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Postnatal development of NMDA receptor-mediated synaptic transmission in cat visual cortex

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Postnatal development of *N*-methyl-D-aspartate (NMDA) receptors in cat visual cortex (area 17) was studied by intracellular recording from cortical cells in slice preparations obtained from kittens aged 0–20 weeks after birth and adult cats. Cells were sampled from layer IV and the lower half of layer II–III, where it is known that most cells receive direct inputs from lateral geniculate nucleus. Excitatory postsynaptic potentials (EPSPs) evoked in cortical cells by white matter stimulation were mediated by both non-NMDA and NMDA receptors, because bath application of a non-NMDA receptor antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX), left slow depolarizing responses which were abolished by an NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid. The contribution of NMDA receptors to the synaptic transmission was assessed by the ratio of the initial rising slope of EPSPs in the presence of DNQX to that in control solution. The NMDA receptor-mediated component of monosynaptic EPSPs was small at 0–2 weeks after birth, steeply increased to the peak value at 5–6 weeks and then declined gradually to the almost initial value by 20 weeks. This time-course agrees with the developmental changes in susceptibility of ocular dominance preference of visual cortical cells to monocular deprivation, suggesting that NMDA receptors play a role in plastic changes in geniculocortical synapses.

INTRODUCTION

Neural responses in the visual cortex are modified by visual experience during the early postnatal period[3,14,40]. Activity-dependent, long-term modification of synaptic transmission has been thought to underlie this modification[12]. Since the activation of *N*-methyl-D-aspartate (NMDA) receptors was found to initiate long-term potentiation (LTP) of synaptic transmission in hippocampus[5], a number of tests have been made to check whether the NMDA receptor is also involved in the modification of visual responses.

This possibility has been supported by an experiment in which the application of NMDA receptor antagonists into visual cortex prevents the modification of ocular dominance preference of cortical cells after monocular deprivation[2,20,32]. It has been further supported by the observation that visual responses are more sensitive to NMDA receptor antagonists during the sensitive period, when visual responses are modified by visual experience, than during the later period, when such modification rarely takes place[8,38]. Therefore, it is likely that during the sensitive period excitatory synaptic transmission in the visual cortex has components mediated by NMDA receptors which are sufficient to induce plastic changes of visual responses.

To further test the involvement of NMDA receptors in visual cortical plasticity, we investigated the postnatal changes in NMDA receptor-mediated components of excitatory synaptic transmission by intracellular recording in cat visual cortex slices. Analysis was made in layer IV and the lower half of layer II–III, where most cells receive direct inputs from lateral geniculate nucleus (LGN)[37]. The results suggest that the contribution of the NMDA receptors to geniculocortical synaptic transmission changes in parallel with the susceptibility of ocular dominance preference of visual cortical cells to monocular deprivation.

MATERIALS AND METHODS

Preparation

Thirty eight kittens at ages of 0–2 (6–14 days, $n = 10$), 5–6 (35–44 days, $n = 10$), 7–10 (49–70 days, $n = 7$) and 16–20 (118–144 days, $n = 11$) weeks and 7 adult cats (older than 1 year) were used. Coronal

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slices (thickness 0.5 mm) were prepared from visual cortex (area 17) under deep anesthesia induced by a mixture of urethane (1 g/kg) and α-chloralose (100 mg/kg) as described previously[22,23]. Slices were perfused with Krebs–Ringer solution (in mM: NaCl 124, KCl 5, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, glucose 10) saturated with a mixture of gas with 95% O_2 and 5% CO_2 at 34°C. The drugs used in the study were 6,7-dinitroquinoxaline-2,3-dione (DNQX: Tocris neuramin) and D,L-2-amino-5-phosphonovaleric acid (APV: Sigma).

Stimulation and recording

Two pairs of bipolar stimulating electrodes made of enameled tungsten wires (interpolar distance 0.3 mm, diameter 0.1 mm) were placed in white matter as described previously[21]. One pair was placed near the border between layer VI and white matter. The other pair was placed in white matter, separated 0.5–0.7 mm from the former electrodes. Constant-current pulses (intensity 0.03–5 mA, duration 100 μs) were applied to the stimulating electrodes at 0.1 Hz.

A glass microelectrode filled with 2 M potassium methylsulfate (electrical resistance 80–160 MΩ) was used for intracellular recording. The electrode was mounted on a three-dimensional oil-driven micromanipulator (Narishige MO-103R), one axis of which was aligned with the cortical columnar axis. Cortical cells were sampled along the columnar axis. A conventional bridge circuit was used to record the membrane potential during current injection (amplitude 0.05–1 nA, duration 200–400 ms) through the recording microelectrode.

Identification of monosynaptic connections

The identification of monosynaptic excitatory postsynaptic potentials (EPSPs) was based on EPSP latency and the central delay, which is the time spent in synaptic transmission after afferent impulses arrived at afferent terminals. EPSPs were considered to be monosynaptic when their latency was almost constant (difference < 0.3 ms) to a wide range of stimulus intensity and the central delay was smaller than 1 ms[23]. When the EPSP latency monotonically became shorter (by more than 1 ms) with the increase of stimulus intensity, the EPSP was considered to be polysynaptic.

