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이학박사학위논문

Functional development of synapse and neuronal circuit in cerebral cortex

대뇌피질의 시냅스 및 신경회로 발달에 관한 연구

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이승현

Abstract

Functional development of synapse and neuronal circuit in cerebral cortex

Lee Seung-Hyun

Interdisciplinary Program in Neuroscience

The Graduate School

Seoul National University

The brain is the most complex organ with about 100 billion nerve cells. The "Cerebral cortex" is the largest suborgan in the brain and is structurally segmented. In addition, the cerebral cortex performs various functions such as sensation, movement, judgment, memory, information processing, and language through the compartmentalized area. For example, V1, the primary sensory cortex, processes and transmits visual information to the other cortex, while the sensory association cortex collects information from the sensory cortex and processes the intermediate information. Thus, the association cortex is related to perception-based judgment functions. What we can see while simultaneously listening and exercising is that the cerebral cortex can perform various functions at the same time. For so called ‘multi-tasking’, the cerebral cortex is developed in such a way that each cortical region forms optimized neural circuits for their specialized function. Dysfunction of the neurons and synapses or neural circuits that composed the cerebral cortex, can causes various and

complicated brain diseases. For example, bipolar disorder, schizophrenia, and autism are known to be caused by dysfunction of the cerebral cortex Understanding the function and development of normal cerebral cortical neurons and neural circuits will not only broaden our understanding of the brain, but will also contribute to the effective treatment of various neuropsychiatric disorders in the future.

Despite the involvement of the cerebral cortex in the regulation of various functions, the basic structure of the neuronal circuits in the cortical areas is substantially the same.
The functional diversity of the cerebral cortex has been understood to be due to the diversity of neuronal circuitry coming from the outside, and it is presumed that they are optimized for the function of each region, but it is not well known whether different cerebral cortex have the same developmental mechanism. The primary sensory cortex, including the V1, undergoes the optimization process of the neural circuit through the experience of the early life in order to secure its inherent function. The specific period of this early life is generally called the 'critical period'. The visual cortex (V1) has the greatest plasticity in the critical period, and at this time, the optimization of neural circuits by experience occurs. Previous studies have shown that in the primary sensory cortex, the intra-cortical synaptic plasticity decreases or disappears after the end of the critical period, and it is known that the development of inhibitory neural circuits plays an important role for termination of critical period. On the other hand, it is important that the association cortex has the ability to collect and process information between different cerebral regions throughout the life rather than optimization of experience-dependent neural circuits. However, there is limited research into whether there is a “critical period” in the development of association cortex and what functional development is occurring at this time. In chapter 1, I have confirmed the structural and functional changes of two different cortical regions with have similar internal circuit structures, the primary visual cortex and the temporal association cortex.

In particular, recent clinical reports have shown that patients with mood and cognitive impairment such as bipolar disorder, autism and schizophrenia show structural and functional abnormalities in cerebral cortex, particularly the prefrontal Cortex (PFC). PFC is an association cortex that by using a perceptual and emotional information provided by each cortex or subcortical regions, known to function as a value-based judgment and selection. Understanding how PFC synapses and associated neural circuits originally function will not only broaden our understanding of the brain, but will also

contribute to the effective treatment of various neuropsychiatric disorders in the future. To identify correlation between behavioral abnormalities and neuronal, synaptic, and neural circuit dysfunctions, I used two mouse models with behavioral anomalies known to be associated with various PFCs Cytoplasmic FMR1-interacting protein 2 (Cyfip2) is an actin-regulatory protein that is expressed in synapses. *Cyfip2*$^{+/-}$ mice exhibit manic-like behavior in response to the mood stabilizer lithium. Manic episode is one of the main features of bipolar disorder, which characterized by increased mood, increased activity and energy, and increased sociability. Although there are clinical reports of structural and functional abnormality of PFC in patients with bipolar disorder, pathophysiological and the drug reaction mechanisms at the neuronal and synaptic levels have not been elucidated before. In chapter 2, I attempted to identify the correlation between neuronal and synaptic dysfunction and behavior in PFC through *Cyfip2* gene loss mouse model showing manic-like behavior.

Depression, anxiety, and social hierarchy are also related to the prefrontal area of the cerebral cortex. *Cereblon* (*CRBN*) deficient mouse model exhibits impaired behavior of depression, anxiety, and social dominance. Follow the previous report, *CRBN* was first identified from the patient with mild intellectual disability (ID), that mutation in *CRBN* genome was found. Deletion or microduplication in various regions of *CRBN* was associated with cognitive and behavioral disturbances. Recent studies have shown that CRBN protein forms a $CRL4^{CRBN}$ E3 ubiquitin ligase complex with cullin-4A (CUL4A), DNA-binding protein 1 (DDB1) and regulator of cullins 1 (ROC1). With the $CRL4^{CRBN}$ complex, acts as a receptor that binds to the protein to be regulated and brings them into the E3 ubiquitin ligase complex. Previous studies using *CRBN* deficient mice have shown that the CRBN protein modulates the secretory probability and cognitive behavior of pre-synaptic cells by regulating BK channel expression on the surface of pre-synaptic cells in the hippocampus. However, depression and anxiety behaviors of *Crbn* KO mice are controlled independent of BK channel in hippocampal region, unlike cognitive behavior. In chapter 3, I investigated changes in the structure and function of excitatory synapses using the neuromuscular junction synapses of the *Crbn* mutant *Drosophila*. In addition, using the *Crbn* KO mouse which showing abnormal mood and social behavior, observed changes in neural circuit and synaptic function in the connected region of the PFC and in the area where signal is transmitted to the corresponding region

These studies, which examined the development of diverse functional cortical and external neural networks and how their dysfunctions appeared in neurons and synapses of neuropsychiatric disorders, have contributed greatly to understanding the structural universality and functional diversity of the cerebral cortex. It is expected that it will be applied to understand the cortex - dependent higher brain function and brain diseases and to establish the future treatment mechanism.

Keywords: Cerebral cortex, critical period, association cortex, medial prefrontal cortex (mPFC), mood disorder, *Cyifp2*, *Cereblon*

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Abbreviations

AMPA	a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
BD	Bipolar disorder
BK_{ca} channel	Large-conductance, Ca^{2+}-activated K^{+} channel
CP	Critical Period
CRBN	Cereblon
CUL4A	Cullin-4A
CYFIP2	Cytoplasmic FMR1-interacting protein2
DDB1	DNA-binding protein 1
Cx	Cortex
EPSC	Excitatory postsynaptic current
GABA	Gamma-aminobutyric acid
ID	Intellectual disability
IMiDs	Immunomodulatory drugs
IPSC	Inhibitory postsynaptic current
KO	knockout

LTD	Long-term depression
MD	mediodorsal thalamus
mPFC	medial Prefrontal Cortex
NAc	Nucleus Accumbens
NMDA	N-methyl-D-aspartic acid
PPR	Paired-pulse ratio
Pr	Probability of release
R_{in}	input resistance
RMP	Resting membrane potential
ROC1	Regulator of cullins 1
SEM	Standard error of mean

General Introduction

What is the Cerebral Cortex?

The cerebral cortex occupies the largest area of the mammalian brain and is responsible for important functions such as sensation, movement, memory, concentration, perception, language, perception, and recognition (Kandel, 2000). The cerebral cortex can be roughly divided into three types according to function. Primary sensory cortex is an area that accepts visual, olfactory, auditory, and somatosensory sensations. The motor cortex is a cortical area that contracts the skeletal muscle when given a stimulus, and is involved in voluntary movement. The association cortex, which is the neocortex except the sensory cortex and the motor cortex, occupies the widest area of the three cortical areas and combines information received from various cortical and subcortical regions (Kiernan and Rajakumar, 2013).

The structural characteristics that the cerebral cortex can have functional diversity can be roughly divided into two at the neuronal and neuronal circuit levels. One is functional, structural compartments to control various functions. Another is an internal neural circuit that connects the input / output to other regions of the brain. Because of these two basic characteristics, the cerebral cortex can control various functions by compartment.

On the other hand, most cerebral cortices have a universal internal structure consisting of six layers. The reason for this is that the cerebral cortex has developed laterally in the cell group with the same origin at the early developmental stage (Budday, 2015; Romero and Borrell, 2015). However, it is relatively less understood whether these different regions of the cerebral cortex have the same developmental mechanism.

