Dissociation of experience-dependent and -independent changes in excitatory synaptic transmission during development of barrel cortex

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A fundamental problem in the study of cortical development is the extent to which the formation and refinement of synaptic circuitry depends upon sensory experience. The barrel cortex is a useful model system to study experience-dependent cortical development because there is a simple mapping of individual whiskers to the corresponding barrel columns in the cortex. We investigated experience-dependent and -independent changes in glutamatergic synaptic transmission in the barrel cortex during the second postnatal week by comparing synaptic responses from whisker-intact mice at postnatal day (P) 7 and P14 with those from whisker-deprived mice at P14. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptor-mediated excitatory synaptic responses were recorded from layer 2/3 pyramidal cells in vitro during voltage-clamp in response to stimulation in layer 4. We observed that the ratio of AMPA- to NMDA-receptor-mediated current (A/N ratio) increased with developmental age. The development of the A/N ratio was unchanged by deprivation of the whisker input throughout the second postnatal week. In contrast, the NMDA-receptor current decay and sensitivity to the NMDA receptor 2B subunit-selective antagonist ifenprodil was affected strongly by such deprivation. These results demonstrate a concurrent dissociation between sensory experience-dependent and -independent changes of glutamatergic transmission in the barrel cortex during the second postnatal week. Furthermore, they suggest that the development of subunit composition of synaptic receptors is dependent on sensory experience, whereas maturation of the synaptic A/N ratio is independent of such experience. Thus, different components of synaptic development may be governed by different developmental rules.

Sensory cortical maps are dynamic representations whose developmental refinement depends on sensory experience (1, 2). Extensive plasticity of these cortical maps is seen during early postnatal development, but the capacity for remapping extends into adulthood in primary sensory cortices (3–5).

The barrel cortex is a useful model system for studying synaptic mechanisms underlying experience-dependent map plasticity because the topographical organization of the whiskers is preserved in the receptive fields of layer 4 cells enabling selective manipulation of sensory experience. Layer 4 neurons in a cortical column (which define one “barrel”) receive input primarily from a single whisker on the animal’s snout. These cortical barrels develop between postnatal day (P) 0 and P5 (6). Previous studies have demonstrated a critical developmental time period for synaptic plasticity at the thalamocortical input pathway to the barrels, with long-term potentiation induction being possible only during the first postnatal week (7). However, the formation and refinement of succeeding cortical circuitry ensues after this early postnatal stage. Layer 4 spiny neurons in barrels send the majority of their axonal projections to basal dendrites of layer 2/3 pyramidal cells in the same functional cortical column (8, 9). Between P8 and P12, large changes in spine and filopodia motility are observed in layer 2/3 cells (10). From the end of the second postnatal week, layer 2/3 pyramidal neurons have matured in their action potential (spike) properties, and layer 4 to 2/3 synapses exhibit N-methyl-D-aspartate (NMDA)-receptor-dependent spike timing-dependent plasticity (11, 12).

NMDA-receptor-mediated responses change during postnatal development. In the visual cortex, NMDA-receptor-mediated excitatory postsynaptic currents (EPSCs) become faster with advancing age (13). This change in kinetic properties is associated with a subunit switch in the NMDA-receptor complex, correlating with an increasing expression of the NMDA receptor (NR) 2A subunit postnatally (14, 15). Both the subunit switch and change in synaptic kinetics depend upon sensory experience in the visual cortex (16). In the barrel cortex, substantial experience-dependent plasticity occurs in layer 2/3 in the course of the second postnatal week (17–19). At a synaptic level, sensory deprivation during the second postnatal week affects both short-term synaptic dynamics (20) and induction of long-lasting synaptic plasticity (21).

To characterize the development of synaptic transmission in layer 4 to 2/3 synapses during the second postnatal week, we investigated the normal maturation of synaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- and NMDA-receptor-mediated glutamatergic transmission. We also investigated to what extent this development depends on sensory experience by depriving the animal of whisker input throughout the second postnatal week. We found that the development of the ratio of AMPA- to NMDA-receptor-mediated synaptic transmission (A/N ratio) progressed independently of sensory experience, whereas the properties of synaptic NMDA receptors depend on such experience during the second postnatal week.

Methods

Experiments were carried out with C57BL/6 mice ranging from P6–P33 in age (supplied by Harlan UK, Bicester, U.K.). All animal procedures were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and under the appropriate licenses according to U.K. Home Office regulations.

