Postnatal development and sensory experience synergistically underlie the excitatory/inhibitory features of hippocampal neural circuits: glutamatergic and GABAergic neurotransmission

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Abstract

During postnatal critical period balance of excitation/inhibition in developing brain is highly regulated by environmental signals. Compared to visual cortex, rare document include effects of sensory experience on hippocampus, which is also bombarded by sensory signals. In this study, basic and tetanized EPSPs were recorded in CA1 area of hippocampus of light- (LR) and dark- reared (DR) rats (at 2, 4 and 6 weeks of age). Also, we assessed age- and activity-dependent changes in the NMDA and AMPA receptors subunit compositions and, GABA producing enzymes. While the sensory deprivation increased amplitude of baseline fEPSPs, it decreased degree of potentiation of post-tetanus responses. Expression of GluA1 and GluA2 subunits of AMPARs was increased across age in DR rats. In contrast to LR rats, mRNA and protein expression of GluN1, GluN2A and GluN2B subunits of NMDARs was decreased in DR ones. Also, dark rearing diminished expression of GABA synthesis enzymes GAD65 and GAD67. These results indicate that, sensory experience adjusts synaptic plasticity and might also affect the balance of excitation/inhibition in the hippocampus.

Keywords: Visual experience; Postnatal development; Hippocampus; Synaptic plasticity; Excitatory/Inhibitory neurotransmission; Rat

1. Introduction

Postnatal critical period encompasses a series of structural and functional changes that finally deliver brain mature. Evidence shows that the critical period is not just an age-related developmental process; further, sensory inputs take a substantial role in the brain maturation (Voss, 2013). Among the sensory signals, visual inputs exhibit a pivotal role in mammalian developing brain (Morishita and Hensch, 2008) and, therefore, role of sensory experience has been extensively studied in visual cortex. It has been shown that monocular deprivation reduces visual evoked potentials in rat visual cortex (Montey and Quinlan, 2011). The effect of sensory experience on plasticity of synaptic transmission and especially long-term potentiation (LTP), an experimental form of synaptic plasticity, has been controversial. Different impact of sensory experience is demonstrated in the visual cortex where both diminished (Berry et al., 1993) and facilitated LTP (Salami et al., 2000b) were reported in the visually deprived animals. Evidence indicates that possibility of LTP induction decreases as the window of critical period closes. In line, it is proved that dark rearing can extend the postnatal critical period and, therefore, prolongs the period of time during which the LTP is inducible (Kirkwood et al., 1995).

Direct and indirect processed signals from sensory cortices are projected to the hippocampus. Especially the mammalian's visual cortex supplies an elementary sensory input to the hippocampus and, thus, a comparable synaptic plasticity is observable in visual cortex and hippocampus (Tsanov and Manahan-Vaughan, 2008). Therefore, the hippocampus, like sensory cortices may undergo a period of postnatal development (Ge et al., 2007). However, limited studies have evaluated dependency of hippocampal circuits' maturation on the environmental signals (Talaie et al., 2010, Talaie and Salami, 2013) and enrichments (Waters et al., 1997).

Glutamate is the main excitatory neurotransmitter in the brain. The ionotropic glutamate receptors consist of several subgroups including NMDA and AMPA receptors. The NMDA and AMPA receptors are heterotetrameric proteins associated by four subunits from the same family which are assembled in different arrangements (Smart and Paoletti, 2012). Composition of AMPA and NMDA receptors' subunits are developmentally regulated (Collingridge et al., 2012). Also, it has been shown that change in sensory experience rearranges composition of NMDA (Quinlan et al., 1999b, Tongiorgi et al., 2003) and AMPA (Goel et al., 2006,

Beston et al., 2010) receptors subunits. These receptors are required for many forms of learning and memory and have a crucial role in synaptic function and plasticity (Abraham and Williams, 2008).

Gama-amino-butyric acid (GABA) mediates the most important inhibitory neurotransmission in the brain. The structure and function of GABAergic neurons were changed during brain development (Koyanagi et al., 2010). Also, researches revealed that, change in visual experience causes change in structure and function of GABAergic neurons in rats' visual cortex (Yazaki-Sugiyama et al., 2009).

The changes in synaptic plasticity of visual cortical neurons are partly contributed to changes in excitatory and inhibitory neurotransmission induced by visual experience. Accordingly, it has been reported that dark rearing reduces NMDA receptor-mediated excitatory postsynaptic currents via decrease in the ratio of GluN2A to GluN2B subunits of cortical NMDA receptors (Philpot et al., 2007). In line, Funahashi et al. revealed that AMPA dependent excitatory post-synaptic currents decrease with age and dark rearing inhibits decrease of AMPA-EPSCs (Funahashi et al., 2013). Also, Keck et al demonstrated that light deprivation results in a rapid and lasting reduction of GABAergic signals in mouse visual cortex (Keck et al., 2011).

Hippocampus is a well-known area of brain involved in memory consolidation. With its good anatomically arranged circuits it has been considered as astonishing part of brain for evaluating LTP (Sloviter and Lomo, 2012). This study was designed to assess the probable mechanisms by which the postnatal development and sensory experience concomitantly develop progression of normal and potentiated synaptic transmission in the CA1 area of hippocampus.

2. Experimental procedures

2.1. Animals and ethics

Eighty four male Wistar rats were used in this study. Control light reared (LR) rats were housed in standard cycles of 12 hr light/12 hr dark. Dark reared (DR) animals were reared in complete darkness from birth through end of experiments. All animals were kept under a temperature of 22 °C and air humidity of 55—60% and free access to food and water *ad libitum*. The experiments were performed on three groups of either LR or DR rats consisting the animals at 2 (2WLR, 2WDR), 4 (4WLR, 4WDR) and 6 (6WLR, 6WDR) weeks of postnatal age (n=8 in each group in electrophysiology experiments, n=6 in each group in RT-PCR and immunoblotting assessments). Previous studies indicated a postnatal critical period for development of neural circuits so that the brain of rats is delivered in matured form around the end of sixth weeks of old (Yang et al., 2009). Protocol of

the experiments was carried out in accordance with the Directive 2010/63/EU on the protection of animals used for scientific purposes.

