

The Effect of Changes in the Visual Experience during Critical Periods of Brain Development on the Synaptic Plasticity of Hippocampal CA1 Neurons in Rats

S.A. Talaei (MSc)¹, M. Salami (PhD)^{*1}

1. Physiology Research Center, Kashan University of Medical Sciences, Kashan, I.R.Iran

Received: Jul 2th 2014, Revised: Nov 26th 2014, Accepted: Jan 21th 2015

ABSTRACT

BACKGROUND AND OBJECTIVE: Changes in the visual experience during the critical periods of brain development lead to the dysfunction of the visual cortex. The visual cortex is a major supplier of sensory input in the mammalian hippocampus. This study aimed to investigate the effects of visual deprivation on the synaptic plasticity of CA1 neurons in this area.

METHODS: This experimental study was conducted on 48 male Wistar rats who had been classified into the two main groups of a 12-12 Light Reared (LR) and Dark Reared (DR) since birth. To perform the experiments, the rats were categorized under 3 subgroups of 2, 4 and 6 weeks of age. Excitatory postsynaptic potentials (EPSP) were recorded for 30 minutes from the dendrites of neurons in the CA1 area. Afterwards, long-term potentiation (LTP) was induced through Tetanic stimulation. Finally, the amplitude of the responses were measured before and after the Tetanic stimulation.

FINDINGS: The amplitude of basic responses in the 2WLR and 6WLR group were 1.28 ± 0.05 Mv and 1.09 ± 0.03 Mv, respectively ($p < 0.0001$) while the range increased from 1.30 ± 0.30 Mv in the 2WDR group to 1.50 ± 0.4 Mv in the 6WDR group ($p < 0.0001$). Upon LTP induction, the highest rise in the amplitude response was observed at the age of 2 weeks in the animals. However, the increasing responses in both groups of LR and DR diminished with age ($p < 0.0001$).

CONCLUSION: Visual deprivation during the critical periods of brain development might lead to an increase in the basal synaptic activity of hippocampal CA1 neurons through an age-related process. Although it does not interrupt the induction of LTP in neurons, it might reduce its intensity and stability.

KEY WORDS: Visual Experience, Synaptic Plasticity, Hippocampus, Critical Period, Wistar Rats.

Please cite this article as follows:

Talaei S.A, Salami M. The Effect of Changes in the Visual Experience during Critical Periods of Brain Development on the Synaptic Plasticity of Hippocampal CA1 Neurons in Rats. J Babol Univ Med Sci. 2015; 17(3):28-34.

***Corresponding Author: M. Salami (PhD)**

Address: Physiology Research Center, Kashan University of Medical Sciences, Kashan, I.R.Iran.

Phone: +98 31 5562 1157

Email: salami-m@kaums.ac.ir

Introduction

Critical period of mammalian brain development is a period which starts at birth and lasts until the maturation of synaptic circuits of the central nervous system. The formation of synaptic circuits during this period is affected by two major physiological procedures: the intrinsic activity of neurons as a result of genetic activity and the interaction with the environment through environmental signals (1). This period takes about 6 weeks in rats' brain (2). The visual system is the most significant sensory input to the mammalian brain. Thus, the signals received by this system play a pivotal role in the maturation of the brain circuits (3). The developmental role of these signals in the critical period has been surveyed in several studies (4,5). In addition, different studies have indicated that changes in these signals dramatically impact on the development of the brain cortex (6-8). A majority of studies have investigated the effects of visual signals on the visual cortex (9,10).

For instance, Goel et al. suggested that visual deprivation during the critical periods of brain development, even for as long as two days, could lead to the impairment of synaptic activity in the visual cortex of rats' brain (11). Hippocampus is a part of the brain's limbic system which is located deeply in the temporal lobe influencing the process of learning and memory (12). It has proven that all the sensory cortices are responsible for sending a part of their signals to this structure which results in the formation of learning and memory after processing of the information (13). Thus, it could be inferred that learning and memory formation in the neural circuits are followed by the changes in the activity of synaptic connections (14). Long-term potentiation (LTP) is a well-known physiological process which is induced through induction of the high-frequency stimulation to the neural circuits and it is considered as a plausible laboratory model for changing synaptic activity, also known as synaptic plasticity (15).

