Network Mechanisms of Spindle-Burst Oscillations in the Neonatal Rat Barrel Cortex In Vivo

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MINLEBAEV M, BEN-ARI Y, KHAZIPOV R. Network mechanisms of spindle-burst oscillations in the neonatal rat barrel cortex in vivo. J Neurophysiol 97: 692–700, 2007. First published November 8, 2006; doi:10.1152/jn.00759.2006. Early in development, cortical networks generate particular patterns of activity that participate in cortical development. The dominant pattern of electrical activity in the neonatal rat neocortex in vivo is a spatially confined spindle-burst. Here, we studied network mechanisms of generation of spindle-bursts in the barrel cortex of neonatal rats using a superfused cortex preparation in vivo. Both spontaneous and sensory-evoked spindle-bursts were present in the superfused barrel cortex. Pharmacological analysis revealed that spindle-bursts are driven by glutamatergic synapses with a major contribution of AMPA/kainate receptors, but slight participation of NMDA receptors and gap junctions. Although GABAergic synapses contributed minimally to the pacing the rhythm of spindle-burst oscillations, surround GABAergic inhibition appeared to be crucial for their compartmentalization. We propose that local spindle-burst oscillations, driven by glutamatergic synapses and spatially confined by GABAergic synapses, contribute to the development of barrel cortex during the critical period of developmental plasticity.

INTRODUCTION

During development, cortical neuronal networks generate particular patterns of activity that participate in cortical development (Ben-Ari 2001; Fox 2002; Katz and Shatz 1996; Khazipov and Luhmann 2006; Moody and Bosma 2005; O’Donovan 1999). The dominant pattern of electrical activity in the neonatal rat neocortex in vivo is a spindle-burst (Hangar et al. 2006; Khazipov et al. 2004b). Spindle-bursts are spatially confined spindle-shape oscillations at alpha-beta frequency, associated with phase-locked neuronal firing and activation of the glutamatergic and GABAergic synapses. Spindle-bursts constitute a self-organizing pattern that persists after deafferentation. In the intact animal, compartmentalized spindle-bursts in somatosensory cortex are triggered by sensory feedback resulting from spontaneous movements in a somatotopic manner (Khazipov et al. 2004b). In visual cortex, spindle-bursts are triggered by spontaneous retinal waves in an eye-specific manner (Hangar et al. 2006). However, the network mechanisms underlying the generation of cortical spindle-bursts are poorly understood. Several patterns of correlated neuronal activity sharing some common features with the spindle-bursts in vivo were previously described in postnatal rod neocortical and hippocampal slices and intact preparations in vitro. These include “synchronized-via-gap-junctions neuronal domains” (Kandler and Katz 1995 1998a,b; Yuste et al. 1992, 1995) and calcium waves (Peinado 2000, 2001), gap-junction– and NMDA-receptor–based cholinergic oscillations (Dupont et al. 2006), giant depolarizing potentials (GDPs), and early network oscillations driven by glutamatergic and excitatory GABAergic synapses (Agmon et al. 1996; Ben-Ari et al. 1989; Garaschuk et al. 1998, 2000; Khazipov et al. 1997; Leinekugel et al. 1997, 1998). Consistent with the in vitro findings, generation of the early hippocampal in vivo pattern of sharp waves (Buhl and Buzsáki 2005; Leinekugel et al. 2002) was also shown to involve excitatory action of γ-aminobutyric acid (GABA) (Sipilä et al. 2006). However, to what extent these developmentally regulated mechanisms contribute to generation of the neocortical in vivo pattern of spindle-bursts remains largely unknown.