Previous studies on cerebral cortical development

To date, studies on the functional development of the cerebral cortex have been mainly focused on primary sensory cortex. The reason is that it was easy to study experience-dependent development around a certain period of time because they have certain type of the input stimuli and can be precisely controlled. The most well-known developmental mechanism is the development of cortical plasticity around the critical period. The critical period is an early “specific time” in life when experience input is strongly modifying the neuronal circuit optimization. Sensory experience during the postnatal “critical period” is essential for normal development of sensory cortical circuits and function (Hubel and Wiesel, 1962).

A more detailed mechanistic study with the visual cortex of cats and monkeys reveals that the input (experience) of a particular period of time coming from the eye is competing with each other, permanently changing the structure of the neural circuit (Hubel and Wiesel, 1970a, 1970B). In a visual cortex study using the rodent model, it was found that when a "monocular deprivation" is performed during a critical period ranging from 21 to 32 days after birth, the neural circuit changes to receive signals from the open eye (Draeger, 1978; Gordon and Syryker, 1996). The above results are evidence that there is a "critical period" in which there is a change in experience-dependent synaptic plasticity in the primary sensory cortex, and the circuit optimized for the experience signal disappears after critical period is terminated. Since the existence of a critical period has been proven, subsequent researches have attempt to reveal the biological mechanism of the critical period. One of the most important factors is inhibitory neurons. Even before the modulators of the critical period were studied, GABAergic neuron had been known to be important for the synaptic plasticity of the cerebral cortex. In the cerebral cortex of the young animal within the critical period, the LTP can be induced successfully in the layer 2-3, whereas not formed in the old animal (Kirkwood and Bear 1994; Kirkwood et al., 1996). These results suggest that the inhibitory circuit of layer 4 might be involved in maturation of synaptic properties during the critical period.

In other words, while inhibition is weak and the synaptic properties can be “plastic” in young cortex, it is impossible to change layer 4 to layer 2/3, the intra-cortical circuit, when the inhibitory circuit of layer 4 is developed.

Several previous studies have shown that development of inhibitory circuit is associated with the initiation / termination of critical periods (Huang, 1999). The visual cortex of the mouse with the development of the inhibitory circuit due to the overexpression of BDNF has been reported to begin early in the critical period and finish early. On the other hand, transgenic animals that inhibit the synthesis of GABA do not open critical period (Fagiolini & Hensch, 2000; Hensch, 1998), and no LTD of visual cortex (Choi, 2002). When long-term administration of benzodiazepine, a GABA-A receptor agonist, to this animal, LTD was restored (Choi et al., 2002) and ocular dominance was reintroduced (Hensch et al., 1998; Fagiolini and Hensch, 2000). Therefore, studies on the functional development in critical period of the cortical circuit correspond to the development of the inhibitory circuit. Therefore, regulatory factors of critical period plasticity have been studied with various factors known to control inhibitory circuit function.

While the primary sensory cortex exhibits the development of inhibitory circuits and optimization of neural circuits (loss of synaptic plasticity), in association cortex, the synaptic plasticity is retained even when the cortex is adult (Bontempi, 1999). The reason for this is presumably due to the function of the cortex that synthesizes and processes various information received from neighboring regions by connecting different cortical regions and subcortical regions throughout life
However, the developmental mechanism of the associative cortical area has a "critical period" that changes the synaptic function on a large scale by "experience" is relatively unknown. In chanpter1, I studied developmental difference of cerebral cortical functional with different functions.

Current studies and neuropsychiatric disorder associated with cerebral cortex

So what is the current direction of the study of cerebral cortical function? Nowadays, the study of the cerebral cortex is attracting attention for two reasons. One is because it is relatively easy to access the

outside by using cranial window and so it is widely used as a model area for studying *in vivo* function such as *in vivo* imaging of neurons and synapses. Another reason is that the relationship between the structure and function of prefrontal cortex (PFC) and various mental illnesses or sociality have been reported through fMRI results in patients with various neuropsychiatric diseases such as major depression disorder, bipolar disorder, schizophrenia (Mochcovitch, 2014; Usnich, 2015; Vargas, 2013; Kaiser, 2016). Therefore, many researchers are paying attention to the function of mPFC. Recent studies with rodent models and post-mortem analysis have shown that psychiatric disorder such as mania and depression are involved in factors such as calcium signaling, circadian rhythm, glutamate receptor singing, mitochondrial function, and ion channel activity (Harrison, 2018 ; Kato, 2007; Mansour, 2005; McClug, 2007; Nurnberger, 2014). Compared to the above molecular genetic studies, studies on the detailed functions of neuronal subtypes and brain regions involved in diseases are relatively inadequate. Therefore, studies on the neuronal function regulation mechanisms and brain regions that regulate abnormal behavior may provide a target for the treatment of future cortical neuropsychiatric disorders.

Purposes

The key purpose of this thesis is to understand the developmental changes of the cerebral cortex, which plays various roles, and the function of the cerebral cortical circuits involved in behavioral abnormalities, at the level of neurons, synapses and neural circuits. To achieve this goal, the researches listed below are addressed in subsequent three chapters.

- To characterize whether there is a 'critical period' for the development of the association cortex, compare the developmental changes of the intracortical neural circuit function of primary visual cortex and temporal association cortex.
- To investigate the correlation between neuronal and synaptic dysfunction and behavioral abnormalities in mPFC through *Cyfip2* mutant mouse model of mania
- To understand the mechanism that control neuronal activity in prefrontal cortical circuit that associated with social dominance and emotion using *CRBN* KO, which shows behavioral abnormalities

This study revealed functional changes in various cerebral cortexes as well as changes in the neuronal activity of mPFC and related circuits associated with neuropsychiatric disorders at neurons, synapses, or circuit levels. These results not only provide a fundamental understanding of cortical development, but also contribute to finding a therapeutic approach.

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Chapter I.
Functional diversity and intra-cortical circuit development of cerebral cortex

Abstract

The cerebral cortex performs a variety of functions despite having a similar internal structure. In general, the primary sensory cortex has a "critical period", a specific period in which neural circuits are optimized by experience given after birth. On the other hand, association cortex, which is responsible for collecting and consolidating information from other cortices, does not require optimization of neural circuits. However, it is poorly revealed whether the association cortex does not have a "critical period" which seen in the primary sensory cortex.In this study, I tried to compare two different cortical regions, (one is the temporal association cortex (TC), which is known to be involved in memory consolidation by receiving information from the hippocampus, another is primary visual cortex (VC or V1) the most research on the critical period) to investigate the difference in structural and functional development before and after the critical period. First, decreased LTD is observed in V1 after critical period, but TC does not. However, the number of inhibitory synapses formed in the cortical layer 4 and layer 2/3 after the critical period and PNN maturation were common in both cortical areas. In addition, inhibitory current increases in intra-cortical synapses were common in both regions. The parameters of basal synaptic function, mEPSC, mIPSC, and intrinsic neuronal excitability, which are independent of neuronal circuit activity, showed marginal differences and showed no clear trend. These results suggest that inhibitory circuit maturation during the critical period may be common to the developing cortex.

Introduction

Cerebral cortex performs various functions by region. The development of cortex is very important for ensuring brain function after birth. In general, the primary sensory cortex is optimized by experience given after birth and the timing of this change is called the "critical period". In other words, the experience given in the critical period is different from the subsequent experience in that it leads to the development of the cortical circuit (Hubel and Wiesel, 1970). The factors that change in the cortex during the critical period are diverse. After the report that the earlier termination of critical period, because of the inhibitory circuit optimization is accelerated in BDNF overexpressed animals, various factors have been suggested to modulate the critical period (Huang, 1999). Furthermore, many studies have been carried out to find the regulatory factors of the critical period. These studies assessed the potential for critical period regulation, by confirming structural changes such as increased parvalbumin positive puncta, formation of perineuronal net, and functional changes such as formation of LTP and LTD in layer 4 to layer 2/3 intra-cortical synapses (Kobayashi, Kirkwood and Bear 1994; Kirkwood, 1996).

