Characterization of Neuronal Migration Disorders in Neocortical Structures. II. Intracellular In Vitro Recordings

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Luhmann, Heiko J., Nikolai Karpuk, Meishu Qu, and Karl Zilles. Characterization of neuronal migration disorders in neocortical structures. II. Intracellular in vitro recordings. J. Neurophysiol. 80: 92–102, 1998. Neuronal migration disorders (NMD) are involved in a variety of different developmental disturbances and in therapy-resistant epilepsy. The cellular mechanisms underlying the pronounced hyperexcitability in dysplastic cortex are not well understood and demand further clinical and experimental analyses. We used a focal freeze-lesion model in cerebral cortex of newborn rats to study the functional consequences of NMD. Intracellular recordings from supragranular regular spiking cells in cortical slices from adult sham-operated rats revealed normal passive and active intrinsic membrane properties and normal stimulus-evoked excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs, respectively). Regular spiking neurons recorded in rat dysplastic cortex showed on average a significantly smaller action potential amplitude, a slower spike rise, and a less steep primary frequency-current relationship. Stimulus-elicited EPSPs in NMD-affected cortex consisted of multiphasic burst discharges, which coincided with extracellular field potentials and lasted 150–800 ms. These epileptiform responses could be recorded at membrane potentials negative to 0 mV, suggesting that NMDA receptors are activated at relatively negative membrane potentials. In comparison with the controls, polysynaptic IPSPs mediated by the γ-aminobutyric acid (GABA) type A and B receptor were either absent or reduced in peak conductance in microgyric cortex by 27% (P < 0.05) and 17%, respectively. However, monosynaptic GABAergic neurons in microgyric cortex get a weaker excitatory input. Our data indicate that the expression of epileptiform activity in NMD-affected cortex rather results from an imbalance between excitatory and inhibitory synaptic transmission than from alterations in the intrinsic membrane properties. This imbalance is caused by an increase in NMDA-receptor-mediated excitation in pyramidal neurons and a concurrent decrease of glutamatergic input onto inhibitory interneurons.

METHODS

Induction of focal cortical NMD

The techniques to induce a focal NMD in rat cerebral cortex are based on a model initially described by Dvorak and Feit (1977) and were similar to those reported in our previous paper (Luhmann and Raabe 1996). Newborn Wistar rats (<24 h, n = 28) were anesthetized by hypothermia, and the skin overlying the frontoparietal cortex was cut with a small scalpel. A copper cylinder of 1 mm in diameter was cooled with liquid nitrogen and placed for 8 s on the calvarium above the parietal cortex. Identical second and third freeze lesions were performed at a distance of 1.5 mm from the first lesion in rostral and caudal direction, respectively. These three lesions resulted in a 4- to 6-mm-long microsulcus in rostrocaudal direction. The wound was closed with histoacryl tissue glue (Braun-Dexon, Melsungen, Germany). Sham-operated rats (n =

INTRODUCTION

An increasing number of clinical observations demonstrate that neuronal migration disorders (NMD), such as microgyria, often are associated with therapy-resistant epilepsy (Chugani et al. 1993a; Fusco et al. 1992; Meencke and Janz 1984; Palmini et al. 1991a,b). The cellular mechanisms underlying this pronounced hyperexcitability and the pharmacoresistance of some forms of cortical dysplasia are not completely understood. Recent electrophysiological in vitro studies on neocortical slices obtained from patients with NMD and drug-resistant epilepsy have demonstrated a higher epileptogenesis in dysplastic cortex (Mattia et al. 1995), indicating that this tissue exhibits some intrinsic factors, which profoundly disturb the normal balance between excitatory and inhibitory mechanisms. At the structural level, these factors may include changes in axonal or dendritic morphology, false synaptic connections, increased electrotonic coupling via gap junctions, or modifications in receptor density. Functional disturbances may be related to changes in intrinsic membrane properties or synaptic network interactions. To address these questions, more detailed studies on adequate animal models may add valuable information on the different factors causing hyperexcitability in dysplastic cortex. We recently reported the expression of severe epileptiform activity in a rat model of focal cortical NMD resembling human microgyria (Luhmann and Raabe 1996). Neocortical slices prepared from these rats revealed spontaneous seizure-like discharges and, on electrical stimulation, propagating epileptiform responses. The aim of the present study is the more detailed electrophysiological and pharmacological characterization of the epileptiform activity in this animal model of cortical NMD using in vitro extracellular and sharp microelectrode recordings.

