Neonatal NMDA Receptor Blockade Disturbs Neuronal Migration in Rat Somatosensory Cortex In Vivo

Glutamate plays an important role in the control of neuronal migration in the developing cerebral cortex. The present study describes changes in the structure and function of the cerebral cortex after transient blockade of N-methyl-D-aspartate (NMDA) receptors during the late period of neuronal migration. Elvax slices containing the NMDA antagonist MK801 were placed over the somatosensory cortex of newborn rats and the drug was released over a period of 2–3 days. After survival times of 1 or 2 weeks, neuroanatomical and in vitro electrophysiological analyses revealed prominent structural and functional alterations in the cortical region underlying the implant. Cortical lamination was disturbed and heterotopic cell clusters were found in layer I of MK801-treated animals. Morphologically identified pyramidal neurons recorded in MK801-treated cortex revealed late NMDA receptor-mediated synaptic inputs and fragile monosynaptic responses at stimulation frequencies >0.2 Hz. Our data indicate that impairment of NMDA receptors during early corticogenesis induces neuronal migration disorders and delays the functional maturation of the developing cortical network.

Keywords: heterotopia, maturation, neonatal, pyramidal neurons, synaptic responses

Introduction

Neuronal migration is an essential process in the early development of the central nervous system. Postmitotic neurons migrate from their site of origin to their final destination, where they differentiate and establish synaptic connections. In the cerebral cortex, the majority of neurons is generated within the ventricular zone and migrate along radial glial fibers in an inside-first gradient into the developing cortex (Angevine and Sidman, 1961; Rakic, 1971, 1988). These radially migrating cells give rise to the glutamatergic pyramidal neurons of the neocortex (Tan et al., 1998). In contrast, most or even all cortical interneurons expressing the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), somatostatin or parvalbumin are generated in the subcortical telencephalon (predominantly the medial ganglionic eminence; Wichterle et al., 2001) and migrate tangentially into the cerebral cortex (Porteus et al., 1994; De Carlos et al., 1996; Ang et al., 2003).

Immature glutamatergic neurons migrating along radial glia into the developing cortex have an important function in the establishment of cytoarchitectonic areas and topographical connectivity maps, as suggested by Rakic in the protomap hypothesis (Rakic, 1988). This radial glial-guided migration is regulated by various cell-cell recognition and adhesion molecules (Cameron and Rakic, 1994), N-type calcium channels (Komuro and Rakic, 1992), GABA receptors (Behar et al., 1998), neurotrophins (Behar et al., 1997) and reelin (Dulabon et al., 2000; for reviews, see De Rouvroit and Goffinet, 2001; Nadarajah and Parnavelas, 2002). Furthermore, activation of N-methyl-D-aspartate (NMDA) receptors also plays a critical role in radial migration (for a review, see Komuro and Rakic, 1998). Neuronal migration of postmitotic granule cells in mouse cerebellar organotypic slice cultures is decelerated by blockade of NMDA receptors and accelerated by enhancement of NMDA receptor activity (Komuro and Rakic, 1993). Neuronal migration of embryonic neocortical neurons in slice cultures is stimulated by activation of NMDA receptors (Behar et al., 1999) and inhibited by blocking NMDA receptors (Hirai et al., 1999). Finally, a significant role of NMDA receptors in regulating neuronal migration in the cerebral cortex has been demonstrated in a mouse model of Zellweger syndrome (Pxr1+/−) (Gressens et al., 2000), indicating that genetically induced alterations in NMDA receptor-mediated calcium mobilization may cause neocortical migration disorders. However, transgenic mice lacking functional NMDA receptors (NMDAR-1 mutants) show a normal neocortical development (Messersmith et al., 1997).

In order to address the question whether a spatially and temporally restricted blockade of NMDA receptors may induce neuronal migration disorders in developing cerebral cortex in vivo, we implanted MK801-containing Elvax slices in the somatosensory cortex of newborn rats (Smith et al., 1995) and analyzed the structural and functional properties of the cortical tissue after 1 or 2 weeks of survival. Our results indicate that blockade of NMDA receptors during the late migration process causes severe cortical malformations and modifications in the functional properties of pyramidal neurons and developing cortical circuits, supporting the hypothesis that NMDA receptors are critically involved in the neuronal migration process of the cerebral cortex.