In the present study, we studied the mechanisms of spindle-bursts in the neonatal rat barrel cortex in vivo using a superfused neocortex preparation initially developed for the adolescent rat hippocampus (Khazipov and Holmes 2003). We found that the physiological pattern of spindle-bursts is preserved in the superfused barrel cortex. Pharmacological profiling of spindle-bursts indicated that spindle-bursts is primarily based on glutamatergic synapses, with a major role of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and a slight contribution of N-methyl-D-aspartate (NMDA) receptors and gap junctions; and that GABAergic synapses are not directly involved in the generation of spindle-burst oscillations, although they play an important role in their spatial compartmentalization.

METHODS

This study followed INSERM guidelines on animal care, with approval from the animal care and use local committee. Wistar rats of both sexes from postnatal day (P) 1 to P7 were used. P0 was the day of birth. During the surgical procedure, rats were anesthetized with a combination of 0.5–1.5 g/kg urethane injected intraperitoneally and ice-cooling. The skin and periosteum were removed from the skull, which was then covered with a layer of dental acrylic, except for two areas about 7 mm in diameter above the right and left barrel cortices (Fig. 1A). The rat was positioned in the stereotaxic apparatus; the skull was attached to the nose (nasal bones) and ear bars (occipital bone) with dental acrylic. A 5-mm-diameter burr hole was drilled in the skull above the right barrel cortex, the dura was cut and removed, and the cortical surface was covered with 0.9% NaCl during the procedure to prevent it from drying. The perfusion chamber was prepared as described previously (Khazipov and Holmes 2003) with some modifications. In brief, a 2-mm-long cylinder was cut from a plastic tube (ID 3.5 mm, OD 4.5 mm) and glued to stretched nylon mesh with cyanoacrylamide glue. The chamber was positioned at the cortical...
surface so that the mesh gently pressed onto the cortex. The chamber was then fixed to the skull by dental acrylic. Chlorided silver wire was inserted into the cerebellum and served as a ground electrode. During recordings, rats were heated by a thermal pad (37°C). The chamber was perfused with oxygenated (95% O2–5% CO2) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 126 NaCl, 3.5 KCl, 2.0 CaCl2, 1.3 MgCl2, 25 NaHCO3, 1.2 NaH2PO4, and 11 glucose (pH 7.4 at a rate of 2 ml/min). Temperature in the chamber was kept at 35–37°C using an automatic temperature controller (TC-344B; Warner Instruments, Hamden, CT). Extracellular field potential recordings (3- to 3,000-Hz band-pass) were performed using arrays of metal electrodes of 50 μm in diameter (California Fine Wire, Grover Beach, CA). Patch-clamp recordings were performed using an Axopatch 200A amplifier (Axon Instruments, Union City, CA) using a patching technique similar to that described in vivo (Leinekugel et al. 2002; Margrie et al. 2002). The pipettes were filled with a solution of the following composition (in mM): 135 Cs-glucuronate (or methylsulfate), 2 MgCl2, 0.1 CaCl2, 1 EGTA, and 10 HEPES (pH 7.25). Membrane potential values were corrected for liquid junction potential of +12 mV. Afferent stimulation was performed by applying electrical pulses (60 V, 50 μs, 0.03 s−1) through pairs of electrodes inserted into the whisker pads and glued to the skin with super glue.

Data were digitized at 10 kHz using a Digidata 1322A interface (Axon Instruments) and analyzed off-line using an Axon package (Axon Instruments), MiniAnalysis (Synaptosoft, Decatur, GA), Origin (Microcal Software, Northampton, MA), and Matlab (The MathWorks, Natick, MA). Group measures are expressed as means ± SE. The statistical significance of differences was assessed with the Student’s t-test. The level of significance was set at P < 0.05.

The drugs gabazine, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphonovaleric acid (D-APV) were purchased from Tocris Neuramin (Bristol, UK), diazepam from Roche (Basel, Switzerland), and all other compounds from Sigma (St. Louis, MO).

