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Distinct Roles of NMDAR and mGluR5 in Light Exposure Reversal of Feedforward Synaptic Strength in V1 of Juvenile Mice after Binocular Vision Deprivation

Xiaoxiu Tie,[a†] Shuo Li,[a†] Yilin Feng,[a] Biqin Lai,[a] Sheng Liu[b*] and Bin Jiang[a*]

[a] *Guangdong Province Key Laboratory of Brain Function and Disease, Zhongshan School of Medicine, Sun Yat-sen University, 74 Zhongshan 2nd Road, Guangzhou 510080, China*

[b] *State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 54 Xianlie South Road, Guangzhou 510080, China*

Abstract—In the visual cortex, sensory deprivation causes global augmentation of the amplitude of AMPA receptor-mediated miniature EPSCs in layer 2/3 pyramidal cells and enhancement of NMDA receptor-dependent long-term potentiation (LTP) in cells activated in layer 4, effects that are both rapidly reversed by light exposure. Layer 2/3 pyramidal cells receive both feedforward input from layer 4 and intra-cortical lateral input from the same layer, LTP is mainly induced by the former input. Whether feedforward excitatory synaptic strength is affected by visual deprivation and light exposure, how this synaptic strength correlates with the magnitude of LTP in this pathway, and the underlying mechanism have not been explored. Here, we showed that in juvenile mice, both dark rearing and dark exposure reduced the feedforward excitatory synaptic strength, and the effects can be reversed completely by 10–12 h and 6–8 h light exposure, respectively. However, inhibition of NMDA receptors by CPP or mGluR5 by MPEP, prevented the effect of light exposure on the mice reared in the dark from birth, while only inhibition of NMDAR prevented the effect of light exposure on dark-exposed mice. These results suggested that the activation of both NMDAR and mGluR5 are essential in the light exposure reversal of feedforward excitatory synaptic strength in the dark reared mice from birth; while in the dark exposed mice, only activation of NMDAR is required.

Key words: visual cortex, feedforward excitatory synaptic strength, dark rearing, dark exposure, light exposure.

INTRODUCTION

Researches on synaptic plasticity in the mouse visual cortex focused on activity-dependent plasticity, one is known as synaptic scaling where a prolonged increase in neural activity globally scales down excitatory synaptic responses, while a chronic decrease in neural activity, on the other hand, scales up the responses (Turrigiano and Nelson,2004; Desai et al., 2002). The other activity-dependent changes occur locally at specific synapses, either as long-term potentiation (LTP) or as long-term depression (LTD) (Bear and Malenka,1994; Kirkwood and Bear,1995; Kirkwood et al., 1995). Layer 2/3 (L2/3) pyramidal cells receive both feedforward (FF) and intra-cortical inputs, and LTP is usually induced by stimulation of specific synapses in the FF pathway from L4 to L2/3. These studies demonstrated that the induction of LTP and LTD in V1 of L2/3 mice follows Hebb's Rule and depends on NMDA receptor (NMDAR) activation (Kirkwood and Bear,1994). Binocular visual deprivation in the form of dark exposure (DE) or dark rearing from birth enhances NMDAR-dependent LTP in L4 to L2/3 input, which can be quickly reversed by light exposure (LE) (Philpot et al., 2001). However, a form of mGluR5-dependent LTP rather than NMDAR-dependent LTP was found in dark-reared (DR) mice after 10–12 h of LE, suggesting that the magnitude of LTP in L2/3 pyramidal cells and its mechanism depend on the history of visual experience (Li et al., 2017). Whether the magnitude of synaptic strength (SS) from the total input in the FF pathway correlates with the magnitude of LTP has not been determined. On the other hand, DE has been found to cause an augmentation of AMPA receptor-mediated miniature EPSC (mEPSC) amplitude in L2/3 pyramidal cells. This is considered homeostatic plasticity, wherein

*Corresponding authors. Address: Guangdong Province Key Laboratory of Brain Function and Disease, Faculty of Forensic Medicine, Zhongshan School of Medicine, Sun Yat-sen University, 74 Zhongshan 2nd Road, Guangzhou 510080, China (B. Jiang).
E-mail addresses: liush87@mail.sysu.edu.cn (S. Liu), Jiangb3@mail.sysu.edu.cn (B. Jiang).
† These authors contributed equally to this work.

Abbreviations: CPP, carboxypiperazin-4-yl-propyl-1-phosphonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LTD, long-term depression; LTP, long-term potentiation; MPEP, 2-methyl-6-(phenylethynyl) pyridine hydrochloride; NMDAR, NMDA receptor.