Materials and Methods

Preparation and Analysis of Elvax Slices Containing MK801

Elvax polymers were manufactured as described previously (Smith et al., 1995; Persico et al., 1997). Briefly, ethylene-vinyl acetate copolymer (Elvax, 40% vinyl acetate, a gift from Erbsloh GmbH, Velbert, Germany) was washed 4–5 times with 95% ethanol during 1 week and subsequently dissolved in methylene chloride to give a 10% solution. Either 4% dimethyl sulfoxide (DMSO; Sigma, Deisenhofen, Germany) or the non-competitive NMDA antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK801) (20 mM in 4% DMSO; RBI, Natick, MA) was added to the Elvax solution. After addition of 1% Fast Green (Sigma) to help visualizing the Elvax slices, the suspension was vortexed for 30 min. The Elvax solution was poured onto a glass slide and covered by a second one. The slides were then clamped together and placed on dry ice for 30 min. Approximately
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100 μm spacing between the slides was provided by a Parafilm frame (American National Can, Greenwich, CT). Elvax was stored at -20°C for 7 days and on the day of surgery 1 x 1 mm Elvax slabs were cut under a binocular microscope. The in vitro release of MK801 was measured by scintillation counting using implants loaded with a mixture of [3H]MK801/MK801 (150,000 labeled/unlabeled; PerkinElmer Life Sciences, Zaventem, Belgium). The implants were incubated in 500 μl GBSS (Gey’s balanced salts solution) at 37°C. The bath solution was exchanged every 24 h and the amount of released radioactivity was measured by scintillation counting.

Animal Preparation
All experiments were conducted in accordance with the national laws for the use of animals in research and approved by the local ethical committee (#23.05-230-3-76/00). Newborn Wistar rats were operated within the first 24 h after birth. In this study, P0 was defined as the first 24 h after birth. Animals were anesthetized by hypothermia and 6–7 mm of the skin overlying the parietal cortex was cut along the midline with a small scalpel. A craniotomy was made over the parietal cortex using a microdrill (Fine Science Tools, Heidelberg, Germany). The dura was carefully opened and an Elvax chip containing DMSO or MK801 was placed on the cortical surface in the region corresponding to the primary somatosensory cortex (Paxinos et al., 1991; Paxinos and Watson, 1998). The cavity was covered with a piece of pericardial membrane (W.L. Gore & Associates, Inc., Putzbrunn, Germany) and the scalp was sutured. After surgery, the animals were warmed up under an infrared lamp and put back into the cages with the mother. Untreated control rats (n = 7) from the same litter, sham-operated control animals treated with DMSO implants (n = 13) and MK801-treated rats (n = 14) were examined at P7 or P14.

Histology
At P7 or P14, animals were anesthetized with Ethane (Abbot Laboratories, Wiesbaden, Germany), decapitated and the brain was quickly removed and fixed for 2–3 h in 4% formaldehyde in phosphate buffer (PB, pH 7.4). After cryoprotection for 24 h in 30% sucrose in PB, coronal sections of 50 μm thickness were cut on a freezing microtome (CM 1325, Leica, Bensheim, Germany) and alternating slices were Nissl-stained or processed for immunohistochemistry. For Nissl-staining, sections were mounted on gelatin-coated slides and air-dried overnight. After incubation in cresylviolet solution for 10 min, slices were dehydrated in increasing concentrations of ethanol, cleared in xylene and coverslipped. Brain slices from 17 animals of both age groups (P7: n = 2 untreated, n = 2 DMSO, n = 5 MK801; P14: n = 5 DMSO, n = 3 MK801) were processed for immunohistochemical staining with anti-NMDA-R1 (Chemicon, Temecula, CA). The sections adjacent to those were processed for visualization of Nissl substance. Free-floating sections were rinsed in 0.01 M PBS for 30 min and the following two pre-incubation steps were performed to prevent unspecific binding: (i) incubation in 0.5% H2O2 for 30 min, afterwards rinsing in PBS for 30 min; and (ii) 1 h incubation in serum-containing buffer [5% NGS (Vector Labs, Burlingame, CA) + 0.1% Triton X-100 (Sigma, Deisenhofen, Germany)]. After pre-incubation, slices were rinsed again in PBS and incubated overnight at 4°C in the dilution of the antisera (25 μg/ml). For negative controls, the sections were incubated in serum containing buffer. The reaction was stopped by rinsing in PBS for 30 min at room temperature. Thereafter, the slices were transferred to the secondary antibody (biotinylated anti-rabbit IgG, diluted 1:200, Vector Labs) for 2 h at room temperature. After rinsing for 30 min, the sections were incubated in the avidin–biotin complex for 1 h (Vectastain Elite kits, Vector Labs). Subsequently they were rinsed once in PBS, twice in TRIS buffer (50 mM, pH 7.2) and transferred to a TRIS buffer solution containing 3.5-diaminobezocine (DAB, Sigma) as chromogen. After an incubation of 10 min, the solution was changed, now additionally containing 30 μl of 30% H2O2 to start the reaction. After 7 min the reaction was stopped by rinsing several times with TRIS buffer. The sections were mounted on gelatin coated slides, air-dried, cleared in xylene and coverslipped.