Unlike primary sensory cortex, sensory associate cortex is responsible for collecting and consolidating information from other cortices. In sensory association cortex, there is no process to optimize the information receive selectively from a particular sensory organ (e.g. to make receive information from two eyes differently). So at least conceptually there is no critical period in these brain regions.For example, V2, V4, IT, and MT all contribute to derive higher-order visual information from visual input coming from the primary visual cortex (V1) (Born and Bradly, 2005; Kolb, 2014; López, 2009 ; Schmid, 2013). However it is not known whether there is a critical period like V1, and how it changes. Nevertheless, both the primary sensory cortex and sensory association cortex share a very similar structure with six layer structures (Meyer, 1999). Therefore, how the

cerebral cortex that exhibits such structural similarities and functional diversity has attracted the attention of many researchers.

I tried to find out whether the sensory associate cortex showed various changes before and after the critical period of the primary sensory cortex. As a model, with V1, which has been the most subject of critical period research, I selected temporal associate cortex (TC), which is known to be involved in memory consolidation by receiving information from hippocampus and forming the same layer structure as V1 To investigate the development of cerebral cortex and the optimization process during the critical period, I compared the structural and functional changes seen in these two cortexes.

Material and Methods

Experimental animals

C57BL6J male mice were 3-week and 2-month-old for the electrophysiological experiments. All mice were purchased from DBL (Eumseong, South Korea) and were breed in an animal facility with a specific pathogen-free (SPF) barrier under a 12h light/dark cycle. Mice were allowed to access to food and water *ad libitum*. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Seoul National University. We also confirm that all methods were performed in accordance with the relevant guidelines and regulations of
Seoul National University and Korean government (Ministry of Science and ICT).

Electrophysiology

Slice preparation

Coronal visual/temporal cortical slices (300 μm) were prepared from 3- week / 2-month-old C57BL/6J mice (male only), modified with previous work (Guo, 2012). Briefly, mice were anesthetized with isoflurane. After decapitation, the brains were rapidly removed and then submerged in ice-cold, oxygenated (95% O_2 and 5% CO_2), low-Ca^{2+}/high-Mg^{2+} dissection buffer containing 5 mM KCl, 1.23 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM dextrose, 0.5 mM $CaCl_2$, 10 mM $MgCl_2$, and 212.7 mM sucrose. Cortical tissues from both hemispheres were sectioned coronally using a vibratome (VT1000P or VT1000S; Leica Biosystems, Germany). The slices were then transferred to a recovery chamber containing oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (ACSF) containing 124 mM NaCl, 5 mM KCl, 1.23 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 26 mM $NaHCO_3$, and 10 mM dextrose and incubated at 28–30°C for at least 30 min before recording.

Whole-cell patch clamp recordings.

After recovery, slices were transferred to a recording chamber where they were continuously perfused with ACSF gassed with 95% O_2/5% CO_2 at a 2 ml/min flow rate. Slices were stabilized for 5 min before the recordings and all of the experiments were performed at room temperature. All recordings were performed in visual or temporal cortex, layer II/III pyramidal neurons were identified by their size and morphology. Recordings were obtained using a EPC-8 amplifier (HEKA), under visual control with differential interference contrast illumination in an upright microscope (Olympus, BX51WI). Patch pipettes (4–6 MΩ) were filled with the following (in mM): 130 Cs-$MeSO_4$, 8 NaCl, 5 TEA-Cl, 10 HEPES, 1 QX-314, 4 ATP-Mg, 0.4 GTP-Na, 0.5 EGTA, 10 Phosphocreatine-Na_2 (for the miniature ESPC (mEPSC), excitatory ESPC (eEPSC), and eIPSC experiments); 130 CsCl, 1.1 EGTA, 2 $MgCl_2$, 0.1 $CaCl_2$, 10 NaCl, 10 HEPES, 2ATP-Na (for mIPSC experiments); 145 K-gluconate, 10 HEPES, 5 NaCl, 0.2 EGTA, 1 $MgCl_2$, 2 ATP-Mg, and 0.1 GTP-Na (for excitability) at a pH of 7.4 and 280–290 mOsm. The extracellular recording solution consisted of ACSF supplemented with picrotoxin (100 μM), TTX (1 μM), and DL-AP5 (50 μM) for mEPSC experiments, picrotoxin (100 μM) for the eEPSC experiment, TTX (1 μM), CNQX (20 μM) and DL-AP5 (50 μM) for the mIPSC experiment, CNQX (20 μM) and DL-AP5 (50 μM) for the eIPSC experiment. Only cells with an access resistance < 20MΩ and an input resistance > 100MΩ were studied. The cells were discarded if the input or the access resistance changed 20%. Data were acquired and analyzed using pClamp 10 (Molecular Devices). Signals were filtered at 3 kHz and digitized at 10 kHz with Digidata 1550B (Molecular Devices),

Stimulation and Pairing-LTD induction

Evoked synaptic responses were elicited by cortical layer IV stimulation (0.2 ms current pulses) using a concentric bipolar electrode placed 200–300μmin front of the postsynaptic pyramidal cell. Test stimulation was applied at intervals of 15 sec to minimize depression resulting from high-frequency stimulation. Compound IPSCs were recorded at 0 mV, and EPSC was recorded at -70 mV. In input-output relationship for measuring I-E balance, the stimulus intensity was varied systemically. At least 4 to 6 responses at each intensity were averaged to calculate the EPSC and IPSC. In pairing-LTD experiment, baseline postsynaptic potential was recorded with half-max stimulation intensity at 15 sec inter-stimulus interval. As a conditioning stimulation for pairing-LTD induction, LFS (1Hz, 200

pulses) was applied on presynaptic axons, and -50 mV holding with voltage-clamp mode for postsynaptic cells.

Statistical analysis

Data analyses and graphical displays were conducted with Prism 5.03 (GraphPad Software, La Jolla, CA, USA). All displayed values represent the mean ± SEM. Significant differences between groups were determined using unpaired Student's t-tests, and to validation of drug effect, multiple comparisons were performed using two-way ANOVA and Bonferroni's multiple comparison post-hoc test

Results

Developmentally declined synaptic plasticity is observed in primary visual cortex, not temporal association cortex of intra-cortical circuit

Many researches that investigate the cortical development during “Critical period”, a major lead-out to define termination of it in primary sensory cortex is loss synaptic plasticity (Kirkwood, 1996; Choi, 2002). Before study the functional development of synapse, I tried to confirm whether the loss of synaptic plasticity in primary visual cortex after "critical period" is reproducible in the C57/BL6J mouse visual cortex. To address it, I measured pairing LTD in mouse primary visual cortex, layer 4 to layer 2-3, intra-cortical circuit. I performed electrophysiological analyses on the acute brain slice from young (3-week-old, critical period) and adult (8 to 12-week-old) mice to compare plasticity between two brain regions.. To induce synaptic plasticity in both cortices, I used pairing-LTD induction protocol that low-frequency stimulation (LFS, 1Hz - 200 pulses) was applied on presynaptic axons, and -50 mV holding with voltage-clamp mode for postsynaptic layer 2/3 pyramidal cells. As I expected, the plasticity of the synapse after critical period was decreased (Figure 1-1a). On the other hand, the “temporal association cortex” was still remains synaptic plasticity in intra-cortical circuit even when the time of visual cortical development is end. Following previous reports, association cortex remained flexible cortex even after developmental stage is over (Bontempi, 1999). So I confirmed that previously reported function is replicated in neocortices of C57BL/6 mouse.

Histological changes of inhibitory circuit during critical period are exhibit in both cortical regions

With results that long-term plasticity of temporal association cortex is maintained throughout the lifetime, I assumed that unlike to visual cortex, temporal cortex have no critical period and developmental changes (mainly maturation of inhibitory circuits) during this time. Various previous

studies demonstrate that the development of inhibitory circuit regulates the onset and termination of the critical period (Hensch, 1998; Huang, 1999). Developmental change of primary visual cortex before and after the critical period is accompanied by functional changes and structural development. In the visual cortex, after the critical period, the maturation of inhibitory cells and the increase of PV-positive inhibitory synapse in pyramidal cells, a principle neuron, have been reported (Kobayashi, 2015). Perineuronal nets (PNNs), known to regulate the maturation of PV^+neuron, is an ECM component that surrounds the PV^+ interneuron as a net and regulates the synaptic input to the PV^+ interneuron. Therefore, the development of PNN structure induces functional maturation of PV^+ interneuron (Beurdeley, 2012; Umemori, 2018). To address whether there is any structural development in temporal cortex, we compare structural markers of inhibitory circuit maturation between visual and temporal cortex. WFA is a marker of PNNs, which surrounds PV^+ interneurons, and the increased WFA intensity means the structural maturation of interneurons. In results, contrary to our expectation, maturation of soma-enwrapping PNN was increased in both visual and temporal cortices without change the number of PV^+ interneurons (Table 1 - 1). This implies that inhibitory neuronal maturation occurs not only in visual cortex but also in temporal cortex after a critical period. Next, we measured the number of inhibitory synapse onto cortical layer 4 and layer 2-3 pyramidal neurons. Following the previous report, inhibitory circuit of layer 4 may be involved in altering synaptic properties during the critical period (Maffei, 2004). In our result, PV-positive puncta onto layer 4 was increased in both cortices, and layer 2-3 show a similar tendency (Table 1 – 1). All of these results demonstrate that the structural development of the inhibitory circuit in the temporal cortex as well as the visual cortex occurs after the critical period.