2.2. Electrophysiology

2.2.1. Surgery and electrode implantation

Rats were anesthetized by urethane (1.5 g/kg, i.p.) and placed in a stereotaxic frame. A large portion of the tissue overlying the hippocampus was carefully removed. Two small holes (1 mm diameter) were drilled in the skull to allow the insertion of stimulating (4.2 mm posterior to bregma, 3.8 mm lateral to the midline) and recording electrodes (3.4 mm posterior to bregma, 2.5 mm lateral to the midline) in the brain. The overlying dura was punctured with a needle. Electrodes were made from Teflon-coated, stainless steel wire (0.008 in outside diameter; A-M systems, USA) exposed only at the tip (tip separation approximately 0.10 mm). The stimulating electrode was bipolar made from twisted wire and the recording electrode was monopolar.

2.2.2. Recordings

The recording and stimulating electrodes were lowered into the CA1 stratum radiatum and the Schaffer's collaterals, respectively. Field extracellular postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum of CA1 area of hippocampus in response to the stimulation of Schaffer's collaterals-commissural pathway. For pre- and post-tetanus recordings the stimulation intensity was adjusted to a level that evoked a 60% of maximum. Pulse stimulus was programmed by a software (Scope for Windows; AD Instruments, Australia) and delivered to a stimulator/isolator unit (WPI-A365, Sarasota, FL, USA). Extracellular recordings were referenced to an indifferent site. Constant current stimulation (200 μ s duration) was programmed by a computer software (Neurotrace, WSI, I. R. Iran) and delivered to a stimulator/isolator unit. The fEPSPs were recorded at 30 s intervals for 30 min period and the baseline data were obtained by averaging the response. The fEPSPs were amplified by a preamplifier (Electromodule, WSI, I.R. Iran), filtered at 1–3000 Hz, digitized (10 points/ms) and stored for offline analysis using "Potentialize" software (WSI, I. R. Iran). Then, LTP was induced by high-frequency stimulation (HFS) of 100 Hz (10 bursts of 10 stimuli, 0.2 ms stimulus duration, and 10 s inter-burst intervals). Following the tetanus, responses to the test pulses were collected continuously for at least 2 h. The size of the fEPSP amplitude was measured from the beginning of negative wave to its peak in mV. LTP was expressed as mean percent increase of amplitude in the post-HFS responses over 120 min against the baseline amplitude; however, the potentiation was interpreted as LTP if the amplitude of fEPSP was increased at

least 20% post-tetanus. It should be noted that eyes of the DR rats were tightly covered on arrival to the experiment room and remained covered during the experiment.

2.3. Tissue collection and storage

The brains from animals aimed for either PCR processing or Western blotting were removed rapidly and hippocampi and dissected immediately. The hippocampal sections were placed in liquid nitrogen and then stored at -80°C until used.

2.4. RT-PCR processing

The relative expression of target mRNA was evaluated by RT-PCR technique. Using a RNA extraction kit (peqGOLD RNA Pure, Peqlab Co., Germany), total RNA of each hippocampus was extracted and assayed by spectrophotometer (BioPhotometer plus, Eppendorf Co., Germany). Firstly, according to standard protocol mentioned in the kit, one µg RNA from each sample was converted to cDNA (Roche, Germany). Then, using specific primer sets (Table 1) the target sequences were amplified by a thermocycler machine (peqSTAR 96X, Peqlab Co., Germany). Using gradient PCR and serial dilution of cDNA sample, PCR optimization for each primer was done (as explained in table 1) as follows: Initial denaturation was performed at 94°C for 2 min, followed by denaturation at 94°C for 30 s, annealing at variable primer-specific temperatures for 45 s and extension at 72°C for 45 s. A further 7 min final extension at 72°C was considered for all mRNAs. The PCR products were identified by agarose gel electrophoresis (2% W/V, stained with ethidium bromide), observed and photographed under an ultraviolet light by gel documentation system (UV Tech, UK). Finally, the bands were analyzed by ImageJ software (Version 1.48, NIH, USA) and ratios of each target mRNA to that of HPRT (as house-keeper gene) was calculated and reported as semi-quantitative expression of the samples.

2.5. Immunoblotting

Western blot analysis was carried out for determination of protein content of the hippocampuses. For this, protein content of hippocampi was extracted by Radioimmunoprecipitation assay buffer (RIPA buffer) containing antiprotease cocktail (Roche, Germany). Total protein quantification was assessed through Bradford assay. Proteins were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis at 120 V for 2 hours, blotted onto a PVDF membrane (Roche, Germany) by semi-dry transfer technique at 10 V for 45 minutes and incubated with different antibodies (purchased from Abcam, Germany) directed against β actin (1:5000),

GluA1 (1:1000), GluA2 (1:500), GluN1 (1:1000), GluN2A (1:1000), GluN2B (1:500), GAD65 (1:1000) and GAD67 (1:2000), diluted in 5% milk PBS-Tween 20 blocking buffer for 12 h at 4°C. Then, the blots were washed in TBST for 30 minutes and were incubated with secondary antibodies (1:3000, horseradish peroxidase-conjugated goat anti-rabbit or mouse antibodies from Abcam, Germany) for 1 h at room temperature. Labeling and development was carried out using the enhanced chemiluminance (ECL) Western blot detection system (AceGlow, Peqlab Co., Germany). Densitometry was done using ImageJ software (Version 1.48, NIH, USA) and each band intensity was normalized against the band intensity of β actin as structural protein.

2.6. Data analysis

Two-way analysis of variance (ANOVA) was applied to determine significance among group concerning change in the amplitude of fEPSPs as between-subjects factor and visual experience as within-subjects factor. Multivariate ANOVA was performed on the data of relative expression of genes and proteins. Tukey's test was used as post hoc test. All data are expressed as Mean $\pm$ S.E.M. The probability levels interpreted statistically significant if P value was less than 0.05.

