037/h0032690

Champagne FA, Curley JP. 2009. Epigenetic mechanisms mediating the long-term effects of maternal care on development. *Neurosci Biobehav Rev* **33:** 593–600. doi:10.1016/j.neubiorev.2007.10.009

Davis JM, Rovee-Collier CK. 1983. Alleviated forgetting of a learned contingency in 8-week-old infants. *Dev Psychol* **19:** 353–365. doi:10.1037/0012-1649.19.3.353

Farooq U, Dragoi G. 2019. Emergence of preconfigured and plastic time-compressed sequences in early postnatal development. *Science* **363:** 168–173. doi:10.1126/science.aav0502

Guskjolen A, Kenney JW, de la Parra J, Yeung BA, Josselyn SA, Frankland PW. 2018. Recovery of "lost" infant memories in mice. *Curr Biol* **28:** 2283–2290. doi:10.1016/j.cub.2018.05.059

Hayne H. 2004. Infant memory development: implications for childhood amnesia. *Dev Rev* **24:** 33–73. doi:10.1016/j.dr.2003.09.007

Heim C, Nemeroff CB. 2001. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* **49:** 1023–1039. doi:10.1016/S0006-3223(01)01157-X

Jacobs WJ, Nadel L. 1985. Stress-induced recovery of fears and phobias. *Psychol Rev* **92:** 512–531. doi:10.1037/0033-295X.92.4.512

Josselyn SA, Frankland PW. 2012. Infantile amnesia: a neurogenic hypothesis. *Learn Mem* **19:** 423–433. doi:10.1101/lm.021311.110

Kagan J. 1984. *The Nature of the Child*. Basic Books, New York.

Kim JH, Richardson R. 2007. Immediate post-reminder injection of gamma-amino butyric acid (GABA) agonist midazolam attenuates reactivation of forgotten fear in the infant rat. *Behav Neurosci* **121:** 1328–1332. doi:10.1037/0735-7044.121.6.1328

Kim JH, Li S, Hamlin AS, McNally GP, Richardson R. 2012. Phosphorylation of mitogen-activated protein kinase in the medial prefrontal cortex and the amygdala following memory retrieval or forgetting in developing rats. *Neurobiol Learn Mem* **97:** 59–68. doi:10.1016/j.nlm.2011.09.005

Kraemer PJ, Randall CK. 1995. Spatial-learning in preweanling rats trained in a Morris water maze. *Psychobiology* **23:** 144–152.

Langston RF, Ainge JA, Couey JJ, Canto CB, Bjerknes TL, Witter MP, Moser EI, Moser MB. 2010. Development of the spatial representation system in the rat. *Science* **328:** 1576–1580. doi:10.1126/science.1188210

Li S, Callaghan BL, Richardson R. 2014. Infantile amnesia: forgotten but not gone. *Learn Mem* **21:** 135–139. doi:10.1101/lm.031096.113

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* **277:** 1659–1662. doi:10.1126/science.277.5332.1659

Madsen HB, Kim JH. 2016. Ontogeny of memory: an update on 40 years of work on infantile amnesia. *Behav Brain Res* **298:** 4–14. doi:10.1016/j.bbr.2015.07.030

Maren S, Phan KL, Liberzon I. 2013. The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci* **14:** 417–428. doi:10.1038/nrn3492

Meaney MJ, Aitken DH, van Berkel C, Bhatnagar S, Sapolsky RM. 1988. Effect of neonatal handling on age-related impairments associated with the hippocampus. *Science* **239:** 766–768. doi:10.1126/science.3340858

Mineka S, Zinbarg R. 2006. A contemporary learning theory perspective on the etiology of anxiety disorders: it's not what you thought it was. *Am Psychol* **61:** 10–26. doi:10.1037/0003-066X.61.1.10

Moriceau S, Sullivan RM. 2006. Maternal presence serves as a switch between learning fear and attraction in infancy. *Nat Neurosci* **9:** 1004–1006. doi:10.1038/nn1733

Newcombe NS, Lloyd ME, Ratliff KR. 2007. Development of episodic and autobiographical memory: a cognitive neuroscience perspective. *Adv Child Dev Behav* **35:** 37–85. doi:10.1016/B978-0-12-009735-7.50007-4

Perris EE, Myers NA, Clifton RK. 1990. Long-term memory for a single infancy experience. *Child Dev* **61:** 1796–1807. doi:10.2307/1130838

Perry R, Sullivan RM. 2014. Neurobiology of attachment to an abusive caregiver: short-term benefits and long-term costs. *Dev Psychobiol* **56:** 1626–1634. doi:10.1002/dev.21219

Poulos AM, Reger M, Mehta N, Zhuravka I, Sterlace SS, Gannam C, Hovda DA, Giza CC, Fanselow MS. 2014. Amnesia for early life stress does not preclude the adult development of posttraumatic stress disorder symptoms in rats. *Biol Psychiatry* **76:** 306–314. doi:10.1016/j.biopsych.2013.10.007

