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The Development of Phasic and Tonic Inhibition in the Rat Visual Cortex

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Gamma-aminobutyric acid (GABA)-ergic inhibition is important in the function of the visual cortex. In a previous study, we reported a developmental increase in $GABA_A$ receptor-mediated inhibition in the rat visual cortex from 3 to 5 weeks of age. Because this developmental increase is crucial to the regulation of the induction of long-term synaptic plasticity, in the present study we investigated in detail the postnatal development of phasic and tonic inhibition. The amplitude of phasic inhibition evoked by electrical stimulation increased during development from 3 to 8 weeks of age, and the peak time and decay kinetics of inhibitory postsynaptic potential (IPSP) and current (IPSC) slowed progressively. Since the membrane time constant decreased during this period, passive membrane properties might not be involved in the kinetic changes of IPSP and IPSC. Tonic inhibition, another mode of $GABA_A$ receptor-mediated inhibition, also increased developmentally and reached a plateau at 5 weeks of age. These results indicate that the time course of the postnatal development of GABAergic inhibition matched well that of the functional maturation of the visual cortex. Thus, the present study provides significant insight into the roles of inhibitory development in the functional maturation of the visual cortical circuits.

Key Words: Development, GABA, Inhibition, Tonic inhibition, Visual cortex

INTRODUCTION

Gamma-aminobutyric acid (GABA)ergic inhibition is critical to normal brain function, since it ensures the temporal fidelity of information flow [1] and regulates the patterns of neuronal network oscillations, which are critical for information processing [2,3]. In the visual cortex, inhibition is reportedly involved in the sculpting of columnar architecture [4], the processing of features of visual input [5], the regulation of the induction of long-term synaptic plasticity [6], and the regulation of the timing of critical period [7]. Since inhibition matures during postnatal development of the visual cortex [8,9], the effect of inhibition on these processes changes during development, and thus inhibitory maturation exerts its effect on the functional development of the visual cortex. For example, whereas inhibitory influence over a certain threshold level is necessary for the induction of long-term synaptic plasticity at the early postnatal developmental stage [10], further development of inhibition suppresses the induction of plasticity [6,11]. Furthermore, the kinetic features of inhibition are known to change during development of the visual cortex [12]. Since slow $GABA_A$ergic inhibition blocks N-methyl-D-aspartic acid receptors (NMDARs) more efficiently [13], changes in the kinetics of inhibition are important in regulating the activation of NMDARs, which is essential for the induction of many forms of synaptic plasticity [14-16]. Thus, it is crucial to investigate the developmental changes in inhibition in order to understand the underlying mechanisms for the developmental decline of long-term synaptic plasticity in the visual cortex [6,17].

Recently, a new modality of GABA receptor type A ($GABA_A$ receptor)-mediated inhibition, called 'tonic inhibition' in contrast to synaptic 'phasic inhibition,' has been extensively studied [18]. Tonic inhibition is mediated either by ambient GABA in extracellular fluid [19,20] or by the spontaneous opening of specific types of $GABA_A$ receptors [21,22], and thus chloride conductance is constantly maintained regardless of the phasic activation of inhibitory synapses. Although it has been suggested that tonic inhibition is important in neuronal information processing [23], the properties of tonic inhibition in the visual cortex have not yet been reported. With the maturation of GABAergic inhibition, tonic inhibition might also change during postnatal development, and may be involved in the development of cortical functions.

Thus, in the present study, we investigated developmental changes in both phasic and tonic inhibition in the rat visual

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ABBREVIATIONS: AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ACSF, artificial cerebrospinal fluid; D-AP5, D-aminopentanoate; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; GABA, gamma-aminobutyric acid; IPSC, inhibitory postsynaptic current; IPSP, inhibitory postsynaptic potential; NMDAR, N-methyl-D-aspartic acid receptor.

cortex. Tonic as well as phasic inhibition progressively increased during postnatal development. Since inhibition is critical in many functional aspects of the visual cortex, information on the development of inhibition will be helpful in understanding the functional development of the visual cortical circuitry and its modification through experience.