The central delay was determined by subtracting the conduction time of the afferent impulses from the EPSP latency. The conduction time was estimated from the conduction velocity of afferent impulses. To calculate the conduction velocity, the distance between the two stimulating electrodes was divided by the difference in latencies of EPSPs evoked by the two electrodes.

Histology

After recording from a cortical cell the recording microelectrode was replaced with another electrode containing 2% Pontamine sky blue, and the recording site for the cell was marked by electrophoretic dye injection according to the micromanipulator readings. The location of the marked sites was determined on histological sections stained with Cresyl violet.

RESULTS

The intracellular study of postsynaptic potentials (PSPs) was conducted in cortical cells which exhibited a resting membrane potential deeper than −50 mV. Although we recorded from cortical cells which responded with either monosynaptic or polysynaptic EPSPs to stimulation of white matter, we present here the analysis of 62 cells which responded with monosynaptic EPSPs. The laminar location of these cells was confirmed to be in layer IV or the lower half of layer II–III by later histological examination. This selection procedure resulted in a relatively low cell sampling rate from each animal.

TABLE I

Sampled cells and passive membrane properties

Layer III indicates the lower half of the layer II–III. The resting membrane potential and input resistance are represented by means ± S.D. Significant difference ($P < 0.05$, t-test) was detected only in input resistance between 0–2 and 7–10 weeks.

Age (weeks)	*Number of cells*			*Resting membrane potential (mV)*	*Input resistance (MΩ)*
	Layer III	*Layer IV*	*Total*		
0– 2	3	8	11	58 ± 7	60 ± 20
5– 6	4	8	12	57 ± 5	49 ± 23
7–10	5	7	12	58 ± 6	42 ± 23
16–20	5	9	14	59 ± 6	43 ± 23
Adult	6	7	13	59 ± 7	44 ± 19

The resting membrane potential of these cells (about −60 mV) was nearly comparable to those obtained from cat visual cortical cells in our previous studies[21–23]. The slightly small value of the resting membrane potential may be partly due to the rather high concentration of K^+ in the perfusing solution used in our studies. While the input resistance was slightly larger at 0–2 weeks than at later developmental stages, the resting membrane potential showed no statistically significant ($P > 0.2$, t-test) differences between different age groups (Table I). This precludes the possibility that the observed differences in effectiveness of NMDA receptor-mediated components in excitatory synaptic transmission were merely reflecting the voltage dependence of NMDA receptor channels[27,30].

Effect of non-NMDA and NMDA receptor antagonists on PSPs

Fig. 1 illustrates the effect of non-NMDA and NMDA receptor antagonists on PSPs in a cell sampled from a 7-week-old kitten. In control solution, this cell showed typical PSPs to stimulation of white matter. Weak stimulation evoked an EPSP. Stronger stimulation evoked a larger EPSP followed by an inhibitory postsynaptic potential (IPSP) and an orthodromic action potential on the rising phase of the EPSP. In accordance with the observation that even a small amplitude EPSP has a slow component sensitive to the NMDA receptor antagonist APV[39] at the resting membrane potential used in our intracellular recording[23], bath application of a non-NMDA receptor antagonist, DNQX (40 μM)[15], left a slowly rising depolarizing response. This depolarization became larger with increasing stimulus intensity and was large enough to elicit an action potential at strong stimulus intensities.

These depolarizing responses were almost blocked by the addition of 100 μM APV, which is known to block NMDA receptors without affecting the non-NMDA receptor-mediated responses in cat visual cortex at this concentration[34]. Therefore, the excitatory synaptic transmission in this cell is thought to be mediated by both non-NMDA and NMDA receptors, as reported for cortical cells sampled from rat and cat visual cortex slices[1,19,23,25,34].

Similar results were obtained in 62 cells which were tested for the effect of DNQX, and in the additional 28 out of the 62 cells which were tested for the effect of DNQX and APV. There was no significant ($P > 0.5$, t-test) difference in resting membrane potential or input resistance before (59 ± 7 mV, 43 ± 17 MΩ) and after application of DNQX and APV (60 ± 8 mV, 43 ± 16 MΩ).

Voltage dependence of PSPs in the presence of DNQX

The slow depolarizing responses recorded in the presence of 40 μM DNQX demonstrated the voltage-dependent characteristics of NMDA receptor-mediated responses. When the membrane potential was changed by current injection through the recording electrode, the amplitude of the response decreased with membrane hyperpolarization (Fig. 2A,B). This voltage dependence was observed in all cells tested ($n = 38$), indicating that the depolarizing responses recorded in the presence of DNQX were mediated by NMDA receptors on the recorded cells.

