Infant Cognitive Training Preserves Learning-Related Prefrontal Circuits for Adult Learning: Learning-Induced Tagging of Dendritic Spines

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Work in various animal models has demonstrated that cognitive training in infancy has a greater effect on adult cognitive performance than pretraining in adulthood. Since the underlying synaptic mechanisms are unclear, the aim of this study was to test the working hypothesis that associative training “preshapes” synaptic circuits in the developing infant brain and thereby improves learning in adulthood. Using a two-way active avoidance (TWA) paradigm, we found that avoidance training during infancy, even though the infant rats were not capable to learn a successful avoidance strategy, improves avoidance learning in adulthood. On the neuroanatomical level we show here for the first time that infant TWA training in the ventromedial prefrontal cortex suppresses developmental spine formation. In contrast in the lateral orbitofrontal cortex, developmental spine pruning is suppressed, possibly by “tagging” activated synapses, which thereby are protected from being eliminated. Moreover, we demonstrate that infant TWA training alters learning-induced synaptic plasticity in the adult brain. The synaptic and dendritic changes correlate with specific behavioral parameters. Taken together, these results support the working hypothesis that infant cognitive training interferes with developmental reorganization and maturation of dendritic spines and thereby “optimizes” prefrontal neuronal circuits for adult learning.

Keywords: cortical development, learning, memory, synaptic plasticity, synaptic tagging

Introduction

Feedback-based learning, that is the ability to use positive or negative performance feedback, is one of the basic features for successfully adapting and optimizing behavioral strategies. In humans and animals, evidence is increasing that this ability matures postnatally. Human adults adjust behavior more successfully when presented with negative feedback than 8- to 11-year-old children (Crone et al. 2004; Crone et al. 2008), and 8- to 9-year-old children perform more inaccurately after receiving negative feedback relative to positive feedback compared with adults (van Duijvenvoorde et al. 2008).

While in humans, a developmental analysis of aversive feedback learning is still at its very beginning (Schlund and Ortu 2010; Schlund et al. 2010), animal studies have shown that young rats display deficits in negative feedback learning tasks. For instance, we and others demonstrated that infant rats show poor learning in a two-way active avoidance (TWA) task, an established aversive feedback learning paradigm in rodents (Izquierdo 1975; Bauer 1978; Myslivecek and Hassmannova 1979; Kudryashova 2006; Schäble et al. 2007; Abraham and Gruss 2010). However, a few studies in rodents revealed that infant cognitive experience appears to induce a long-lasting and stable memory trace, which is recruited during adult learning. When trained on an aversive associative task during infancy, animals perform better in the same learning task as adults compared with non-pretrained adults (Schäble et al. 2007; Gruss et al. 2010; Sarro and Sanes 2011).

So far, surprisingly little is known about the underlying neural mechanisms for the developmental differences in avoidance learning (Haggblom et al. 1974; Steimer and Driscoll 2003; Kudryashova et al. 2009). Furthermore, the functional neuronal circuits, which mediate aversive feedback learning, are not yet well defined (Stevens 2009). Moreover, the host of literature on learning-induced structural neuronal and synaptic changes in the adult brain is in striking contrast to the relative paucity of studies analyzing learning-induced synaptic changes during brain development. This is particularly surprising, since the critical importance of the perinatal environment on brain development is well documented (Kolb et al. 2012). Although the ontogeny of learning has been studied in a variety of learning paradigms (Hyson and Rudy 1984; Moye and Rudy 1987; Sullivan and Leon 1987; Doupe et al. 2004), only few studies have analyzed the effects of early learning on adult performance (Schäble et al. 2007; Abraham and Gruss 2010; Gruss et al. 2010; Sarro and Sanes 2011) and even less is known about the underlying synaptic mechanisms. All neocortical regions (sensory and motor regions) are influenced by early experience, but the developmental profiles as well as the impact of the early environment (and the specific components of the early environment) differ significantly for specific cortical regions. The focus of this study was laid on the impact of early cognitive environment on those cortical regions, which are known to be essential for associative learning. The orbitofrontal cortex has been shown to be engaged in behavioral flexibility and in the evaluation of contingencies between conditioned and unconditioned stimuli, tasks, which are essential components of TWA learning. Ventromedial prefrontal cortex (vmPFC) regions, such as the infralimbic (IL) and prelimbic (PL) areas mediate the fear conditioning aspects, which represent an essential part in TWA training. Thus, we addressed the questions (i) does learning in infancy result in different or similar synaptic changes compared with learning in adulthood, (ii) what are the long-term structural synaptic consequences of infant learning, (iii) what is the impact of infant training on synaptic plasticity in adulthood, and (iv) are there region-specific differences in learning-induced changes in developmental synaptic reorganization in the prefrontal cortex?
Materials and Methods