Containing organized neural circuits, the hippocampal structures, especially the CA1 area, are a proper reference for investigating this process (16). Moreover, a critical period of development is known to occur for the hippocampus which is similar to that of the sensory cortices. It has been suggested that certain environmental changes such as sensory deprivation (17), living in crowded environments and an increase in the sensory inputs to the brain (18), could bring about changes in the performance of its neurons. Given the

fact that part of the visual signals enter the hippocampal formation and that transformed visual signals could bring about changes in the structure and function of the visual cortex, it is probable that modified visual messages could also affect the structure and function of the hippocampus during the critical period of brain development.

Therefore, this study aimed to investigate the impact of different periods of visual deprivation during the critical period of brain development on the synaptic plasticity of the hippocampal CA1 area in rats.

Methods

This experimental study was conducted on 48 male Wistar rats. Animals were kept in the standard animal house and sufficient access to water and food. Protocol of the experiments was reviewed and approved by the Ethical Committee of Kashan University of Medical Sciences. With regards to the light conditions, the animals were divided into two main groups of Light Reared (LR) and Dark Reared (DR). The LR animals had been preserved under normal light conditions (i.e 12 hours of light and 12 hours of dark) while the DR rats had been preserved in complete darkness from birth to the end of the experiment. Moreover, each of the main groups was divided into 3 subgroups (N=8). The first subgroup included rats of 2 weeks of age (2WLR, 2WDR), the second included rats of 4 weeks of age (4WLR, 4WDR) and the third subgroup included rats of 6 weeks of age (6WLR, 6WDR). Half an hour before the beginning of the tests, the rats were transferred to the laboratory where they were anesthetized by an intraperitoneal injection of Urethane (1.5g per kg of body weight).

The animals in the DR group were initially anesthetized by drugs in the darkroom and their eyes were shut tightly upon which they entered the laboratory. After fixating their head in a Stereotaxic apparatus (Stoelting USA), 0.5 ml of 1% lidocaine was injected under their head skin in addition to local anesthesia so that the scalp would be removed from the skull by an easy cut. Following that, the scalp was removed from the back of the neck to the vicinity of the nose while all other tissues were completely pushed in order for the skull to appear. After determining the Bregma, Lambda and midline on the scalp, the locations of electrodes were highlighted by Stereotaxic Atlas (Paxinos and Watson) (19). The stimulating electrode

was located on the axon of the Schaffer's collaterals of CA3 neurons at the coordinates of D=2.4 mm, LR=3.8 mm, AP=-4.2 mm and the recording electrode was located on the dendrites of CA1 neurons at the coordinates of D=2.5 mm, LR=2.5 mm, AP=-3.4 mm. The electrodes were both Bipolar, Teflon-coated and made of stainless steel with a diameter of 0.005 inches (AM Systems USA).

The correct location of the electrodes was detected via Electrophysiological recording. To make sure, the accuracy of the placement of electrodes was examined by applying paired pulse. The 20% increase of the amplitude of the second response compared to the first one was indicative of the correct placement of the stimulating and recording electrodes. In response to the stimulation of the Schaffer's collaterals, the EPSPs were recorded, and amplified 2000x by an amplifier (WSI, A3308).

Then, they were given to the Data Acquisition Board (AD Instruments, Australia) where they were converted into digital data and recorded. About 30 minutes after the first recording of the responses and the time when the amplitude responses remained unchanged with the steady intensity of the stimulation, the Input/Output curve was drawn. The electrical stimulation intensity in which 60% of the maximum amplitude of the response was obtained was selected as the stimulation intensity for the continuation of the trial as well as the tetanic stimulation.

Furthermore, stimulations at a frequency of 0.1 Hz were applied for 100 ms with a delay of 5 ms. Afterwards, EPSPs were recorded for 30 minutes. In order to induce the LTP on the tested neuronal circuits, High Frequency Stimulation (HFS) was applied. The stimulation pattern consisted of 10 trains including ten 200 Hz stimulation frequencies with an interval of 2 ms. The duration of each stimulating pulse was 0.1 ms. After the induction of tetanic stimulation, the process of stimulation and recording continued for 2 hours. The Scope for Windows software (Australia, PowerLab) was used for measuring the stimulation and recording as well as the analysis of the responses.

In order to compare the two groups, the percentage of changes in the amplitude responses were evaluated in mV before and after the tetanic stimulation. Any increase by 20% in the amplitude of the responses after the tetanic stimulation was considered as an LTP induction criterion (20). The collected data were analyzed by One-way ANOVA and Tukey's post-analysis and $p < 0.05$ was considered significant.