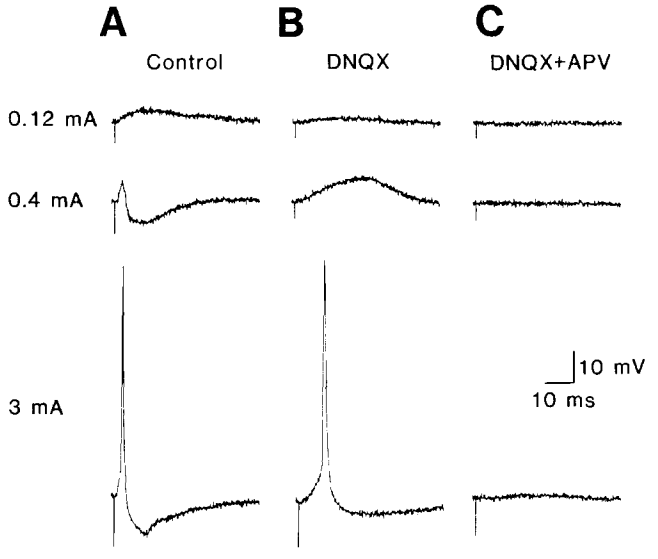

Fig. 1. Effects of non-NMDA and NMDA receptor antagonists on PSPs. A: single trace of postsynaptic potentials evoked by white matter stimulation in a layer IV cell sampled from a 7-week-old kitten. The intensity of stimulation was 0.12 (top), 0.4 (middle), and 3 mA (bottom). B and C: similar to A but 30 min after bath application of 40 μM DNQX and 15 min after application of 100 μM APV in addition to DNQX. Time and voltage calibrations are common to all traces.

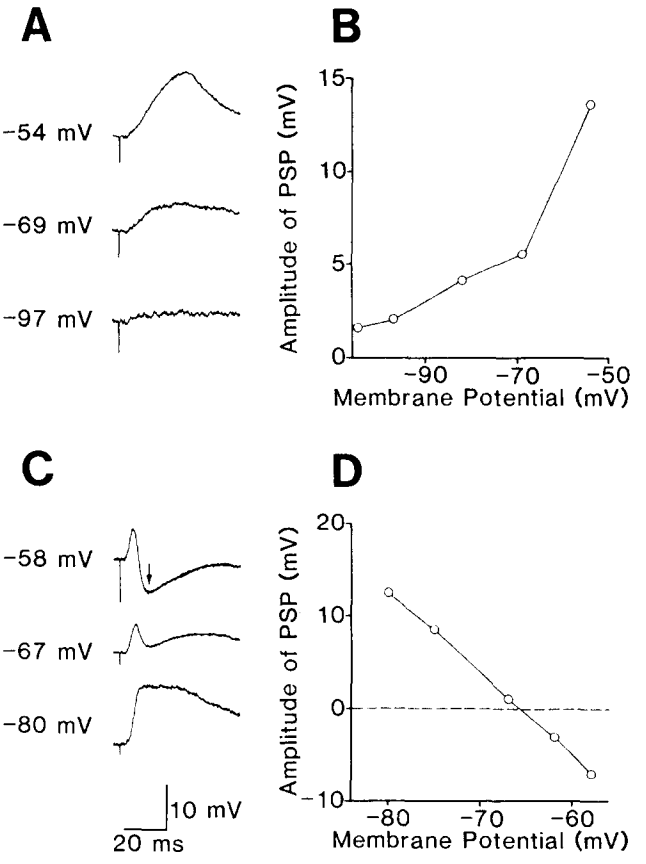

Fig. 2. Voltage dependence of PSPs under blockade of non-NMDA receptors. A: average traces ($n = 5$) of EPSPs evoked by white matter stimulation in a layer IV cell while the membrane potential was changed to the value indicated to the left of each trace. Age, 5 weeks old. B: amplitude of EPSP (ordinate) plotted as a function of membrane potential (abscissa). The data were obtained from the cell shown in A. C: similar to A but EPSPs followed by IPSPs which were recorded from another layer IV cell (5 weeks old). D: amplitude of IPSP (ordinate), which was measured at the time indicated by an arrow in C, plotted as a function of membrane potential (abscissa). Time and voltage calibrations are common to all traces.

White matter stimulation at strong intensities often produced hyperpolarizing responses preceded by the NMDA receptor-mediated EPSPs (Fig. 2C,D). These hyperpolarizing responses reversed to depolarizing responses when the membrane potential was hyperpolarized below -58 to -69 mV, indicating that they were IPSPs. Since these IPSPs were abolished by adding 100 μM APV (not shown), we believe that NMDA receptor-mediated EPSPs in inhibitory cells presynaptic to the recorded cell were strong enough to evoke an action potential.

Contribution of NMDA receptor-mediated component to the EPSP

Application of DNQX did not change the onset of monosynaptic EPSPs (< 0.4 ms), while it abolished or delayed the onset of polysynaptic EPSPs (Fig. 3). This assures that the identification of mono- and polysynaptic EPSPs is reliable and that the monosynaptic EPSPs

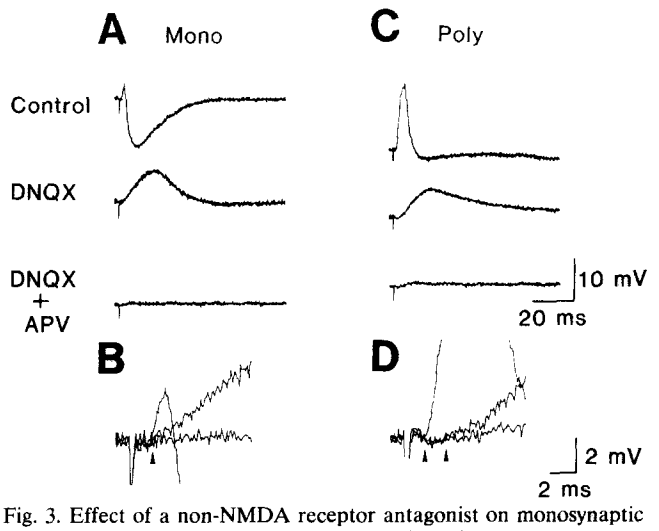

Fig. 3. Effect of a non-NMDA receptor antagonist on monosynaptic and polysynaptic EPSPs. A: average traces ($n = 5$) of PSPs evoked by white matter stimulation in a layer IV cell (8 weeks old), which responded with monosynaptic EPSPs, before (top), after application of 40 μM DNQX (middle), and after application of 40 μM DNQX and 100 μM APV (bottom). B: superimposed traces in A at expanded time and voltage scales. The arrow head indicates the onset of EPSP. C and D: similar to A and B but polysynaptic EPSPs recorded from a layer II–III cell. Time and voltage calibrations are common to A and C, and B and D, respectively.