Subjects and Experimental Groups
Female Wistar rats (strain Schönwalde) from the breeding colony at the Leibniz Institute for Neurobiology Magdeburg, Germany, were used in all experiments. All animals were reared under normal animal facility conditions [temperature: 21 ± 2°C; humidity: 55 ± 5%; with free access to food and water under an artificial 12:12-h light–dark cycle (light on at 06:00 AM); husbandry, comprising cage cleaning, was done once a week]. Pregnant females were checked for litters daily, and at postnatal day 3 (P3) litters were standardized to 10 female pups per dam. At P24, pups were weaned and housed in groups of 4 littersmates in translucent standard laboratory cages type IV (E. Becker & Co. GmbH, Castrop-Rauxel, Germany) until used in the experiments.

The experimental protocols were approved by an ethics committee of the government of the state Saxony-Anhalt according to the German guidelines for the care and the use of animals in laboratory research (§8, Abs.1, 25.05.1998), and all experiments were performed in accordance with the European Communities Council Directive of November 1986 (86/609/EEC).

Prior to starting the learning experiments, the animals were randomly assigned to one of the following treatment groups:

1. Nontrained infants (n): Animals left untrained were reared undisturbed with their family (n = 11).
2. Trained infants (t): Animals were trained from P17 to P21 as described above in detail (n = 11).
3. Nontrained as infants as well as adults (nn): Animals were left untrained until used in the Golgi experiment (n = 7).
4. Trained as infants, but not trained as adults (nt): Animals were trained from P17 to P21, but not in adulthood (P80–P84) (n = 7).
5. Nontrained as infants, but trained as adults (nt): Animals were not trained as infants (P17–P21), but trained in adulthood (P80–P84) (n = 7).
6. Trained as infants as well as adults (tt): Animals were trained as infants (P17–P21) and retrained in adulthood (P80–P84) (n = 8).

Two-way Active Avoidance (TWA) Training
Animals were trained in a 5-day training procedure as infants (P17–P21) and/or adults (P80–P84). Only one subject per litter was used for each experimental group to prevent potential confounds resulting from litter effects.

All experiments were conducted in fully automated shuttle boxes (TSE Systems, Germany) located in ventilated and sound-protected cubicles. The experiments were carried out between 08:00 AM and 02:00 PM. Each of the 5 consecutive training days started with a 3-min habituation period to allow the animals to explore the learning environment. Then, the animals were exposed to 50 trials daily using the following parameters: conditioned stimulus (CS): 2.4-kHz tone, 80 dB, 5-s maximal duration; unconditioned stimulus (UCS): 0.6-mA foot shock, 15-s maximal duration; intertrial interval (ITI): 20 s. The following behavioral parameters were automatically recorded: number of avoidance, escapes, as well as escape latencies. An avoidance response was recorded when the rat moved to the opposite compartment during the CS, but prior to UCS (i.e. within 5 s). An escape response was recorded when the rat moved to the opposite compartment during CS + UCS (i.e. within maximal 15 s). At the end of each trial, that is, after an avoidance or escape response, CS or CS + UCS were terminated, and the ITI started. After each animal, the shuttle box was cleaned with 70% ethanol (Roht, Germany) to remove odor cues.