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32) were operated in the same way with the exception that the copper cylinder was not cooled. Animals survived for 1–4 mo before they were used for in vitro electrophysiological experiments.

In vitro slice preparation and recording techniques

The techniques for preparing and maintaining rat neocortical slices in vitro were similar to those described previously (Luhmann and Raabe 1996; Luhmann et al. 1995). Rats were anaesthetized deeply with ether and decapitated with a small-animal guillotine. A tissue block including the primary somatosensory cortex was rapidly removed and stored for 1–2 min in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 3 KCl, 1.25 NaH2PO4, 1.8 MgSO4, 1.6 CaCl2, 26 NaHCO3, and 10 glucose. This ACSF had a pH of 7.4 when saturated with 95% O2-5% CO2. The tissue block was glued to the cutting platform of a Campden vibratome and submerged in oxygenated ice-cold ACSF. Coronal slices were cut at a nominal thickness of 400 μm, trimmed to smaller pieces and transferred to the interface-type recording chamber (34 ± 35°C), to an incubation-storage chamber (32 ± 33°C) or immediately fixed in 4% paraformaldehyde for later histological examination (see further).

Slices were allowed to recover for ≥1 h before recording began. Intracellular recordings were performed in the primary somatosensory cortex at a cortical depth between 100 and 400 μm from the pial surface, corresponding to layers II/III in the normal cortex. In microgyric cortex, recordings were obtained from cells in the same cortical depth located 200–800 μm lateral from the pseudosulcus (Figs. 1 and 2C). Simultaneous extra- and intracellular recordings were performed only in microgyric cortex (n = 13). Extracellular field potentials were obtained close to the intracellular recording site with ACSF-filled electrodes (2–5 MΩ). Intracellular microelectrodes were pulled with a horizontal puller (Brown-Flaming P-87, Sutter Instruments, Novato, CA), backfilled with 2 M potassium acetate (pH 7.4) and varied in resistance between 60 and 100 MΩ. For morphological identification of the recorded neuron, 1–2% biocytin (Sigma) was added to the pipette solution and injected into the cells (n = 19) by depolarizing current pulses (for details, see Schröder and Luhmann 1997). Intracellular electrodes were connected to a high-impedance amplifier (IR-283, Neuro Data Instruments, New York, NY). The signals were displayed on an oscilloscope and stored on-line at 10 kHz on a computer by use of the Wintida software (HEKA Instruments, Lam-brecht, Germany). For orthodromic synaptic stimulation, a bipolar stimulating electrode was positioned 300–600 μm lateral to the recording site (Fig. 1). Previous studies have shown that this stimulus protocol reliably evokes excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs, respectively) in supragranular neurons (Howe et al. 1987). Electrical stimuli of 200-μs duration and 2- to 30-V amplitude were delivered to the stimulating electrode at a frequency of ≤0.1 Hz. EPSPs were elicited with low-intensity electrical stimulation. IPSPs were evoked by raising the stimulus intensity to twice the threshold to activate an action potential at resting membrane potential.

Histological procedures

The location, size, and type of the lesion was studied in more detail by resectioning the 400-μm-thick fixed slices after cryoprotection with 30% sucrose on a freezing microtome at 60°C. These sections were mounted on glass slides and Nissl stained to identify laminar boundaries by use of a Zeiss Axioskop (Fig. 1). Slices containing biocytin-filled cells were fixed overnight in 4% paraformaldehyde, washed in 0.1 M phosphate buffer, and cryoprotected with 30% sucrose. Resected slices were processed with ABC peroxidase reagent (PK-4000 kit, Vector labs) and intensified with OsO4 (for details, see Schröder and Luhmann 1997) (Fig. 2).