themselves include NMDA receptor-mediated components.

Fig. 4 shows representative examples of the NMDA receptor-mediated component of monosynaptic EPSPs at each developmental stage. White matter stimulation was applied at a stimulus intensity which was subthreshold for evoking orthodromic action potentials in control solution. At all developmental stages the NMDA receptor-mediated component, which was usually truncated by IPSPs, was unmasked after the application of DNQX, as shown in the top and middle traces of Fig. 4A–D. The NMDA component was largest at 5 weeks, although it was present at all developmental stages.

The NMDA receptor-mediated component was estimated by the ratio of the initial rising slope of EPSPs in the presence of DNQX to that in control solution. This method of estimation is more reliable than measuring the amplitude or area of the PSPs since the later phase of PSPs may contain polysynaptic components as well as responses mediated by voltage-gated channels opened by EPSPs. Furthermore, the late phase of the

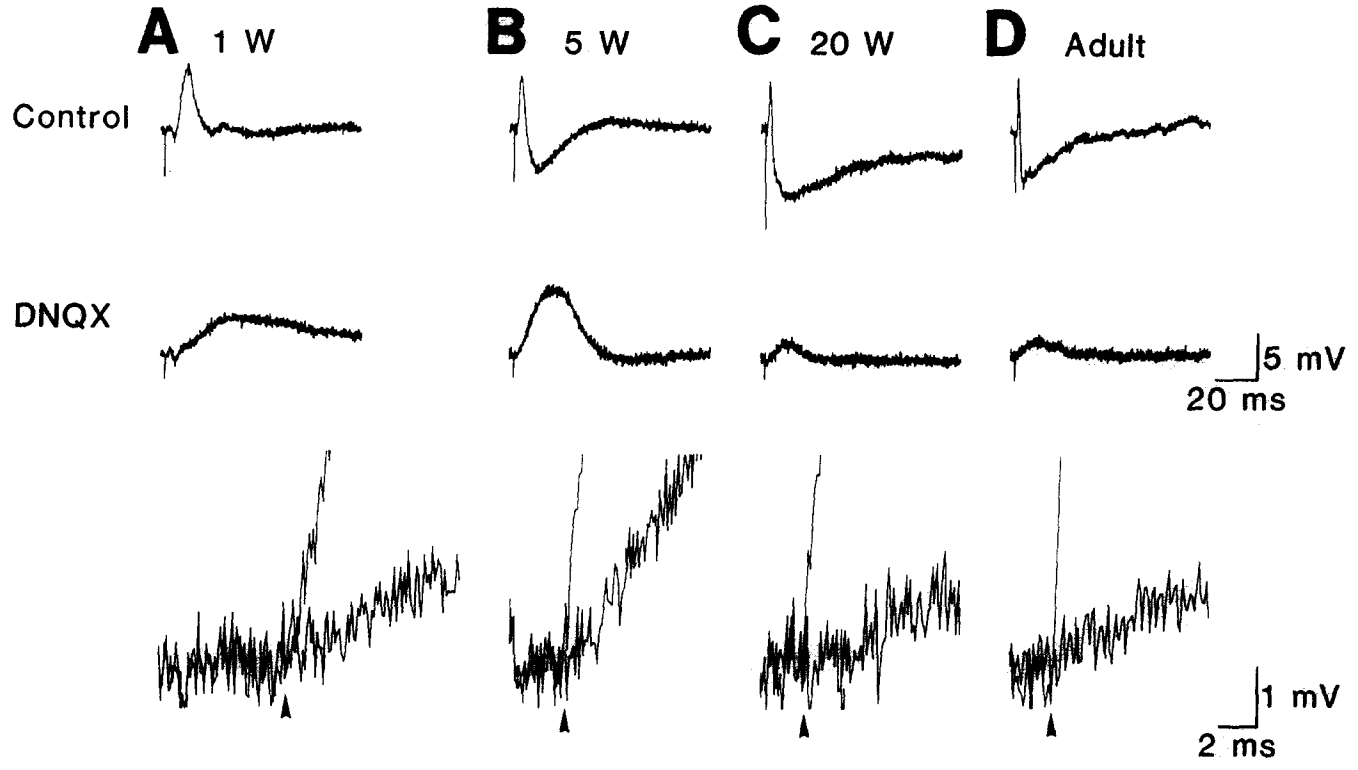

Fig. 4. The representative example of NMDA receptor-mediated EPSPs at each developmental stage. A: average traces ($n = 5$) of PSPs evoked by white matter stimulation in a layer IV cell sampled from a 1-week-old kitten before (top) and 30 min after application of 40 μM DNQX (middle). Bottom traces represent the superimposed responses before and after application of DNQX at expanded time and voltage scales. To make clear the onset of EPSPs (arrow head) extracellular responses recorded after withdrawal of the recording electrode were subtracted from the intracellular responses. B–D: similar to A but layer IV cells sampled from 5-and 20-week-old kittens, and an adult cat, respectively. Time and voltage calibrations of 20 ms and 5 mV are common to top and middle traces, and those of 2 ms and 1 mV to bottom traces, respectively.