Quantitative Neuromorphology
Infants and adults were decapitated at P24 or P87, respectively. The brains were removed from the skull and immersed in Golgi-Cox solution for 14 days, then dehydrated and embedded in 8% Celloidin. Serial transverse sections (150 µm) were prepared (Fig. 1) and mounted on glass slides and processed as described in Murmu et al. 2006, using a modified Golgi-Cox protocol by Glaser and Van der Loos (1981). Using the rat brain atlas (Paxinos and Watson 1998) for anatomical orientation, spine numbers, spine densities, dendritic length, and dendritic ramification of layer II/III pyramidal neurons of the pregenual vmPFC (Fig. 1), which consists of the PL and the IL regions. In addition, these parameters were quantified in the lateral subdivision of the orbitofrontal cortex (LO, Fig. 1). All neurons were reconstructed under a 100x objective, using a computer-based neuron tracing system (NEUROLUCIDA®; MicroBrightField, Williston, VT, USA). The motor stage of the microscope allows a quantitative 3-dimensional analysis of complete dendritic trees. Neurons selected (Fig 1) for analysis had to satisfy the following criteria: (1) Location of soma within layer II/III and within the boundaries of the vmPFC and LO, (2) complete staining of apical and basal dendritic trees, (3) primary apical dendrites had to branch regularly in a series of bifurcating branches divided into primary, secondary, tertiary etc. The length of the dendritic trees was measured by tracing the entire dendrite while counting dendritic spines. Dendritic protrusions were considered as spines if their head was clearly separated from the dendritic shaft by a neck (mushroom type) or if they could be classified as stubby spines. Spine frequency (the number of visible spines per µm) was calculated for each neuron by dividing the total number of spines counted through the total dendritic length. The results were expressed as mean spine frequencies per total neuron and additionally as spine frequencies per branch order. In addition to the calculation of total dendritic length, a 3-dimensional version of a Sholl analysis (Sholl 1953) was performed. In the present study, the number of intersections with concentric spheres at 50-µm intervals, and dendritic length per radial distance was calculated in order to obtain more detailed information about changes in the complexity of the dendritic trees. Neurons in the left and right hemispheres were analyzed separately (n = 2 per hemisphere). All measurements were conducted by an experimenter, who was unaware of the experimental condition of the animals.

- Infant groups:
  1. Lateral LO: nontrained control (n): n = 6; infant training/no adult training (tn): n = 7; adult training (nt): n = 7; infant + adult training (tt): n = 8.
- Adult groups:
  1. Lateral LO: nontrained control (nn): n = 6; infant training/no adult training (tn): n = 7; adult training (nt): n = 7; infant + adult training (tt): n = 7.

Due to methodological inadequacies, some of the brains of the behaviorally tested animals were not included in the quantitative analysis.

Statistical Analysis
Using SPSS (version 15.0; SPSS Inc., Chicago, USA) the behavioral data were analyzed with the General Linear Model for repeated measures analysis of variance (ANOVA) followed by, if applicable, post-hoc least significant difference multiple comparisons tests (LSD). For comparison of infant training performance, a 3 x 5-ANOVA with treatment (t; tn; tt) as the main factor, and day of training (day 1–5) as repeated-measurement factor was used. For comparison of adult training performance, a 2 x 5-ANOVA with treatment (nt; tt) as main factor, and day of training (day 1–5) as repeated-measurement factor was used. For a detailed day-by-day-analysis, univariate analysis of variance (ANOVA) followed by, if applicable, post-hoc least significant difference multiple comparisons tests (LSD) was carried out. For all analyses, Bonferroni confidence interval adjustment for multiple comparisons was used. All tests were two-tailed and the level of significance was set at P < 0.05. All data are presented as mean ± SEM. For total spine densities, a 2-way repeated-measures ANOVA (GraphPad Prism version 5.00, GraphPad Software, San Diego, CA, USA) treatment x hemisphere was applied for infant as well as adult groups. Since no hemisphere differences or interactions, hemisphere x treatment were detected, the values for the hemispheres were pooled resulting in one value per brain region/animal to
avoid inflation of degrees of freedom. One-way ANOVA with Bonferroni post-hoc test for selected pairs of treatment groups was performed using the GraphPad Prism.