Sensory Deprivation. For sensory deprivation experiments, P6 mice were anaesthetized on ice and had all large whiskers on the left side of the snout plucked after topical application of EMLA cream (5% lidocaine, prilocaine; Astra Pharmaceuticals, Kings Langley, U.K.). Mice were returned to their litter and reared under normal conditions but were checked for whisker regrowth every 2–3 days until they were killed at P13–P15 or P26–P33.

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, N-methyl-D-aspartate; A/N ratio, ratio of synaptic AMPA- to NMDA-receptor-mediated current; EPSC, excitatory postsynaptic current; NR, NMDA receptor; Pn, postnatal day n; CPP, (RS)-3-(2-carboxypropyl)azetin-4-yl-propyl-1-phosphonic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; I–V, current-voltage.

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Mice exhibiting detectable whisker regrowth were anesthetized with a 1:1:20 mixture of Hypnorm (Janssen-Cilag, Bucks, U.K.), Hypnovel (Roche), and water in a dosage of 1.0 ml/kg i.p. before replucking commenced.

**Slice Preparation.** Thalamocortical slices (300–400 \( \mu \)m thick) were made from the right somatosensory cortex by using the method described by Agmon and Connors (22) after cervical dislocation. Slices were maintained at room temperature in a submerged-style holding chamber for at least 1 h, superfused with artificial cerebrospinal fluid containing 126 mM NaCl, 3 mM KCl, 1.25 mM NaH2PO4, 2 mM MgSO4, 2 mM CaCl2, 26 mM NaHCO3, and 10 mM glucose (pH 7.2–7.4), and bubbled with carbogen gas (95% O2, 5% CO2). All recordings were made with an AxoPatch-1D or a GeneClamp-500 amplifier (both from Axon Systems, Everett, WA) in barrel regions of layer 4 cortex. The stimulating electrode (S) was placed in layer 4, and the recording electrode (R) was placed in layer 2/3, and the stimulating electrode (S) was placed in layer 4. The recording electrode (R) was placed in layer 2/3, and the stimulating electrode (S) was placed in layer 4. (A) Example traces showing the average evoked synaptic current responses from a range of postsynaptic holding potentials from 0 to +40 mV in the presence of the AMPA-receptor antagonist CNQX (10 \( \mu \)M). Current amplitudes for the fast component (gray line, open circles) are plotted against respective holding potentials. (B) Example trace showing average evoked synaptic current responses from a range of postsynaptic holding potentials from 0 to +40 mV in the presence of the AMPA-receptor antagonist CNQX (10 \( \mu \)M). Current amplitudes for the slow component (black line, filled circles) are plotted against respective holding potentials. (C) Fast AMPA-receptor-mediated (black line, filled circles) and slow NMDA-receptor-mediated (gray line, open circles) peak currents were plotted as a function of membrane holding potential. (D) Example trace showing the average evoked synaptic current responses from a range of postsynaptic holding potentials from 0 to +40 mV in the presence of the NMDA-receptor antagonist CPP (20 \( \mu \)M). Current amplitudes for the fast component (black line, filled circles) are plotted against respective holding potentials. (E) Example trace showing average evoked synaptic current responses from a range of postsynaptic holding potentials from 0 to +40 mV in the presence of the AMPA-receptor antagonist CNQX (10 \( \mu \)M). Current amplitudes for the slow component (gray line, open circles) are plotted against respective holding potentials. (Vertical scale bars, 50 pA; horizontal scale bars, 100 ms.)

**Electrophysiological Protocol.** Whole-cell patch-clamp recordings were made from layer 2/3 pyramidal neurons under visual guidance by infrared differential interference contrast microscopy at room temperature. Patch pipettes (4–6 M\( \Omega \)) were pulled from standard wall borosilicate tubing and were filled with intracellular solution containing 140 mM CsCl, 0.2 mM EGTA, 10 mM Hepes, 2 mM ATP-Mg, 0.3 mM GTP, 5 mM QX-314, and 5 mg/ml biocytin. Voltage clamp recordings were made with an AxoPatch-1D or a GeneClamp-500 amplifier (both from Axon Instruments, Foster City, CA). No corrections were made for liquid junction potential or series resistance. Overall, the mean access resistance was 23 ± 2 M\( \Omega \), and the mean input resistance was 139 ± 10 M\( \Omega \). Cells were identified by their location and shape during recording, and, in addition, some cells were histologically processed for biocytin-labeling after recording to confirm their anatomical identity and position in layer 2/3. EPSCs were evoked by extracellular stimulation (50 \( \mu \)s in duration, 5–50 \( \mu \)A, 0.1 Hz) by using a monopolar stainless steel electrode (A-M Systems, Everett, WA) in barrel regions of layer 4 cortex. The minimum amount of current necessary to evoke a reliable monosynaptic response at a holding potential of −100 mV was applied.