Functional development of the inhibitory circuit during the critical period is observed in temporal association cortex as well as primary visual cortex.

By histological evidence, I found structural development of inhibitory neuronal circuits was also observed in temporal cortex where plasticity did not disappear even after development. This is a result of overestimating the hypothesis that synaptic plasticity will last a lifetime because the association cortex does not have a "critical period".

Next, I attempted to define whether the functional characteristics of intra-cortical synapse in the temporal cortex also change around the critical period, and show only structural development regardless of the function change. I studied how developmental stage regulates the strength of inhibitory synapse in layer 2/3 of the rodent visual cortex by using the magnitude of the maximal IPSC to quantify the total inhibitory input converging onto a given cell (Ling and Benardo, 1998, 1999). The amplitude of IPSCs evoked with layer 4 stimulation saturates as the stimulus intensity is increased. The IPSC/EPSC ratio also saturates, and its maximal value is used to compare the balance of inhibition and excitation between different cells. Changes in the intensity of synaptic inhibition have been suggested to establish the timing of the critical period for experience-dependent plasticity (Komatsu, 1983; Kirkwood and Bear, 1994; Hensch, 1998; Huang, 1999). I observed the I-E balance as a functional development index of TC and V1 intra-cortical circuit function. As expected in the visual cortex, an increase in I-E balance is observed after the critical period. In contrast, I-E balance was also increased in the temporal cortex, where functional development of the inhibitory circuit was not expected (Fig. 1-2).

Following the result above (Figure 1-1), that temporal cortex, which is an area of association cortex that maintains synaptic plasticity even after development is completed, plays a role of consolidating sensory information into associative memories. Therefore, the temporal cortex has assumed that there will be a factor to fine-tune synaptic function to maintain synaptic plasticity despite inhibitory development. Before understanding this, I preform to analyze the characteristics of basic synapse and neuronal function electrophysiologically. As a result, both visual and temporal cortices showed no significant change in neuronal excitability at layer 2/3 pyramidal cells around the critical period (Figure 1-3). The amplitude of visual mEPSC increased marginally. (Figure 1-4). The mIPSC was slightly altered in both cortical areas (Figure 1-5). Taken together, the synapses of TC and V1 shows similar structural and functional changes while development despite distinct different functions.

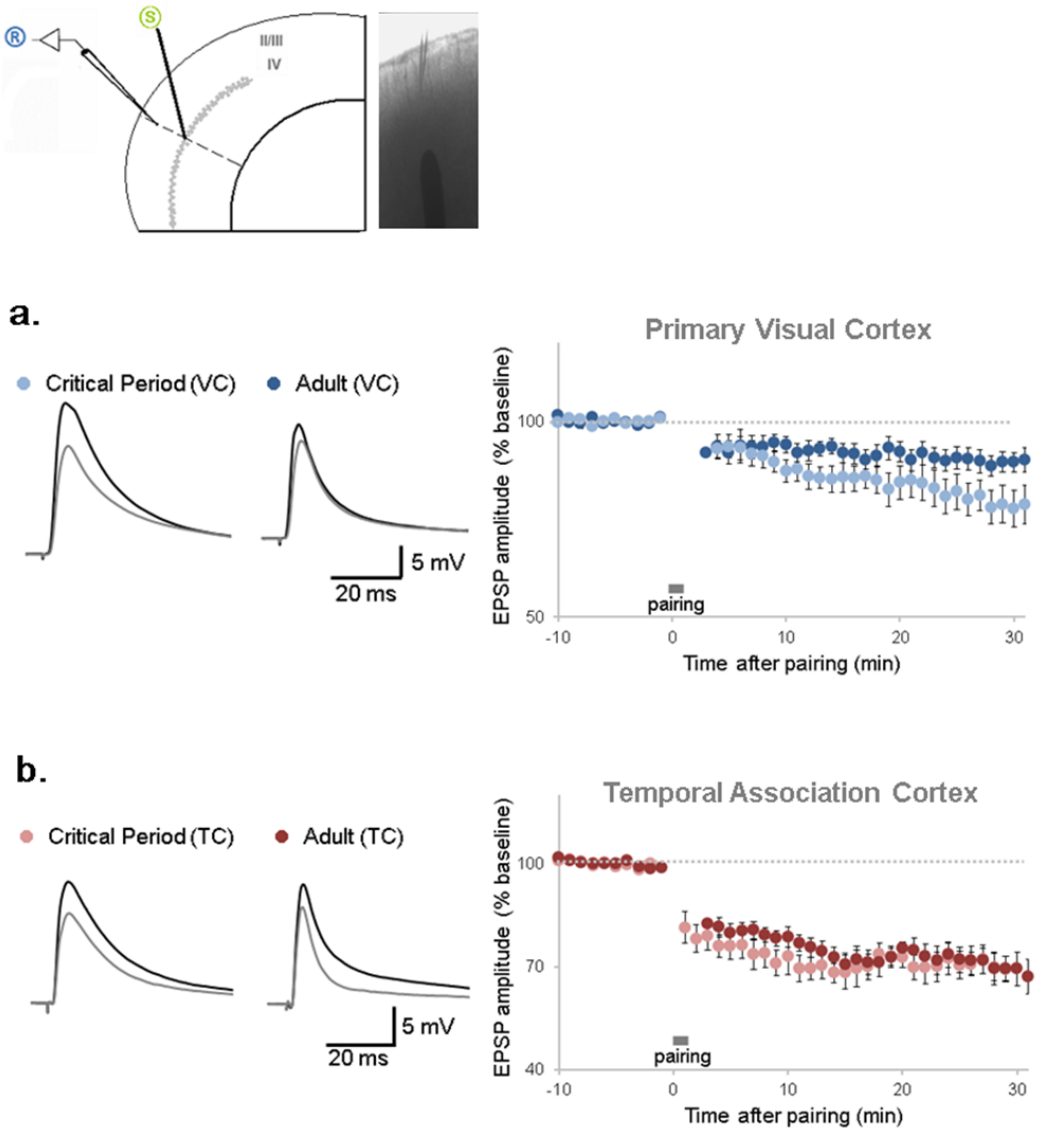

Figure 1-1. Developmentally declined LTD in primary visual cortex, not temporal association cortex intra-cortical circuit.

Pairing-LTD measured primary visual cortex (V1, VC) and temporal association cortex (TC) at 3-week-old and 8 to 12-week-old C57BL/6J mice.
(a-b, left) Representative traces before (black) and 30 min after conditioning (gray).
(a-b, right) Time course of mean amplitudes of evoked-EPSP before and after conditioning stimuli (pairing) at acute slice of VC and TC.
All data shown mean±SEM.

	Visual Cortex (Young => Adult)	Temporal Cortex (Young => Adult)
PV^+ Cell #	No Change	No Change
WFA	Increase	Increase
PV^+ Puncta (L 4)	Increase	Increase
PV^+ Puncta (L 2/3)	No Change (Slight Increase)	Increase

Table 1-1 . Histological changes in visual and temporal cortex.