Findings

The test results revealed a significant difference between the amplitude of all groups in the basic responses as well as before and after the LTP induction. Moreover, the analysis of data on the responses recorded in the CA1 region of the rats' hippocampus in the LR group showed the mean amplitude of the responses in the 2WLR group to be 1.28 ± 0.05 mV which decreased in the other two groups as of 1.17 ± 0.04 in the 4WLR group and 0.50 ± 0.28 mV in the 6WLR group reaching 1.09 ± 0.03 mV.

The results were also indicative of a significant difference in the amplitude response between the groups of 2WLR and 4WLR ($p = 0.02$) (fig 1).

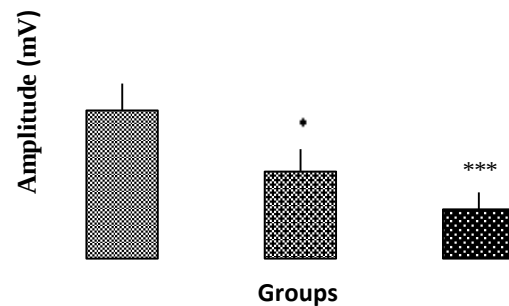


Figure 1. Comparison of the mean amplitude responses in different groups of animals grown in the natural light cycle of 12 hours (N=8 in each group)

The difference of amplitude between groups of 2WLR and 4WLR ($p < 0.05$). The difference of amplitude between groups of 2WLR and 6WLR ($p < 0.001$).

In addition, the difference of the amplitude response was found to be significant between the groups of 2WLR and 6WLR ($p < 0.0001$). LTP induction leads to a significant increase in the amplitude of the fEPSP and the difference before and after the LTP induction was considered as significant in all the groups ($p < 0.0001$). Along with aging and reaching the final maturation of the brain circuits, the increasing amplitude dropped after the Tetanic stimulation in all groups.

As a result, the maximum and the minimum increase were observed in the 2WLR and 6WLR rats, respectively (Figure 2). However, the difference between the amplitude responses after the LTP induction was considered as significant in all groups ($p < 0.0001$).

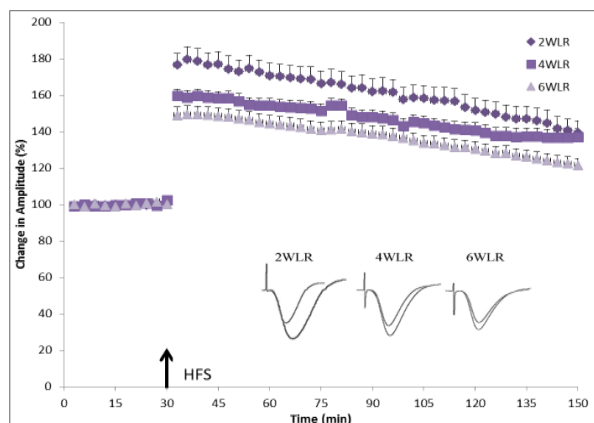


Figure 2. Tetanic stimulation in fEPSP as recorded in the hippocampal CA1 region of the rats in natural light-dark cycle induced by LTP.

The difference between the amplitude responses in all groups after LTP induction ($p < 0.0001$)

The analysis of the data based on the amplitude responses of the animals grown in 24 hours of darkness indicated that with the age, the responses experienced an increasing trend. The mean amplitude responses in the 2WDR group was 1.30 ± 0.03 mV, while it reached 1.41 ± 0.03 and 1.50 ± 0.04 mV in the next two groups, respectively. Furthermore, the results revealed a significant difference in the amplitude response between the groups of 2WDR, 4WDR and 6WDR ($p < 0.0001$) (Figure 3). However, the difference in the basic amplitude response was not considered as significant between the two groups of 4WDR and 6WDR. For another thing, enhancing the responses in all the three aforementioned groups after the LTP induction was higher than 20% as recorded during 2 entire hours. amplitude after applying high frequency stimulation in the perforant path of the hippocampal CA1 of the animals grown in the 24 hours of darkness. However, the increase in the amplitude of all responses was lower after the LTP induction compared to the same-age rats who were grown in the light. According to statistical analysis of the data, the difference before and after the LTP induction was found to be significant in all groups ($p < 0.0001$). Furthermore, comparison of the data in the groups of animals revealed that with age, the enhancement of the responses reduced (Figure 4).