METHODS

Slice preparation

Visual cortical slices were prepared from Sprague-Dawley rats of either sex (postnatal 3 to 12 weeks, Orientbio Inc., Korea), maintained under standard conditions (23±1°C, 12/12 hours light/dark cycle). Animal care and surgical procedures were approved by the Ethics Committee of the Catholic University of Korea, consistent with the National Institutes of Health guidelines for the care and use of laboratory animals. Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and the brains were quickly removed and placed in ice-cold dissection medium. Coronal sections of the occipital cortex (300 μm in thickness) were prepared on a vibrotome (Campden Instruments, Leics, UK) and were allowed to recover in a submerging chamber for 40 min at 37°C. The slices were maintained at room temperature prior to recording. The dissection and storage medium consisted of 125 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 2 mM $MgSO_4$, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 10 mM D-glucose, bubbled with carbogen (95% O_2/5% CO_2). The slices were transferred to a recording chamber containing artificial cerebrospinal fluid (ACSF, 1.5~2 ml/min) (125 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 10 mM D-glucose), bubbled with carbogen and maintained at 32~33°C.

Electrophysiological recording

Recording electrodes (3~4 MΩ) were pulled from borosilicate glass pipettes (1B150F-4, World Precision Instruments, Inc., Sarasota, FL, USA) using a micropipette puller (MODEL P-97, Sutter instrument Co., Novato, CA, USA). Whole-cell responses were recorded using a whole-cell patch-clamp recording technique with an EPC9 amplifier (HEKA Elektronik, Lambrecht, Germany) and Pulse 8.31 software (HEKA Elektronik) or with an EPC8 amplifier and pClamp 9.0 software (Axon Instruments, Foster City, CA, USA). In an experimental set to evaluate the development of the inhibitory-to-excitatory ratio, the intracellular solution was composed of (in mM): 130 K-gluconate, 10 KCl, 4 Mg-ATP, 10 Na_2-phosphocreatine, 0.3 Na_3-GTP and 10 HEPES (pH 7.25 with KOH). Pyramidal neurons in layer 2/3 of the primary visual cortex were visually identified using IR-DIC video-microscopy with an upright microscope (BX51-WI fitted with a 40×/0.80NA water immersion objective, Olympus, Tokyo, Japan), and their regular spiking patterns were confirmed. Typical access resistance was 15~20 MΩ. Membrane potentials were not corrected for about 14 mV junction potential. Input resistance was measured by the injection of a negative current pulse (−80 pA), which evoked 3~17 mV hyperpolarization, depending on the age of the animals. Membrane time constant was measured from the decay of the membrane response evoked by a small hyperpolarizing current pulse, fitted with a single exponential function: $V(t)=Aexp(-t/\tau)+C$. Decay of synaptic responses was also analyzed using the same function. Membrane potential and current were both measured by switching between current- and voltage-clamp modes. Synaptic responses were evoked by the application of extracellular stimulation in layer 4 beneath the recorded cell. Excitatory events were measured at a holding potential of −75 mV in normal ACSF, and inhibitory events were subsequently recorded at a holding potential of 0 mV in the presence of the NMDAR antagonist D-aminopentanoate (D-AP5, 50 μM) and the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μM). To evaluate tonic inhibition, a CsCl-based internal solution, containing (in mM) 145 CsCl, 4 Mg-ATP, 10 Na_2-phosphocreatine, 0.3 Na_3-GTP, 10 HEPES and 3 QX-314 (pH 7.25 by CsOH), was used. In this experiment, DNQX (20 μM), D-AP5 (50 μM), and the $GABA_B$ receptor antagonist CGP52432 (1 μM) were applied to suppress neurotransmitter receptors other than $GABA_A$ receptors. The amplitude of tonic inhibition was analyzed as the difference between the holding currents measured before and after the application of the $GABA_A$ receptor antagonist bicuculline (10 μM) while the membrane potential was clamped at −75 mV. The holding current was calculated from 100 msec epochs containing no obvious spontaneous synaptic events, taken every four seconds during an 80-second period [24].

Chemicals

D-AP5, DNQX, bicuculline, and CGP52432 were purchased from Tocris (Bristol, UK). The other chemicals were purchased from Sigma (St. Louis, MO, USA).

Statistical analysis

Data were expressed as the mean±SE. Statistical comparisons were performed using paired or unpaired two-tailed Student's *t*-test unless otherwise specified. One-way ANOVA, followed by a Tukey's post-hoc test, was also used for multi-group comparison (Systat v11, SYSTAT Software, Inc., Richmond, CA, USA). The level of significance was set at $p<0.05$.