**Data Acquisition and Analysis.** Data were acquired with an ITC-16 AD board (Instrutech, Mineola, NY) by using custom-made...
A/N Ratio Increases During the Second Week of Postnatal Development. To determine the developmental profile of synaptic glutamatergic currents, evoked EPSCs were recorded from slices prepared from normal, whisker-intact mice between ages P6 and P15. Amplitudes of the fast and slow components were measured, and I–V plots were constructed (Fig. 1B and C). The fast component showed a linear I–V relation at all ages (Fig. 1C), whereas the slow component showed a curvilinear relationship characteristic of NMDA-receptor-mediated currents (Fig. 1C).

To confirm that these were AMPA- and NMDA-receptor-mediated currents, respectively, CPP and CNQX were added to isolate the AMPA- and NMDA-receptor-mediated currents, respectively. By using 20 μM CPP, the pharmacologically isolated AMPA-receptor-mediated current and labeled group. To confirm that this increase occurred gradually over development, a separate set of experiments was carried out at P9 and P12 that revealed A/N ratios of 0.82 ± 0.07 (n = 7) and 1.22 ± 0.36 (n = 8), respectively. An ANOVA showed a significant effect of developmental age on A/N ratio, with post hoc t tests showing a significant increase in ratio at later ages relative to P6–P8 (Fig. 2C).
Development of A/N Ratio Is Independent of Sensory Experience. To examine whether this developmental increase in the A/N ratio depends on sensory experience, we measured the A/N ratio in P13–P15 littermate mice whose whiskers had been plucked from P6 (Fig. 2). In slices taken from these whisker-deprived mice, the A/N ratio was 1.23 ± 0.33 (n = 7), which was not significantly different from their whisker-intact littersmates of the same age (P13–P15, 1.43 ± 0.38; P > 0.05) but was significantly different from P6–P8 mice (0.51 ± 0.10; P < 0.05) (Fig. 2 B and C). By using the estimated peak NMDA currents at 20 ms (see Methods), the recalculated A/N ratio differed by <4% from that of their age-matched littersmates. Thus, after a 1-week period of sensory deprivation during early postnatal life, the increase in A/N ratio still occurred. We conclude that the developmental increase in A/N ratio can occur independently of sensory experience.

Sensory Whisker Experience Decreases the Ifenprodil-Sensitive Component of the Synaptic NMDA-Receptor-Mediated Current. To examine a possible change in the subunit composition of the NMDA receptor, the overall contribution of NR2B-containing NMDA receptors was estimated by using the NR2B subunit-selective antagonist, ifenprodil (Fig. 3A). During normal postnatal development, 3 μM ifenprodil reduced the NMDA-receptor-mediated current by 53% (C.I. 43–61%) at P6–P8 (control, n = 9; ifenprodil, n = 5) but only by 14% (C.I. 0–34%) at P13–P15 (control, n = 15; ifenprodil, n = 10) (Fig. 3B). This difference was statistically significant (bootstrap test, P < 0.01), indicating that the NR2B component of the synaptic NMDA-receptor-mediated current in layer 2/3 of the barrel cortex decreased substantially during the second postnatal week (Fig. 3). In contrast, in slices from P13–P15 mice whose whiskers were plucked from P6, the ifenprodil sensitivity did not decrease with age (Fig. 3B); ifenprodil still significantly reduced the NMDA-receptor-mediated current by 49% (C.I. 24–71%; control, n = 7; ifenprodil, n = 6), which is not significantly different from P6–P8 [bootstrap test; P > 0.05] but is significantly different from P13–P15 whisker-intact mice (bootstrap test; P < 0.05). These results suggest that the decrease in the NR2B component of the NMDA-receptor-mediated current during normal development over the second postnatal week is experience-dependent.