3. Results

3.1. Synaptic transmission in the CA3/CA1 pathway

Developmental changes in pre- and post-HFS responses of the LR and DR rats were assessed through *in vivo* recordings in anesthetized animals. The baseline and tetanized fEPSPs were recorded in response to the test stimulus, determined by means of input/output curves (figure 1). Statistics indicated that the light deprivation effectively influenced the age-dependent changes in activity of the neuronal circuits ($F_{11,2388}=44.360$; $P<0.0001$).

3.1.1. Baseline recordings

The amplitude size of fEPSPs recorded in the CA1 area of hippocampus of the LR animals diminished by about 15% across age so that the magnitude of the baseline responses considerably decreased from 1.28 ± 0.05 mV in the 2WLR rats to 1.09 ± 0.03 mV in the 6WLR rats ($P=0.024$). The light deprivation noticeably reversed the effect of age on the baseline activity where the amplitude size of the fEPSPs increased by about 15% from 1.30 ± 0.03 mV in the 2WDR group to 1.50 ± 0.04 mV in the 6WDR group ($P=0.018$). Negligible difference was observed between the animal groups at the second and forth weeks of age in either LR or DR groups. Comparison of the data taken from the LR and DR rats indicated that the different effect of aging on the synaptic transmission rises as the brain developing proceeds. Whereas the amplitude size of fEPSPs is almost

similar in all two-week aged groups, pronounced differences appear between the rats at the forth ($P=0.003$) and sixth week of age ($P<0.0001$). Figure 2 demonstrates age-dependent changes in the fEPSP amplitude in the LR and DR animals.

3.1.2. Induction of LTP in the CA3/CA1 pathway

The tetanic stimulation of the Schaffer collaterals resulted in a prominent and maintained LTP in the fEPSPs recorded in all groups of the LR rats ($P<0.0001$). The mean potentiation was the highest in the 2WLR rats ($61.38\pm13.74\%$) and displayed a significant gradual decrease over age where the mean value of the post-HFS enhancement was declined to $47.61\pm8.11\%$ and $37.09\pm8.64\%$ in the 4WLR and 6WLR, respectively ($P<0.0001$ between 2WLR and 6WLR groups). The post-tetanus fEPSPs in the DR groups also fulfilled our criterion of LTP, however, the level of potentiation was significantly lower than the LR groups ($P<0.0001$). The developmental decrease in degree of LTP was also visible in the DR animals so that the highest LTP in the 2WDR rats ($34.96\pm5.83\%$) diminished to $24.94\pm3.83\%$ and $20.88\pm5.56\%$ in the 4WDR and 6WDR rats, respectively ($P=0.036$ between 2WDR and 6WDR groups). Comparison of the induced LTP between the LR and their DR counterparts indicated that the dark rearing effectively reduced plasticity of the synaptic transmission in all groups ($P<0.0001$). The present findings are in accordance to those reported previously (Talaie et al., 2010). Figure 3 illustrates the time course of pre- and post-HFS recordings in the CA1 area of hippocampus of the LR and DR animals.

3.2. Expression of mRNA

Expression of the mRNAs encoding two subunits of the AMPA receptors (GluA1 and GluA2), three subunits of the NMDA receptors (GluN1, GluN2A and GluN2B) and two isoforms of key GABA producing enzymes (GAD65 and GAD67) were considered in the studies using RT-PCR technique. Multivariate ANOVA revealed a considerable difference between relative expression of the mRNAs in the LR and DR testing groups ($F_{5,30}=31.115$; $P<0.0001$).

3.2.1. AMPA receptors

3.2.1.1. GluA1 subunits

The relative expression of mRNA encoding GluA1 subunit of the AMPA receptors showed a substantial reduction (of about 43%) over the postnatal life of the LR animals; the values are 1.71 ± 0.15 and 0.98 ± 0.05 for

the 2WLR and 6WLR rats, respectively ($P<0.0001$). In the DR animals, in contrast, we observed a slight but statistically significant increase in the mRNA expression of the subunit where the relative values increased (by about 16%) from 1.94 ± 0.08 in the 2WDR rats to 2.25 ± 0.12 in the 6WDR animals ($P=0.044$). Evaluation of the data taken from the LR and DR groups revealed that the sensory deprivation during the postnatal critical period substantially enhances occurrence of the expression of the GluA1 subunit in the DR rats compared to their LR counterparts ($P<0.0001$).

3.2.1.2. GluA2 subunits

The mRNA of another subunit of AMPA receptors, GluA2, displayed an increasing pattern of expression with age in hippocampus of both LR and DR animals. Relative mRNA expression of the GluA2 subunit raised from 0.80 ± 0.10 in the 2WLR rats to 1.49 ± 0.12 in the 6WLR animals ($P<0.0001$). Such an increased form of mRNA expression was also seen in the DR rats where the lowest and highest expressions occurred in the 2WDR (1.54 ± 0.15) and 6WDR (1.91 ± 0.09) groups, respectively ($P=0.024$). Importantly, amount of the enhanced mRNA expression was more pronounced in the LRs (by about 88%) rather than the DRs (by about 24%). However, the relative expression of GluA2 subunit mRNA had a value of about two folds in the 2WDR compared to 2WLR rats and substantial elevated expression was visible in the three ages of DR groups compared to their LR counterparts ($P<0.0001$). Therefore, it seems that expression of the mRNA in the very young animals is sufficiently affected by sensory experience although age also takes a key role during development. Figure 4-A depicts the developmental trend of mRNA expression level of AMPA receptor subunits in the LR and DR groups.

3.2.2. NMDA receptors

3.2.2.1. GluN1 subunit

The mRNA encoding the GluN1 subunit of the NMDA receptors was expressed increasingly across age. The values are 0.78 ± 0.15 , 1.58 ± 0.13 and 2.43 ± 0.11 for the 2WLR, 4WLR and 6WLR rats, respectively ($P<0.0001$). The same pattern of relative expression was observed in the DR groups with 0.63 ± 0.11 , 1.72 ± 0.19 and 1.73 ± 0.11 for the 2WDR, 4WDR and 6WDR rats, respectively ($P<0.0001$). From these data it is understandable that age positively affect the expression of the GluN1 subunit; an almost three times increase in the mRNA expression in both the LR and DR experimental subjects. The visual deprivation had a slight but significant negative effect only at the sixth week of age ($P=0.001$).