RESULTS

Developmental changes in passive membrane properties

Since passive membrane properties affect the amplitude and time course of synaptic events [25], developmental changes in input resistance and membrane time constant were initially investigated in the pyramidal cells in layer 2/3 of the visual cortex of 3- ('3 weeks,' n=9), 5- ('5 weeks,' n=9), 8- ('8 weeks,' n=9), and 12-week-old rats ('12 weeks,' n=9). Input resistance was determined by measuring the deflection of membrane potential by hyperpolarizing current pulse (−80 pA), and membrane time constant was measured by fitting the decay of membrane potential from the deflection (Fig. 1). Input resistance decreased from 3 to 8 weeks of age (136.6±18.9 MΩ, 88.8±9.6 MΩ, 69.8±8.1 MΩ, and 64.0±7.6 MΩ for '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, $p<0.001$ between '3 weeks' and '5 weeks,' $p<0.05$ between '5 weeks' and '8 weeks'). Membrane time constant also decreased from 3 to 5 weeks of age and reached a plateau thereafter (15.6±1.1 msec,

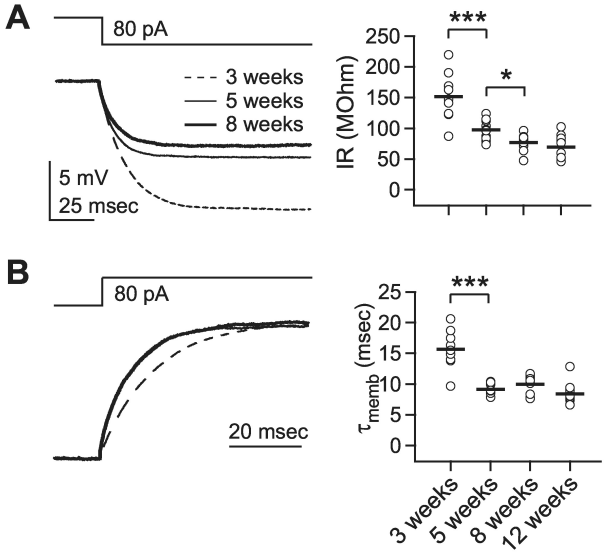

Fig. 1. Developmental changes in the passive membrane properties. Passive membrane properties were measured by injection of a small negative current (−80 pA). (A) Developmental changes in input resistance. Left panel: upper trace shows the command current and lower traces show the averaged responses of membrane potential in layer 2/3 pyramidal cells at 3- ('3 weeks,' dashed line), 5- ('5 weeks,' thin solid line), and 8-week-old rats ('8 weeks,' thick solid line). The right panel plots individual data (symbols) and averages (thick solid lines). IR: input resistance. (B) Developmental changes in membrane time constant. Left panel: upper trace shows the command current and lower traces show normalized responses of membrane potential in layer 2/3 pyramidal cells. The right panel plots individual data (symbols) and averages (thick solid lines). τ_{memb}: membrane time constant. *$p<0.05$, and ***$p<0.001$ between groups linked by lines.

