The postnatal environment can counteract prenatal effects on cognitive ability, cell proliferation, and synaptic protein expression

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SPECIFIC AIMS

To elucidate whether environmental conditions during prenatal and postnatal periods affect cognitive functions of adult offspring and structural and biochemical alterations in their brains, we investigated respective effects and combined effects of stress and/or enriched environments during pre- or postnatal periods on spatial learning and memory, cell proliferation, and synaptic changes in adulthood through a longitudinal study.

PRINCIPAL FINDINGS

1. Pre- or postnatal effects of stress and enriched environments on spatial memory ability, dentate gyrus cell proliferation, and markers of synaptic plasticity in the brain

Male pups born to mothers housed in stressful (S), enriched (E), or control (C) conditions before birth were sorted and housed in stressful (S), enriched (E), or control (C) conditions after birth. In a Y-maze (Fig. 1A) and a water maze test (Fig. 1B) to verify the effect of the housing condition, spatial cognitive function in EC and CE was significantly improved but spatial memory in SC and CS was significantly impaired compared with that in CC.

Proliferation of progenitor cells in the granular cell layer (GCL) was examined by staining for 5-bromo-2′-deoxyuridine (BrdU) -positive cells. Mitotic figures were evident in sections from every animal (Fig. 1C). One day after the last injection of BrdU (Fig. 1O), proliferation of progenitor cells was increased in EC or CE but decreased in SC compared with that in CC (Fig. 1E–H). However, there was no difference between CS and CC (Fig. 1J). At 4 wk after the last injection of BrdU (Fig. 1P), mature cells were found throughout the GCL and appeared ovoid or round (Fig. 1D); the pattern of cell proliferation was similar to that observed 1 day after BrdU injection (Fig. 1J–N). The majority of BrdU-positive cells were neuron (63–75%) in each group. The CE group had a significantly higher percentage of surviving BrdU-positive cells that colabeled for Calbindin.

We examined the expression levels of two independent synaptic markers—neuronal cell adhesion molecule (NCAM) and synaptophysin (SYP)—in the brain of the adult offspring. NCAM and SYP immunoreactivity and BDNF levels were significantly increased in EC and CE but decreased in SC and CS in the cortex and hippocampus compared with CC.

2. Effects of pre- and postnatal environment on spatial memory ability and markers of synaptic plasticity in cross-housing groups

In the Y-maze (Fig. 2A), EE showed significantly more alternation than ES, and SE showed significantly more alternation than SS. Water maze task (Fig. 2B) showed that EE had a significantly shorter latency than ES (1st, 3rd, 4th, 5th sessions) and SE had a significantly shorter latency than SS (4th session). EE had a significantly shorter latency than SE (4th session) and ES had a significantly shorter latency than SS (2nd session).

In the cortex (Fig. 2C, E) and hippocampus (Fig. 2D, F), EE showed significantly higher immunoreactivity of NCAM (140 and 180 kDa isomers) than ES, and SE showed higher immunoreactivities of NCAM than SS in the cortex. ES showed higher immunoreactivities of NCAM than SS. EE showed significantly higher levels of SYP immunoreactivity than ES or SE, and SE or ES showed higher levels of SYP immunoreactivity than SS in the cortex (Fig. 2G, J) and hippocampus (Fig. 2H, J).

EE showed significantly increased BDNF levels in the cortex (Fig. 2K) and hippocampus (Fig. 2L) compared
Figure 1. Effects of pre- or postnatal stress and enriched environments on spatial learning and memory performance and cell proliferation. A) Spontaneous alternation behavior during a 10-min session in the Y-maze. Data are presented as the % of correct alternation to total arm entries (mean±SE, n=7–8 per group). $^*P<0.05$, $^{**}P<0.01$ vs. CC group, by post hoc analysis. B) Spatial learning in the Morris water maze. Data are presented as latency (mean±SE, n=7–8 per group) to find the platform over 5 consecutive days of testing. $^*P<0.05$ CC vs. EC group; $^{**}P<0.05$ CC vs. SC group; $^{***}P<0.05$ CC vs. CE group; $^{***}P<0.05$ CC vs. CS group, by post hoc analysis. Cell proliferation and survival in the granule cell layer (GCL) of hippocampus were determined. Representative photomicrographs of proliferating (C, 1000×, scale bar=20 μm; E–I, 200×) and mature BrdU-positive cells (D, 1000×; N, 200×). Proliferating cells were localized to the subgranular zone (SGZ) and often appeared in clusters. Mature cells are found throughout the GCL and appear ovoid or round. The number of BrdU-positive cell 1 day after BrdU injection (O) and 4 wk after BrdU injection (P). Cells were counted in 8 sections (4 rats/group). Data are presented as number of BrdU-positive cell (mean±SE). $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. CC group, by post hoc analysis.