Sensory Whisker Experience Is Associated with Faster NMDA Current Decay. In whisker-intact animals, the decay time constant of the NMDA-receptor-mediated current became significantly faster with age in the course of the second postnatal week [from 139 ± 21 ms at P6–P8 (n = 11) to 89 ± 11 ms at P13–P15] (Fig. 3), with a significant linear correlation between NMDA decay time constants and postnatal age (P6–P15, R = −0.34, P < 0.05, n = 41). To test whether the NMDA-receptor kinetics are associated with the subunit composition of NMDA receptors, we estimated the decay time constant of the NMDA-receptor-mediated current in the presence and absence of ifenprodil. In both P6–P8 and P13–P15 whisker-deprived mice, ifenprodil significantly reduced the NMDA decay constant from 139 ± 21 ms (n = 11) to 71 ± 12 ms (n = 4) and 132 ± 13 ms (n = 7) to 73 ± 17 ms (n = 6), respectively (P < 0.05, t test for both comparisons) (Fig. 3C). In contrast, no such effect of ifenprodil was observed on the faster NMDA current decay recorded in slices from P13–P15 whisker-intact mice (control, 89 ± 11 ms, n = 15; ifenprodil, 84 ± 16 ms, n = 9). Thus, the effect of ifenprodil at different developmental ages was consistent with the hypothesis that NR2B-containing receptors are responsible for the slower phenotype.

Sensory Deprivation Does Not Permanently Alter NMDA-Receptor Subunit Composition. To investigate whether the whisker deprivation causes either a delay or a permanent change in the NR2B component of the NMDA-receptor-mediated current, another group of mice were whisker-deprived for 21 days. As with the 7-day deprivation, whisker plucking on one side of the snout began at P6 and was continued until the mice were killed between P26 and P33,
as described in Methods. Whisker deprivation did not alter the ifenprodil sensitivity of NMDA-receptor-mediated currents in P26–P33 mice. Thus, the estimated ifenprodil-sensitive current was only 26% in P26–P33 whisker-deprived mice (control, n = 8; ifenprodil, n = 6), which was not significantly different from either age-matched whisker-intact mice or P14 whisker-intact mice (P > 0.05, bootstrap test), suggesting that NR2B-containing receptors make only a minor contribution to the NMDA current in these animals.

This conclusion was strengthened by comparing the NMDA decay time constants. These constants were both faster than at younger ages, and no significant difference was detected in the NMDA decay time constants between control and ifenprodil conditions in either whisker-intact or -deprived mice. The NMDA decay time constants in the whisker-intact animals were 54 ± 10 ms (n = 7) in control and 45 ± 6 ms (n = 7) in ifenprodil-treated (P = 0.24, t test) mice. The NMDA decay time constants in the whisker-deprived animals were 51 ± 6 ms (n = 10) in control and 56 ± 12 ms (n = 8) in ifenprodil-treated (P = 0.35) mice. Thus, prolonged whisker deprivation appears to delay the NMDA receptor subunit change rather than permanently alter the subunit composition.

Discussion

These findings demonstrate a previously undescribed dissociation between the effects of sensory experience on the postnatal maturation of different, simultaneously measured components of evoked excitatory synaptic currents in layer 2/3 cells in barrel cortex. We report, first, that the A/N ratio increases with developmental age during the second postnatal week. This increase also occurs during sensory deprivation. Second, during the same developmental period and in the same synapses, we observed changes in NMDA-receptor-mediated responses, consistent with a reduction in NR2B-containing NMDA receptors. These changes, however, are dependent on sensory experience, because NMDA-receptor-mediated synaptic responses from mice with 1 week’s whisker deprivation were significantly different in both their kinetic properties and ifenprodil sensitivity from those recorded in age-matched, whisker-intact animals. Thirdly, this change was not permanent because after 21 days of whisker deprivation, no significant difference was observed in NMDA-receptor-mediated currents from their age-matched, whisker-intact littermates. Thus, we have shown a concurrent dissociation of experience-dependent and -independent changes in synaptic glutamatergic transmission in layer 2/3 pyramidal cells during early postnatal cortical development.

Sensory Experience-Dependent Maturation of A/N Ratio. Over the course of the second postnatal week, our experiments demonstrated an increase in the A/N ratio in both whisker-intact and -deprived mice. The A/N ratio is likely to be an important determinant of both short-term synaptic integration and long-term synaptic plasticity and appears to be conserved across areas in mature neocortex (28). These factors suggest that the development of the A/N ratio is likely to be tightly regulated. It is possible that the more complex dendritic geometry at P14 may result in imperfect space-clamp conditions and, thus, distort the waveform of EPSC components measured. However, this effect cannot explain the increase in A/N ratio that we observed with age; because it is the amplitude of the faster AMPA component that would be attenuated more strongly by the increase in dendritic filtering with age (8).