1) WFA (Wisteria floribunda agglutinin): a marker of Perineuronal net (PNN).
2) PV+ puncta: Synapse of PV+ interneuron forming in pyramidal cell

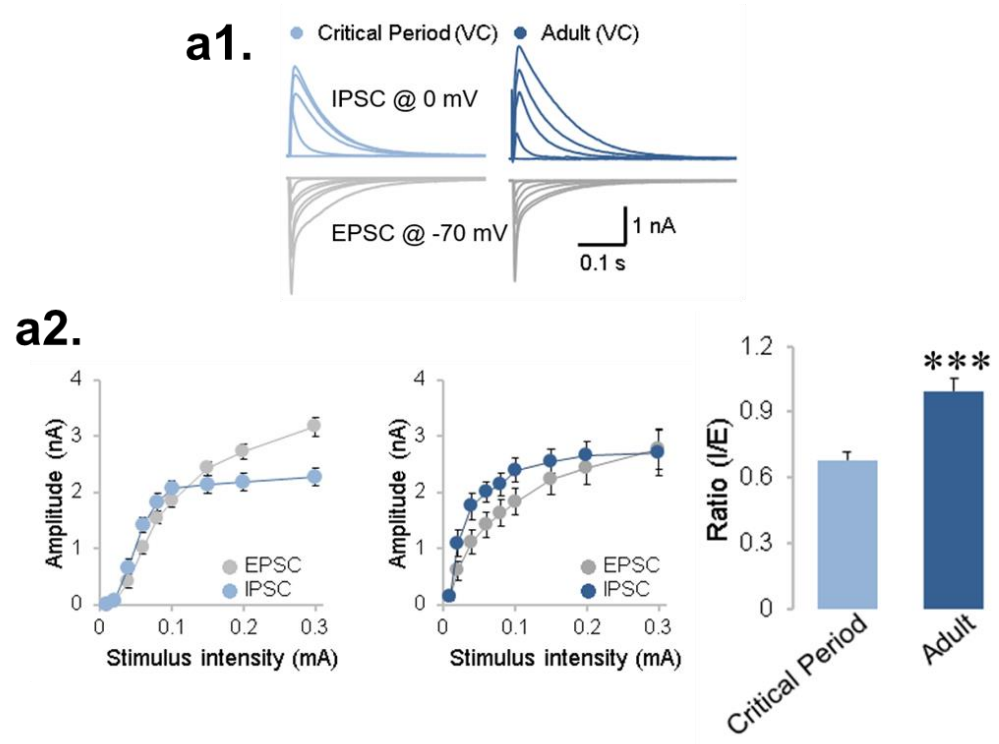
a1.
Critical Period (VC)
Adult (VC)
IPSC @ 0 mV
EPSC @ -70 mV
1 nA
0.1 s
a2.
Amplitude (nA)
Stimulus intensity (mA)
EPSC
IPSC
Ratio (I/E)
***
Critical Period
Adult
b1.
Critical Period (TC)
Adult (TC)
IPSC @ 0 mV
EPSC @ -70 mV
1 nA
0.1 s
b2.
Amplitude (nA)
Stimulus intensity (mA)
EPSC
IPSC
Ratio (I/E)
**
Critical Period
Adult

Figure 1-2. Increased I-E balance in visual and temporal cortex.

Developmental changes in the balance of excitation and inhibition.
(a1 and b1) Representative traces of response evoked by a stimulus series of increasing intensity in a cell from 3-week-old mice (a1) and a 8 to 12-week-old mice (b1). (a2 and b2, left) The input–output relationship for the IPSC (colored) and EPSC (gray) is shown in for 3-week-old mice and in 8 to 12-week-old mice. (a2 and b2, right) Developmental changes in the maximal IPSC/EPSC ratio.
Measure EPSCs at – 70 mV holdings (gray symbol), and IPSCs at 0 mV holdings (colored symbol)
All data shown mean ± SEM. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; n.s., Not significant by independent Student's *t* test.

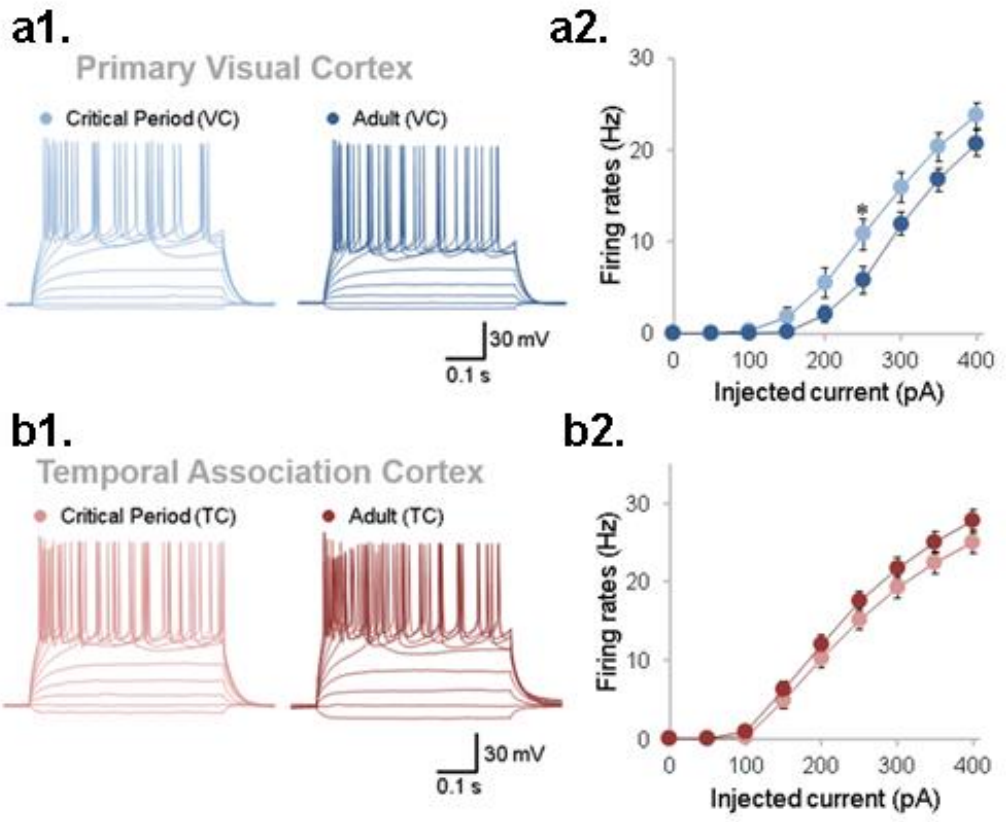

Figure 1-3. Excitability was not altered during a critical period in both cortices.

Intrinsic neuronal excitability of the cortical layer 2/3 pyramidal cells shows no significant change in VC and TC.

(a1 and b1) Representative traces from young (3-week-old, light color) and adult (8 to 12-week-old, dark color) cells.

(a2 and b2) Firing rates of layer 2/3 pyramidal neurons from V1 (a2) and TC (b2) by performing current clamp recordings in response to series of incremented current injection.

All data shown mean ± SEM.

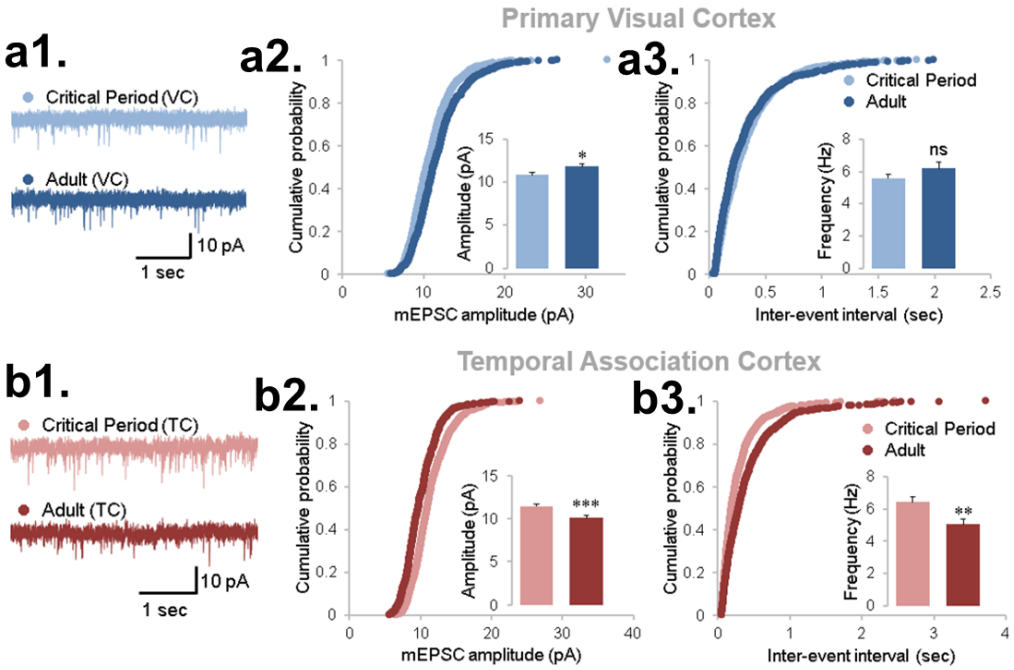

Figure 1-4. Excitatory synaptic transmission changes in visual and temporal cortex during a critical period.