3.2.2.2. *GluN2A subunit*

The relative expression of the GluN2A subunit mRNA demonstrated a developmental increase of about 2.8 fold in the mRNA expression of the subunit. The values are 0.23 ± 0.03 , 0.64 ± 0.14 and 0.88 ± 0.09 for the 2WLR, 4WLR and 6WLR, respectively ($P < 0.0001$). However, keeping animals in complete darkness declined (by more than half) the relative expression of the mRNA from 0.29 ± 0.02 in the 2WDR rats to 0.12 ± 0.02 in the 6WDR animals ($P < 0.0001$). Comparison of the data reveals that the mRNA expression of the GluN2A subunit is age-dependent in both the LR and DR groups, however, as is reflected in a considerable difference (by more than 7 fold) between the 6WLR and 6WDR rats ($P < 0.0001$) the visual experience demonstrates a more effective impact when the DR animals get aged.

3.2.2.3. *GluN2B subunit*

The mRNA expression of GluN2B subunits occurred age-independent where slight decrease in the relative expression of the subunit over age was not statistically significant. The values are 0.94 ± 0.11 and 0.79 ± 0.08 for the 2WLR and 6WLR animals, respectively. An analogous pattern of reduced mRNA expression was also observable in the dark reared animals, however, the variation between the 2WDR (0.84 ± 0.14) and 6WDR (0.46 ± 0.07) rats was considerable ($P = 0.023$). The visual experience-dependent decrease in mRNA expression of the GluN2B subunit in the 6WDRs was statistically noticeable when compared with their LR counterparts ($P = 0.045$). Figure 4-B represents the age- and sensory experience-dependent changes in mRNA expression level of NMDA receptor subunits in the LR and DR rats.

3.2.3. Glutamate decarboxylase enzymes

3.2.3.1. *GAD65 enzyme*

The relative expression of the mRNA encoding GAD65 enzyme in the LR animals was increased (by about 86%) over age; from 1.27 ± 0.15 in the 2WLRs to 2.36 ± 0.13 in the 6WLRs ($P < 0.0001$). The mRNA expression in the DR rats resembled that in their LR counterparts so that we observed a 2-fold increase in the expression of GAD65 enzyme mRNA; values are 0.37 ± 0.06 and 0.74 ± 0.14 in the 2WDR and 6WDR rats, respectively ($P < 0.0001$). A substantial effect of visual inputs on the mRNA expression of the enzyme is observable in considerable difference between the LR and DR animals; whether in the second (by about 3.5 times, $P < 0.0001$) or six week (by about 3.5 times, $P < 0.0001$) of postnatal age.

3.2.3.2. *GAD67* enzyme

Appearance of GAD67 enzyme mRNA was also increased (by about 80%) in an age-dependent manner in the LR groups where the values of the mRNA expression are 1.43 ± 0.08 , 2.05 ± 0.13 and 2.56 ± 0.10 in the 2WLR, 4WLR and 6WLR rats ($P < 0.0001$). The mRNA expression in the DR animals was also positively age-sensitive so that it increased (up to about 50%) from 0.45 ± 0.03 in the 2WDR rats to 0.52 ± 0.08 and 0.68 ± 0.09 in the 4WDR and 6WDR animals ($P < 0.0001$). The effectiveness of the environmental signals on expression of the GAD67 enzyme mRNA was more pronounced than developmental factors. Expression of the enzyme was higher by a factor of three in the two-weeks aged DR rats compared to their LR counterparts and further increased (up to 4-fold) through fourth and sixth week of age ($P = 0.0001$). Figure 4-C describes how the postnatal critical period underlies the mRNA expression level of glutamate decarboxylase enzymes in the LR and DR experimental subjects.

3.3. Expression of protein

Western blotting method was used to evaluate the relative expression of target proteins for the NMDA and AMPA receptor subunits, and GABA synthesizing enzymes. Multivariate analysis of variance showed a significant difference between expression of all target proteins in the LR and DR testing groups ($F_{5,30} = 26.870$; $P < 0.0001$).

3.3.1. *AMPA receptor*

3.3.1.1. *GluA1* subunits

A developmental slight decrease (by about 25%) in the expression of GluA1 subunit of AMPA receptors was found in the LR animals, however, the changes was noticeable ($P = 0.004$) only in the 6WLR rats (1.40 ± 0.09) when compared to the 2WLR animals (1.07 ± 0.04). The visual deprivation reversed the postnatal alteration in appearance of the GluA1 subunit where expression of the subunit increased slightly (by about 21%) from 1.66 ± 0.06 in the 2WDRs to 2.01 ± 0.06 in the 6WDR rats ($P = 0.003$). Comparison of the data from three groups of LR and DR animals indicated that the protein expression was significantly higher in all groups of the DRs ($P < 0.0001$).

3.3.1.2. *GluA2 subunits*

The expression of GluA2 subunit protein in the LR groups was highly age-dependent. The GluA2 subunits were appeared increasingly (by about 190%) over early postnatal life; from 0.77 ± 0.05 to 2.21 ± 0.10 in the 2WLR and 6WLR rats, respectively ($P < 0.0001$). Hence, aging takes a substantial role in production of the GluA2 subunits in the LR groups. Although expression of the GluA2 protein in the DR animals was also enhancing across age, however, the rising pattern was much smaller (by about 16%) compared to their LR counterparts; the values are 2.29 ± 0.11 and 2.65 ± 0.09 in the 2WDR and 6WDR rats, respectively ($P = 0.01$). The most effect of the sensory experience appears in the two-week groups so that the protein expression was prominently higher (by about 3.5 times) in the visual deprived animals compared to the light reared ones ($P < 0.0001$). Figure 5-A shows the effect of age and sensory experience on composition of NMDA receptor subunits in the LR and DR groups.