Considering a criterion of 20% increase in the amplitude response after the induction of LTP, it could be inferred that despite the induction in the 6WDR group, it only lasted about half an hour. Statistical analysis of the results showed a significant difference between the amplitude response after LTP induction in

2WDR and 6WDR groups ($p < 0.0001$) while the rest of the comparisons were not proven to be significant.

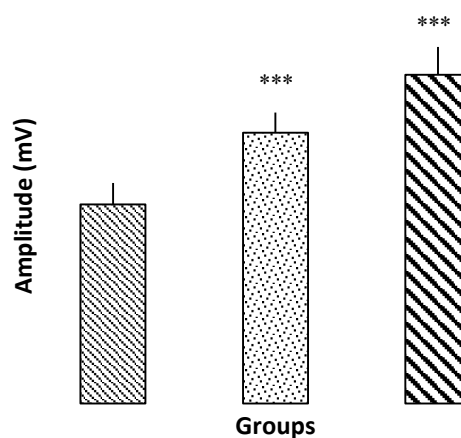


Figure 3. Comparison of the mean amplitude in different groups of animals grown in the darkness for 24 hours (N=8 in each group).

Differences in the amplitude responses between the groups of 2WDR, 4WDR and 6WDR ($p < 0.0001$).

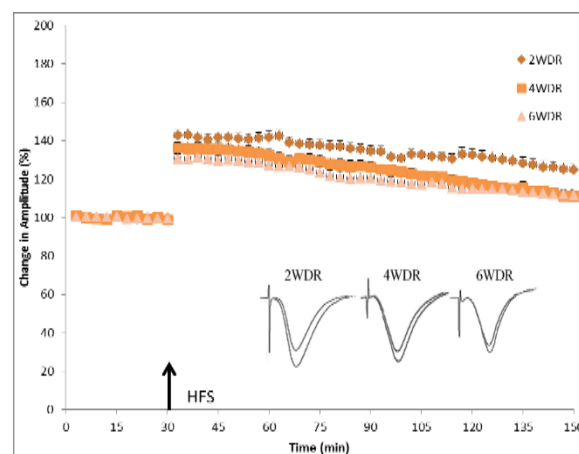


Figure 4. Following the Tetanic stimulations in the recorded fEPSP of the hippocampal CA1 area of the rats grown in complete darkness, a significant increase was observed in the amplitude response and the induction of LTP in all the groups.

The difference between the amplitude response and LTP induction between the groups of 2WDR and 6WDR ($p < 0.0001$).

Discussion

The results of the present study indicated that with age and approaching the end of the critical period of brain development, the extent of post-synaptic potentials might decrease in the hippocampal CA1 while visual deprivation is likely to add to this range through a

time-dependent process. Moreover, the enhancement of field potentials is likely to diminish through an age-related process after a high-frequency stimulation of the circuits in this area. Although deprivation of the visual signals during this critical period did not seem to interrupt the induction of LTP in the circuits, it led to a considerable decrease in the size and stability of synaptic response enhancement. Eckert et al. proved that an increase in the environmental signals received by the brain during the critical period of development could result in the improvement of memory and LTP induction in the CA1 region of the rats' hippocampus through crowding the environment and after that (18). In another study, it was suggested that crowding the environment results in the enhancement of the basic responses as well as facilitating the LTP induction in the brain slices prepared from the rats' hippocampal CA1 area (21).

In their research, Talaei et al. proved that changes in the visual experience, even after puberty, could also impair the LTP induction in the rats' CA1 and dentate gyrus (22). What is the cause of the observed changes? In the answer, it could be stated that during the critical period of brain development the amount and type of various Neurotransmitter activities constantly change due to the intrinsic activity of the neurons and making contact with the environment in order to reach maturation and stability. Glutamate is the major excitatory neurotransmitter in the mammalian hippocampus (23) and GABA is the major inhibitory neurotransmitter (24).