Pryce CR, Rüedi-Bettschen D, Dettling AC, Weston A, Russig H, Ferger B, Feldon J. 2005. Long-term effects of early-life environment manipulations in rodents and primates: potential animal models in depression research. *Neurosci Biobehav Rev* **29:** 649–674. doi:10.1016/j.neubiorev.2005.03.011

Pugh CR, Rudy JW. 1996. A developmental analysis of contextual fear conditioning. *Dev Psychobiol* **29:** 87–100. doi:10.1002/(SICI)1098-2302(199603)29:2<87::AID-DEV1>3.0.CO;2-H

Richardson R, Riccio DC, Axiotis R. 1986. Alleviation of infantile amnesia in rats by internal and external contextual cues. *Dev Psychobiol* **19:** 453–462. doi:10.1002/dev.420190506

Rovee-Collier C. 1990. The "memory system" of prelinguistic infants. *Ann N Y Acad Sci* **608:** 517–536. doi:10.1111/j.1749-6632.1990.tb48908.x

Rovee-Collier C. 1999. The development of infant memory. *Curr Dir Psychol Sci* **8:** 80–85. doi:10.1111/1467-8721.00019

Rovee-Collier C, Cuevas K. 2009. Multiple memory systems are unnecessary to account for infant memory development: an ecological model. *Dev Psychol* **45:** 160–174. doi:10.1037/a0014538

Rovee-Collier C, Hayne H. 1987. Reactivation of infant memory: implications for cognitive development. *Adv Child Dev Behav* **20:** 185–238. doi:10.1016/S0065-2407(08)60403-6

Rovee-Collier CK, Sullivan MW, Enright M, Lucas D, Fagen JW. 1980. Reactivation of infant memory. *Science* **208:** 1159–1161. doi:10.1126/science.7375924

Rudy JW, Stadler-Morris S, Albert P. 1987. Ontogeny of spatial navigation behaviors in the rat: dissociation of "proximal"- and "distal"-cue-based behaviors. *Behav Neurosci* **101:** 62–73. doi:10.1037/0735-7044.101.1.62

Schacter DL, Moscovitch M. 1984. Infants, amnesics, and dissociable memory systems. In *Advances in the study of communication and affect, Vol. 9 Infant memory* (ed. Moscovitch M), pp. 173–215. Plenum Press, New York.

Spear NE, Parsons PJ. 1976. Analysis of a reactivation treatment: ontogenetic determinants of alleviated forgetting. In *Processes in animal memory* (ed. Medin DL, et al.), pp. 136–165. Erlbaum, Hillsdale, NJ.

Spear NE, Smith GJ. 1978. Alleviation of forgetting in preweanling rats. *Dev Psychobiol* **11:** 513–529. doi:10.1002/dev.420110602

Sroufe LA, Egeland B, Kreutzer T. 1990. The fate of early experience following developmental change: longitudinal approaches to individual adaptation in childhood. *Child Dev* **61:** 1363–1373. doi:10.2307/1130748

Stanton ME. 2000. Multiple memory systems, development and conditioning. *Behav Brain Res* **110:** 25–37. doi:10.1016/S0166-4328(99)00182-5

Stanton ME, Murawski NJ, Jablonski SA, Robinson-Drummer PA, Heroux NA. 2021. Mechanisms of context conditioning in the developing rat. *Neurobiol Learn Mem* **179:** 107388. doi:10.1016/j.nlm.2021.107388

Tan HM, Wills TJ, Cacucci F. 2017. The development of spatial and memory circuits in the rat. *Wiley Interdiscip Rev Cogn Sci* **8**. doi:10.1002/wcs.1424

Travaglia A, Bisaz R, Sweet ES, Blitzer RD, Alberini CM. 2016. Infantile amnesia reflects a developmental critical period for hippocampal learning. *Nat Neurosci* **19:** 1225–1233. doi:10.1038/nn.4348

Travaglia A, Steinmetz AB, Miranda JM, Alberini CM. 2018. Mechanisms of critical period in the hippocampus underlie object location learning and memory in infant rats. *Learn Mem* **25:** 176–182. doi:10.1101/lm.046946.117

Tsai TC, Huang CC, Hsu KS. 2018. Infantile amnesia is related to developmental immaturity of the maintenance mechanisms for long-term potentiation. *Mol Neurobiol* **56:** 907–919. doi:10.1007/s12035-018-1119-4

Weiss C, Disterhoft JF. 2015. The impact of hippocampal lesions on trace-eyeblink conditioning and forebrain-cerebellar interactions. *Behav Neurosci* **129:** 512–522. doi:10.1037/bne0000061

Received May 10, 2021; accepted in revised form July 5, 2021.