Behavioural Brain Research 207 (2010) 515–519

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

Abrupt emergence of long-lasting memory in the pre-weanling rat

Solène Languille[a,b,*], Paulette Richer[a,b], Bernard Hars[a,b]

[a] Univ Paris-Sud, UMR 8620, Orsay F-91405, France
[b] CNRS, Neurobiologie de l'Apprentissage, de la Mémoire et de la Communication, UMR 8620, Orsay F-91405, France

ARTICLE INFO

Article history:
Received 11 August 2009
Received in revised form 20 October 2009
Accepted 26 October 2009
Available online 3 November 2009

Keywords:
Memory
Forgetting
Ontogenesis
Infantile amnesia
Conditioned aversion

ABSTRACT

During the course of ontogenesis does long-lasting memory emerge progressively or abruptly, and when? To examine this question, rat pups were conditioned at different ages (3-, 10-, 12-, 15- or 18-day-old) and tested at different retention intervals: from 3 days to 1 year. Conditioned aversion memory established before 12-day-old lasts for only 1 week, but when acquired after 15 days, memory survives for more than 1 year. This defines a short temporal window of 3 days for sudden emergence of a remote memory. Our result offers a precise temporal target to explore the mechanisms involved in long maintenance of memory.

Many studies have shown that memory is forgotten more rapidly in young animals than in adults, which corresponds to the so-called infantile amnesia [7]. This rapid rate of forgetting has been extensively demonstrated in 18–30-day-old rats, however few studies have analyzed the evolution of resistance to forgetting during post-natal development [5,8,24]. One constraint is to use a memory test that can be learned by pups of all ages. Conditioned taste aversion (CTA), which consists of associating a novel taste with a malaise, is distinguishable from other types of memory in pre-weanling rats because of its biological relevance for the organism [5]. CTA involves sensory and motor systems of suckling which are well developed in newborn [15,23], meaning it is possible to test it in very young rodent pups. Indeed, we have previously shown that rat pups from 3- to 18-day-old (P3/P18) can acquire and express a CTA memory 3 days later [18]. Moreover, although CTA is reputed to be time resistant in the adult [16,20]; it is not known whether it is as robust when acquired at pre-weaning age. Here, we have studied the influence of age on the kinetics of long-term retention of CTA acquired before weaning: at P3, P10, P12, P15 and P18. The experiments focus on whether long-term memory resistance appears abruptly or varies during the nursing period, and whether early forgetting persists or whether it recovers after some weeks?

We have used a paradigm of CTA where the conditioned stimulus (CS; peach-aromatized milk) was presented via a surrogate nipple for 10 min and paired 30 min later with an injection of LiCl (150 mM, 2% body weight, i.p.) which causes an immediate malaise (unconditioned stimulus, US) (previous description in [12,18]). At each age, pups were submitted to two conditions: either conditioned (CS–US) or unconditioned (CS. . .US); for the latter, the CS and the US were separated by a long delay (≈8 h). To ensure independence of the data, only one pup from a litter was assigned to a given condition.

Regardless of the age of conditioning, each pup was tested at one single retention interval. Pups conditioned at P3, P10 or P12 were tested at 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks or 5 weeks (except during the immediate post-weaning period). Pups conditioned at P15 or P18 were tested at 3 days, 2 weeks, 3 weeks, 9 weeks or 52 weeks. When testing occurred before weaning (at the 21st post-natal day), the peach-aromatized milk was presented in the morning for a 10-min session. During testing, as for conditioning, the attractiveness of aromatized milk was assessed by the time spent attached to the nipple. The total time of attachment to the nipple was measured on-line by two experimenters who were blind to the pretreatment of the pups. An aversion index 1 was calculated as the relative time spent attached to the nipple: (time during testing/total time during conditioning and testing) × 100. Thus, the more the pups refused the flavored milk nipple (aversion), the lower the score was. When testing occurred after weaning, this session was conducted following 3 days of water deprivation (excluding a test in the week of weaning). During deprivation, rats were habituated to drink water from two graded pipettes for 10 min/day in individual testing cage in an experimental room at about 10:00, and had free access to water for 15 min in the home cages at 16:00. On the day of testing, all rats were

* Corresponding author at: Laboratoire de Neurobiologie de l'Apprentissage, de la Mémoire et de la Communication, CNRS UMR 8620, Université Paris-Sud, Bât. 446, Orsay F-91405, France. Tel.: +33 1 69 15 49 77; fax: +33 1 69 15 77 26.
E-mail address: solene.languille@u-psud.fr (S. Languille).