There are two main receptors NMDA and AMPA, Glutamate is engaged in its activities. In the early stages of life, the only glutamatergic neurons expressed are NMDA receptors while other synaptic circuits and glutamate receptors are not expressed. These circuits are commonly referred to as AMPA Silent (25). Along with the development of the mammalian nervous system, AMPA receptors are also expressed in the glutamatergic circuits resulting in the maturation of the circuits. For instance, within 2 to 3 weeks after birth, almost half the glutamatergic hippocampal circuits exit the AMPA Silent mode (26). Thus, the expression of AMPA receptor subunits begin to increase in the rats' brain through an age-related process until their adolescence

(27). In addition, it has been proven that visual deprivation could lead to an increased expression of the receptor subunits in the rats' visual cortex (28, 29). It has been established that in the early stages of life, the expression of NR2B, one of the receptor subunits of NMDA, is more abundant in the mammalian brain and along with the development of the brain, the expression of NR2A subunit gradually increases taking precedence to the expression of NR2B during adolescence (30).

The switch between the two isoforms of NR2 subunit specifically requires the activity of the neuronal circuits as well as the sensory experience. In their research, Philpot et al. claimed that visual experience is vital to the evolutionary changes of the NMDA receptor subunits in the visual cortex (31). It has also been found that in animals deprived of seeing, the switching process does not occur and exposing them to light for only an hour is likely to ignite this process (32). With reference to the inhibitory neurotransmitter GABA, it has been confirmed that GABAergic neurons of the rats' hippocampus practice stimulatory activities during the animals' infancy acquiring their inhibitory properties along with the maturation of the nervous system (33).

Furthermore, it has been suggested that changes in the visual experience might alter the structure and function of GABAergic neurons in the visual cortex of rats (34, 35). From the findings of the current study it could be concluded that changes in the visual experience during the critical period of brain development occur through a time-dependent process. By altering the stimulatory and inhibitory neurotransmitters in the rats' brain, visual experience could increase the basal synaptic transmission in the hippocampal CA1 neurons of rats and although it does not interrupt the induction of LTP in neurons, it might reduce the intensity and stability of the induction.

Acknowledgments

Hereby, we extend our deepest gratitude to the Deputy of Research, Kashan University of Medical Sciences for the financial support of this research (grant no. 9133).

References

1. Morishita H, Hensch TK. Critical period revisited: impact on vision. *Curr Opin Neurobiol.* 2008;18(1):101-7.
2. Rozas C, Frank H, Heynen AJ, Morales B, Bear MF, Kirkwood A. Developmental inhibitory gate controls the relay of activity to the superficial layers of the visual cortex. *J Neurosci.* 2001;21(17):6791-801.
3. Hensch TK. Critical period mechanisms in developing visual cortex. *Curr Top Dev Biol.* 2005;69:215-37.
4. Fagiolini M, Hensch TK. Inhibitory threshold for critical-period activation in primary visual cortex. *Nature.* 2000;404(6774):183-6.
5. Hooks BM, Chen C. Critical periods in the visual system: changing views for a model of experience-dependent plasticity. *Neuron.* 2007;56(2):312-26.
6. Heynen AJ, Yoon BJ, Liu CH, Chung HJ, Hugarir RL, Bear MF. Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation. *Nat Neurosci.* 2003;6(8):854-62.
7. He HY, Hodos W, Quinlan EM. Visual deprivation reactivates rapid ocular dominance plasticity in adult visual cortex. *J Neurosci.* 2006;26(11):2951-5.
8. Baroncelli L, Sale A, Viegi A, Maya Vetencourt JF, De Pasquale R, Baldini S, et al. Experience-dependent reactivation of ocular dominance plasticity in the adult visual cortex. *Exp Neurol.* 2010;226(1):100-9.
9. Fathollahi Y, Salami M. The role of N-methyl--aspartate receptors in synaptic plasticity of rat visual cortex in vitro: effect of sensory experience. *Neurosci Lett.* 2001;306(3):149-52.
10. Jaffer S, Vorobyov V, Kind PC, Sengpiel F. Experience-dependent regulation of functional maps and synaptic protein expression in the cat visual cortex. *Eur J Neurosci.* 2012;35(8):1281-94.
11. Goel A, Xu LW, Snyder KP, Song L, Goenaga-Vazquez Y, Megill A, et al. Phosphorylation of AMPA receptors is required for sensory deprivation-induced homeostatic synaptic plasticity. *PLoS One.* 2011;6(3):e18264.
12. Bird CM, Burgess N. The hippocampus and memory: insights from spatial processing. *Nat Rev Neurosci.* 2008;9(3):182-94.
13. Neves G, Cooke SF, Bliss TV. Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. *Nat Rev Neurosci.* 2008;9(1):65-75.
14. Rebola N, Srikumar BN, Mülle C. Activity-dependent synaptic plasticity of NMDA receptors. *J Physiol.* 2010;588(1):93-9.
15. Kullmann DM, Lamsa KP. Long-term synaptic plasticity in hippocampal interneurons. *Nat Rev Neurosci.* 2007;8(9):687-99.
16. Jin SX, Feig LA. Long-term potentiation in the CA1 hippocampus induced by NR2A subunit-containing NMDA glutamate receptors is mediated by ras-GRF2/Erk map kinase signaling. *PLoS One.* 2010;5(7):e11732.
17. Uysal N, Ozdemir D, Dayi A, Yalaz G, Baltaci AK, Bediz CS. Effects of maternal deprivation on melatonin production and cognition in adolescent male and female rats. *Neuro Endocrinol Lett.* 2005;26(5):555-60.
18. Eckert MJ, Bilkey DK, Abraham WC. Altered plasticity in hippocampal CA1, but not dentate gyrus, following long-term environmental enrichment. *J Neurophysiol.* 2010;103(6):3320-9.
19. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th ed. Academic Press; 2007. p.31.
20. Talaei SA, Sheibani V, Salami M. Light deprivation improves melatonin related suppression of hippocampal plasticity. *Hippocampus.* 2010;20(3):447-55.
21. Malik R, Chattarji S. Enhanced intrinsic excitability and EPSP-spike coupling accompany enriched environment-induced facilitation of LTP in hippocampal CA1 pyramidal neurons. *J. Neurophysiol.* 2012;107(5):1366-78.
22. Talaei SA, Salami M. Sensory experience differentially underlies developmental alterations of LTP in CA1 area and dentate gyrus. *Brain Res.* 2013;1537:1-8.
23. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, et al. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev.* 2010;62(3):405-96.
24. Jelitai M, Madarasz E. The role of GABA in the early neuronal development. *Int Rev Neurobiol.* 2005;71:27-62.
25. Kerchner GA, Nicoll RA. Silent synapses and the emergence of a postsynaptic mechanism for LTP. *Nat Rev Neurosci.* 2008; 9(11):813-25.
26. Liao D, Hessler NA, Malinow R. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature.* 1995;375(6530):400-4.