3.3.2. NMDA receptors

3.3.2.1. *GluN1 subunits*

Western blotting showed that the protein expression of GluN1 subunits of the NMDA receptors is mildly alternating with age. The lowest expression was seen in the 2WLR rats (0.64 ± 0.04) and increased (by about 90%) to 1.22 ± 0.05 in the 6WLR animals ($P < 0.0001$). An almost similar pattern of the subunit expression was observable in the DR group where the GluN1 subunit protein was elevated (by about 80%) from 0.79 ± 0.05 to 1.41 ± 0.05 in the 2WDRs and 6WDRs, respectively ($P < 0.0001$). Comparison of the data from the LR and DR groups indicates that deprivation of the DR animals from normal light enhances the GluN1 expression in all testing groups. Statistics reveals differences between the two- ($P = 0.038$) four- ($P = 0.04$) and six- ($P = 0.009$) weeks old rats.

3.3.2.2. *GluN2A subunits*

The expression of GluN2A subunit protein of the NMDA receptors was developmentally elevated (by about 82%) with age in the LR groups. The target protein expression began with 0.71 ± 0.08 in the 2WLR rats and enhanced to 1.29 ± 0.04 in the 6WLR animals ($P < 0.0001$). We found that the GluN2A subunit protein expression was sensitive to the dark rearing. The GluN2A subunit was appeared by 0.66 ± 0.04 in the 2WDR rats and decreased (by about 40%) to 0.40 ± 0.05 in the 6WDR group ($P = 0.006$). Comparison of the data from The LR and DR groups appeared that while the protein expression in the 2WDR animals was not statistically different

compared to their LR counterparts it was considerably smaller (by about one third) in the animals at sixth week of postnatal age ($P<0.0001$).

3.3.2.3. *GluN2B subunits*

Western blot processing demonstrated a negligible increased in the expression level of GluN2B subunit of the NMDA receptors in the LR animals. The alterations between groups of LR rats were not statistically significant. The negative role of sensory experience in expression of the subunit was observable where the protein expression reduced (by about 25%) from 0.65 ± 0.05 in the 2WDR animals to 0.49 ± 0.04 in the 6WDR rats ($P=0.01$). Function of the visual inputs led to a significant difference between the 6-weeks old LR and DR groups ($P=0.04$). Figure 5-B elucidates the visual experience-related alterations in configuration of NMDA receptor subunits of the LR and DR animals during the postnatal period of life.

3.3.3. Glutamate decarboxylase enzymes

3.3.3.1. *GAD65 isoform*

The relative expression of protein of isoform GAD65 of glutamate decarboxylase enzyme showed a rising pattern over postnatal development of the LR groups. The level of expression increased (by about 140%) from 0.85 ± 0.05 in the 2WLR animals to 2.04 ± 0.08 in the 6WLR rats ($P<0.0001$). The expression of GAD65 protein in the DR animals was also age-related where we observed a robust increase (by about two times) in production of the enzyme across age; from 0.41 ± 0.06 in the 2WDR rats to 1.23 ± 0.09 in the 6WDR animals ($P<0.0001$). The visual experience-dependent decrease in expression of GAD65 in the DR rats was statistically noticeable when compared with their age-matched LR counterparts ($P<0.0001$).

3.3.3.2. *GAD67 isoform*

The GAD67 isoform of the glutamate decarboxylase enzyme showed to be expressed highly based on an age-dependent manner in both the LR and DR animal groups. The protein expression for the enzyme was developmentally enhanced (by about 2.5 times) in the LR groups; the values are 0.97 ± 0.06 , 2.17 ± 0.09 and 2.45 ± 0.04 in the 2WLR, 4WLR and 6WLR rats ($P<0.0001$). The increasing pattern of GAD67 protein level in the DR groups resembled that in their LR counterparts, however, the alternations were more vigorous (more than 4 times) so that expression of the enzyme protein was changed from 0.37 ± 0.06 to 1.21 ± 0.09 and 1.59 ± 0.16 in the 2WDR, 4WDR and 6WDR animals, respectively ($P<0.0001$). Expression of the target protein for GAD67 was also highly visual experience dependent. The protein expression in all groups of the DR animals was

significantly lower than their age-matched LR counterparts ($P < 0.0001$). Figure 5-C explains that the postnatal development and environmental signals cooperatively affect synthesis of glutamate decarboxylase enzymes in the LR and DR rats.

4. Discussion

The present study evaluated sensory experience-dependent changes in synaptic transmission during a postnatal critical period and probable mechanisms in the level of excitatory and inhibitory neurotransmission. Our results demonstrated that the light deprivation reverses the developmental decrease in basic excitatory synaptic transmission where the amplitude size of baseline fEPSPs was increased in the DR rats across age. On the other hand, the dark rearing resulted in a diminished synaptic plasticity. However, the age-dependent reduction in the LTP magnitude was similarly kept in both dark and normally reared groups.

Cortical–hippocampal networks (Hoffman and McNaughton, 2002) as well as cortical–cortical connections (Graham and Willshaw, 1999) play a key role in memory processing. It is demonstrated that the hippocampus and the motor cortex, parts of brain not directly involved in vision, are influenced by dark rearing (Corvetto et al., 2006). Experience-dependent changes have been reported in evoked responses recorded in the hippocampus (Moser, 1995, Gruart et al., 2006). Consistently, it is reported that theta burst stimulation of lateral geniculate nucleus, the thalamic nucleus projecting to visual cortex, increases amplitude of population spikes recorded from dentate gyrus (Tsanov and Manahan-Vaughan, 2008). Our previous works indicated that the synaptic activities of the hippocampal formation are remarkably responsive to light deprivation. We showed that the dark rearing decreased the magnitude of LTP in the CA1 area of hippocampus (Talaie et al., 2010). Also the visual deprivation differently influenced the basic synaptic activity and occurrence of LTP in both the dentate gyrus and CA1 area of young rats, driven by the entorhinal cortex (Talaie and Salami, 2013). On the other hand, Waters et al. reported an inconsistent result where they found that the developing hippocampus is insensitive to environmental signals (Waters et al., 1997). However, the pattern of tetanic stimulation, the stimulating and recording sites, the type of recorded response, and the period of postnatal life, via developmental increase in inhibitory with respect to excitatory responses, affect incidence and level of synaptic potentiation (Rozas et al., 2001, Desai et al., 2002). The fact that LTP has been considered as a key mechanism of learning and memory (Abraham and Williams, 2008) raises the question if the sensory deprivation affects cognition as well as LTP. Using various approaches of behavioral assessments, we have shown impact of visual sensory experience on some aspects of learning and memory (Salami, 2007, Salami et al., 2008, Salami et al., 2012).