For Sholl analysis, a 2-way repeated-measure ANOVA (GraphPad Prism) with treatment and distance from the soma as factors was followed by Bonferroni post-hoc test. The significance level was set at $P < 0.05$.

For the correlation analysis between TWA performance and spine densities/numbers and dendritic parameters, a Pearson correlation analysis was used, and the significance level was set at $P < 0.05$.

**Preparation of Figures**

Digital images were processed by using Adobe Photoshop 10.01 (Adobe Systems Inc., USA) and assembled into montages. For the photomicrographs, only general adjustments of color, contrast, and brightness were made. The neurons displayed in Figure 1 were reconstructed from serial images (0.65 µm steps in $z$-axis), with a Zeiss AxioImager.Z1 microscope equipped with a motorized stage using the Axiovision software (AxioVs40V 4.8.2.0). High-power images of dendritic segments were reconstructed from serial images taken in 0.25 µm steps in $z$-axis.

**Results**

**Infant Training Performance**

In the 3 groups, which were trained as infants (t, tn, and tt), no difference was observed with respect to the number of avoidances, escapes, and escape latencies. For all 3 groups, an effect of the day of training was found on the number of avoidances ($F_{4,92} = 7.210$, $P < 0.001$) and escape latencies ($F_{4,92} = 19.840$, $P < 0.001$), with increased numbers of avoidances and decreased escape latencies. In addition, no
interaction between treatment × day of training was observed. These results emphasize that the different experimental groups (t, tn, and tt) show comparable (low) learning during infant training (Fig. 2, left).

**Beneficial Effect of Infant Training on Adult Learning Performance**

In adults, an effect of training on avoidances ($F_{1,13} = 7.611, P = 0.016$) and escapes ($F_{1,13} = 8.364, P = 0.013$), an effect of day of training on avoidances ($F_{4,52} = 17.841, P < 0.001$), escapes ($F_{4,52} = 14.924, P < 0.001$), and escape latency ($F_{4,52} = 2.858, P = 0.032$), as well as an interaction of treatment × day of training on avoidances ($F_{4,52} = 3.235, P = 0.019$) and escapes ($F_{4,52} = 2.671, P = 0.042$) were observed. A detailed day-by-day analysis (Fig. 2, right) revealed in the infant + adult training (tt) group enhanced the numbers of avoidances on training day 1 ($F_{1,13} = 4.856, P = 0.046$), day 2 ($F_{1,13} = 9.835, P = 0.008$), and day 3 ($F_{1,13} = 12.274, P = 0.004$) as well as decreased the numbers of escapes on training day 1 ($F_{1,13} = 8.693, P = 0.011$), day 2 ($F_{1,13} = 10.785, P = 0.006$), and day 3 ($F_{1,13} = 12.274, P = 0.004$) compared with the animals, which received training only as adults (nt).

**Age-, Region-, and Training-Specific Changes of Spine Densities**

**Orbitofrontal Cortex**

Total spine densities: One-way ANOVA revealed significant differences in spine density between the treatment groups for the apical ($F_{5,44} = 8.589, P < 0.0001$) and basal ($F_{5,44} = 10.06$,

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Figure 2. Behavioral results: the number of avoidances (top), escapes (middle), and escape latencies (bottom) during infant (left) and adult (right) TWA training. Note that in the left column tt (open circles) are the same animals, which were retrained as adults (tt, filled circles). *$P < 0.05$. For abbreviations of the experimental groups see Materials and Methods.
dendrites. Compared with the adult control group (nn), post-hoc analysis revealed significantly elevated spine densities on the apical dendrite of the adult trained (nt) (P < 0.0001), infant training/no adult training (tn) (P < 0.001), and infant + adult training (tt) (P < 0.0001) groups (Fig. 3A). The same effect was found for the basal dendrites (Fig. 3B). No significant difference in spine density was observed between the infant control (n) and infant trained (t) groups.