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neurons in L2/3 adjust their activities to compensate for prolonged periods of visual input loss (Goel and Lee, 2007). In addition, it is reported that L4 synapses comprise ~5% of the total synapses onto L2/3 neurons, and intra-cortical lateral inputs constitute the majority of the excitatory synapses to these neurons. While recordings of mEPSCs in L2/3 cells reflect the overall inputs onto these cells, it is difficult to distinguish whether the FF pathway from L4 to L2/3 is augmented or not. Recordings of evoked strontium-desynchronized miniature excitatory postsynaptic currents (evoked Sr^{2+}-mEPSCs) allow the determination of quantal synaptic response size (Goel and Lee, 2007; Petrus et al., 2015). However, the amplitude of evoked Sr^{2+}-mEPSCs, which represents the FF excitatory inputs from L4 to L2/3, was found to be unaltered in adult mice, while lateral intra-cortical inputs on L2/3 were strengthened, and the amplitude of mEPSCs recorded in this layer increased after 1 week of DE (Goel and Lee, 2007; Petrus et al., 2014; Petrus et al., 2015). These observations suggested that visual deprivation produced diverse adjustments due to differences in age and pathway inputs onto L2/3 cells. To determine how different types of visual deprivation alter the FF pathway in juvenile mice, we recorded evoked Sr^{2+}-mEPSCs in L2/3 pyramidal cells to examine the changes in FF excitatory synaptic strength (FF-SS) in DR or DE young mice with subsequent LE. We found that DR decreased FF-SS, which was completely reversed by 10–12-h LE. Blockade of NMDAR or inhibition of mGluR5 *in vivo* prevented the effect of LE in juvenile mice. Interestingly, NMDAR-mediated reversal of FF-SS by LE occurred in both DE mice and mice reared in the dark from birth, whereas mGluR5-mediated reversal of FF-SS by LE only occurred in mice reared in the dark from birth, and the latter negatively correlated with NMDAR-dependent LTP. These results suggested that NMDAR and mGluR5 play distinct roles in the effect of LE reversal of FF-SS in L2/3 cells after DR or DE.

EXPERIMENTAL PROCEDURES

Animals

Wild-type C57BL/6J mice between postnatal day (P) 19 and P26 were used. All animals were fed *ad libitum*. Control, normally reared (NR) mice were raised in 12-h light/dark cycles. DR mice were kept in a darkroom from birth, in which feeding and cage cleaning were performed by wearing infrared visors. Dark exposure (DE) or light exposure (LE) was performed by placing subjects in a darkroom or on the normal light/dark cycle, respectively. All animal experimental procedures were approved by the Animal Care and Use Committees of Sun Yat-sen University and were performed in accordance with the guidelines of the National Institutes of Health on animal care and the ethical guidelines. The minimum number of animals was used in each experiment.

Visual slice electrophysiology

Coronal slices (300 μm thick) containing V1 were prepared as described previously (Li et al., 2017) using a microslicer (Vibratome 3000) in ice-cold cutting solution composed of the following (in mM): 212.7 sucrose, 3 KCl, 1.25 NaH_2PO_4, 3 $MgCl_2$, 1 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose, bubbled with 95% O_2/5% CO_2. Brain slices were immediately transferred to artificial cerebrospinal solution fluid (ACSF) at 35 °C for at least 30 min before the recordings. The ACSF was composed of the following (in mM): 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 dextrose, bubbled with 95% O_2/5% CO_2. All recordings were performed at 31 °C. L2/3 pyramidal neurons were identified visually under infrared differential interference contrast optics based on their morphological properties, such as triangular somas and prominent apical dendrites, as described previously (Li et al., 2017).

LTP induction

Whole-cell voltage-clamp recordings were used to evoked EPSCs from pyramidal cells in L2/3. Excitatory responses in pyramidal cells in L2/3 were evoked by a concentric bipolar stimulating electrode with a tip diameter of 125 μm (FHC) placed in L4. The distance between the stimulating and recording electrodes was limited to within 80–120 μm. Patch pipettes (3–4 mΩ) were filled with the internal solution composed of the following (in mm): 120 Cs-methylsulfonate, 10 HEPES, 10 Na-phosphocreatine, 5 lidocaine *N*-ethyl bromide (QX-314), 4 ATP, and 0.5 GTP; pH 7.2–7.3. The osmolarity of the internal solution was 270–285 mOsm. Only cells with series resistances < 20 mΩ and input resistances > 100 mΩ were included in the analysis. Cells were excluded if their input resistances changed > 15% or if their series resistances changed > 10% over the experiment. Recordings were filtered at 3 kHz and digitized at 10 kHz using the Igor Pro software (WaveMetrics). To induce LTP, a pairing protocol was employed as following: depolarization to 0 mV, 2-Hz stimulation, and 360 pulses. To suppress excessive polysynaptic activity in the presence of picrotoxin (50 μm), the concentration of divalent cations in the recording ACSF was elevated to 4 mm Ca^{2+} and 4 mm Mg^{2+} to reduce recruitment of polysynaptic responses. A test pulse was delivered at 0.05 Hz to monitor the baseline amplitude for 10 min before and for 25–35 min following the paired protocol.

Drugs were applied through the perfusion of ACSF to selectively block the respective type of glutamate receptors, and interleaved control recordings were made in slices without drug from the same animals. We used the following drugs as selective antagonists: DL-APV (Sigma) at 100 μm for NMDAR and 2-methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP; Tocris Bioscience, Avonmouth, Bristol, United Kingdom) at 10 μm for mGluR5.

Evoked Sr^{2+}-miniature EPSC measurements

To measure the amplitude of quantal AMPA-EPSCs (Sr^{2+}-mEPSCs), Ca^{2+} was replaced with Sr^{2+} (3 mm) in ACSF to drive asynchronous glutamate release. DL-APV (50 μm) and picrotoxin (50 μm) were added to the