R E S U L T S

To study the mechanisms of spindle-bursts in the barrel cortex of neonatal rats (postnatal days P1–P7), we used a technique of superfused neocortex in vivo that was originally developed for adult hippocampus (Khazipov and Holmes 2003). The photograph and the experimental setup are schematically shown in Fig. 1A. In brief, under anesthesia, a round window was cut in the skull above the barrel cortex and dura was removed. A cylinder with fine mesh at its bottom was gently pressed onto the surface of the barrel cortex (mesh prevents pulsations and allows application of drugs directly onto the cortical surface). In addition to the ease of pharma-

![Figure 1](https://example.com/image1.png)

**FIG. 1.** Spindle-bursts in the superfused barrel cortex of neonatal rat in vivo. A: scheme (top) and photograph (bottom) of the experimental preparation. Perfusion chamber is a cylinder with fine mesh at its bottom. It is placed at the surface of barrel cortex so that mesh gently presses on the cortex to prevent pulsations. This construction allows application of drugs directly on the cortical surface. Pair of stimulation electrodes is implanted into the whisker pads. B: local field potential recordings (3- to 3,000-Hz band-pass) from the postnatal day 4 (P4) rat superfused barrel cortex (BCx); below are shown the corresponding time–frequency analysis and multiple unit activity (MUA). Spindle-bursts are marked with asterisks. C: spindle-burst marked by asterisk on B (top trace) is shown on an expanded timescale. D: action potentials of individual cortical neuron recorded concomitantly in current-clamp mode during the spindle-burst oscillation (depth, 600 μm). E–G: spindle-burst occurrence (E), duration (G), and normalized power (F) in the superfused and intact barrel cortices. Pooled data from 13 P2–P7 rats; total 1,165 spindle-bursts.
cological manipulations, this technique is also convenient for placement of electrodes. The contralateral barrel cortex remained intact and served as a control.

We first determined whether the normal physiological pattern of spindle-bursts and sensory-evoked responses are preserved in the superfused barrel cortex by comparing the activity in the barrel cortex superfused with normal ACSF with the intact contralateral barrel cortex using local field potential recordings (3- to 3,000-Hz band-pass). Spontaneous activity in both superfused and intact barrel cortices was similar and characterized by highly discontinuous periods of activity. Virtually all multiple unit activity (Fig. 1, B and C) and action potentials of individual cortical neurons recorded concomitantly in current-clamp mode (n = 9 cells; Fig. 1D) were synchronized to intermittent spindle-shape oscillations. These were similar to the spindle-bursts described previously in the primary somatosensory cortex for areas of body representation (Khazipov et al. 2004b) as well as in visual cortex (Hangaram et al. 2006). Spindle-bursts had similar durations of 344 ± 15 and 370 ± 15 ms and peak power of oscillations at 15 ± 3 and 21 ± 3 Hz, and reversed in polarity at about 1 mm depth (in the superfused and intact barrel cortices) (Fig. 1, F and G; n = 13 rats; P2–P7). Spindle-bursts occurred more frequently in the superfused barrel cortex (4.2 ± 0.4 min⁻¹) than in the intact barrel cortex (2.5 ± 0.4 min⁻¹; P < 0.05; Fig. 1E).

Sensory potentials evoked by electrical stimulation of the contralateral whisker pad occurred with a similar delay of 38.3 ± 1.4 and 39.5 ± 3.5 ms and had amplitudes of 792 ± 49 and 593 ± 12 μV in the superfused and intact barrel cortices, respectively (Fig. 2A; n = 17 rats; P2–P7). Relatively long latencies of sensory responses are typical for the immature animals and human and they probably reflect the lack of myelination (Khazipov et al. 2004b; Pihko and Lauronen 2004). Transcortical current source analysis of the myelinization (Khazipov et al. 2004b) as well as in visual cortex (Hanganu et al. 2005) did not reveal any difference between the stimulation-evoked and spontaneous spindle-bursts in the superfused barrel cortex (Fig. 3, A and B; n = 17 rats). Power-spectrum (Fig. 3C) and spindle-burst duration analysis (Fig. 3D) did not reveal any difference between the stimulation-evoked and spontaneous spindle-bursts in the superfused barrel cortex. Thus the physiological patterns of spindle-bursts and sensory-evoked cortical responses are preserved in the superfused barrel cortex preparation.