The α-amino-3 hydroxy-5-methyl-4-isoxazole propionate receptor (AMPAR) mediated miniature excitatory synaptic current (mEPSC) in VC and TC.

(a1 and b1) Representative traces from young (3-week-old, light color) and adult (8 to 12-week-old, dark color) cells.

(a2 and b2) Amplitude of mEPSC from VC and TC layer 2/3 pyramidal neurons.

(a3 and b3) Frequency of mEPSC from VC and TC layer 2/3 pyramidal neurons. All data shown mean ± SEM. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. n.s., Not significant by independent Student's t test.

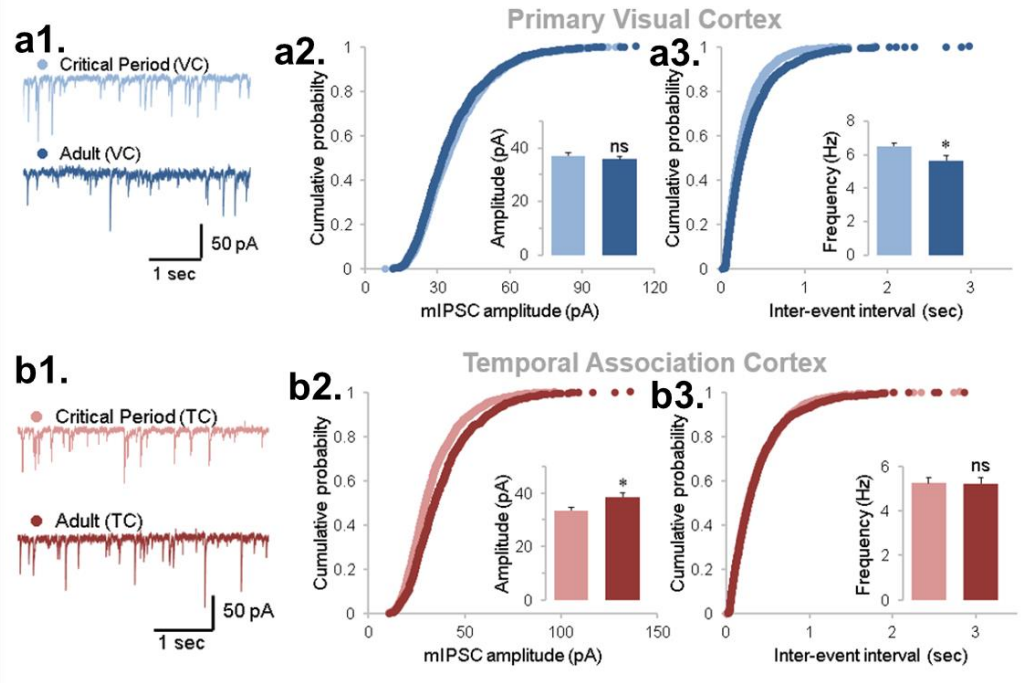

Figure 1-5. Inhibitory synaptic transmission changes in primary visual and temporal association cortex during a critical period.

The $GABA_A$ receptor mediated miniature inhibitory postsynaptic current (mIPSC) in VC and TC. (a1 and b1) Representative traces from young (3-week-old, light color) and adult (8 to 12-week-old, dark color) cells.

(a2 and b2) Amplitude of mEPSC from VC and TC layer 2/3 pyramidal neurons.

(a3 and b3) Frequency of mEPSC from VC and TC layer 2/3 pyramidal neurons. All data shown mean ± SEM. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; n.s., Not significant by independent Student's *t* test.

Discussion

Through this study I found that there were no differences in the structural and functional development of the inhibitory circuit in the two different cortical regions. As reported previously, above results also show that TC maintains plasticity, unlike V1, where intra-cortical plasticity decreases or disappears after a critical period. Therefore, I expect that structural changes to be different in both cortices (TC expects no critical period), I also examined the indicators of known cortical development: the number of inhibitory pavalbumin-positive neurons, development of PNNs, and changes in inhibitory synapse number in TC. As a result, they show similar patterns of structural development of inhibitory circuit in both cortices. In order to confirm whether the functional maturation of each cortical region occurs like structural change, I measured the I-E balance, which is an indicator of inhibitory circuit maturation. Unlike what I expected, an increase in inhibitory current was observed in TC as well as V1. Therefore, I examined the regulation of micro-function in synapses, and examined the characteristics of neurons and synapses constituting the intra-cortical circuit. As a result, it was confirmed that changes in the intrinsic and basal neural and synaptic functions by indicators of basic synaptic function such as excitability and miniature EPSC and mIPSC. These data showed marginal differences without a clear tendency. Together, I found that even a temporal association cortex which do not have 'conceptual' critical period, the development of structural and functional inhibitory circuits occurs equally as that of primary visual cortex.

Our remaining results, except LTD induction results, show that TC as well as V1 exhibited almost the same structural and functional change. So why are these changes coming? This question allows us to consider several possibilities. The first hypothesis is that TC has a critical period and takes same visual activity at the same time as V1 and showing an experiences-dependent change. This is unlikely, considering that TC is involved in memory consolidation. It is hard to see that TC's circuit is optimized by receiving visual input at a certain time considering the nature of memory consolidation, which requires constant input and retrieval of different inputs throughout the lifetime from other brain regions including the hippocampus. Another possibility is that these changes are common to

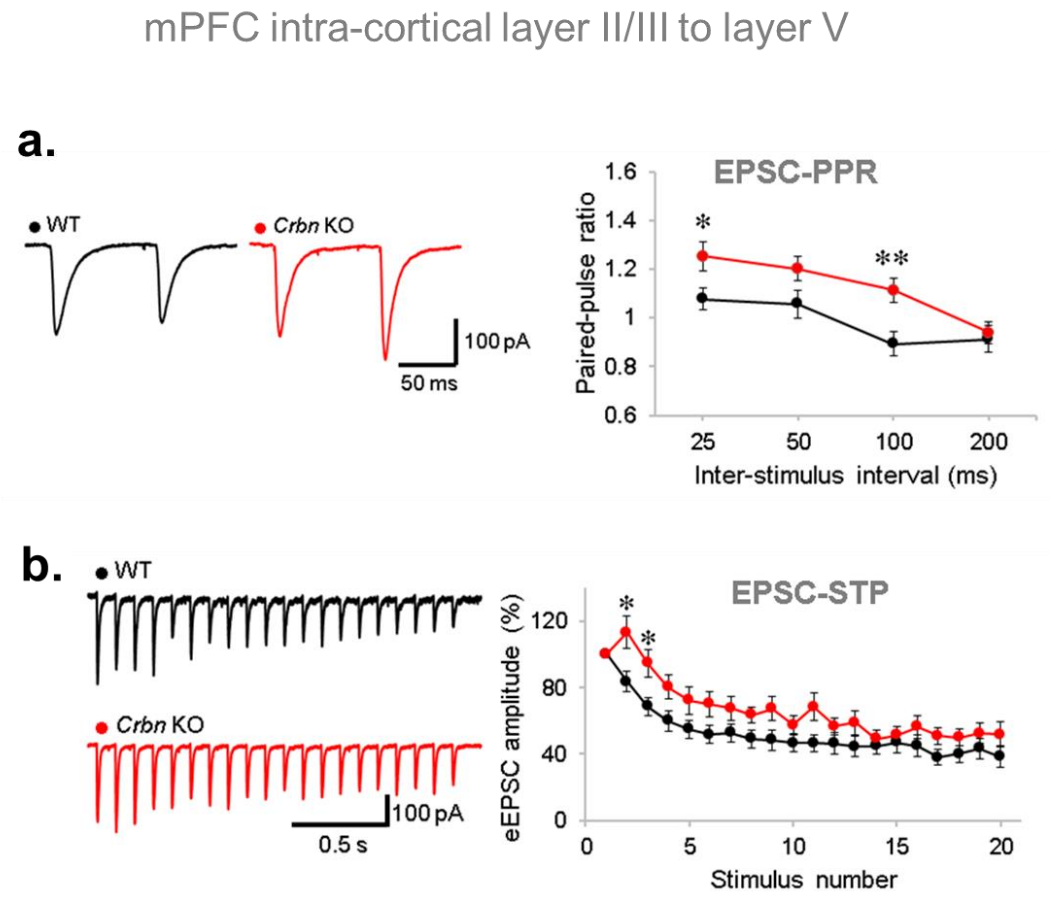

Figure 3-10. Increased excitatory presynaptic release probability in mPFC intra-cortical layer 2/3 to layer 5 circuit in *CRBN* KO mice.