**Sholl analysis**

Main effects: Apical spine density 2-way ANOVA revealed main effects for treatment (F5,176 = 8.451, P < 0.0001) distance from the soma (F4,176 = 154.9, P < 0.0001) and an interaction treatment x distance (F20,176 = 2.458, P = 0.0009). For basal spine density, similar main effects were detected for treatment (F3,88 = 8.541, P < 0.0001) and distance from the soma (F2,88 = 103.6, P < 0.0001). No treatment x distance interaction could be observed (F10,88 = 1.642, P = 0.108).

Post-hoc analysis for factor treatment: Between the 2 infant age groups, no effect of treatment could be observed either for apical, or for basal spine density. Between the 4 adult age groups, significant differences in apical spine density were found between the adult control group without any training (nn) and 2 adult trained groups [adult training (nt): P < 0.01–0.001; infant + adult training (tt): P < 0.01–0.0001, Fig. 3A,B], this effect was found for nearly all Sholl segments (Fig. 3C). Similar effects were found for the basal dendrite for all 3 adult groups trained at different time points [adult training (nt): P < 0.01–0.0001; infant training/no adult training (tn): P < 0.05; infant + adult training (tt): P < 0.001–0.0001, Fig. 3D].

No statistically significant differences were observed in dendritic length and complexity for apical and basal dendrites.

**Ventromedial Prefrontal Cortex**

**Total spine densities:** One-way ANOVA revealed significant differences in spine density between the treatment groups for the apical (F5,38 = 3.54, P = 0.01) and basal (F5,38 = 6.296, P = 0.0002) dendrites. "Post-hoc analysis” for the apical dendrites revealed no significant differences in spine density between the infant (n) and adult (nn) control groups without training. Significant differences were found between the adult control group without any training (nn) and infant training/no adult training (tn) group (P < 0.05) with higher spine density on the apical dendrites of the adult nontrained (nn) group (Fig. 4A). On the basal dendrites (Fig. 4B), a significant decrease in spine density was observed in the infant training/adult no training (tn) group compared with the untrained (nn) control group (P < 0.001). In addition, the adult untrained (nn) control group displayed significantly higher spine density on the basal dendrites compared with the infant untrained (n) control group (P < 0.001).

**Sholl analysis**

Main effects: Two-way ANOVA for apical spine density revealed main effects for distance from the soma (F4,144 = 88.78,}
and an interaction treatment × distance ($F_{20,144} = 2.283$, $P < 0.0027$). No effect for treatment could be detected ($F_{5,144} = 1.886$, $P = 0.1211$). For basal spine density, similar main effects were detected for treatment ($F_{5,72} = 5.236$, $P = 0.001$), distance from the soma ($F_{2,72} = 27.32$, $P < 0.0001$), and for treatment × distance interaction ($F_{10,72} = 5.399$, $P < 0.0001$).

Post-hoc analysis for factor treatment: Between the 2 infant age groups (n and t), a significant effect of treatment was found only for basal spine densities, with a higher spine density in the trained (t) group on the dendritic segment between 110 and 160 µm distance from soma ($P < 0.05$; Fig. 4). Between the 4 adult groups, no significant differences in basal spine density were found between the untrained control group (nn) and the 3 trained groups (Fig. 4D). Between the 4 adult groups, no significant differences in basal spine density were found between the untrained control group (nn) and the 3 trained groups (Fig. 4D). In contrast, the infant training/no adult training group (nt) showed significantly lower spine densities on the apical dendritic segments between 210 and 260 µm ($P < 0.05$) compared with the adult untrained control group (nn) (Fig. 4C). On the basal dendrite, the spine densities between 110 and 160 µm distance from the soma displayed significantly lower spine densities in the infant untrained control group (n) and all adult groups ($P < 0.001$).

No statistically significant differences were observed in length and complexity for apical and basal dendrites.