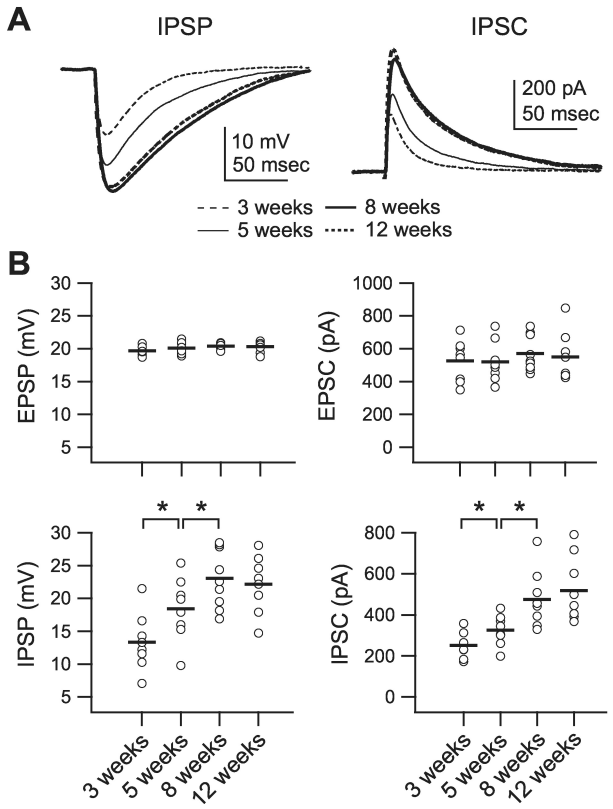

Fig. 2. Development of phasic inhibition. Inhibitory postsynaptic potential (IPSP) and inhibitory postsynaptic current (IPSC) were recorded at 0 mV of membrane potential in the presence of DNQX (20 μM) and D-AP5 (50 μM) at stimulation intensities that evoked 20 mV depolarization at −75 mV membrane potential. (A) Averaged IPSPs (left traces) and IPSCs (right traces) showing differences between groups. IPSPs and IPSCs were recorded from slices of 3- ('3 weeks,' thin dashed lines), 5- ('5 weeks,' thin solid lines), 8- ('8 weeks,' thick solid lines), and 12-week-old rats ('12 weeks,' thick dashed lines). (B) Individual data (symbols) and averages (thick lines) for excitatory postsynaptic potential (EPSP), excitatory postsynaptic current (EPSC), IPSP, and IPSC for each of the groups indicated in the lower panels. *$p<0.05$ between groups linked by lines.

9.2±0.3 msec, 9.9±0.5 msec, and 8.4±0.6 msec for '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, $p<0.001$ between '3 weeks' and '5 weeks'). Thus, passive membrane properties in the visual cortex changed during postnatal development in such a way that synaptic activities would deflect membrane potential more quickly.

Development of phasic inhibition

In a previous study, we reported the development of synaptically evoked phasic inhibition during the critical period from 3 to 5 weeks of age in the rat visual cortex [11]. To further evaluate the development of inhibition, in the present study we extended the time period to 12 weeks of age. To evaluate the development of inhibition relative to excitation, inhibitory postsynaptic potential (IPSP) and inhibitory postsynaptic current (IPSC) were measured at the stimulus intensity that evoked a depolarizing excitatory postsynaptic potential (EPSP) of 20 mV at −75 mV membrane potential (Fig. 2B, upper left panel). Excitatory postsynaptic current (EPSC) evoked in all age groups at the same stimulus intensity with 20 mV EPSP did not differ among the groups (525.0±44.4 pA, 520.1±43.8 pA, 570.4±36.2 pA, and 548.5±52.7 pA for '3 weeks', '5 weeks', '8 weeks,' and '12 weeks', respectively, p=0.84 by ANOVA) (Fig. 2B, upper right panel). Although there could be some inhibitory components in EPSPs and EPSCs, they were considered to be excitatory since inhibitory components would be minimal at −75 mV membrane potential, which was close to the chloride equilibrium potential in this experiment (E_{Cl}=−68 mV). The amplitude of stimulus current pulse applied to evoke 20 mV depolarization was also similar among the groups (131.4±9.3 μA, 146.2±8.7 μA, 142.3±5.9 μA, and 149.7±10.4 μA for '3 weeks', '5 weeks', '8 weeks,' and '12 weeks', respectively, p=0.246 by ANOVA). Thus, excitatory strength did not differ among the age groups, despite the decrease in input resistance with age (Fig. 1). Other active properties, such as voltage-dependent ion channels, might also be involved in the developmental changes [26]. By contrast, both IPSPs and IPSCs increased from 3 to 8 weeks of age (IPSPs 13.3±1.5 mV, 18.4±1.7 mV, 23.1±1.5 mV, and 22.2±1.6 mV at '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, $p<0.05$ between '3 weeks' and '5 weeks,' $p<0.05$ between '5 weeks' and '8 weeks;' IPSCs 250.7±22.0 pA, 325.3±26.3 pA, 475.9±43.9

pA, and 527.7±56.4 pA at '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, $p<0.05$ between '3 weeks' and '5 weeks,' $p<0.05$ between '5 weeks' and '8 weeks'). These results suggest that phasic inhibition increases developmentally and reaches the adult level at 8 weeks of age.