We further explored the pharmacological profile of spontaneous and sensory evoked activity in the superfused barrel cortex. Tetrodotoxin (TTX, 2 μM), a blocker of the voltage-gated sodium channels, completely and reversibly suppressed spontaneous spindle-bursts (Fig. 4, A and E), sensory-evoked potentials, and sensory-evoked spindle-bursts (Fig. 5D; n = 3 rats). AMPA/kainate receptors antagonist CNQX (20 μM) completely and reversibly eliminated spontaneous spindle-bursts (Fig. 4, A and E), reduced sensory field potentials evoked by contralateral whisker pad stimulation to 8.8 ± 0.3% of control values (Fig. 5, A and B), and blocked sensory-evoked spindle-bursts, which was also evidenced by a reduction in the power of activity at the dominant frequency of the evoked spindle-burst oscillations (18 ± 2 Hz) from 49.4 ± 12 Hz in the superfused barrel cortex (4.2 ± 0.4 min⁻¹) than in the intact barrel cortex (2.5 ± 0.4 min⁻¹; P < 0.05; Fig. 1E).

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16.4 to 0.15 ± 0.03 μV² in the time frame of 300–1,000 ms after sensory stimulation (Fig. 5, A and D; n = 10 rats; P2–P7). Spindle-bursts and sensory-evoked responses fully recovered 25–40 min after washout of CNQX (Fig. 5B). The amplitude of sensory responses in the intact barrel cortex was not affected by the CNQX applied to the superfused barrel cortex (Fig. 5B). Consistent with the extracellular data, whole cell recordings revealed complete suppression of the EPSCs and spindle-bursts (Fig. 5C; n = 3 cells; depth 450–600 μm). Although spindle-burst oscillations were virtually completely suppressed by CNQX, bursts of multiple units were still efficiently evoked by sensory stimulation and occurred spontaneously (Fig. 5A). This residual activity persisted in the presence of a high concentration of bath-applied CNQX (50 μM; n = 9) and after ipsilateral intraventricular CNQX injection (1 μl of 10 mM CNQX, n = 4). The mechanism underlying this residual activity at present remains unknown. The NMDA receptor antagonist D-APV (80 μM) did not significantly affect sensory potentials and only modestly affected spindle-bursts. The occurrence of spontaneous spindle-bursts (5.1 ± 0.8 min⁻¹; n = 9) and the probability of evoking a spindle-burst (0.8 ± 0.1; n = 5) was not significantly modified by D-APV; however, the duration of spindle-bursts was slightly shortened (from 292 ± 14 to 264 ± 19 ms), reduced in power (33 ± 14 to 8 ± 3 μV²), and the oscillation frequency was slightly increased (Figs. 4, B–E and 5D; n = 9 rats; P2–P7). Taken together, these results suggest that generation of spindle-burst oscillation is based on glutamatergic synapses with the major contribution of AMPA/kainate receptors and slight participation of NMDA receptors.