Cortical slices containing intracellularly biocytin labeled cells (see below) were processed by a modification of the technique described by Horikawa and Armstrong (Horikawa and Armstrong, 1988) (for details see Schröder and Luhmann, 1997). Slices were fixed in 4% paraformaldehyde for at least 24 h subsequently to the experiment, rinsed, and were incubated 60 min with 0.5% H2O2 and 0.8% Triton-X to inhibit endogenous peroxidases. An overnight incubation with an avidin–coupled peroxidase (ABC kit, Vector Labs) was followed by a pre-incubation in 0.5 mM DAB and a subsequent reaction in DAB and 0.015% H2O2. Staining was intensified by a treatment with 0.15% Oso4. Afterwards slices were rinsed, dehydrated slowly through alcohol and propyleneoxide, and embedded in Durcupan (Fluka, Buchs, Switzerland).

Slice Preparation and In Vitro Electrophysiology
For electrophysiological examination, sham-operated DMSO-treated and MK801-treated rats were sacrificed at postnatal days 13–17. The animals were deeply anesthetized with enflurane (Ethane, Abbott, Wiesbaden, Germany) and decapitated. The brain was quickly removed and stored for 2–3 min in ice-cold artificial cerebrospinal fluid (ACSF) consisting of (in mM) 124 NaCl, 26 NaHCO3, 1.25 NaH2PO4, 1.8 MgCl2, 1.6 CaCl2, 5 KCl, 10 glucose; equilibrated with 95% O2/5% CO2 (pH 7.4, osmolarity 336 mOsm). Two to three neocortical slices (thickness 400 μm) including the region under the EVA implant were cut on a vibratome (Pelco 101, TPI, St Louis, MO) and were transferred to an incubation chamber filled with equilibrated ACSF at 32°C in which they recovered for at least 1 h before recording began. The slices were transferred into a submerged recording chamber mounted on the fixed stage of an upright microscope with differential interference contrast optics (Axioskop, Zeiss, Jena, Germany) and were superfused with ACSF at 32°C. Pyramidal neurons in the region under the Elvax implant were visualized and recorded in whole-cell patch-clamp mode as described previously (Kilb et al., 2002; Luhmann et al., 2000). Patch-pipettes were pulled from borosilicate tubing (20 μm outside diameter, 1.16 mm inside diameter; Science Products, Hofheim, Germany) using a vertical puller (PP-83, Narishige, Tokyo, Japan) and were filled with a solution containing (in mM) 117 K-glucuron, 13 KCl, 2 MgCl2, 11 EGTA, 10 HEPES, 2 Na2-ATP, 0.5 Na-GTP (pH adjusted to 7.4 with KOH and osmolality to 306 mOsm with sucrose). The patch pipettes were connected to the headstage of a discontinuous voltage-clamp/current-clamp amplifier (SECO5L, NPI, Tamm, Germany). Signals were amplified, low-pass filtered at 3 kHz, visualized on an oscilloscope (TD8210, Tektronix, Beaverton, OR), digitized online by an AD/DA-board (ITC-16, Heka, Lampedrecht, Germany), recorded and processed with WINTIDA 4.11 software (Heka). The intrinsic membrane properties were analyzed under current clamp conditions directly after obtaining whole-cell conditions. For the determination of the input current, voltage relation, and the active membrane properties, hyperpolarizing and depolarizing current pulses were injected from a holding potential of −70 mV. The spike amplitude was measured from the spike threshold and the spike width was determined at the half-maximal spike amplitude. All potentials were corrected for liquid junction potentials (9.8 mV). To investigate the synaptic inputs onto the cell, a bipolar tungsten stimulation electrode (3–5 MOhm, FHC, Brunswick, NJ) was positioned in the white matter medially to the recorded cell. Voltage pulses of 50 μs duration and 2–70 V in amplitude were delivered to the stimulation electrode at a frequency of 0.2 Hz except where noted. CNQX and DL-APV were obtained from Tocris (Balwin, MO) and were used from a stock solution in DMSO. Maximal DMSO concentration in the superfusate was 0.1%. In all experiments 0.5% bicytin (Sigma, Deisenhofen, Germany) was added to the pipette solution to label the cells on which a whole-cell configuration was established.