**Developmental Changes of Dendritic Spines in the LO and vmPFC**

In the LO, total spine densities on the apical ($P < 0.01$) and basal ($P < 0.0001$) dendrites decreased during postnatal development (comparing the infant (n) with adult (nn) untrained group; Fig. 3A,B). This effect was observed along the apical dendritic segments between 10 and 110 µm and 210–260 µm distance from soma [range of $P$-values between $<0.05$ and $<0.01$ and along the entire basal dendrite ($P < 0.01$); Fig. 5A,B].

In the vmPFC, total spine densities on the apical dendrite did not change during development (comparing the infant (n) with adult (nn) untrained group). However, Sholl analysis revealed an increase in apical spine density between 60–110 µm ($P < 0.05$), 110–160 µm ($P < 0.05$), and 210–260 µm ($P < 0.001$) during development (Fig. 5C). In contrast, total spine density along the basal dendrites increased ($P < 0.001$) during postnatal development (Fig. 4A,B). Sholl analysis revealed that this spine increase was found on the dendritic segments between 110 and 160 µm ($P < 0.001$; Fig. 5C,D).

**Correlation Between TWA Performance and Synaptic Changes**

In the LO of the adult training (nt) group, a significant negative correlation was found for basal spine number and total number of failures ($R = -0.79$, $P = 0.04$).
In the vmPFC of the adult training (nt) group, a significant positive correlation was found between basal spine number and total number of avoidances ($R = 0.85$, $P = 0.02$), basal dendritic length and avoidances on the last day of training ($R = 0.78$, $P = 0.04$), and basal dendritic length and maximal number of avoidances ($R = 0.77$, $P = 0.04$). Furthermore, in the adult training (nt) group, a significant negative correlation was found between basal dendritic length and escapes on the last day of training ($R = -0.78$, $P = 0.04$) and the minimum number of escapes ($R = -0.77$, $P = 0.04$). In addition, a negative correlation was found between the basal spine numbers and the total number of escapes ($R = -0.84$, $P = 0.02$).

**Discussion**

On the behavioral level, we confirmed earlier findings, which showed that avoidance training during infancy, even though the infant rats were not able to learn an avoidance strategy, improves avoidance learning in adulthood (Schäble et al. 2007; Gruss et al. 2010). On the brain structural level, we show here for the first time for pyramidal neurons located in layer II/III of the LO and vmPFC that (1) infant TWA training suppresses the developmental formation of spine synapses in the vmPFC and spine pruning in the LO; (2) infant TWA training alters learning-induced synaptic plasticity in the adult brain; and (3) synaptic and dendritic changes correlate with specific behavioral parameters during learning.

It was pointed out (Kolb et al. 2012) that the mPFC in the rat is analogous to the dorsolateral and medial frontal cortex of primates, and the rat LO is analogous to primate orbital regions, but still little is known about how these prefrontal subareas change with early learning experience. The prefrontal cortex is essentially involved in working memory, an executive function that mediates problem-solving (Senn et al. 2004), which in humans starts to occur in infants around the age of 7–12 months (Anderson 2002; De Luca and Leventer 2008). Around the age of 3–5 years, children start to improve cognitive flexibility, goal-directed behaviour, and planning (De Luca and Leventer 2008); however, they still continue to make errors related to these emerging abilities (Espy 2004). In humans, the protracted development of prefrontal cortical regions is manifest in gross morphology as well as in neuronal fine structure and synaptic reorganization. Prefrontal gray matter seems to increase after birth and reaches a maximum between 4 and 12 years of age and thereafter decreases gradually (Giedd et al. 1999). The increase in gray matter is paralleled dramatic reduction in synaptic density (Huttenlocher 1979; Petanjek et al. 2011), which is in line with the view that synaptic pruning represents an essential developmental principle to achieve a selective specialization of cortical networks.