Data analysis and pharmacology

Neurons were analyzed in their intrinsic membrane properties and their synaptic inputs. The resting membrane potential was determined by measuring the difference between the intra- and extracellular recorded potential. The neuronal input resistance was calculated according to Ohm’s law from the ratio of the voltage deflection (10–20 mV) versus the injected current. The spike amplitude was measured from the resting membrane potential baseline to the peak of the first action potential elicited by suprathreshold current injection. This action potential was differentiated with respect to time to determine the maximum rate of spike rise and fall. The primary f-I slope was calculated from a plot of the first interspike interval versus the magnitude of the injected current. Stim-
FIG. 2. Morphology of intracellular biocytin-filled upper layers pyramidal neurons in Nissl-stained coronal sections of parietal cortical slices from sham-operated (A and B) and freeze-lesioned (C and D) rat. Filled arrows point to position of labeled cells and pseudosulcus is marked by an open arrow. Scale bar in A indicates 500 μm for A and C, and scale bar in B corresponds to 100 μm in B and D.

ULUS-EVOKED EPSPs were measured at resting membrane potential in their peak amplitude, and the presence of any epileptiform activity was quantified by calculating the recurrent excitation index (REI) according to the following formula (for details, see Mittmann et al. 1994)

\[
\text{REI} = 1 - \frac{(\text{EPSP1} - \text{EPSP2})}{\text{EPSP1}}
\]

(where EPSP1 = peak amplitude of 1st EPSP and EPSP2 = peak amplitude of largest EPSP after EPSP1). Whereas for a normal monophasic EPSP, this parameter would be 0, a REI > 0 would indicate a pathophysiological response pattern consisting of at least two EPSPs. Stimulus-evoked IPSPs were studied in their reversal potential and peak conductance by linear regression analysis (for details, see Connors et al. 1988; Luhmann et al. 1995). The NMDA antagonist dl-2-amino-5-phosphonovaleric acid (APV, Sigma) and the (+)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) antagonist 6-nitro-7-sulfamoyl-benzo(f)quinoxaline-2,3-dione (NBQX, Novo Nordisk) were dissolved in ACSF in a concentration of 300 and 100 μM, respectively, and applied locally on the slice surface close to the recording site via a broken micropipette with a tip diameter of 5–10 μm.

For statistical analysis, the Student's t-test was performed on the data. Values throughout this report are given as means ± SE.

RESULTS

Anatomy of microgyric cortex and intracellular labeling

A focal freeze lesion performed on the parietal cortex of the newborn rat reliably produced a malformation that resembled in its structure the pathology of the human microgyric cortex (Aicardi 1991; Kuzniecky and Barkovich 1996; Taylor et al. 1971). The irregular cortical architecture at the site of the lesion can be described best by the existence of a pseudosulcus and a loss of the normal six-layered lamination (open arrow in Fig. 1). This malformation was microscopi-
TABLE 1. Intrinsic membrane properties in normal and microgyric rat cortex

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>NMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential, mV</td>
<td>-83 ± 1 (51)</td>
<td>-80.4 ± 1.7 (49)</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>39.9 ± 2.1 (50)</td>
<td>41.6 ± 1.9 (49)</td>
</tr>
<tr>
<td>Spike amplitude, mV</td>
<td>109 ± 1.6 (51)</td>
<td>100.2 ± 2 (49)**</td>
</tr>
<tr>
<td>Maximum rate of spike rise, V/(s)</td>
<td>276 ± 8.8 (51)</td>
<td>208.2 ± 10 (47)**</td>
</tr>
<tr>
<td>Maximum rate of spike fall, V/(s)</td>
<td>79.8 ± 2.5 (51)</td>
<td>75.2 ± 3.2 (47)</td>
</tr>
<tr>
<td>(dV/dt) ratio</td>
<td>3.51 ± 0.09 (51)</td>
<td>2.93 ± 0.15 (47)**</td>
</tr>
<tr>
<td>Primary (f-I) slope, Hz/(nA)</td>
<td>156 ± 10.2 (30)</td>
<td>93.5 ± 11 (30)**</td>
</tr>
</tbody>
</table>

Passive and active intrinsic membrane properties in upper layer regular spiking cells in parietal cortical slices from adult sham-operated rats (control) and age-matched animals with focal neuronal migration disorder (NMD). All data are expressed as means ± SE and number of cells are given in parentheses. Statistically significant differences are indicated by * \(P < 0.05\), ** \(P < 0.01\), and *** \(P < 0.001\).