LTP is long-lasting increase of synaptic transmission which induce after high frequency stimulation of synapses (Kerchner and Nicoll, 2008). First step in LTP induction is activation of AMPARs after releasing of glutamate from pre-synaptic terminal which cause calcium entry to the post-synaptic terminal and producing EPSP. High-frequency stimulation induces EPSPs summation and increases calcium ions in post-synaptic terminal (Miyamoto, 2006). Some types of hippocampal LTP, like LTP of in the Schaffer's collateral pathway is NMDAR dependent (Ireland and Abraham, 2008). In this form of LTP, due to calcium accumulation and increasing of post synaptic potential, the magnesium blockade of NMDARs is removed and LTP is developed (Molnár, 2011).

AMPARs which assembled by two identical heterodimers with GluA1 and GluA2 are the most principal AMPAR subtype in hippocampal pyramidal neurons (Smart and Paoletti, 2012). The GluA2 subunit determines critical biophysical properties of AMPARs and strongly influences receptor assembly and trafficking (Isaac et al., 2007). At the birth, post-synaptic terminals of glutamatergic circuits have only NMDAR and called AMPA silent synapses (Kerchner and Nicoll, 2008). Expression of AMPARs increases across brain development. For example, at 2 or 3 weeks of age about half of hippocampal glutamatergic synapses are not AMPA silent (Liao and Malinow, 1996). Composition of AMPAR subunits changes during development. Pickard and her colleagues demonstrated that during postnatal development, expression of GluA2 subunit increases in hippocampal slices (Pickard et al., 2000). We showed that, postnatal development differently affected the AMPA subunits GluA1 and GluA2 in the LR animals where the mRNA expression of the subunits was decreased in the former and increased in the latter. Effect of dark rearing on composition of AMPARs subunits were evaluated in some studies. In one study it has been shown that dark rearing increases expression of GluA1 subunit in mouse visual cortex (Goel et al., 2006), and in another study has been revealed that monocular deprivation increases GluA2 expression in cat visual cortex (Beston et al., 2010). We also showed that 6 weeks dark rearing increased expression of both AMPARs subunits particularly the GluA2, in hippocampus. Increasing expression of AMPARs causes increase of AMPARs dependent currents. Goel et al. have shown that increased expression of GluA1 subunit elevates miniature excitatory post synaptic currents (mEPSC) in mouse visual cortex (Goel et al., 2006). It has been demonstrated that, AMPA dependent excitatory post-synaptic currents decrease with age and dark rearing inhibits decrease of AMPA-EPSCs (Funahashi et al., 2013). Elevated amplitude of basic responses in dark reared animals in our study also, in part is due to increased expression of both AMPARs subunits in hippocampus.

NMDA receptors are another ionotropic channels of glutamate and have a pivotal role in occurrence of neocortical (Salami et al., 2000a) and hippocampal (Yashiro and Philpot, 2008) LTP. These receptors are assembled as an obligate heterotetramer composed of two GluN1 and two GluN2 subunits in many region of mammals' brain especially in hippocampus. To date, one isoform of GluN1 and four isoforms of NR2 (A-D) were identified (Paoletti et al., 2013). Composition of NMDARs changes during development. The GluN1 is dominant subunit of NMDARs during critical period and expression of this subunit in layer IV of visual cortex rises across development (Mower and Chen, 2003). Cao et al. demonstrated that expression of GluN1 protein increased in rats' visual cortex during postnatal development (Cao et al., 2000). In our study, the GluN1 subunit of NMDA receptors was expressed increasingly across age in both LR and DR groups; however, the light deprivation declined the degree of mRNA expression. GluN2A and GluN2B subunits are predominant NMDARs subunits in forebrain (Yashiro and Philpot, 2008). It is proved that both GluN2A and GluN2B subunits are fundamental for induction of LTP in CA1 area of hippocampus (Jin and Feig, 2010). At birth, expression of GluN2B is much more than GluN2A and when mammals' brain get matured expression of GluN2A is more than GluN2B (Liu et al., 2004). In our study, expression of GluN2A subunit was 1.8 fold than expression of GluN2B in hippocampus of 6WLR rats. Obviously, switching between these two isoforms of GluN2 needs neuronal circuits' activity and sensory experience (Philpot et al., 2007). During development of visual cortex, expression of GluN2A gradually increases to become the most prevalent subunit in adult ferret. Also, it is a correlation between decline of developmental plasticity in visual cortex and reduction in expression of GluN2B subunit in layer IV of visual cortex (Erisir and Harris, 2003). The GluN2A/GluN2B ratio decreases in visual cortex of dark reared animals and also in correlate, the slower kinetic of NMDA dependent current has been seen in visual cortex (Quinlan et al., 1999a). Dark rearing pronouncedly decreases expression of GluN2A subunit in all layer of rats' visual cortex (Tongiorgi et al., 2003). We also, showed that the GluN2A/GluN2B ratio deeply decreased in hippocampus of dark reared rats and because the LTP induction in CA1 neurons is NMDA dependent, decreased post-HFS potentiation in these animals in part, may be referred to the GluN2A/GluN2B ratio reduction.