Developmental changes in the kinetics of synaptic events

Expression of $GABA_A$ receptor subunits has been shown to change during development in the rat visual cortex [12]. Since the time course of $GABA_A$ receptor-mediated responses is dependent on the composition of subunits [27], the kinetics of phasic inhibition would also change during postnatal development. To address this issue, changes in the peak time and the decay time constant of IPSP and IPSC were investigated in 3- to 12-week-old rats (Fig. 3). The peak time was measured as the time difference between electrical stimulation and the peak of the response, and the decay time constant was obtained by single exponential fitting. Peak time increased during development (IPSPs 10.7±0.4 msec, 11.9±1.0 msec, 16.2±1.1 msec, and 15.3±2.1 at '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, $p<0.01$ between '5 weeks' and '8 weeks;' IPSCs 5.9±0.3 msec, 7.2±0.5 msec, 9.1±0.5 msec, and 8.3±

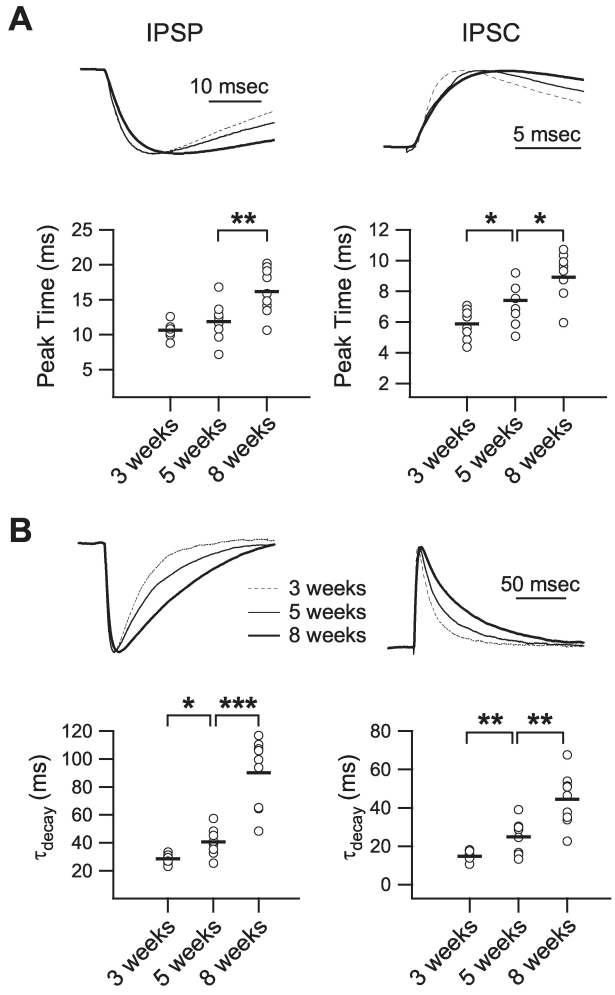

Fig. 3. Developmental changes in the kinetics of phasic inhibition. (A) Peak time of IPSP (left panel) and IPSC (right panel). Upper traces show normalized IPSPs and IPSCs of 3- ('3 weeks,' dashed line), 5- ('5 weeks,' thin solid line), and 8-week-old rats ('8 weeks,' thick solid line) with an extended time scale. Lower panels plot individual data (symbols) and averages (thick lines) for the peak time of IPSPs and IPSCs. (B) Decay time constant of IPSP (left panel) and IPSC (right panel). Upper traces show normalized IPSPs and IPSCs of 3- (dashed line), 5- (thin solid line), and 8-week-old rats (thick solid line). Lower panels plot individual data (symbols) and averages (thick lines) for the decay time constant of IPSPs and IPSCs. τ_{decay}: decay time constant. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ between groups linked by lines.