Statistics
If not otherwise noted, all values are expressed as mean±standard error of the mean. For statistical analysis a Kolmogorov–Smirnov test (Systat 10, SSI, Point Richmond, CA) and χ² test were used, results were designated significant at a level of P < 0.05.

Results
In Vitro Release of MK801 from Elvax Slices
The time course of MK801 release from Elvax slices containing tritiated MK801 dissolved in DMSO was measured for 9 days at
Neonatal NMDA Receptor Blockade Causes Neuronal Migration Disorders

None of the untreated control rats (n = 7) or sham-operated controls treated with DMSO implants (n = 10) showed any obvious alterations in cortical architecture (Fig. 2A,B). Nissl-stained sections from P7 and P14 animals revealed the typical six-layered organization of the cerebral cortex. In contrast, all MK801-treated rats (n = 12) analyzed at P7 (n = 5) or P14 (n = 7) showed severe cortical malformations. These MK801-induced neuronal migration disorders were characterized by severe disturbances in the formation of cortical layers, focal cortical dysplasias and heterotopia within the upper layers (Figs 2C,D and 3). These cortical malformations were restricted to the site of the Elvax implants. Therefore the observed effects were caused by MK801 application on the neocortex during the first 2–3 postnatal days.

The distribution of NMDA receptors was studied in nine control animals (two untreated and two DMSO-treated at P7; five DMSO-treated at P14) and eight MK801-treated rats (five at P7 and three at P14) by immunohistochemistry with the antibody against the NMDA-R1 receptor subunit (Fig. 4). In agreement with previous observations in rat cerebral cortex (Rema and Ebner, 1996; Hagemann et al., 2003), dense neuronal NMDA-R1 staining was observed in layers II–VI of the control cortex (Fig. 4A). In MK801-treated cortex a laminar disorganization and groups of NMDA-R1 immunopositive cells could be detected in layer I (Fig. 4B–D). In agreement with our observations in Nissl-stained sections (Figs 2C,D and 3), these modifications in the cortical architecture were restricted to the site of the MK801-containing Elvax implants. However, both experimental groups revealed the same cellular NMDA-R1 pattern with intense staining of the cell bodies and apical dendrites (Fig. 4E).

Electrophysiological Alterations in MK801-treated Cortex

Whole-cell recordings were performed from 11 neurons in DMSO-treated P14-17 control rats and from seven neurons in MK801-treated P14-15 animals. All neurons recorded in controls and MK801-treated cortex could be identified as pyramidal neurons following histological processing for biocytin (Fig. 5A,B). We could not detect any obvious differences in the somatodendritic structure of biocytin-labeled cortical pyramidal neurons between sham-operated and MK801-treated rats. The average resting membrane potential of the neurons recorded in the DMSO-treated animals (−68.4 ± 2.1 mV, n = 11) was not significantly different from that recorded in the MK801-treated rats (−74.1 ± 2.2 mV, n = 7). The input resistance and membrane time constant were also not significantly different between DMSO-treated (345 ± 42 MOhm, 38.5 ± 4.8 ms) and MK801-treated animals (220 ± 59 MOhm, 31.7 ± 3.8 ms). Upon membrane depolarization above a threshold of −45.2 ± 1 mV (n = 11), repetitive action potentials with regular firing pattern, an average amplitude of 51.6 ± 2.5 mV and a duration of 2.0 ± 0.1 ms were elicited in the DMSO-treated animals (Fig. 5C). In contrast, action potentials in the MK801-treated animals had a significantly (P < 0.001) larger amplitude of 64.7 ± 1.5 mV (n = 7), while the action potential threshold (−46.9 ± 0.8 mV) and duration (1.7 ± 0.1 ms) were not significantly different from the DMSO-treated controls (Fig. 5D).