GABA is main inhibitory neurotransmitter in mammals' brain. Glutamic acid decarboxylase (GAD) enzyme catalyzes decarboxylation of glutamate to GABA (Bachtar and Stagg, 2014). The structure and function of GABAergic neurons were changed during brain development (Koyanagi et al., 2010), so that, these neurons has an excitatory action in early postnatal development of rats' hippocampus, and get inhibitory role at maturation (Tyzio et al., 2008). It has been shown that, change in visual experience causes change in structure and function

of GABAergic neurons in rats' visual cortex. Using in vivo intracellular recording, it has been revealed that visual deprivation increased GABA transmission in pyramidal neurons of mice visual cortex (Yazaki-Sugiyama et al., 2009). Also, removal of visual signals in adult mice causes a rapid and lasting decrease in number of inhibitory cell spines and buttons in visual cortex (Keck et al., 2011). We also showed that, rearing rats in complete darkness deeply suppressed increasing pattern of GAD65- and GAD67- expression in hippocampus during critical period. These results may be a reason for increasing pattern of amplitude of fEPSPs recorded from CA1 neurons of dark reared rats across age. Also, we demonstrated that, post-HFS amount and stability of potentiation in CA1 neurons of dark reared rats decreased across development. It has been shown that prolonged dark rearing at birth extends the critical period of brain development and delays maturation of visual cortex (Fagiolini and Hensch, 2000). The developmental decrease in magnitude of LTP has been shown on layer II/III of visual cortex and referred to maturation of intracortical inhibitory transmission (Rozas et al., 2001). Rearing adult rats in darkness also, decreased in functional inhibitory synaptic density and post-TBS inhibitory post-synaptic currents (IPSPs) (Huang et al., 2010).

5. Conclusions

In conclusion, change in visual experience during critical period of brain development alters basic and tetanized responses of CA1 area of rats' hippocampus. This readjustment in synaptic activity, at least in part, could be due to an imbalance between excitatory and inhibitory neurotransmission via rearrangement of AMPA and NMDA receptors' subunits and delaying maturation of GABA synthesizing system.

6. Author Contributions

S.A. Talaei and M. Salami designed the experimental procedures and analyzed the data. S.A. Talaei and A. Azami collected the data. All the authors drafted the manuscript. M. Salami supervised the project and edited the manuscript.

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Table 1- Primers used for RT-PCR

Gene	Sequence (5'-3')	Annealing temperature (°C)	Product size (bp)	Cycles	NCBI Reference Sequence
HPRT	F GGTCCATTCCTATGACTGTAGATTTT R CAATCAAGACGTTCTTTCCAGTT	60	172	33	NM_012583.2
GluN1	F AGTGGAACGGAATGATGGGCGA R ACTGAAGCGGTCCAGCAGGTA	57	262	26	NM_001270602
GluN2A	F GAGCCAGATGACAACCACT R TCTTGAGGATGTCGATGCAG	58	200	31	NM_012573.3
GluN2B	F CCAAGAGGAGGAAACAGCAG R TGAGGCGAGTTCTCCTTTGT	56	184	28	NM_012574.1
GluA1	F TCCACGTGATCGAAATGAAA R GTCATTGCCCTCAAACCTGGT	58.5	202	28	NM_031608.1
GluA2	F GGCCTGTAATCCTGGACTGT R ACACCAGGGAATCGTCGTAG	59	197	28	NM_017261.2
GAD65	F CTGTGTACGGGGCTTTTGAT R TGCATCAGTCCCTCCTCTCT	59.5	231	29	NM_012563.1
GAD67	F CACAACTCAGCGGCATAGA R CTGGAAGAGGTAGCCTGCAC	59.4	149	26	NM_017007.1

Figure legends

Figure 1. An example of input/output curve in the 6WLR and 6WDR rats. The neuronal circuits displayed a substantial decreased stimulus intensity in the DR animals to evoke the baseline fEPSPs.

Figure 2. Synergistically effect of postnatal development and dark rearing on mean amplitude of fEPSPs recorded from CA1 area of rats' hippocampus. The amplitude size of fEPSPs faded by about 15% across age (* $P=0.024$, between 2WLR and 6WLR groups). Reversely, light deprivation caused an about 15% increase in amplitude size of fEPSPs (** $P=0.018$, between 2WDR and 6WDR groups). Also, statistical comparison revealed a difference between 4WLR and 4WDR groups (# $P=0.003$) and between 6WLR and 6WDR ones (\$ $P<0.0001$).

Figure 3. Summary of field potential recording in the CA1 area of hippocampus of normal light reared (A) and dark reared (B) rats comparing mean $\pm$ SEM percentage changes in amplitude of fEPSPs at different time before and after of application of high frequency stimulation (HFS, arrow). HFS induced LTP in the fEPSPs of CA1 area of all the LR ($P<0.0001$) and the DR groups ($P<0.0001$). The potentiation was highest in 2WLR rats and decreased over age ($P<0.0001$ between 2WLR and 6WLR groups). The same gradual decreasing pattern was also seen in the DR rats where the mean value of post-HFS potentiation was $34.96\pm 5.83\%$ in 2WDR rats and diminished to $24.94\pm 3.83\%$ and $20.88\pm 5.56\%$ in 4WDR and 6WDR rats, respectively ($P=0.036$ between 2WDR and 6WDR groups). Comparison of the induced LTP between the LR and their age-matched DR groups indicated that the dark rearing effectively reduces the synaptic plasticity ($P<0.0001$). Inset is representative traces of fEPSPs recorded from CA1 area of hippocampus of each group before and after of HFS.