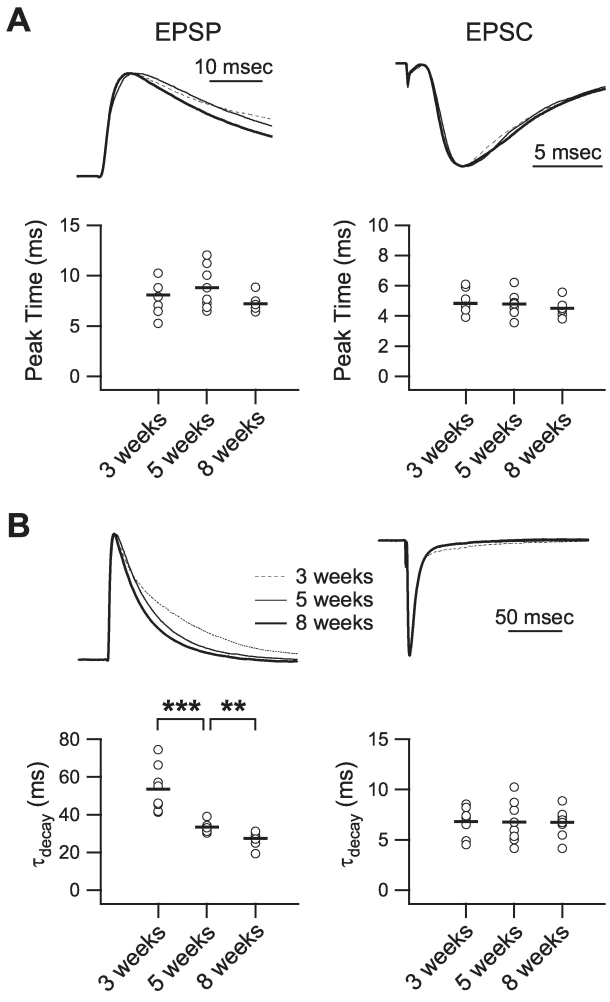

Fig. 4. Developmental changes in the kinetics of excitation. (A) Peak time of EPSP (left panel) and EPSC (right panel). Upper traces show normalized EPSPs and EPSCs of 3- ('3 weeks,' dashed line), 5- ('5 weeks,' thin solid line), and 8-week-old rats ('8 weeks,' thick solid line) with extended time scale. Lower panels plot individual data (symbols) and averages (thick lines) for the peak time of EPSPs and EPSCs. (B) Decay time constant of EPSP (left panel) and EPSC (right panel). Upper traces show normalized EPSPs and EPSCs of 3- (dashed line), 5- (thin solid line), and 8-week-old rats (thick solid line). Lower panels plot individual data (symbols) and averages (thick lines) for the decay time constant of EPSPs and EPSCs. τ_{decay}: decay time constant. **$p<0.01$, and ***$p<0.001$ between groups linked by lines.

0.9 at '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, $p<0.05$ between '3 weeks' and '5 weeks,' $p<0.05$ between '5 weeks' and '8 weeks') (Fig. 3A). The decay time constant in both IPSPs and IPSCs also increased from 3 to 8 weeks of age (IPSPs 28.5±1.2 msec, 40.6±3.5 msec, 90.2±8.1 msec, and 85.4±10.3 msec at '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, $p<0.05$ between '3 weeks' and '5 weeks,' $p<0.001$ between '5 weeks' and '8 weeks;' IPSCs 14.8±0.8 msec, 24.8±3.2 msec, 44.4±4.5 msec, and 42.7±5.9 msec at '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, $p<0.01$ between '3 weeks' and '5 weeks,' $p<0.01$ between '5 weeks' and '8 weeks') (Fig. 3B). Thus, activation and inactivation of phasic inhibition in pyramidal cells of layer 2/3, evoked by electrical stimulation of layer 4, slowed during postnatal development.

Changes in inhibitory kinetics may also affect the kinetics of excitatory synaptic responses, so changes in the kinetics of EPSP and EPSC were also investigated (Fig. 4). In contrast to the inhibitory events, the decay time constant of EPSP decreased with development (53.5±4.2 msec, 33.3±0.9 msec, 27.5±1.2 msec, and 25.2±0.8 msec at '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, $p<0.001$ between '3 weeks' and '5 weeks,' $p<0.01$ between '5 weeks' and '8 weeks') (Fig. 4B, left panels). Other kinetic properties of excitatory components showed no developmental changes (decay time constant of EPSCs 6.8±0.5 msec, 6.7±0.7 msec, 6.7±0.4 msec, and 6.6±0.6 msec at '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, p=0.997 by ANOVA; peak time of EPSPs 8.1±0.6 msec, 8.8±0.7 msec, 7.2±0.2 msec, and 7.5±0.2 msec at '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, p=0.139 by ANOVA; and peak time of EPSCs 4.9±0.3 msec, 4.8±0.3 msec, 4.5±0.2 msec, and 5.1±0.2 msec at '3 weeks', '5 weeks', '8 weeks,' and '12 weeks,' respectively, p=0.187 by ANOVA). Because the kinetics of EPSCs did not change during postnatal development, the faster decay in EPSPs at 5 and 8 weeks of age, compared with that at 3 weeks of age, appears to be resulted from the increased amplitude and slowed decay of IPSC during this period (Fig. 3). Thus, excitatory synaptic transmission might mature before 3 weeks of age and be maintained stably during and after the critical period in the rat visual cortex.