0166-4328/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2009.10.035

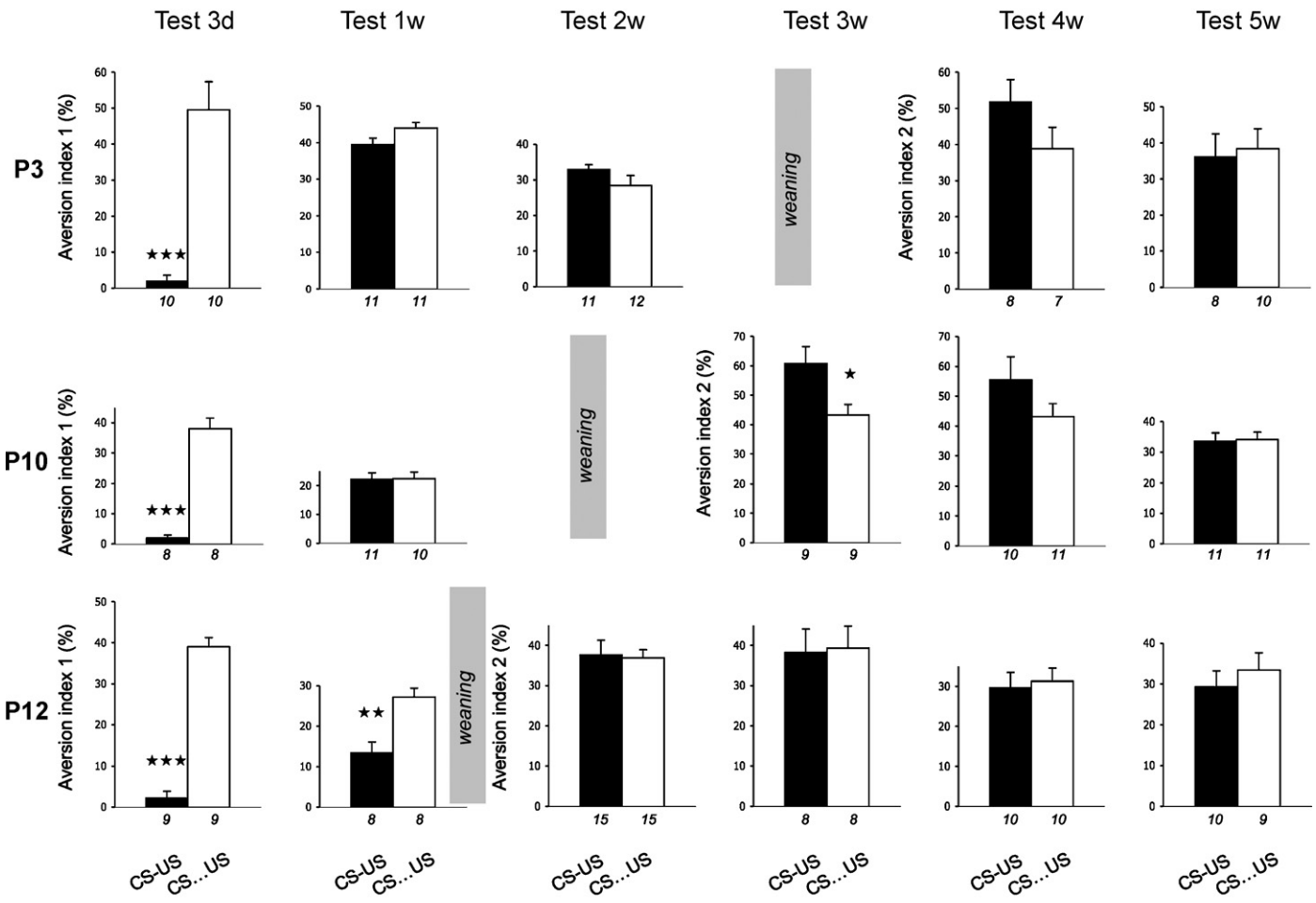

Fig. 1. Memory of conditioned taste aversion as function of age (P3, P10 and P12) and retention interval. Training was at different ages before weaning: P3, P10 and P12. Testing was at different intervals: 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks and 5 weeks. The lower the index, the more the aversion. Pups of CS–US group (■) received aromatized milk (CS) paired with malaise (US) induced by LiCl and those of CS. . .US group (□) were submitted to an unpaired condition (≈8 h between CS and US). The aversion is expressed at 3 days in all cases, but disappears at 1 week for P3 and P10, and at 2 weeks for P12. $^{***}p < .001$; $^{**}p < .01$ and $^{*}p < .05$.

exposed for 10 min to two pipettes filled with peach-aromatized water. During testing, as for habituation, fluid intake was measured with a precision of 0.1 ml. For individual animals, the aversion index 2 was expressed as fluid intake relative to water consumption: (fluid intake during testing/(fluid intake during testing + mean water intake during last 2 days of habituation)) × 100. As for pups, this score was thus defined to minimize the effect of individual variability in fluid consumption and the lower the score the stronger the aversion. Data from both males and females were combined for all experiments as *t*-test confirmed there were no sex differences in any of measures reported here.

Pups conditioned at P3 expressed a clear aversion when compared with the unconditioned pups at a 3-day retention test interval ($t(18) = 8.98$; $p < .0001$; Fig. 1). When testing occurred later (1 week, 2 weeks, 4 weeks or 5 weeks after conditioning), there was no significant difference between groups ($p > .05$ for each interval). Aversion memory formed at P3 is no longer expressed 1 week after acquisition.