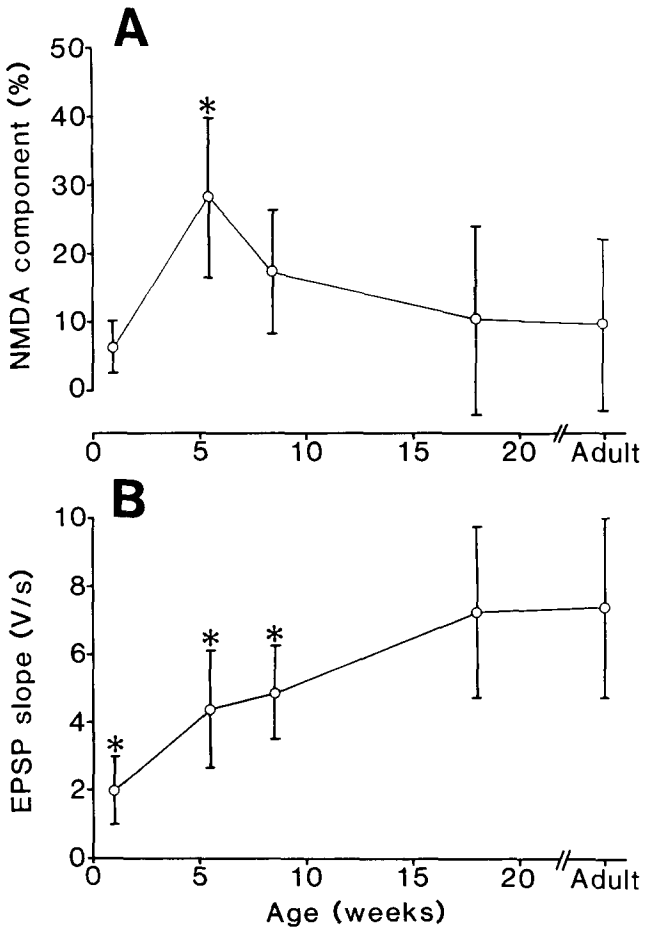

Fig. 5. Contribution of the NMDA receptor-mediated component to EPSPs. A: percentage (mean ± S.D.) of the EPSP slope in the presence of 40 μM DNQX to that in control solution (ordinate) plotted against the age (abscissa). Asterisks indicate that the value is significantly ($P < 0.05$, t-test) different from the adult value. B: EPSP slopes in control solution, which were used in the estimation of the NMDA component in A, plotted against the age.

NMDA receptor-mediated component may be more dependent on the size of EPSPs than the initial phase because of the voltage dependence of NMDA receptor channels[27,30].

The rising slope estimation was made for EPSPs with about 70% amplitude of threshold depolarization for evoking orthodromic action potentials in control solution. There was no significant ($P > 0.3$, t-test) difference in the threshold potentials between different age groups (12 ± 3, 12 ± 2, 12 ± 4 and 12 ± 4 mV for 0–2, 5–6, 7–10 and 16–20 weeks and 11 ± 5 mV for adults). Since there was no significant ($P > 0.1$, t-test) difference in the ratio between cells sampled from layer IV and the lower half of layer II–III at each age group, the postnatal changes in both layers are presented together in Fig. 5A. The percentage of the NMDA receptor-mediated component was smallest at 0–2 weeks, reached a peak value at 5–6 weeks, and then decreased to a value slightly larger than the initial value by 20 weeks.

Although the ratio of the initial rising slope of EPSPs in the presence of DNQX to that in control solution was relatively insensitive to the size of EPSPs, it is still possible that its postnatal change was due to a difference in the magnitude of the EPSP slope used to estimate the ratio. To test this possibility, we compared the rising slope of EPSPs recorded in control solution between different age groups (Fig. 5B). The control slope increased monotonically with age. It was smallest at 0–2 weeks, increased to more than twice the initial value at 5–6 weeks and reached the adult level by 16–20 weeks. This indicates that the contribution of the NMDA receptor-mediated component indeed decreased after 5–6 weeks, since a larger control EPSP slope could have resulted in a larger value for the contribution of the NMDA component.

However, it is still possible that the increase in the NMDA receptor-mediated component from 0–2 weeks to 5–6 weeks could be due to the increase in the control EPSP slope. Therefore, we compared the contribution of NMDA receptor-mediated component at 0–2 weeks with that estimated by using weak stimulation at 5–6 weeks. The weak stimulation at 5–6 weeks evoked control EPSPs with rising slopes (1.9 ± 0.6 V/s, $n = 8$) comparable to those at 0–2 weeks (Fig. 5B). The contribution of the NMDA receptor-mediated component estimated from the smaller EPSPs at 5–6 weeks ($22 \pm 7\%$) was still significantly ($P < 0.001$, t-test) larger than that at 0–2 weeks, indicating that the increase from 0–2 to 5–6 weeks was not due to a difference in the rising slope of EPSPs in control solution between the two age groups.

