# Training-induced synaptic maturation in dorsal hippocampus during infantile age

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## Abstract

Episodic memories formed during the first postnatal period are rapidly forgotten, a phenomenon known as 'infantile amnesia'. Interestingly, previous work in Alberini Lab shows that the memory during the infantile amnesia period are not lost and can be reinstated by reminder later in life. The infantile memories are stored using mechanisms involving the dorsal hippocampus (dHC). Further, training during this period increases in markers of excitatory synapses. in dorsal hippocampus that last for at least 48 hours after receiving the training. Therefore, we are proposing to study how the long-term memory is formed and stored during infantile learning. We focused on learning-induced changes in synapses hippocampus using immunohistochemistry with pre-synaptic marker synaptophysin and post-synaptic marker PSD95. We have found that there is increasing synaptic number, size and also connections, indicating that the hippocampus does develop by training during the period when they experience infantile amnesia.

## Background Introduction

**Infantile Amnesia** is the inability of adults to recollect early episodic memories, which is associated with the rapid forgetting that occurs in childhood. It has been suggested that infantile amnesia is due to the underdevelopment of the infant brain, which would preclude memory consolidation, or to deficits in memory retrieval.

**Inhibitory avoidance task** (a type of fear learning). Fear learning is the process of gathering information about the internal and external environment in situations that evoke fear. Fear learning is the first step toward creating memories for fearful events (fear memories), which are robust and represent a long-lasting record of the acquired information that is capable of modifying behavior when retrieved. Like other forms of learning, fear learning (fear memory acquisition) is followed first by memory consolidation, a period of time when memories are still labile and can be modulated (enhanced or impaired), and then by memory storage, involving the participation of hippocampus (McGaugh, 2000).

## Materials and Methods

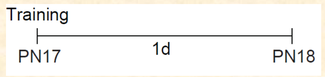



Fig. 1 Training paradigm

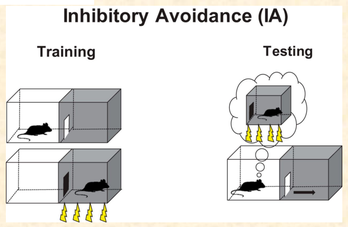
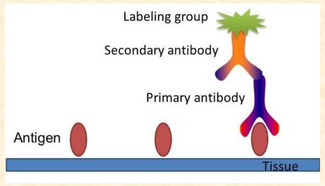
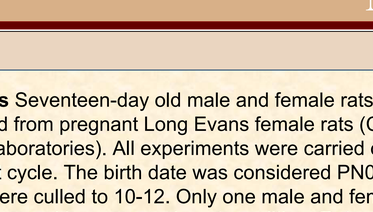



Fig. 2 Inhibitory avoidance task demonstration

**Animals** Seventeen-day old male and female rats were obtained from pregnant Long Evans female rats (Charles River Laboratories). 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 female per litter was used in any experiment condition. Rats were weaned to at PN21. All animals in different groups were perfused at 18-day old. Naive, trained and shock-only groups have 3 rats each.



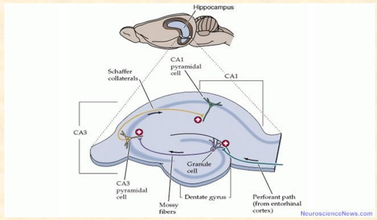

**Immunohistochemistry** Rats were perfused transcardially with ice-cold 0.1-M PBS for 3 min and then followed by 4% (wt/vol) paraformaldehyde (PFA) for 15 min. Brains were postfixed overnight in 4% (wt/vol) PFA, followed by a 2- to 3-d cryoprotection in 30% (wt/vol) sucrose/ PBS at 4 °C. The fixed brains were cut into 30-μm coronal sections using a cryostat and stored in at -20℃ in cryoprotectant (25% Ethylene Glycol, 25 % Glycol in PB, pH = 7.4). Brain sections were stained by two primary antibodies: mouse anti-PSD95 (1: 500, Thermo Fisher, MA1-045), and rabbit anti-Synaptophysin (1: 800, Abcam, ab14692) and subsequently incubated with anti-mouse Alexa-Fluor 488–conjugated and anti-rabbit Alexa-Flour 647 secondary antibody (1: 1,000; Fisher Scientific).







**Confocal imagining** and synaptic puncta quantification Images were captured on an Olympus VS120 fluorescent microscope for preview. Then images were captured using a Leica SP5 confocal microscope with 63x/1.40 Oil DIC M27 objective and 2.5 zoom with a single step at the depth of 4 micrometers from the surface. Three non-overlapping images (size 1024 x 1024) were taken for each area (upper blade of dentate gyrus, lower blade of dentate gyrus, CA1, CA2, and CA3) by randomly going around the area. The number of presynaptic, postsynaptic and co-localized puncta was analyzed using ImageJ.




