Thesis summary

Development of the connections between fast-spiking interneurons and pyramidal neurons in mouse visual cortex

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Introduction

Fast-spiking inhibitory interneurons (FS neurons), a major population of inhibitory neurons in the neocortex, have important roles in cortical function. It was previously reported that FS neurons preferentially form reciprocal connections with adjacent pyramidal neurons, sending inputs to the FS neurons in the visual cortex and prefrontal cortex. The reciprocal pairs of FS and pyramidal neurons often make strong synaptic connections with each other. These reciprocal connections are considered to contribute to controlling excitatory-inhibitory balance and inducing high-frequency oscillation in the neocortex. Thus, the development of reciprocity in FS neurons and pyramidal neurons seems important for the maturation of cortical functions. However, the developmental process and mechanisms remain unclear.

Visual experience during postnatal development is crucial for the maturation of visual cortical functions. The N-methyl-D-aspartate receptors(NMDARs) are key molecules involved in activity-dependent synaptic modification underlying experience-dependent refinement of visual response properties and neural connectivity. At present, it remains unknown how reciprocity in FS and pyramidal cell pairs is established. Particularly, the dependence on visual experience/NMDARs and the difference in maturation mechanisms between one-way and reciprocal connections or between excitatory and inhibitory synapses is not known.

In this study, I investigated the development of reciprocal connections between

FS and pyramidal cells, and their dependence on visual experience and NMDARs in mouse primary visual cortex. The proportion of pairs reciprocally connected increased soon after eye opening in postnatal development in association with a specific increase in the amplitude of unitary inhibitory postsynaptic currents (IPSCs) and excitatory postsynaptic currents (EPSCs) in the reciprocal pairs. The visual experience was required for this reciprocal pair-selective potentiation of IPSCs, but not EPSCs. Furthermore, NMDARs on FS neurons but not on pyramidal cells contributed to the potentiation of IPSCs, while the potentiation of EPSCs does not depend on NMDARs.

Material and methods

Animals

C57BL/6 mice or C57BL/6 background transgenic mice of either sex at postnatal day (P) 21-26 were used for the experiments. For the developmental study, mice at P10-13 and P14-16 were also used. To label inhibitory neurons, the vesicular GABA transporter (VGAT)-Venus mice, expressing Venus fluorescent protein driven by mouse VGAT promoter, were used in mice at all tested ages. The PV-Cre mice expressing Cre recombinase driven by the endogenous parvalbumin (PV) promoter were crossed with Ai14 mice that express tdTomato protein depending on Cre to generate PV-tdTomato mice. PV-tdTomato mice were used to visualize the PV-positive interneurons at P15-16 and P21-26. Some of the mice were reared in a completely dark room from before birth

up to the time just before the preparation of slices at P21-26. For the GluN1 conditional knock-out (cKO) experiments, GluN1-flox mice were used.

Virus injections

For the cKO of GluN1, I injected 100 nl of adeno-associated virus (AAV) carrying Cre recombinase under three promoters to GluN1-flox mice at P0 or P1. For cortex-specific knock out, transcranial injection to the posterior part of one hemisphere in the neocortex was performed.

Whole-cell recordings

Mice were deeply anesthetized with isoflurane and their whole brain was removed. Coronal slices (300 μm-thick) were prepared from the primary visual cortex. Whole-cell recordings were simultaneously obtained from pyramidal neurons and FS neurons, which were identified by the shape of soma and the expression of a fluorescent marker protein, in layer 2/3 of slices. For recordings of synaptic currents, a K-gluconate-based internal solution was used. The connections between neuron pairs were assessed by applying pairs of brief depolarizing current pulses to evoke action potentials in one of the cells and recording postsynaptic currents from the other cell. To record IPSCs from pyramidal cells and EPSCs from FS neurons, the membrane potentials were clamped at -40 mV and -70 mV, respectively.

Results

I first analyzed the normal development of the synaptic connections between pyramidal cells and FS neurons in layer 2/3 of visual cortex. The proportion of pairs reciprocally connected increased just after eye opening. This was due to the increase in the proportion of inhibitory connections. The amplitude of unitary IPSCs, which was recorded from pyramidal neurons in response to an action potential in FS neurons, significantly increased from P10-13 to P14-16 and thereafter remained unchanged. Comparing the IPSC amplitude between one-way and reciprocally connected pairs, there was no significant difference at P10-13. The amplitude in reciprocal pairs was larger than in one-way inhibitory connected pairs at P14-16, and these reciprocity-dependent strong IPSCs were maintained up to P21-26. The amplitude of unitary EPSCs also increased from P10-13 to P14-16, but subsequently decreased at P21-26. No significant difference was found in the EPSC amplitude between reciprocally and excitatory one-way connected pairs at P10-13. At P14-16 and P21-26, the EPSC amplitude in reciprocal pairs was considerably larger than in one-way excitatory connected pairs. These results demonstrated that the unitary IPSCs and EPSCs in reciprocal pairs were selectively strengthened after eye-opening, although this strengthening did not occur in one-way connected pairs.

Mechanisms for the establishment of high reciprocity and reciprocity-dependent potentiation of synaptic connections were explored. First, I examined the effects of

visual deprivation using dark reared mice from birth until the time just before slice preparation at P21-26. Dark rearing did not affect the proportion of connections in FS and pyramidal neuron pairs. However, the potentiation of IPSCs in reciprocal pairs was impaired by dark rearing. The potentiation of EPSCs in reciprocal pairs occurred in dark reared mice, similar to age-matched normal mice.

Next, I examined the effect of the deletion of NMDARs. To this end, a GluN1-flox mouse line was used to perform conditional knock-out (cKO) of *GluN1*, the essential subunit of NMDARs. Cre recombinase was induced by three different promoters: CMV for the ubiquitous KO, CaMKII for the pyramidal neuron-specific KO, and mDlx for the inhibitory neuron-specific KO. None of these cKO of *GluN1* affected the proportion of synaptic connections in FS and pyramidal neuron pairs. However, the specific potentiation of IPSCs in reciprocal pairs was inhibited by CMV and mDlx, but not CaMKII dependent KO of *GluN1*, demonstrating that NMDARs on inhibitory cells were important to establish reciprocity-dependent potentiation of IPSCs. These three types of *GluN1* cKO did not affect the potentiation of EPSCs in reciprocally connected pairs, indicating that the potentiation did not depend on NMDARs.

Discussion

The current study showed that both IPSCs and EPSCs in reciprocally connected FS and pyramidal neuron pairs were specifically potentiated soon after eye opening. Visual experience and NMDARs on inhibitory cells were necessary for the reciprocity-dependent potentiation of IPSCs, but not for the potentiation of EPSCs. These results suggest that the strength of inhibitory and excitatory connections, depending on the connectivity of FS neurons and pyramidal neurons, is modified by different mechanisms during development. This study provides new insight into the developmental mechanisms of connection specificity between FS neurons and pyramidal neurons in the visual cortex.