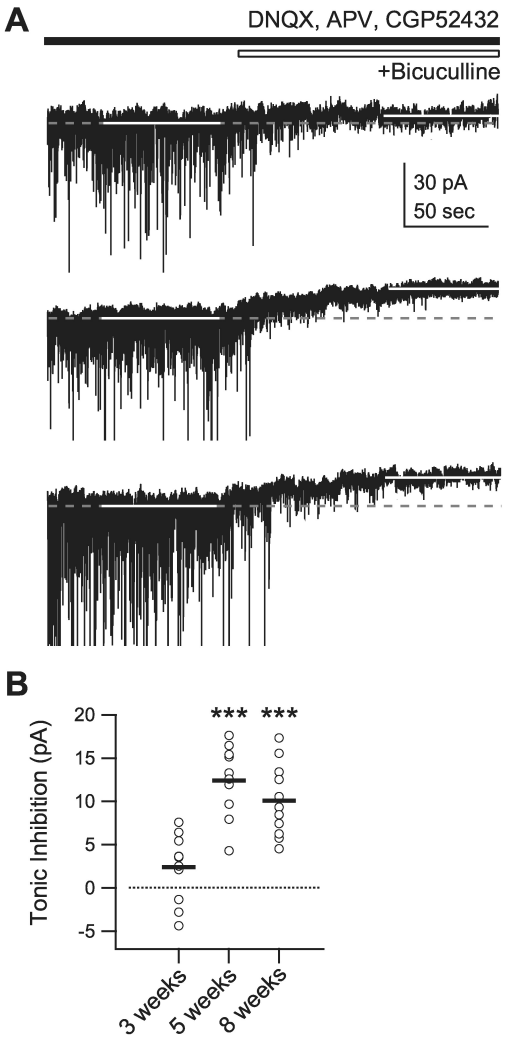

Fig. 5. Developmental changes in tonic inhibition. Tonic inhibition was measured as the difference between the holding current before and after the application of the $GABA_A$ receptor antagonist bicuculline (10 μM) while membrane potential was clamped at −75 mV. (A) Current traces at the holding potential of −75 mV before and after the application of bicuculline for 3- (top trace), 5- (middle trace), and 8-week-old rats (bottom trace). White solid lines indicate periods at which holding currents were measured. Grey dashed lines indicate holding currents measured before the application of bicuculline. (B) Individual data (symbols) and averages (thick lines) of the changes in holding currents for 3- ('3 weeks'), 5- ('5 weeks'), and 8-week-old rats ('8 weeks'). ***$p<0.001$ vs. '3 weeks'.

Development of tonic inhibition

Although tonic inhibition is another important mode of $GABA_A$ receptor-mediated inhibition, to the best of our knowledge it has never been reported in the visual cortex. We therefore investigated the developmental changes in tonic inhibition. Tonic inhibition was detected as the difference between the holding current before and after application of the $GABA_A$ receptor antagonist, bicuculline (10 μM) (Fig. 5). In 3-week-old rats, the holding current did not differ significantly either before or after the application of bicuculline (2.4±1.2 pA before and after bicuculline, n=11, p=0.07) (Fig. 5A, top trace). By contrast, a significant degree of tonic inhibition could be detected in 5-week-old rats (12.4±1.2 pA, n=11, $p<0.001$ vs. '3 weeks') (Fig. 5A, middle trace). In 8-week-old rats, tonic inhibition did not differ from that of 5-week-old rats (10.1±1.3 pA, n=11, p=0.19 vs. '5 weeks') (Fig. 5A, bottom trace). These results suggest that, in the rat visual cortex, tonic inhibition is also regulated developmentally before phasic inhibition is.