In all investigated cells, monosynaptic responses could be evoked by electrical stimulation of the underlying white matter. In the DMSO-treated control animals, the postsynaptic currents (PSCs) revealed an onset latency of 4.1 ± 0.5 ms (n = 11) and a maximal amplitude of −205 ± 46.2 pA. Both, the average onset latency (4.9 ± 0.4 ms, n = 7) and maximal amplitude (−109.7 ± 7.3 pA) of the PSCs recorded in MK801-treated animals were not significantly different when compared to the DMSO controls. Orthodromic synaptic stimulation elicited in 7/11 cells recorded in DMSO-treated control animals only a monosynaptic fast response (Fig. 6A). In the remaining four neurons, additional polysynaptic responses as described previously in neocortical slices from juvenile rats (Luhmann and Prince, 1990b) could be observed. The percentage of neurons showing a prominent polysynaptic input was significantly larger in MK801-treated animals (6/7, P < 0.05, χ²-test). These polysynaptic responses showed onset latencies of 100–200 ms and lasted for >200 ms (Fig. 6B). In the MK801-treated animals the amplitude of the polysynaptic PSCs (analyzed at the lowest stimulus intensity to elicit mono- and polysynaptic PSCs) amounted to 386 ± 138% (n = 6) of the monosynaptic PSCs’ amplitude, while in the DMSO-treated animals the polysynaptic PSCs were significantly (P < 0.001) smaller, reaching only 83 ± 18% of the monosynaptic PSCs’ amplitude. Bath application of 60 μM APV had no effect on the amplitude of monosynaptic PSCs in DMSO-control animals (n = 4) or MK801-treated animals (n = 2), but completely blocked the polysynaptic PSCs in MK801-treated animals (n = 2) (Fig. 6C). Bath application of 10 μM CNQX blocked or profoundly suppressed monosynaptic (n = 6) and polysynaptic
(n = 2) responses recorded in MK801-treated animals (Fig. 6D), as well as monosynaptic responses (n = 8) in DMSO-treated rats.

Monosynaptic responses recorded in MK801-treated cortex also revealed a higher sensitivity to increasing stimulus frequencies when compared to the age-matched control cortex (Fig. 7). PSCs recorded in MK801-treated cortex were significantly reduced (P < 0.001) at stimulation frequencies of >0.2 Hz, whereas PSCs in control cortex revealed only a minor

Figure 2. Digital photographs of 50 μm Nissl-stained coronal sections from a P7 untreated control rat (A), a P7 DMSO-treated control animal (B) and two P7 rats treated with an Elvax implant loaded with MK801 (C, D). Note normal cortical lamination in both control animals and disorganized cortical architecture with heterotopia in upper layers (arrows in C) in the MK801-treated rats. The rectangle in (C) marks position of Figure 3A. Scale bars = 500 μm.
suppression in this frequency range. These data indicate that synaptic responses in MK801-treated cortex preserve functional properties that are characteristic for the immature cortex.

**Discussion**

Our findings indicate that NMDA receptors play a crucial role in the neuronal migration process. Transient blockade of NMDA receptors in vivo causes structural and functional abnormalities in the developing cerebral cortex. Cortical layering is disturbed and heterotopic cell clusters accumulate in layer I. These structural alterations are accompanied by functional modifications at the single cell and cortical network level. Pyramidal neurons in MK801-treated cortex showed larger action potentials and received more often a polysynaptic input mediated by NMDA receptors. Monosynaptic responses recorded in MK801-treated cortex were more fragile than those recorded in age-matched sham-operated control cortex and could not follow stimulation frequencies of >0.2 Hz. These data indicate that NMDA receptor blockade during the neonatal period does not only interfere with the cortical migration process but also hinders the functional maturation of the cortical network.