Figure 4. Effect of postnatal dark rearing on age-related mRNA expression of AMPA and NMDA receptors' subunits and GAD enzyme isoforms in rats' hippocampus. A- Expression of GluA1 subunit showed a substantial reduction (by about 43%) over the postnatal life in the LR animals (* $P<0.0001$ between 2WLR and 6WLR groups). In contrast, postnatal dark rearing increased (by about 16%) the expression of GluA1 subunit (# $P=0.044$, between the 2WDR to 6WDR groups). Comparison of data taken from age-matched groups showed that dark rearing substantially enhances the expression of GluA1 in rats' hippocampus ($P<0.0001$ for all comparisons). Expression of the GluA2 subunit mRNA showed an age-dependent increase in hippocampus of both the LR and the DR animals (** $P<0.0001$ between 2WLR and 6WLR groups and ### $P=0.024$ between 2WDR and 6WDR groups). The relative expression of GluA2 subunit mRNA was substantially elevated in all groups of the DR groups compared to their LR counterparts ($P<0.0001$ for all comparisons). B- Expression of

mRNA encoding GluN1 subunit had an increasing pattern in light- and dark-reared groups across age (* $P<0.0001$ between 2WLR and 6WLR groups and # $P<0.0001$ between 2WDR and 6WDR animals). Comparing data taken from age-matched groups showed that the visual deprivation had a slight but significant negative effect only at the sixth week of age (\$ $P=0.001$). The relative expression of GluN2A subunit mRNA increased about 2.8 fold from 2WLR to 6WLR rats (** $P<0.0001$). In contrast, expression of GluN2A mRNA declined by more than half from 2WDR to 6WDR rats (## $P<0.0001$). Although expression of this subunit in 2WLR and 2WDR rats was the same, but the relative expression of GluN2A mRNA was more than 7 fold in 6WLR rats when compared to 6WDR ones (\$\$ $P<0.0001$). However, expression of GluN2B did not change in the LR rats during postnatal development, but noticeably decreased from 2WDR to 6WDR rats (### $P=0.023$). Also, the visual experience-dependent decrease in mRNA expression of GluN2B subunit in the 6WDRs was statistically noticeable when compared with their LR counterparts (\$\$\$ $P=0.045$). C- Expression of the mRNA encoding GAD65 enzyme was increased across age in both the LR and the DR rats (* $P<0.0001$ between 2WLR and 6WLR and # $P<0.0001$ between 2WDR and 6WDR groups). Data analysis also showed that expression of GAD65 mRNA suppressed by dark rearing when the LR rats compared to the age-matched DR ones ($P<0.0001$ for all comparisons). Expression of GAD67 mRNA like GAD65 mRNA was also positively age-sensitive and increased in both the LR and the DR hippocampus across postnatal development (** $P<0.0001$ between 2WLR and 6WLR and ## $P<0.0001$ between 2WDR and 6WDR groups). Comparison of the data also demonstrated that expression of GAD67 mRNA in the DR rats' hippocampus was lower than the age-matched LR counterparts ($P<0.0001$ for all comparisons).

Figure 5. Expression of hippocampal AMPA and NMDA receptors' subunits and GAD enzyme isoforms in hippocampus of the LR and the DR rats. A- Statistical chart shows that expression GluA1 subunit decreased (by about 25%) across age in the LR animals (* $P<0.004$ between 2WLR and 6WLR groups). The visual deprivation reversed expression pattern of GluA1 where expression of this subunit increased (by about 21%) from 2WDR to 6WDR animals (# $P=0.003$). Comparison of the data from three groups of LR and DR animals indicated that the protein expression was significantly higher in all groups of the DRs ($P<0.0001$). The relative expression of another subunit of AMPA receptors, GluA2, also displayed an increasing pattern with age in hippocampus of both the LR and the DR animals (** $P<0.0001$ between 2WLR and 6WLR groups and ## $P=0.01$ between 2WDR and 6WDR groups). Between groups comparison also revealed that expression of GluA2 subunit was higher in the hippocampus of 2WDR and 6WDR rats than their age-matched light reared

ones ($P < 0.0001$ for both comparisons). B- Expression of GluN1 subunit increased in both the light- and dark-reared groups during postnatal development (* $P < 0.0001$ between 2WLR and 6WLR groups and # $P < 0.0001$ between 2WDR and 6WDR animals). Comparison of the data from the LR and DR groups indicated that light deprivation enhanced GluN1 expression in all testing groups. (\$ $P = 0.038$ between the two-week old rats, @ $P = 0.04$ between the four-week old animals, and ^ $P = 0.009$ between the sixth-week old groups). The relative expression of GluN2A subunit increased about 80% from 2WLR to 6WLR rats (** $P < 0.0001$). In contrast, expression of GluN2A had a decreasing pattern from 2WDR to 6WDR rats (## $P = 0.006$). Also, between groups comparison demonstrated that expression of this subunit was lower in 4WDR and 6WDR rats than their age-matched counterparts ($P < 0.0001$ for both comparison). The relative expression of GluN2B subunit did not changed in the normally light reared rats during postnatal development. Light deprivation decreased expression of GluN2B from 2WDR to 6WDR rats (### $P = 0.01$), so that, led to a significant difference between the 6-weeks old LR and DR groups (\$\$\$ $P = 0.04$). C- Expression of GAD65 enzyme was increased across age in the hippocampus of LR rats (by about 1.4 fold, * $P < 0.0001$ between 2WLR and 6WLR) and in the hippocampus of DR animals (by about 2 fold, # $P < 0.0001$ between 2WDR and 6WDR groups). The visual experience-dependent decrease in expression of GAD65 in the DR rats was statistically noticeable when compared with their age-matched LR counterparts ($P < 0.0001$ for all comparisons). Expression of another isoform of glutamate decarboxylase enzyme, GAD67, was also positively age-sensitive and increased in the LR rats (by about 2.5 times, ** $P < 0.0001$ between 2WLR and 6WLR) and in the DR ones (by about more than 4 times, ## $P < 0.0001$ between 2WDR and 6WDR groups) across postnatal development. Also, the protein expression in all groups of the DR animals was significantly lower than their age-matched LR counterparts ($P < 0.0001$ for all comparisons).

Figure 1

Figure 2

Figure 3

Figure 3-A

Figure 3-B

Figure 3-C

Figure 4-A

Figure 4-B

Figure 4-C

Figure 5-A

Figure 5-B

Figure 5-C