## Result & Discussion

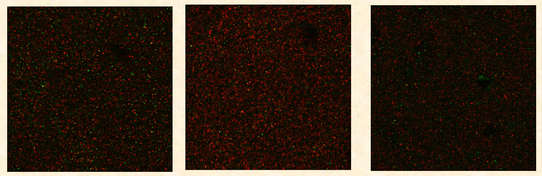


**Fig 1. Sample image from the confocal imaging** 63x oil, 2.5 zoom
(a) Naïve CA3 sample image
(b) Trained CA3 sample image
(c) Shock-only CA3 sample image

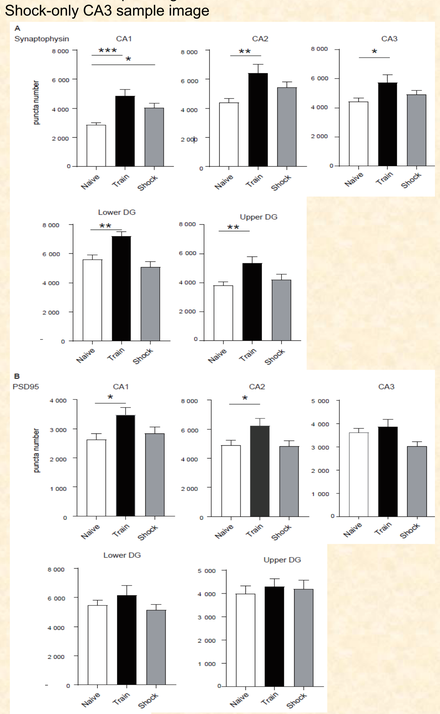



**Figure 2. Both pre-synapses (Synaptophysin marked) and post-synapses (PSD95 marked) puncta numbers increase after training.**
Experiment schedule is shown above the panel. Rats were under normal condition (naïve, white), trained with IA (black), or shocked (grey) at PN17 (n=3/group) and euthanized after 1 day (d) at PN18. 3 brain slices at nearly the same bregma were chosen for immunohistochemistry analysis (N=9/animal). 3 non-overlapping images were randomly taken for each region (N'=27/region). Data are expressed region wise. Error bar is ± s.e.m. One-way ANOVA followed by Dunnett's post hoc tests, *p < 0.05, **p < 0.01, and ***p < 0.001.

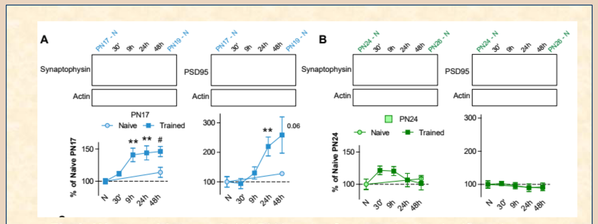



**Fig 3. Western blot result** (A) PN17 rats. Synaptophysin significantly increases 9hr after training and remains higher after 48hr. PSD95 significantly increases 24hr after training and remains higher after 48hr. (B) PN24 rats. No significant change in either synaptophysin or PSD95 was observed.

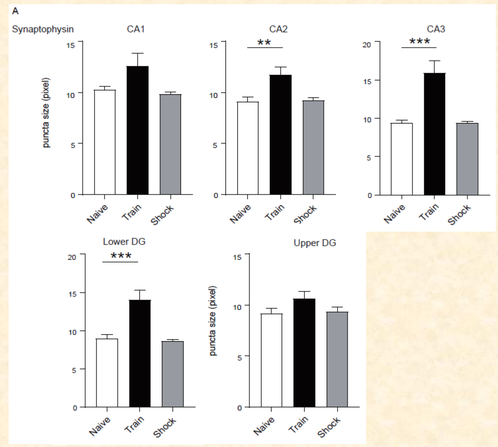



**Figure 3. The size of pre-synapses (Synaptophysin marked) were grown larger after training.**
Rats were under normal condition (naïve, white), trained with IA (black), or shocked (grey) at PN17 (n=3/group) and euthanized after 1 day (d) at PN18. 3 brain slices at nearly the same bregma were chosen for immunohistochemistry analysis (N=9/animal). 3 non-overlapping images were randomly taken for each region (N'=27/region). ImageJ Measure was used for counting the area. Data are expressed region wise. Error bar is ± s.e.m. One-way ANOVA followed by Dunnett's post hoc tests, *p < 0.05, **p < 0.01, and ***p < 0.001.

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