Pups conditioned at P10 expressed a clear aversion when compared with unconditioned pups at a 3-day retention test interval ($t(14) = 9.19$; $p < .0001$). When testing occurred 1 week, 4 weeks or 5 weeks after conditioning, there was no significant difference between groups ($p > .05$ in each case). Pups conditioned at P10 showed an apparent forgetting of aversion memory from 1-week post-acquisition, as did P3 conditioned pups. At a 3-week retention interval, the conditioned pups expressed preference for the CS compared with unconditioned pups ($t(16) = -2.52$; $p < .05$), suggesting that conditioned pups had forgotten the aversion but they remembered the CS marked by the US. Such preference has been previously reported 25 days after a garlic-illness association at P10 [24]. This observation is in agreement with a multidimensional view of the memory trace where the target memory appears to be lost when the CS still has a transient (for 1 week) impact on the behavioral output. This suggests that different components of the memory trace could be differentially resistant to forgetting.

Pups conditioned at P12 expressed aversion when compared with unconditioned pups at an interval of 3 days and 1 week (3 days: $t(16) = 12.25$; $p < .0001$, 1 week: $t(14) = 3.95$; $p < .01$). When testing occurred later (2 weeks, 3 weeks, 4 weeks or 5 weeks after conditioning), there was no significant difference between groups ($p > .05$ in every cases). Aversion memory established at P12 is still expressed 1 week after conditioning, but no longer at 2 weeks.

For each retention interval, pups conditioned at P15 or P18 expressed aversion when compared with unconditioned pups (Fig. 2): 3 days ($t(13) = 11.42$; $p < .0001$. $t(14) = 13.37$; $p < .0001$), 2 weeks ($t(12) = 7.14$; $p < .0001$. $t(12) = 7.41$; $p < .0001$), 3 weeks ($t(12) = 3.60$; $p < .01$. $t(14) = 3.87$; $p < .01$), 9 weeks ($t(15) = 2.35$; $p < .05$. $t(25) = 3.59$; $p < .01$), and 52 weeks ($t(19) = 2.62$; $p < .01$. $t(17) = 3.43$; $p < .01$). Aversion at P15 and at P18 was similar at each retention delay, suggesting that retention did not evolve between P15 and P18. The main point here is that, despite a progressive reduction in aversion with time, pre-weaned rats remembered CTA 1 year later. It is currently accepted that CTA is a robust form of learning that can be retained for several weeks, months and