Figure 2. Effects of pre- and postnatal environments on spatial learning and memory performance and expression of NCAM, SYP and BDNF in cross-housing groups. A) Spontaneous alternation behavior during a 10 min session in the Y-maze (A). Data are presented as the % of correct alternation to total arm entries (mean±SE, n=10–11 per group). $^*P<0.05$, $^{**}P<0.01$ vs. EE group; $^{**}P<0.05$ vs. SS group, by post hoc analysis. Spatial learning in the Morris water maze (B). Data are presented as latency (mean±SE, n=7–8 per group) to find the platform over 5 consecutive days of testing. $^*P<0.05$, $^{**}P<0.01$ EE vs. ES group; $^{**}P<0.05$ ES vs. SS group; $^{***}P<0.01$ EE vs. SS group; $^{***}P<0.001$ EE vs. SS group, by post hoc analysis. No differences in the average swim-speed were observed at any time point. Western immunoblot analysis of NCAM (C–F) and SYP (G–J) in the cortex (C, E, G, I) and hippocampus (D, F, H, J) of the cross-housing groups (n=6 per group). Data are presented as optical density unit measures (mean±SE, E, F, I, J). $^*P<0.05$, $^{**}P<0.01$ vs. EE group; $^{***}P<0.001$ vs. SS group, by post hoc analysis. (K, L) BDNF protein expression levels in the cortex (K) and in the hippocampus (L) of the cross-housing groups by ELISA. 4 rats per group were used (15–20 plate/rat). Data are presented as amount of protein (pg BDNF/μg total protein, mean±SE) $^*P<0.05$, $^{***}P<0.001$ vs. EE group; $^{**}P<0.01$ vs. SS group, by post hoc analysis.
with ES or SS. The BDNF level of SE was significantly increased vs. that of SS.

CONCLUSIONS AND SIGNIFICANCE

Our results showed that pre- or postnatal stress reduced proliferation of progenitor cells in the GCL and learning and memory performance whereas pre- or postnatal enriched environment increased them. Recent studies have revealed that newly generated cells derived from progenitor cells in GCL might mediate synaptic plasticity, alter cognitive functions, and be required to replace dying neurons, suggesting a compensatory adaptive response to neuronal or synaptic loss.

SYP is a synaptic vesicle protein that acts as a specific marker and is involved in neuronal transmission. The NCAM level is a reliable index of synaptic density. In our study, pre- or postnatal enriched environment increased the expression levels of NCAM and SYP, whereas pre- or postnatal stress decreased the expression level of both synaptic markers in the cortex and hippocampus. These data indicate that environmental experiences during pre- or postnatal period induce morphological and/or structural changes in the adult brain and suggest that the level of synaptogenesis or synaptic survival by the pre- or postnatal experience could contribute to experience-related functional effects through the regulation of neurotransmitter release, synaptic stabilization, and strength.

BDNF has been reported as an attractive candidate that translates experience-dependent neuronal activity into structural and functional changes in the neuronal populations under the stressful or enriched conditions. BDNF plays an essential role in the acquisition of spatial memory and enhancing long-term potentiation in the hippocampus. We found that the BDNF expression levels as well as the immunoreactivity for SYP and NCAM in the cortex and hippocampus were consistent with behavioral alterations, suggesting that BDNF may partially contribute to the experience-dependent cognitive alterations by mediating the structural and/or functional plasticity in the adult brain.

Our longitudinal cross-housing study revealed that environmental conditions during postnatal period, regardless of prenatal conditions, affect learning and memory performance in the Y-maze and the water maze. These results indicate that the effects of the prenatal environments on cognitive function can be attenuated significantly by the postnatal environments. In the adult brain, the dominant effects on synaptic structural changes appeared to be more apparent in the cortex than in the hippocampus. Although further studies are needed, our data strongly suggest that the postnatal environment might be more crucial for the permanent memory storage associated with the cortical network. We found that the behavioral alterations appeared to be consistent with BDNF expression in cross-housing groups. These data allow us to propose a pathophysiological path where the dominant effect of postnatal environment over the prenatal manipulations on spatial learning and memory may result from the synaptic plasticity partially mediated by BDNF.

In humans and animal models, the offspring of mothers experiencing stress during pregnancy were reported to display long-term behavioral abnormalities throughout life, inducing structural and morphological changes in the brain. Our results, however, show that these detrimental effects of prenatal stress can be counteracted by enriched housing after birth, which indicates postnatal environmental manipulation can cure some cognitive impairments induced by prenatal stress. These beneficial effects were attenuated by postnatal stress, implying that prenatal care is necessary for adequate brain functioning but may be greatly modified by postnatal conditions. Our findings reveal that 1) either prenatal or postnatal stress or enrichment condition affects cognitive ability and some brain functions in the adult life and 2) postnatal stress or enrichment environment could greatly influence the effects of prenatal stress or enrichment condition.