Cells in which white matter stimulation did or did not evoke antidromic action potentials developed differently. For the antidromically activated cell group, the contribution of the NMDA receptor-mediated component was estimated only from cells which responded with antidromic action potentials at a stimulus intensity higher than that evoking orthodromic action potentials. The NMDA receptor-mediated component decayed earlier in the antidromically activated cells (triangles in Fig. 6) than in the non-antidromically activated cells (circles), although the difference was not statistically significant ($P > 0.05$, t-test). It is possible that the stimulating electrode failed to activate axons of efferent cells, because the axons of efferent cells might have been cut in making the slice preparations or because they might have run far from the stimulating electrode. Therefore, the non-antidromically activated cells may include some efferent cells and consequently, the significance of a difference between the

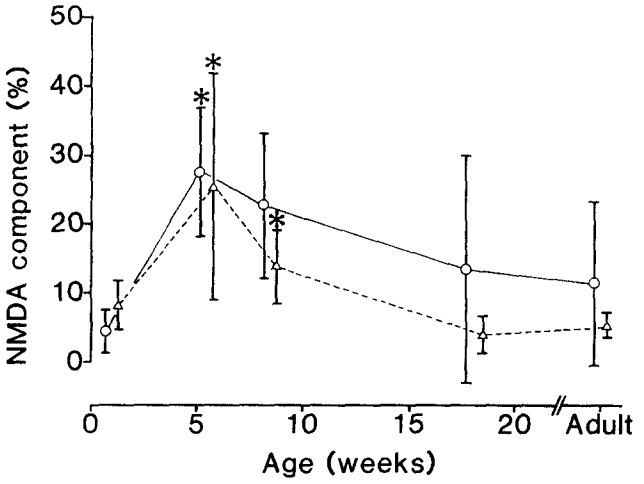

Fig. 6. Contribution of the NMDA receptor-mediated component to EPSPs for antidromically and non-antidromically activated cells. The contribution of NMDA receptor-mediated component was plotted against age for antidromically activated (△) and non-antidromically (○) activated cells. Asterisks indicate that the value is significantly ($P < 0.05$, t-test) different from the adult value. Number of antidromically activated cells are 5, 4, 7 and 6 for 0–2, 5–6, 7–10 and 16–20 weeks and 5 for adults. Number of non-antidromically activated cells are 6, 8, 5 and 8 for 0–2, 5–6, 7–10 and 16–20 weeks and 8 for adults.

two groups of cells may become smaller. In considering this possibility it is likely that efferent cells lose the NMDA receptor-mediated component earlier than non-efferent cells.

Time-course of NMDA receptor-mediated EPSPs

In addition to the changes in the contribution of NMDA receptors to the synaptic transmission, the time-course of NMDA receptor-mediated EPSPs also changed with age. The time-course was examined in the presence of DNQX using white matter stimulation that evoked small amplitude (0.8–4 mV) EPSPs followed by no IPSPs. The rise time was largest at 0–2 weeks and decreased to the adult value by 7–10 weeks (triangles in Fig. 7A). A similar change occurred in half width of the EPSPs (circles in Fig. 7A).

This developmental change is very similar to that in the membrane time constant of visual cortical cells[21], suggesting that the change in the time-course of the NMDA receptor-mediated EPSP merely reflects the change in time constant. This possibility was supported by the observation that the rise time and half width normalized by the time constant showed no significant ($P > 0.1$, t-test) difference between age groups (Fig. 7B).

Effectiveness of NMDA receptor-mediated EPSPs

The effectiveness of NMDA-receptor mediated EPSPs was further assessed by the ability to evoke orthodromic action potentials in the presence of DNQX. Orthodromic action potentials were evoked in most of cells at 5–10 weeks while only a few cells exhibited