In the next series of experiments we studied the contribution of GABAergic synapses to the generation of spindle-bursts by applying the GABA[subscript A] receptor antagonist gabazine. To control the efficacy of gabazine in the superfused cortex, we studied its action on the GABA[subscript A]-PSCs recorded with low-chloride solution at 0 mV holding potential. Gabazine (40 μM) completely blocked spontaneous and sensory-evoked GABA[subscript A]-PSCs (n = 5 neurons; depth 450–600 μm; Fig. 6B). Gabazine did not significantly change the amplitude of the early negative peak of the sensory-evoked field potentials [control: 450 ± 69 μV; gabazine: 496 ± 78 μV (110 ± 6%); n = 5; P > 0.05; P3–P5; Fig. 6B]. This is in keeping with the fact that the early part of the sensory-evoked potential is purely glutamatergic thalamocortical EPSP and the GABAergic conductance is delayed (Figs. 2C and 6B, **; see also above and Agmon and O’Dowd 1992; Agmon et al. 1996; Moore and Nelson 1998). However, gabazine induced the appearance or strongly enhanced (to 929 ± 6%; n = 5) the late negative peak of the sensory EPSP, which probably reflects the intracortical spread of excitation (Fig. 6B, **). Blockade of GABA[subscript A] receptors by gabazine also
significantly increased spontaneous cortical activity by almost doubling the occurrence of spontaneous spindle-bursts, as well as increasing the probability of evoking a spindle-burst by stimulation to 0.96 ± 0.02 (n = 10; P < 0.05; Fig. 6, A, E, and F). The dominant frequency of spindle-burst oscillations was not significantly modified by gabazine (Fig. 6C), although the power increased from 16 ± 5 to 24 ± 6 μV^2 for spontaneous and from 9 ± 2 to 22 ± 5 μV^2 for sensory-evoked spindles, and the duration of spindle-bursts increased from 323 ± 13 to 658 ± 41 ms (n = 10; P2–P6; P < 0.01, Fig. 6D). Superfusion with the NKCC1 antagonist bumetanide (20 μM), which negatively shifts the GABA_A reversal potential in neonatal cortical neurons (Yamada et al. 2004), did not appreciably affect the occurrence and the power-frequency characteristics of spindle-bursts (n = 6; Fig. 6F). Consistent with these results, systemic intraperitoneal injection of bumetanide (0.6 g/kg) did not affect spindle-bursts (n = 3; data not shown). Application of the positive allosteric GABA_A modulator diazepam (70 μM), which transiently increases the frequency of the hippocampal GDPS in vitro (Khalilov et al. 1999), reduced the frequency of spindle-burst occurrence by twofold without any effect on the frequency of spindle-burst oscillations and their power (Fig. 6F; n = 5; P2–P6). These results suggest that GABAergic transmission is not necessarily required for pacing the rhythm of spindle-burst oscillations but that it plays an inhibitory role at the network level.

Horizontal compartmentalization is a salient feature of spindle-burst (Khazipov et al. 2004b). Therefore in the next experiment we tested a hypothesis that GABAergic synapses are involved in compartmentalization of spindle-bursts. Using four site recordings from the barrel cortex with a separation distance between the electrodes of 500 μm, we found that spindle-bursts are predominantly local events with a relatively low coefficient of cross-correlation of activity between the recording sites (Fig. 7A; n = 5; P2–P7) in keeping with the results obtained in the body representation areas of somatosensory cortex (Khazipov et al. 2004b). In the presence of gabazine (40 μM), spindle-bursts were synchronously recorded from several (and often all four) recording sites, accompanied with a strong increase in the cross-correlation between recording sites (Fig. 7B). To verify whether surface application of gabazine does not abolish the potential deep burst generators we administered gabazine intraperitoneally (0.3 mg/kg) and found a similar increase in the occurrence of spindle-bursts and their cross-correlation over large cortical areas (n = 6; data not shown). Thus although GABA_A receptors are not instrumental for pacing the rhythm of spindle-bursts, they appear to play a critical role in the compartmentalization of spindle-burst activity—probably by the mechanism of surround inhibition.