Excitatory synaptic interactions

Whereas orthodromic synaptic stimulation in control cortices elicited a normal EPSP and biphasic IPSP, comparable stimuli to the microgyric cortex evoked severe epileptiform responses. A typical sequence of normal EPSPs recorded in 29 of 29 cells in sham-operated cortex in response to increasing stimulus intensities is illustrated in Fig. 4A. At resting membrane potentials between -80 and -90 mV, orthodromic synaptic stimulation elicited an initial monophasic EPSP of 30–80 ms in duration (Fig. 4A, 1–4) that, at higher stimulus intensity, reached the threshold for activation of one fast action potential (Fig. 4A5). For all recordings shown in Fig. 4A, 1–4, the REI amounts to 0. Analogous protocols in NMD-affected cortices evoked at low stimulus intensities in 26 of 43 cells (60%) a long-lasting (150–800 ms) multiphasic EPSP, which triggered one or several action potentials at latencies >100 ms (Figs. 4B, 1–4, and 5, A–C). Higher stimulus intensities elicited an early action potential that was followed by a prolonged epileptiform EPSP (Figs. 4B5 and 5D). This long-lasting EPSP could evoke several action potentials (Figs. 5D and 6C1). The estimated REI of neurons recorded in microgyric cortex was 1.64 ± 0.2 \((n = 16)\), suggesting the existence of a second EPSP with a larger amplitude in the majority of neurons. Simultaneous intra- and extracellular recordings of stimulus-evoked synaptic activity in microgyric cortex revealed normal responses in 2 of 13 cases and clear epileptiform activity in the remaining 11 dual recordings (Fig. 5). In these 11 cases, the late intracellular bursts coincided with large extracellular responses, suggesting that these events were generated by a large neuronal network. In the remaining 17 of the 43 cells (40%) recorded in NMD cortex, orthodromic synaptic stimulation at resting membrane potential did not evoke any polysynaptic epileptiform activity. However, these neurons showed long-lasting EPSPs on depolarization (Fig. 6B1).

The voltage dependence and pharmacology of these unusual EPSPs recorded in microgyric cortex was studied in more detail by recording EPSPs at different membrane potentials and sequential application of antagonists to the NMDA and AMPA receptor. In sham-operated cortex, stimulus-evoked EPSPs showed the typical voltage dependence with a second component being activated at more depolarized potentials (Fig. 6A1). This second component was blocked by APV, indicating that this response was mediated by NMDA receptors (Fig. 6A2). The remaining early response was blocked by addition of NBQX, suggesting that AMPA receptors mediated this component (Fig. 6A3). In cortical slices from age-matched rats showing a focal NMD, stimulus-evoked EPSPs recorded at depolarized potential consisted of a large and long-lasting APV-sensitive component (cf. -60 mV trace in Fig. 6B1 with -62 mV trace in Fig. 6B2) and an early NBQX-sensitive response (cf. Fig. 6B2 with 6B3). In neurons that showed strong polysynaptic epileptiform responses at resting membrane potential, low-intensity stimulation elicited long-lasting (>100 ms) burst discharges at all potentials between -54 and -112 mV (Fig. 6C1). Application of APV completely blocked these epileptiform responses, indicating a participation of NMDA receptors in generating this polysynaptic activity (Fig. 6C2). Addition
FIG. 3. Intracellular recordings from an upper layer regular spiking neuron in the primary somatosensory cortex of an adult sham-operated rat (A) and an age-matched animal with neuronal migration disorder (B). A1 and B1: voltage response to injection of 2 hyperpolarizing and depolarizing current pulses at a resting membrane potential of −92 and −90 mV, respectively. A2 and B2: 1st action potential of the cell’s response shown in A1 and B1 to injection of a suprathreshold depolarizing current pulse. A3 and B3: action potentials shown in A2 and B2 differentiated with respect to time. Note slower rate of action potential rise and fall of the cell recorded in the microgyric cortex.

FIG. 4. Stimulus-evoked excitatory postsynaptic responses in a regular spiking neuron recorded in a sham-operated rat (A) and an age-matched animal with neuronal migration disorder (B) at a resting membrane potential of −82 and −88 mV, respectively. Stimulus intensity in A and B was increased gradually from trace 1 to 5 to show responses with a comparable initial amplitude. Note delayed discharge in B3 and B4 and long-lasting response in B5. Stimulus artifacts are erased.
of NBQX blocked the remaining early EPSP (Fig. 6C3). The functional role of NMDA receptors in microgyric cortex was analyzed in more detail by recording monosynaptic EPSPs in NBQX-containing solution \( (n = 13) \). Under these conditions, a late voltage-dependent and APV-sensitive \( (n = 6) \) response can be recorded at membrane potentials between \(-70\) and \(-95\) mV (Fig. 7, A and B), indicating that NMDA receptors are activated over a large voltage range in NMD cortex (Fig. 7C, ○).