*Figure 5.* Sholl analysis of synaptic changes in the lateral LO, and vmPFC in response to infant and adult TWA training. For significance levels see Figures 1 and 3.
In rats, the orbital prefrontal cortex develops earlier than the medial prefrontal cortex (van Eden and Uylings 1985), and the immaturity of these regions is also reflected by the synaptic changes, which we observed between infancy and adulthood in the LO (decrease of spine density) and in the vmPFC (spine increase) independent of TWA training. It is tempting to speculate that the infant rats’ disability to learn a successful avoidance strategy might be due to the functional immaturity of these prefrontal regions, including their synaptic integration into limbic circuits.

What do infant rats learn during TWA training, which they might remember after several months during adult training? We showed that infant training requires the contingency of the CS–UCS, and that the infant rats develop a goal-oriented escape strategy (Gruss et al. 2010). For learning to occur, the outcome of a learning trial must be unexpected (Kamin 1969), that is, during training the animal makes prediction errors and learns about the discrepancy between the actual and predicted outcome. In the TWA paradigm, the CS serves as predictor of the UCS, and once the CS–UCS association has been formed the transition from an escape to an avoidance strategy that requires the ability to integrate the prediction error into the learning process, and the animal will gradually shorten the time difference between CS onset and behavioral response (moving to the other compartment). We speculate that, due to the immaturity of the underlying neuronal pathways, the infant rat might either not yet be capable to establish a prediction error, and/or or not be able to integrate the information derived from the prediction error into the ongoing learning process, and thus might get stuck with an (suboptimal) escape strategy.

**Differential Learning-Induced Synaptic Changes in the Infant and Adult Brain**

As pointed out previously, not only the developmental profiles of the 2 prefrontal regions differ, but also the synaptic “substrate” for TWA learning, are significantly different in the infant and adult brain, which consequently results in differential synaptic changes during TWA training. While the infant LO did not undergo changes in spine density during TWA training, in the adult LO spine density increased. In the infant vmPFC, spine density almost doubled on the distal segments of the basal dendrites during TWA training, whereas in the adult vmPFC spine density did not change. This differential learning-induced synaptic plasticity might also be related to the specific roles of these prefrontal regions in learning and memory. The LO is engaged in behavioral flexibility, for example, switching between different problem-solving strategies, and in the evaluation of contingencies between CS and UCS in order to optimize behavioral strategies that maximize reward and minimize punishment (Ongur and Price 2000; O’Doherty 2003; Izquierdo et al. 2004; Kringelbach and Rolls 2004; Schoenbaum and Roesch 2005). The synaptic pathways mediating these higher-order executive functions, including control and inhibition of inappropriate behavioral and emotional responses and decision-making, appear not yet to be fully functional in the infant brain, which may explain why the infant rats not only learn to escape but also fail to learn an active avoidance strategy.

The vmPFC regions, that is, the IL and PL areas, are more likely involved in the fear conditioning aspects (Sotres-Bayon et al. 2006), which represents the first learning step during TWA training, and in learning to escape. The IL is essentially involved in both, behavioral flexibility such as rule shifting, and in conflict solving (Rich and Shapiro 2007; Oualian and Gisquet-Verrier 2010), and it modulates behavioral and neuroendocrine functions in response to stressful situations. The PL is essential for working memory functions, for the expression of learned fears (Heidbreder and Groenewegen 2003; Gabbott et al. 2005; Vertes 2006) and it is involved in behavioral flexibility (Marquis et al. 2007; Ragozzino 2007). In adult rats, the PL is involved in the production of fear responses (Sotres-Bayon and Quirk 2010), which depending on the learning paradigm includes freezing or escape strategies. There is a short time window (P18–P25) during which the recruitment of the PL in fear learning changes dramatically (Li et al. 2012), which may indicate that this prefrontal cortical region is hooked up to the fear pathways during this time.