DISCUSSION

The present study investigated the developmental changes in various aspects of $GABA_A$ receptor-mediated inhibition. Since the relative strength of inhibition compared with excitation is more important than the development itself [28], we investigated the development of phasic inhibition relative to the same amount of excitation in different age groups. We found a developmental increase in the inhibitory-to-excitatory ratio (I/E ratio), which confirmed our previous findings [11]. Inhibition increased during post-

natal development from 3 to 8 weeks and reached a plateau at 8 weeks of age. Thus, the development of inhibition in the rat visual cortex appears to be completed at around 8 weeks of age. In contrast, in a report from Morales et al. [9], the I/E ratio did not increase from 5 to 8 weeks. Since the I/E ratio was calculated at the maximum amplitude of inhibition achieved by electrical stimulation, the discrepancy might originate from differences in the experimental methods. Ocular dominance plasticity in the visual cortex was induced until 5 weeks of age when it was measured by unit recording [29], but it could be induced until about 7 weeks of age when measured by sweep visually evoked potential [30]. Functional maturation of the visual cortex can be assessed by visual acuity and receptive field properties [29], and inhibition has proven to be important in these processes [31]. Since these features develop until postnatal day 45 [29], the present results regarding the development of inhibition reflects well the time course of the functional maturation of the rat visual cortex.

$GABA_A$ receptors are pentamers of nineteen subunits, and manifest different kinetics depending on the composition of subunits [18,32]. Since the expression of subunits changes during development, the kinetics of $GABA_A$ receptor-mediated synaptic events also changes with development [12,33]. Spontaneous IPSC (sIPSC) was reported to show faster kinetics during postnatal development in the rat visual cortex [12]. In contrast, in the present study, phasic inhibition showed slower kinetics with development. Various types of inhibitory interneurons make inhibitory synapses with different kinetics [34-36]. Furthermore, slow sIPSC was also reported in the rat visual cortex, although it was very rare compared to fast sIPSC [37]. Because spontaneous activities differ among various types of inhibitory interneurons [38], if these slow IPSC-evoking interneurons generate spontaneous firing more rarely than fast IPSC-evoking interneurons, sIPSC recording might have failed to detect the developmental changes in the kinetics of evoked IPSC in the present study. Thus, the discrepancy with respect to the kinetics might result from the properties of inhibitory interneurons that mediate slow GABAergic responses. Anatomical maturation of inhibitory circuits, such as the number of interneurons, boutons and synaptic contacts, is known to be completed at around 5 weeks of age in rodents [39-43]. Increases in the I/E ratio and tonic inhibition between 3 and 5 weeks might, therefore, largely depend on the developmental increase in inhibitory synaptic transmission itself. By contrast, the increased I/E ratio during 5 to 8 weeks, along with the changes in kinetics, might be explained by changes in the composition of $GABA_A$ receptor subunits. Although the detailed differences among various subtypes of inhibitory interneurons having different kinetics, and the changes in the composition of receptor subunits, remain to be elucidated, our results suggest that the developmental decrease in the induction of NMDAR-dependent synaptic plasticity might result from the increased suppression of NMDAR by the developmental increase in both I/E ratio and slowed GABA responses, since slower inhibition more efficiently suppresses NMDAR [13].

The changes in the kinetics of inhibition could also result from the changes in the passive membrane properties. Since the membrane time constant became shorter with development, however, the changes in inhibitory kinetics cannot be a mere reflection of the passive properties. Moreover, the developmental change in the kinetics of excitation was opposite to that of inhibition. In contrast to the inhibitory kinetics, only the decay time constant of EPSP changed. A portion of this change might reflect the changes in the subunit composition of NMDAR [44-46].

Tonic inhibition is also an important $GABA_A$ receptor-mediated inhibition that modulates neuronal gain [47] and offset [48], and thus controls neuronal output patterns and synaptic integration [49]. These modulations would be important in visual feature processing, but the properties of tonic inhibition in the visual cortex have not yet been reported. In the present study, tonic inhibition was not detected in 3-week-old rats, but increased significantly in 5-week-old rats. Although the functional implication of the developmental increase in tonic inhibition is still unclear, it might be involved in the functional development of the visual cortex and in the developmental decline of synaptic plasticity. Future study of the roles of tonic inhibition in the visual cortex would enrich our understanding of the function and postnatal development of the visual cortical circuits.

In the present study, we discovered many developmental changes in $GABA_A$ receptor-mediated inhibition in the rat visual cortex. The influence of inhibition increases with development, both in strength and duration. The time course of these changes matches well the developmental decline of synaptic plasticity, as well as the development of signal processing capability in the visual cortex. Thus, our results concerning the developmental changes of phasic and tonic inhibition provide valuable information to assist in the understanding of the importance of inhibition in the functional development of the visual cortex.

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