In the rat barrel cortex, short-term sensory deprivation impairs the developmental increase in A/N ratio by preventing the insertion of GluR1 subunits into the synapse (26). Although this “AMPAfication” mechanism occurs over a short-term course of sensory experience, we demonstrated that after a prolonged period (7 days) of sensory deprivation, the A/N ratio in deprived animals is not significantly different from that in whisker-intact mice. This finding suggests the possibility that an alternative experience-independent mechanism for increasing AMPA-receptor-mediated responses exists and is recruited during the prolonged absence of sensory stimulation. Our results do not give any information as to whether the A/N ratio increase is due to an increase in presynaptic glutamate release or postsynaptic receptor density. Indeed, in hippocampal cultures, changes in glutamate concentration were observed to be the main cause of variability in EPSC size, and after long-term potentiation, glutamate concentration increased at previously “silent” synapses (29, 30). Thus, the increase in the A/N ratio also might be explained by a developmental increase in glutamate available in the synaptic cleft.

Experience-Dependent Alteration in NMDA-Receptor-Mediated Synaptic Responses. By the end of the second postnatal week, NMDA-receptor-mediated responses showed significantly faster decay times and were significantly less sensitive to the NR2B-selective antagonist, ifenprodil, as compared with evoked synaptic responses recorded from 1-week-old animals. These findings are consistent with reports from both somatosensory and visual cortex, where NMDA-receptor-mediated EPSCs have faster decay and a decreased NR2B-containing component with increasing age (31, 32). Similar but earlier changes also are observed in thalamocortical synapses on layer 4 neurons in the barrel cortex, whereby NMDA-receptor-mediated responses become shorter in duration and the NR2A component increases with increasing postnatal age (7, 33, 34).

Previous experiments in layer 4 to 2/3 synapses of visual cortex have shown that the developmental switch in NMDA receptor subunits observed after eye opening is impaired if the rat is deprived of visual experience during this time, with a greater NR2B-sensitive component persisting in rodents reared in the dark (16). Here, we observed an experience-dependent change in the NR2B component of synaptic NMDA-receptor-mediated responses in layer 4 to 2/3 of barrel cortex despite no change in overall A/N ratio from the same synapses. From P7 and P14 whisker-deprived animals, more than half of the total NMDA-receptor-mediated response was blocked by ifenprodil, an NR2B subunit-selective antagonist, compared with a significantly lower proportion at P14 in whisker-intact animals.

In contrast to this experience-dependence of the properties of synaptic NMDA responses at the end of the second postnatal week, no such differences were observed at P28 after a longer period of sensory deprivation. Thus, the effect of sensory deprivation at this cortical synapse is not permanent but rather delays the alteration in receptor subunit composition. Although a critical period exists for layer 4 barrel formation and thalamocortical plasticity in the first postnatal week (7), our results suggest that there is a sensitive time window for changes in NMDA-receptor composition between layers 4 and 2/3 during the second postnatal week but that these experience-induced alterations can be modified later over time.

What might be the functional consequences of this change in NMDA receptor subunit composition? If spike timing-dependent plasticity is a synaptic mechanism contributing to cortical map formation and plasticity in layer 4 and 2/3 neurons, the kinetics of the NMDA-receptor-mediated responses at the synapses between them might play an important role in governing these changes. Although the developmental change in NMDA-receptor responses in the thalamocortical barrel pathway does not regulate the critical period for induction of long-term potentiation or barrel formation (33, 34), the persistence of synaptic and cortical map plasticity reported at later ages in layer 2/3 (4, 11, 35) might be influenced to a greater extent by changes in NMDA-receptor-mediated responses. Indeed, between layers 4 and 2/3 both induction of long-term
potentiation and an expansion of layer 2/3 after pairing-induced stimulation with the underlying barrel column occurs in an NMDA-receptor-dependent manner (11, 36). Moreover, at the synaptic level, developmental regulation in the signaling pathways utilized by synaptic plasticity mechanisms has been reported in hippocampal and thalamocortical pathways (37–39). Thus, different NMDA receptor subunits could be linked to distinct intracellular signaling cascades (40, 41).

In conclusion, our results have disclosed that during postnatal development of glutamatergic transmission at a cortico-cortical synaptic pathway in the barrel cortex, it is possible to dissociate a concurrent change in the NMDA-receptor-mediated component dependent on sensory experience from the normal experience-independent maturational increase in the A/N ratio. These results further suggest that the overall synaptic strength is regulated by different developmental rules from those governing the precise receptor-subunit composition at the synapse.

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