Several studies using intact cortex and cortical slices in vitro indicated an important role for gap junctions in the generation of correlated neuronal activity during the early postnatal period (Dupont et al. 2006; Kandler and Katz 1995, 1998a,b; Peinado 2000, 2001; Yuste et al. 1992, 1995). Therefore we studied the effect of the gap-junction blocker mefloquine (50 μM) (Cruikshank et al. 2004) on spindle-bursts. Mefloquine increased the occurrence of spontaneous spindle-bursts to 146 ± 33% (n = 7; P1–P3; P < 0.05) without affecting the power and frequency of the spindle-burst oscillations (Fig. 4E) and probability of evoked spindle-bursts (Fig. 5D). An increase in the occurrence of spontaneous spindle-bursts to 157 ± 14% was also observed after injection of mefloquine (25 mM, 100 nl) into the cortex (depth 1,000 μm; n = 4 rats; P2–P3; P < 0.05). These results suggest that spindle-bursts significantly differ in their generation mechanisms from the early patterns of cortical activity in vitro that are blocked by the gap-junction antagonists (Dupont et al. 2006; Kandler and Katz 1995, 1998a,b; Peinado 2000, 2001; Yuste et al. 1992, 1995).

**DISCUSSION**

The principal findings of the present study can be summarized as follows: 1) spindle-bursts are the principal pattern of activity in the barrel cortex during the first week of postnatal life; like spindle-bursts in the body representation areas, spindle-bursts in the barrel cortex can be evoked by sensory stimulation; 2) the principal properties of spindle-bursts are preserved in the superfused cortex preparation that enables easy pharmacological manipulations and recordings; 3) generation of spindle-burst oscillations requires AMPA/kainate receptor activity at glutamatergic synapses; NMDA receptors and gap junctions are slightly involved in the generation of spindle-bursts; and 4) GABAergic synapses importantly con-
tribute to the compartmentalization of spindle-bursts by surround inhibition.

We used the novel methodological approach of superfused neocortex in vivo to provide a stable platform for electrophysiological recordings and pharmacological manipulation. Advantages of the present technique compared with conventional in vivo recordings can be summarized as follows: 1) the cortex is exposed and the recording electrodes can be placed under visual control as easily as in the intact cortex or cortical slices in vitro and 2) rapid pharmacological manipulations, including application and washout of drugs, are possible. This is a distinct advantage over other in vivo techniques that require systemic or local injections. An important finding of the present study is that the physiological pattern of spindle-bursts is preserved in the superfused neocortex in vivo. The reason for the higher frequency of occurrence of spindle-bursts in the superfused cortex is unknown, but it may be attributable to the washing out of urethane from the superfused cortex and/or some difference in the composition of ACSF from that of the physiological cerebrospinal fluid. Other than the higher occurrence, the characteristics of spindle-bursts and sensory-evoked activity in the superfused barrel cortex were not significantly different from that of the intact cortex. Thus the preparation of the superfused neocortex combines the in vitro approaches with the in vivo situation, thus enabling one to study the physiological pattern of activity that appear to be much more complex in the intact brain than in isolated slices (Steriade 2001).

Our results suggest that the generation of spindle-bursts in the neonatal barrel cortex is primarily based on synapses between glutamatergic cells, with the major contribution of the AMPA/kainate receptors. Because the glutamatergic synapses on cortical neurons are mainly provided by the intracortical connections and thalamocortical input, at least two models of spindle-burst oscillations can be proposed: intracortical and thalamocortical. In the intracortical model, the spindle-burst is a local oscillation generated in the network of interconnected cortical glutamatergic neurons. Because the GABA_A antagonists did not significantly affect the frequency of spindle-burst oscillations, neuronal inhibition probably comes from an afterhyperpolarization mediated by the voltage- and calcium-dependent potassium channels. In the thalamocortical model, spindle-bursts are generated by a rhythmic thalamocortical input provided by oscillation in thalamic neurons, similar to the mechanism of adult sleep spindles (Steriade et al. 1993). The spatial confinement of spindle-bursts in the latter model could be explained by the relative sparsity of long-range intracortical and corticothalamic connections, which are important for synchronization of sleep spindles over the entire cortex (Contreras et al. 1996). This is aided by efficient shunting surround inhibition of depolarizing GABA. The two models are not incompatible and both thalamic and cortical oscillators may resonate given that their intrinsic frequencies are close.