**Role of NMDA Receptors in Neuronal Migration**

The critical role of NMDA receptors in neuronal migration has been previously documented for granule cells in the developing mouse cerebellum using slice cultures (Komuro and Rakic, 1993). Further support for this hypothesis came from migration studies using neocortical cell dissociates and slice cultures (Behar et al., 1999; Hirai et al., 1999; Hirasawa et al., 2003) and from a mutant mouse lacking the Pxr1 import receptor for peroxisomal matrix proteins, in which the neocortical migration defect was caused by NMDA receptor dysfunction (Gressens et al., 2000).
Figure 5. Morphology of biocytin-labeled pyramidal neurons recorded in P14 DMSO-treated control cortex (A1, A2) and P14 MK801-treated rat cortex (B1, B2). The cells shown in (A2) and (B2) are marked by arrow in (A1) and (B1), respectively. Note the cortical malformation in (B1). Action potential discharge pattern elicited by injection of depolarizing current pulses in a pyramidal neuron recorded in a somatosensory cortical slice from a P14 DMSO-treated control (C) and a P14 MK801-treated rat (D). The first action potential from (C1) and (D1) is shown at higher gain in (C2) and (D2), respectively.
In the weaver mutant mouse the migratory failure of cerebellar granule neurons has been also related to an impairment of the NMDA receptor due to the lack of the 2B subunit (Liesi et al., 1999). However, neither transgenic mice lacking the essential R1 subunit of the NMDA receptor (Messersmith et al., 1997) nor mice with a deletion of the NMDAR1 gene restricted to excitatory neocortical neurons (Iwasato et al., 2000) show any obvious abnormalities in the formation of the cortical layers. Since knockout mice may develop compensatory mechanisms, we investigated the role of NMDA receptors in cortical neuronal migration in an in vivo rat model, in which NMDA receptors are transiently blocked during the early neonatal period by local application of MK801 to the primary somatosensory cortex. This manipulation resulted in neuronal migration disorders resembling cortical malformations described in other experimental models (Jacobs et al., 1996; Luhmann and Raabe, 1996; Luhmann et al., 1998; Rafiki et al., 1998; Roper, 1998; Defazio and Hablitz, 2000) and in humans (for reviews, see Gleeson and Walsh, 2000; Monuki and Walsh, 2001). Since these alterations were restricted to the site of the MK801 application, the neuronal migration disorders observed in this in vivo model most likely result from a functional impairment of NMDA receptors during the early postnatal period.

Disturbances in cortical migration do not only arise during conditions of NMDA receptor inhibition or blockade, but also when NMDA receptors are excessively stimulated (Marret et al., 1996; Kihara et al., 2002) or when the extracellular magnesium concentration is reduced (Komuro and Rakic, 1993), suggesting that the cortical migration process may be distorted by any alterations in NMDA receptor function. Since previous studies in different species have demonstrated that neuronal migration is regulated by the intracellular calcium concentration (Komuro and Rakic, 1992, 1996; Horgan and Copenhaver, 1998), the reduction or rise in the calcium influx through NMDA receptors most likely represents one important mechanism to regulate neuronal migration disorders observed in this model. Other modulatory effects of the NMDA receptor may be mediated by somatostatin (Yacubova and Komuro, 2002), GABA and glycine which have a depolarizing action during early development of the cerebral cortex (Luhmann and Prince, 1991; Kilb et al., 2002; Flint et al., 1998; Owens et al., 1996) and which activate voltage-dependent calcium channels (Yuste and Katz, 1991). A modulatory influence of GABA on neuronal migration has been already demonstrated in cortical cell culture systems (Behar et al., 1996, 1998, 2000) and a similar effect under in vivo can be expected. It may well be that NMDAR1 mutant mice show no disturbances in cortical neuronal migration because migrating neurons in these animals compensate for the lack of calcium influx through NMDA receptors by using these alternative routes of intracellular calcium rises (Messersmith et al., 1997).