**Infant TWA Training Has Long-Term Effects on Adult Learning-Induced Synaptic Plasticity**

How is the “memory trace” stored within prefrontal neuronal circuits? We show here for the first time that infant associative learning interferes with developmental synaptic reorganization in prefrontal brain regions. We discovered that these developmental synaptic events in both the LO and vmPFC go in the opposite direction (Fig 6), and that in both brain regions synaptic development is altered by the infant learning process. While in the vmPFC infant TWA training appears to suppress the developmental formation of new spine synapses [comparing infant training (tn) and naive nontreated control (nn) groups, Fig 6], it suppresses developmental pruning of spine synapses in the LO. In view of the remarkable stability of the memory formed during infant learning (several months), it is tempting to speculate that, during infant TWA training, specific synaptic subpopulations in the LO might be “tagged” (compare Fig. 6) and thereby protected from developmental pruning and maintained until adulthood. These “preshaped” synaptic circuits may reflect the neuronal substrate for a long-lasting “memory trace,” which during adult TWA training can be reactivated and thereby accelerate avoidance learning. Frey and Morris (1997) introduced the term “synaptic tagging” as a possible mechanism for LTP-induced changes in synaptic efficacy and specificity. Synaptic tagging places a local molecular tag at specific, potentiated synapses (Redondo and Morris 2011), which lasts for at least 1–2 h or longer and thereby confers long-lasting changes in synaptic specificity. Assuming that synaptic tagging occurs not only in the adult, but also in the infant brain, LTP-related mechanisms during infant learning might trigger molecular synaptic events, which protect specific synaptic subpopulations (those which are activated during infant learning) in the LO from developmental pruning (Fig. 6A,C). The molecular factors, which are currently discussed in relation to synaptic tagging, and which might be also involved in the structural changes observed in our study, include a rapid LTP-induced formation of filamentous actin (F-actin) in dendritic spines (Hayashi et al. 2012) and the formation of a complex formed by the Ca2+/calmodulin-dependent protein kinase II (CaMKII) and the NMDA-type glutamate receptor (NMDAR), which induces a cascade involving densin, delta-catenin, and N-cadherin (see review by Sanhueza and Lisman 2013). Even though some of these molecular tags appear to be transient, they still might guide the pronounced synapse turnover during...
specific developmental time windows and thereby induce long-term structural changes in synaptic composition.

In contrast, TWA training in animals without previous infant TWA training encounters a reduced density of spine synapses in the LO. Due to the lack of recruitable “preshaped” synaptic circuits, the adult “naïve” animals may have to establish new synaptic connections during adult TWA training, which may explain the “slower” avoidance learning in the non-to pre-trained adult animals.

How specific are these training-induced synaptic changes? Does infant training just provide an “enriched environment,” which is known to result in long-term synaptic changes in the brain (Greenough et al. 1987; Rosenzweig and Bennett 1996), or do these synaptic changes represent specific, long-lasting structural memory traces? The latter view is strongly supported by a recent, very elegant study using an acoustic aversive learning paradigm in gerbils (Sarro and Sanes 2011), which demonstrated that (1) animals which received avoidance training as infants using amplitude modulations (AM) as CS displayed better AM detection thresholds as adults, as opposed to animals, which received only AM stimulation, and (2) that the effect of infant training is stimulus-specific, that is, infants trained to a frequency modulation detection task show impaired adult performance on an AM detection task, and (3) that these animals did not recover from this learning deficit.
Neuronal Structural Changes Correlate with Learning Performance

Correlating neuronal parameters with TWA learning success in each individual animal revealed some interesting correlations for basal dendrites in animals trained only as infants (tn) or only as adults (nt). Those animals with the highest number of avoidance responses during infant TWA training displayed as adults higher spine densities in the LO and vmPFC. This supports the interpretation that specific spine subpopulations are "tagged" during infant training and encode the behavioral strategy (Fig. 6). Along the same line, the best adult avoidance learners displayed the highest spine numbers in the vmPFC, which again supports the interpretation that in the good adult learners developmental spine formation was less suppressed following infant TWA training (Fig. 6). Finally, poor avoidance learning was associated with fewer spine numbers in the LO as indicated by a negative correlation between the basal spine numbers and the number of failures. With respect to dendritic length and complexity, we found that good avoidance learners displayed larger basal dendritic trees in the vmPFC, which indicates that in these animals a larger synaptic input area is available for learning.

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References