In both the present and previous studies whole cell recordings revealed GABAergic synaptic currents phase-locked with the local field potential oscillations (Hanganu et al. 2006; Khazipov et al. 2004b), suggesting that interneurons are activated during spindle-bursts. However, because blockade of GABA_A receptors did not significantly affect the frequency of oscillation it seems that GABAergic interneurons play only a minor role in pacing of the rhythm of oscillation. This differs

FIG. 7. Effect of the GABA_A antagonist gabazine on the compartmentalization of spindle-bursts. A: field-potentiel recordings from the superfused barrel cortex with a 4-microelectrode array (500-μm separation distance between the electrodes) in control conditions and (B) in the presence of gabazine (40 μM). Spindle-burst marked by black asterisk on A (left) is shown on an expanded timescale in the middle. On the right, cross-correlation analysis of spindle-bursts at 4 recordings sites (pooled data from 6 rats).
from the adult brain in which the major patterns of activity are significantly influenced by interneurons (Freund and Buzsáki 1996; Fuentealba and Steriade 2005). GABAergic inhibition undergoes significant developmental changes during the first postnatal week, during which time GABA—acting by GABA$_A$ receptors—depolarizes immature neocortical neurons because of elevated intracellular [Cl$^-$]$_i$ (LoTurco et al. 1995; Luhmann and Prince 1991; Owens et al. 1996; Yamada et al. 2004; Yuste and Katz 1991). Interestingly, in the hippocampus, GDPs in vitro and sharp waves in vivo are blocked by the NKCC1 and Katz 1991). Interestingly, in the hippocampus, GDPs in vitro emphasized the role of several developmentally regulated mechanisms of neuronal synchronization in the developing cortex, including 1) gap junctions (Dupont et al. 2006; Kandler and Katz 1995, 1998a,b; Peinado 2000, 2001; Yuste et al. 1992, 1995), 2) NMDA receptors (Ben-Ari et al. 1989; Dupont et al. 2006; Leinekugel et al. 1997), and 3) depolarizing GABA (Ben-Ari et al. 1989; Garaschuk et al. 1998, 2000; Khazipov et al. 1997, 2004a; Leinekugel et al. 1997; Sipilä et al. 2005, 2006). Our results suggest that the generation of the in vivo neocortical pattern of spindle-bursts relies on a rather “mature” mechanism based on AMPA/kainate-receptor–mediated synaptic transmission. It should be noted that AMPA/kainate antagonists were also efficient in suppressing some types of cortical network activity in the neonatal period, including spontaneous—but not stimulation-evoked—GDPs (Ben-Ari et al. 1989; Bolea et al. 1999; Khazipov et al. 1997; Lamasa et al. 2000), polysynaptic events in barrel cortex evoked by thalamic stimulation (Agmon et al. 1996), and neocortical early network oscillations (Garaschuk et al. 2000).

In the rodent somatosensory cortex, activity-dependent cortical plasticity is maximal over an early “critical” postnatal developmental period, which is characterized by enhanced synaptic plasticity and by the potential for profound alterations of anatomical and functional organization of the barrel cortex by manipulation of the sensory input (Crair and Malenka 1995; Erzurumlu and Kind 2001; Feldman et al. 1998, 1999; Fox 2002; Fox and Wong 2005; Katz and Crowley 2002; Katz and Shatz 1996; Van der Loos and Woolsey 1973). Glutamate receptor blockade during the first postnatal week was previously shown to disrupt the topographic refinement of thalamocortical connectivity and columnar organization of the barrel cortex (Elias et al. 2003; Fox et al. 1996) and impairs formation of the intracortical connectivity (Dagnew et al. 2003). We propose that local spindle-burst oscillations, driven by glutamatergic synapses and compartmentalized by GABAergic synapses, contribute to development of the barrel cortex during the critical period of developmental plasticity.

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REFERENCES


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