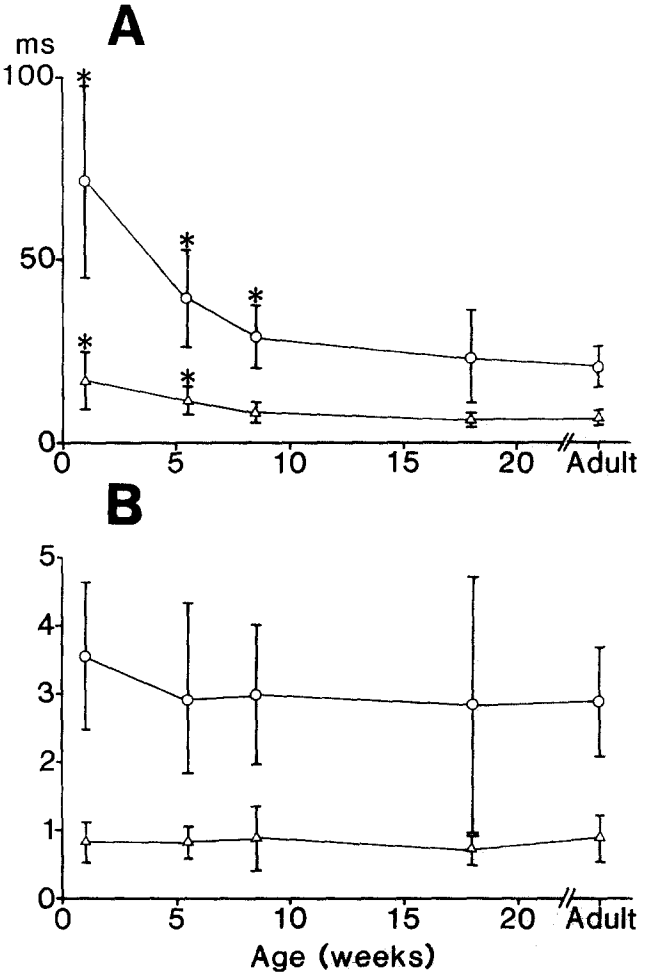

Fig. 7. Time-course of NMDA receptor-mediated EPSP. A: 10–90% rise time (△) and half width of EPSP recorded in the presence of 40 μM DNQX (○) plotted against age. B: similar to A but 10–90% rise time and half width divided by membrane time constant. Asterisks indicate that the value is significantly ($P < 0.05$, t-test) different from the adult value.

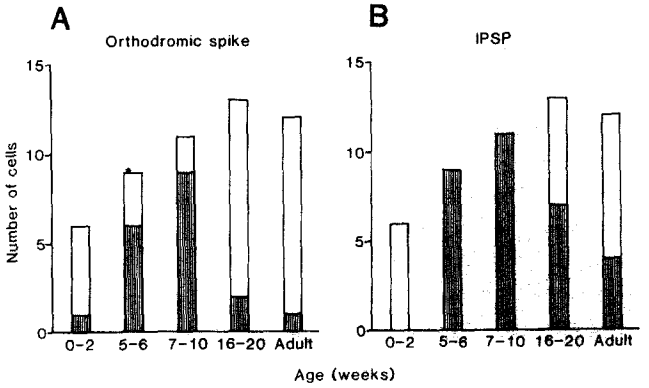

Fig. 8. Effectiveness of NMDA receptor-mediated EPSPs. A: number of cells which responded with (shaded columns) and without orthodromic action potentials (open columns) to white matter stimulation in the presence of 40 μM DNQX. B: number of cells which responded with (shaded columns) and without IPSPs (open columns) to white matter stimulation in the presence of 40 μM DNQX. Cells responded with antidromic action potentials to weak stimulation were omitted from analysis, because the antidromic action potentials prevented us from identifying the presence of IPSPs and determining whether the EPSP was large enough to evoke orthodromic action potentials.

spikes at earlier or later developmental stages (Fig. 8A).

The effectiveness of NMDA receptor-mediated EPSPs in inhibitory neurons presynaptic to the recorded cells was assessed by the presence or absence of the IPSP in the cells recorded in the presence of DNQX. The proportion of cells which responded with an IPSP changed with age in a way similar to that of cells which responded with orthodromic action potentials (Fig. 8B). However, a substantial number of cells showed IPSPs even in adults, whereas orthodromic action potentials were rarely evoked in adults.

DISCUSSION

Neural connections with the NMDA receptor-mediated component

The present study demonstrates a transient increase of the NMDA receptor-mediated component during postnatal development. This developmental change occurred similarly in cells recorded from layer IV and the lower half of layer II–III. Although these cells are known to receive direct geniculate inputs[37], the sources of monosynaptic inputs are uncertain because many other input fibers are also known to innervate cortical cells, especially those in layer II–III[9,26,37]. In spite of the input diversity it is likely that the transient increase of the NMDA receptor-mediated component in layer IV cells may mainly reflect those in geniculocortical synaptic transmission, because the main afferents to layer IV cells originate from LGN cells[24,33]. However, since it is known that layer VI cells which project to LGN send axon collaterals to layer IV[9,26], this connection might have contributed to monosynaptic EPSPs. However, these axons have a slower conduction velocity than the geniculate axons, so it is likely that the initial part of the EPSPs evoked by white matter stimulation in layer IV cells is mediated mostly by geniculocortical synapses[7]. Since we measured the initial slope to assess the contribution of the NMDA receptors to excitatory synaptic transmission, it is also likely that our estimation mainly reflects geniculocortical synaptic transmission.

All tested cells responded with an IPSP after the application of DNQX at 5–10 weeks. Therefore, synaptic excitation of inhibitory cells has a component mediated by NMDA receptors that is strong enough to elicit action potentials at these ages. Even in older animals, when most monosynaptic EPSPs have lost the capability to elicit action potentials in the condition where non-NMDA receptors were blocked by DNQX, about one-third of tested cells responded with an IPSP, suggesting that inhibitory cells have a large NMDA receptor-mediated component even in the later developmental stages. This is not contradictory to the finding that non-efferent cells have a larger NMDA receptor-mediated component than efferent cells in the later developmental stage, since inhibitory cells, if we recorded from them, are thought to be included in the population of non-efferent cells. Another possible explanation for the common occurrence of IPSPs is that inhibitory cells elicit action potentials even to weak excitatory inputs. We think more direct experiments are necessary to test these possibilities.