(a) Excitatory PPR in *Crbn* KO mPFC layer 5 pyramidal neurons is slightly increased. (Left) Representative traces. (Right) PPR is plotted against indicated ISIs (right).

(b) Excitatory short term plasticity (STP) induced by 20-pulse train stimulation in *Crbn* KO mPFC layer 5 neurons. Representative traces (left) and the amplitudes of EPSCs are plotted against stimulus number (right).

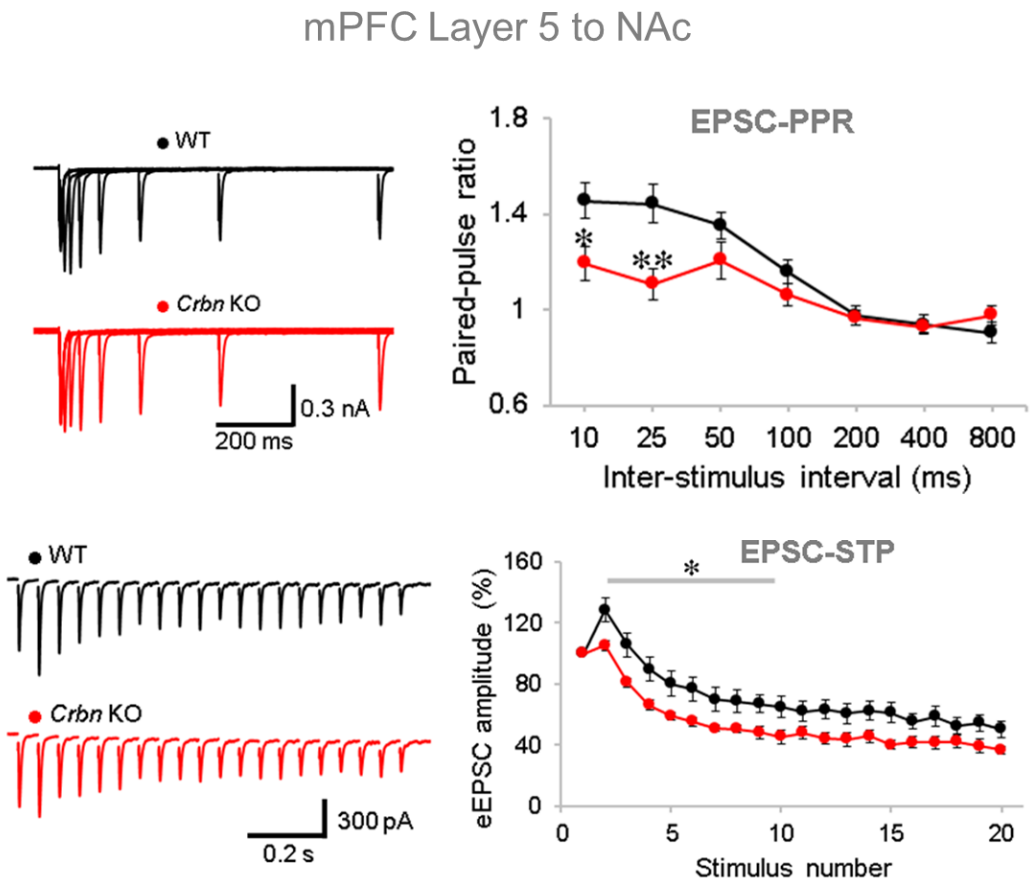

Figure 3-11. Increased excitatory presynaptic release probability in mPFC-NAc circuit in *CRBN* KO mice.

(a) Excitatory PPR in *Crbn* KO NAcc medium spiny neurons (MSNs) is slightly increased. (Left) Representative traces. (Right) PPR is plotted against indicated ISIs (right).

(b) Excitatory short term plasticity (STP) induced by 20-pulse train stimulation in *Crbn* KO mPFC layer 5 neurons. Representative traces (left) and the amplitudes of EPSCs are plotted against stimulus number (right).

Discussion

In this study, I report anxiety/depression and subordinate social dominance behaviors of *Crbn* KO mice that previously unknown. Using Crbn KO fly, I found that CRBN regulates the presynaptic neurotransmitter release probability of excitatory synapse independent of synaptic structure. Crbn has been identified as a mild intellectual-disability (ID) related gene. Following the previous report, Crbn is known to maintain cognitive behavior and excitatory synaptic function by regulating surface expression of the BK channel in hippocampus (Choi, 2018). Therefore, when the BK_{ca} channel was blocked with the drug, the cognitive dysfunction of *Crbn* KO was restored. However, depression / anxiety behaviors are regulated independently of the BK channel blocker.

Unlike cognitive dysfunction restored by BK_{ca} channel blocker, Paxilline, depression / anxiety like behaviors are not reversed. This suggests that there will be another molecular mechanism independent of the BK channel that regulates the above behavioral patterns. CRBN forms $CRL4^{CRBN}$ E3 ubiquitin ligase complex and mediates different functions through interaction with various substrates such as BK_{ca} channel, AMPKα subunit, CLC-2 channel, and IKZF1 (Hohberger, 2009; Jo, 2005; Lee, 2013; Lu, 2014). Therefore, it may be interesting to trace the molecular mechanism of *Crbn* KO mice showing depressive / anxious behavior in the future.

To find brain regions that mediate behavioral abnormalities in Crbn KO mice, I screened areas showing differences in neuronal activity after tube dominance test for social confrontation stimuli. This is consistent with previous reports that the neuronal activity of the MDT-mPFC circuit is directly related to the social hierarchy (Wang, 2011; Zhou, 2017). Many studies before have identified mPFC as a brain region that regulates mood and social dominance (Riga, 2014; Russo and Nestler, 2013; Fujii, 2009; Mah, 2004; Rudebeck, 2007; Wang, 2011, Zhou, 2017). The neuronal function of mPFC was analyzed electrophysiologically in *Crbn* KO mice, which showed behavioral abnormalities known to be regulated by mPFC. As a result, it was confirmed that the excitatory presynaptic function changed in the input circuit of the mPFC and the intra-cortical circuit and output circuit to NAc.

One question to be asked here is how the various modes of behavior, such as that shown by *Crbn* KO, can be controlled in the mPFC domain. The association cortex, mPFC, is an area that regulates diverse and complex higher brain functions by connecting various functional sub-brain regions. Especially, mPFC forms an excitatory output circuit in subcortical regions such as NAc, LHb, DRN, VTA and amygdala to control not only mood such as depression, anxiety, fear, addiction but also social hierarchy (Riga,2014; Russo and Nestler, 2013; Wang, 2014; Zhou, 2018). In other words, various mood and sociability related behaviors are commonly associated with mPFC, but detailed prefrontal circuits that control these behaviors are relatively unknown. The mechanism by which mPFC can regulate these diverse behaviors is understood to be due to the variety of upstream / downstream neuronal circuits. However, there are less understood that of detailed circuit-behavior correlates on how different kinds of behaviors (eg, depressive behavior and social dominance) are controlled differently. Crbn KO has various behavioral patterns known to be mPFC circuit dependent. In addition, we confirmed that the neuronal activity of the surrounding region, which is in circuit with mPFC, is changed. It is interesting to note that the mPFC is associated with a subset of behavioral phenotypes in *Crbn* KO mice.