With the exception of the disturbances in cortical architecture and the accumulation of neuronal cell clusters in layer I, the NMDAR1 immunohistochemistry staining did not reveal any obvious differences between the controls and the MK801-treated animals. The cellular and the overall cortical staining pattern for NMDAR1 was very similar as described previously in developing and mature rat cerebral cortex (Monyer et al., 1994; Aoki et al., 1994; Rema and Ebner, 1996; Hagemann et al., 2003). These data indicate that the transient and localized blockade of NMDA receptors does not induce any obvious alterations in the number or distribution of NMDA receptors. However, changes in receptor affinity, subunit composition or calcineurin-dependent regulation of NMDA receptor channel openings (Lieberman and Mody, 1994) cannot be excluded.

Transient NMDA receptor blockade during early cortical development does not only disturb neuronal migration, but also interferes with apoptotic processes occurring in rodent cerebral cortex during the early postnatal period (Ikonomidou et al., 1999). It cannot be excluded that NMDA receptor blockade prevents the apoptotic degeneration of transient neurons.

Figure 6. Synaptic responses recorded in a P14 DMSO-treated control (A) and P14 MK801-treated rat (B–D). Electrical stimulation of the white matter elicits a monosynaptic fast EPSP in the control (A). Synaptic activation of a neuron recorded in MK801-treated cortex elicits a fast monosynaptic response followed by a delayed polysynaptic response lasting >200 ms (B). The late polysynaptic response recorded MK801-treated animals can be blocked by the NMDA receptor antagonist APV (C) and AMPA/kainate receptor blockade with CNQX blocks the monosynaptic and the late polysynaptic responses (D).
cortical neurons, thereby generating ectopic cell clusters as they have been demonstrated in CPP32-deficient mice with decreased apoptosis (Kuida et al., 1996).

Neonatal NMDA Receptor Blockade Slows Functional Maturation

Application of MK801 to the neonatal somatosensory cortex did not only induce structural modifications in the cortical architecture, but also functional alterations in the single cell and network properties. It has been previously shown that synaptic responses recorded in immature cerebral cortex often contain a late NMDA receptor-mediated component (Luhmann and Prince, 1990b; Agmon and O’Dowd, 1992; Carmignoto and Vicini, 1992) due to developmental changes in the voltage-dependence of the NMDA receptor and subunit composition (Burgard and Hablitz, 1994; Sheng et al., 1994) or due to the delayed maturation of the intracortical GABAergic system (Luhmann and Prince, 1990a). In agreement with these earlier studies we recorded in about one-third of the pyramidal neurons in control cortices a late NMDA receptor-mediated synaptic response. In contrast, almost every neuron in MK801-treated cortex displayed a large polysynaptic response which could be blocked by an NMDA receptor antagonist. These data indicate that the cortical network of MK801-treated animals is preserved in a more immature functional state and that NMDA receptors are activated more easily. A developmental delay in the functional maturation of NMDA receptors has been also observed in rat superior colliculus, which was treated with an NMDA receptor antagonist released from an Elvax implant during the neonatal period (Colonnese et al., 2003). At the age of P12–P20, these animals revealed an increase in the amplitudes and durations of NMDA receptor-mediated synaptic currents suggesting that the current flow through the NMDA receptor is a critical signal in the developmental regulation of its own activity (Colonnese et al., 2003). Further support for the hypothesis that the functional maturation of the neocortical network is delayed in MK801-treated animals comes from our observation that monosynaptic responses recorded in MK801 rats are more fragile and significantly decrease in amplitude during stimulation frequencies of >0.2 Hz. Interestingly, action potential amplitudes were larger in MK801-treated animals when compared to the age-matched controls. Since action potential amplitude of neocortical neurons increases during the neonatal period (McCormick and Prince, 1987), this result may indicate that pyramidal neurons in MK801-treated rats develop some intrinsic mechanisms to compensate for the functional immaturity of glutamatergic synaptic properties.

Since NMDA receptors are modulated by a number of drugs, e.g. ethanol (Yamakura and Shimoji, 1999), drug-induced disturbances in NMDA receptor function during the period of neuronal migration may induce permanent and deleterious alterations in the structure and function of the developing cerebral cortex (Barth, 1987; Liesi, 1997). It remains to be studied to what extent low drug concentrations may interfere with the cortical migration process and whether even subtle migration disorders are linked to cognitive impairments.

Notes

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