Comparison with other receptor-mediated synaptic transmission

The efficiency of non-NMDA and NMDA receptor-mediated synaptic transmissions is likely to develop with different time-courses. We have assessed the efficiency of excitatory synaptic transmission as a whole by the rising slope of EPSPs evoked in cat visual cortical cells by supramaximal stimulation of white matter[21]. The EPSP efficiency increased steeply from its smallest value at 1 week after birth to about twice this value by 5 weeks and then continued to increase slowly to the adult level. The present study demonstrates that the NMDA receptor-mediated component increases from about 5% to about 30% during the first 5 weeks, suggesting that the efficiency of EPSPs mediated by both non-NMDA and NMDA receptors increased during this period. Thereafter the percentage of the NMDA receptor-mediated component decreased substantially while the EPSP efficiency slightly increased as a whole[21], suggesting that during the later developmental period the efficiency of the non-NMDA receptor-mediated component increased slightly while the efficiency in the NMDA receptor-mediated component decreased.

The NMDA receptor-mediated component also developed differently from the inhibitory synaptic transmission in cat visual cortex. The efficiency of IPSP increased during the initial 5 weeks while there was no significant changes thereafter[21]. It has been reported that a delayed maturation of the inhibitory system manifests transiently polysynaptic NMDA receptor-mediated activity in developing rat neocortex[25]. The same phenomenon could be observed in the developing cat visual cortex. However, we found a pronounced NMDA receptor-mediated component in monosynaptic EPSPs in a restricted period of development, indicating that the change occurred at least partly in the NMDA receptor-mediated component itself. In addition, it is unlikely that the postnatal change in the NMDA receptor-mediated component merely reflects changes in the passive membrane properties of visual

cortical cells because they develop with different time-courses[21].

Comparison with in vivo and binding studies

The present study supports the findings that visual responses are more sensitive to NMDA receptor antagonists in immature than in mature animals[8,38]. Visual responses in layer IV cells are very sensitive to APV in young kittens and lost their sensitivity with age. By contrast, layer II–III cells at all ages are as sensitive to APV as layer IV cells of young kittens. From these observations it has been suggested that NMDA receptors are present in geniculocortical synapses during the sensitive period and are lost with age, while they are present in intracortical synapses of layer II–III at all ages. The present study supports this supposed postnatal change of NMDA receptors in geniculocortical synapses.

Although APV sensitivity of visual responses in the initial phase soon after birth has not been reported, the density of APV-sensitive [^{3}H]glutamate binding sites has been reported for the entire period of postnatal development[4]. The binding sites in layer IV steeply increased from 2 to 4 weeks after birth and gradually declined to almost the initial level in adults. This time-course agrees well with that of NMDA receptor-mediated components in monosynaptic EPSPs.

Altogether, these three different evaluations of NMDA receptors strongly suggest that the time-course of postnatal changes of NMDA receptors in geniculocortical synapses corresponds with the profile of the sensitive period of ocular dominance preference of visual cortical cells[16,31].

Functional role of NMDA receptors

The demonstration that the application of an NMDA receptor antagonist to visual cortex blocked the effect of monocular deprivation on ocular dominance preference of cortical cells suggested the involvement of the NMDA receptor in visual cortical plasticity[2,20]. This hypothesis was further supported by the postnatal changes of NMDA receptors in geniculocortical synapses, which parallel the time-course of susceptibility of ocular dominance preference to monocular deprivation[16,31]. Therefore, NMDA receptors may play a role in plastic changes in geniculocortical synaptic transmission.

However, visual responses of layer II–III cells are sensitive to APV even in animals older than 1 year[8,28], when modifiability of ocular dominance preference has been lost[6]. In addition, our present study demonstrated that some layer IV cells still retained a rather large NMDA receptor-mediated component in monosynaptic EPSPs even in adults. NMDA receptors in adults may function in normal signal transmission[28] rather than activate plastic changes. However, since even adult visual cortical cells reveal some capacity to modify their responses when a part of the retina has been damaged[10,13,17], NMDA receptors in adult visual cortex still can be involved in plasticity.

The NMDA receptor has the ability to detect correlated activity in pre- and postsynaptic cells[27,30]. Indeed, induction of LTP in most hippocampal pathways requires the activation of NMDA receptors[5,11,29]. This led us to test the involvement of NMDA receptors in LTP in the visual cortex. The experimental results are contradictory. Although LTP in rat visual cortex requires activation of NMDA receptors[1,19], LTP in kitten visual cortex requires activation of postsynaptic Ca^{2+} channels but not of NMDA receptors[22,23]. Therefore, it is likely that NMDA receptors play some roles in cat visual cortical plasticity but through processes other than LTP of excitatory synaptic transmission.

Rauschecker and Hahn have reported that ketamine/xylazine anaesthesia soon after monocular visual experience prevents ocular dominance shift of visual cortical cells[32]. Since ketamine is an NMDA receptor antagonist, they suggested that NMDA receptors might act to consolidate modified synaptic activities to structural changes after visual experience. Another possible role of NMDA receptors in plasticity is to modify inhibitory synaptic transmission, since it is reported that postsynaptic Ca^{2+} increase due to activation of NMDA receptors or Ca^{2+} channels can modify inhibitory synaptic transmission[18,36]. Selective responsiveness of visual cortical cells are known to be modified by visual experience during the sensitive period[3,14] and inhibition is hypothesized to contribute to this selectivity[35]. Further experiments will be necessary to elucidate the function of NMDA receptors in the visual cortical plasticity.

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