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국문초록

우리의 뇌는 약 천억 개의 신경세포들로 구성된 가장 복잡한 기능을 하는 기관이다. ‘대뇌피질’은 이러한 뇌를 구성하는 하위기관 중 가장 넓은 면적을 차지하며 구조적으로 구획화되어 있다. 또한 대뇌피질은 구획화된 영역을 통해 감각, 운동, 판단, 기억, 정보의 가공, 언어 등 다양한 기능을 수행한다. 예를 들면, 일차 감각피질인 시각피질(Visual Cortex)은 시각 정보를 가공하여 다른 피질에 전달하는 반면, 연합피질인 측두엽피질 (Temporal Association Cortex)은 감각피질로부터 받은 정보를 수집하여 중간정보를 가공하는 대뇌피질 영역으로서, 지각기반 판단 기능에 연관되어 있다. 우리가 보면서 동시에 말하고, 들으면서 운동할 수 있는 것은 대뇌피질이 동시에 다양한 기능을 수행할 수 있기 때문이다. 대뇌피질은 이러한 멀티태스킹(multi-tasking)을 위해 각각 기능에 최적화된 영역들이 해당 기능에 특화된 신경회로를 이루게끔 발달된다. 대뇌피질을 구성하는 신경세포와 시냅스, 또는 신경회로의 기능이상은 다양하고 복잡한 뇌질환의 원인이 된다. 예를 들어 조울증, 조현병, 자폐증 등이 대뇌피질의 기능 이상에서 기인하는 것으로 알려져 있는 정신질환이다. 따라서 정상적인 대뇌피질 신경 및 신경회로의 기능과 발달을 이해하는 것은 뇌에 대한 우리의 이해를 넓혀 줄 뿐만 아니라 장차 다양한 신경정신질환의 효과적인 치료법을 도출하는데 기여할 것이다.

대뇌피질이 다양한 기능 조절에 관여함에도 불구하고, 영역 내 신경회로의 기본 구조는 영역 구분 없이 상당히 동일하다. 대뇌피질의 기능적 다양성은 외부에서 들어오는 신경회로가 가지는 다양성 때문으로 이해되어 왔으며, 각각 영역의 기능에 최적화되어 발달될 것으로 추정되지만, 이들 서로 다른 대뇌피질간 과연 동일한 발달기전을 가지는지는 잘 알려져 있지 않다. 시각피질을 포함한 일차감각피질은

고유의 기능을 확보하기 위해 생애 초반의 경험을 통한 신경회로의 최적화 과정을 거친다. 이러한 생애 초기 특정시기를 일반적으로 '결정적 시기(Critical Period)' 라고 한다. 시각피질은 critical period 에 가장 큰 가소성을 가지며, 이 시기에 경험에 의한 신경회로의 최적화가 일어난다. 기존 연구들을 통해 일차감각피질에서는 critical period 가 종료된 이후에는 피질 내(intra-cortical) 시냅스의 가소성이 감소하거나 소실된다는 것이 밝혀졌으며, 이에 억제성 신경회로의 발달이 중요한 역할을 한다는 것이 알려져 있다. 반면, 연합피질은 경험의존적 신경회로의 최적화보다는, 서로 다른 대뇌 영역간의 정보를 수집하여 가공하는 기능이 생애 전반에 걸쳐 일어나는 것이 중요하다. 그러나 연합피질의 발달에 '결정적 시기'가 있는지, 이 시기에 어떠한 기능적 발달이 일어나는지에 대한 연구는 부족하다. 1 장에서는 유사한 내부 신경회로 구조를 가지는 서로 다른 두 피질 영역, 일차감각피질인 시각피질과, 연합피질인 측두엽피질의 발달상 신경세포의 구조 및 기능 변화를 확인하였다.

대뇌 피질의 기능이상은 다양한 신경정신질환을 야기한다. 특히 최근의 여러 임상학적 보고들에 의하면, 조현병, 자폐증, 조울증 등과 같은 기분 및 인지장애 환자들은 대뇌피질 중에서도 특히 전전두엽피질(prefrontal Cortex, PFC)의 구조 및 기능이상을 보임이 알려져 있다. PFC 는 각 피질에서 제공된 지각정보를 감정정보와 함께 종합하여 가치에 기반을 둔 판단과 선택의 기능을 한다고 알려진 연합피질영역이다. 따라서 PFC 의 시냅스 및 연관된 신경회로가 원래는 어떠한 기능을 하는지 이해하는 것은 뇌에 대한 우리의 이해를 넓혀 줄 뿐만 아니라, 장차 다양한 신경정신질환의 효과적인 치료법을 도출하는데 기여할 것이다. 본 연구자는 다양한 PFC 와 연관되어있다고 알려진 행동학적 이상을 보이는 두 가지 마우스 모델을 사용하여 신경세포와 시냅스, 신경회로의 기능이상을 확인하였다. Cytoplasmic FMR1-interacting protein 2(Cyfip2) 는 시냅스에 발현하는 actin-조절자 단백질이며, *Cyfip2* 유전자 손실 마우스는 정신안정제인 lithium 에 반응하는 manic-like 행동을 보인다. 조증(manic episode)은 양극성 장애의 주된 특징 중 하나로, 기분이 고조되고, 활동성과 에너지의 증가와 사교성의 증가를 특징으로 하는 장애이다. 양극성 장애를 가진 환자군에서

PFC 의 구조 및 기능이상을 보인다는 임상학적 보고들이 있으나, 신경세포 및 시냅스 수준에서의 병태생리학적 기전과 약물반응기전에 대해서는 거의 밝혀진 바가 없다. 이러한 중요성에 착안하여 2 장에서는 manic-like 행동을 보이는 Cyfip2 유전자 손실 마우스 모델을 통해, PFC 에서의 신경세포 및 시냅스 기능이상과 이상행동과의 상관관계를 확인하였다.

우울, 불안과 같은 기분장애와 사회성, social dominance 역시 대뇌피질의 전전두엽 영역과 연관되어 있음이 최근 많은 연구들을 통해 밝혀지고 있다. *Cereblon* (*CRBN*) 결손 마우스 모델은 subordinate 한 dominance behavior 를 보이며, 우울, 불안 장애 행동을 보인다. *Cereblon* (*CRBN*)은 IQ 50~70 사이의 경도 지적장애(mild intellectual disability)를 보이는 환자들에서 돌연변이가 있음이 최초로 발견되었으며, CRBN 유전체 다양한 영역의 결손이나 microduplication 이 인지 및 행동장애와 연관이 있음이 보고된 바 있다. CRBN 단백질은 DNA-binding protein 1(DDB1), cullin-4A(CUL4A), regulator of cullins 1(ROC1)과 함께 $CRL4^{CRBN}$ E3 ubiquitin ligase complex 를 형성하며 위 복합체에서 CRBN 은 조절을 받을 단백질과 결합하여 이들을 E3 ubiquitin ligase complex 로 데려오는 receptor 역할을 함이 밝혀졌다. 최근 *CRBN* 유전자 결손 마우스를 이용한 연구를 통해 CRBN 단백질이 BK_{ca} channel 과 $AMPK_{\alpha}$의 인산화를 통해 해마의 Schaffer collateral – CA1 시냅스의 기능 및 cognitive behavior 를 조절한다는 것을 밝힌 바 있다. 그러나 *CRBN* KO 마우스의 우울, 불안행동 및 social dominance behavior 는 인지행동과는 달리 해마영역의 BK channel 과 무관하게 조절된다. 3 장에서는 *CRBN* mutant 초파리의 근신경접합부를 사용하여 흥분성 시냅스의 구조 및 기능의 변화를 확인하였으며, Social dominance 및 기분장애 행동 이상을 보이는 *CRBN* 유전자 결손 마우스를 이용하여 PFC 및 해당영역에 신호를 주고 받는 주변영역과 구축하는 신경회로 및 시냅스 기능변화를 관찰하였다.

다양한 기능을 하는 대뇌피질 내/외부 신경망의 발달과, 그 기능이상이 신경정신질환의 신경세포 및 시냅스에서 어떻게 나타나는지를 확인한 본 연구들은 대뇌피질의 구조적 보편성과 기능적 다양성을 이해하는 데 크게 기여할 뿐 아니라 피질 의존적인 고등 뇌기능 및 뇌질환을 이해하여 향후 치료기전을 확립하는 데 주요하게 응용될 것으로 기대된다.

주요어: 대뇌피질(cerebral cortex), 결정적 시기(critical period), 연합피질(association cortex), medial prefrontal cortex (mPFC), 기분장애, *Cyfip2*, *Cereblon*

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