27. Durand GM, Zukin RS. Developmental regulation of mRNAs encoding rat brain kainate/AMPA receptors: A northern analysis study. *J Neurochem.* 1993;61(6):2239-46.
28. Goel A, Jiang B, Xu LW, Song L, Kirkwood A, Lee HK. Cross-modal regulation of synaptic AMPA receptors in primary sensory cortices by visual experience. *Nat Neurosci.* 2006;9(8):1001-3.
29. Goel A, Lee HK. Persistence of experience-induced homeostatic synaptic plasticity through adulthood in superficial layers of mouse visual cortex. *J Neurosci.* 2007;27(25):6692-700.
30. Liu XB, Murray KD, Jones EG. Switching of NMDA receptor 2A and 2B subunits at thalamic and cortical synapses during early postnatal development. *J Neurosci.* 2004;24(40):8885-95.
31. Philpot BD, Sekhar AK, Shouval HZ, Bear MF. Visual experience and deprivation bidirectionally modify the composition and function of NMDA receptors in visual cortex. *Neuron.* 2001;29(1):157-69.
32. Quinlan EM, Olstein DH, Bear MF. Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. *Proc Natl Acad Sci U S A.* 1999;96(22):12876-80.
33. Tyzio R, Minlebaev M, Rheims S, Ivanov A, Jorquera I, Holmes GL, et al. Postnatal changes in somatic γ -aminobutyric acid signalling in the rat hippocampus. *Eur J Neurosci.* 2008;27(10):2515-28.
34. Keck T, Scheuss V, Jacobsen RI, Wierenga CJ, Eysel UT, Bonhoeffer T, et al. Loss of sensory input causes rapid structural changes of inhibitory neurons in adult mouse visual cortex. *Neuron.* 2011;71(5):869-82.
35. Yazaki-Sugiyama Y, Kang S, Câteau H, Fukai T, Hensch TK. Bidirectional plasticity in fast-spiking GABA circuits by visual experience. *Nature.* 2009;462(7270):218-21.

.