**Inhibitory synaptic transmission**

Suprathreshold orthodromic synaptic stimulation elicited in layers II/III neurons in sham-operated cortex a normal biphasic IPSP, consisting of the fast \((f-)\) IPSP with a reversal potential of \(-66 \pm 2\) mV, followed by a late \((l-)\) IPSP, which reversed polarity at \(-83 \pm 1.9\) mV \((n = 29)\) (Fig. 8A1). Application of APV neither significantly affected the average reversal of the \(f\)-IPSP \((-68 \pm 3.2\) mV) nor of the \(l\)-IPSP \((-87 \pm 4.2\) mV, \(n = 6)\) (Fig. 8A2). In microgyric cortex, orthodromic stimulation elicited a \(f\)-IPSP with a similar reversal potential \((-64.1 \pm 1.9\) mV), but a significantly \((P < 0.05)\) smaller peak conductance \((44.5 \pm 4.3\) nS, \(n = 23)\; Fig. 8B1) when compared with the sham-operated controls \((60.6 \pm 5.5\) nS, \(n = 29)\). The \(l\)-IPSP recorded in NMD-affected cortices did not significantly differ in its reversal potential \((-79.1 \pm 2.6\) mV) or conductance \((15.8 \pm 2.3\) nS, \(n = 23)\) from the \(l\)-IPSP in controls \((19.1 \pm 1.9\) nS, \(n = 29)\).

These data indicate that \(\gamma\)-aminobutyric acid-A (GABA\(_A\))-receptor–mediated inhibition was significantly impaired in microgyric cortex. As in controls, application of APV did not significantly affect the reversal potential of the \(f\)-IPSP \((-65 \pm 2.9\) mV) and \(l\)-IPSP \((-84 \pm 2.6\) mV, \(n = 13)\) recorded in cortical slices with NMD (Fig. 8B2). Figure 8C1 illustrates a cell in microgyric cortex recorded in ACSF with no apparent hyperpolarizing \(f\)- or \(l\)-IPSP. Addition of APV uncovered a weak biphasic IPSP (Fig. 8C2), indicating that a strong NMDA component contributed to the postsynaptic response in normal ACSF. For a more detailed analysis of the GABAergic function in sham-operated and microgyric cortex, we measured the peak conductances of both IPSPs in ACSF (so-called polysynaptic IPSPs; see row 1 in Fig. 8) and after blockade of ionotropic glutamate receptors with APV and NBQX (monosynaptic IPSPs; see row 3 in Fig. 8).

In eight cells recorded in sham-operated rats, the conductance of the \(f\)- and \(l\)-IPSP in ACSF amounted to \(60.9 \pm 9.6\) and \(24.6 \pm 5.3\) nS, respectively. After addition of APV and NBQX, two of these eight cells showed no PSP, indicating that in these neurons the stimulating electrode did not activate the monosynaptic inhibitory input. In the remaining six cells, the conductance of the monosynaptic \(f\)- and \(l\)-IPSP was \(16.7 \pm 1.6\) and \(7.5 \pm 0.5\) nS, respectively (Fig. 8A3). In microgyric cortex, the polysynaptic \(f\)- and \(l\)-IPSP recorded in six cells in ACSF amounted to \(41.5 \pm 6\) and \(21.5 \pm 6.5\) nS, respectively. As in controls, two cells in microgyric cortex revealed no or only a very weak monosynaptic IPSP when APV and NBQX were added to the bathing solution (Fig. 8B3). However, the remaining four cells showed a monosynaptic \(f\)- and \(l\)-IPSP conductance \((17.1 \pm 3.3\) and \(8.2 \pm 1.5\) nS, respectively; Fig. 8C3), which was very simi-
FIG. 6. Voltage dependence and pharmacology of excitatory postsynaptic potentials (EPSPs) recorded in normal sham-operated cortex (A) and cortex displaying neuronal migration disorder from 2 animals (B and C). A1–C1: responses to low-intensity orthodromic stimulation in control bathing solution. A2–C2: responses to identical stimulus intensities as in control, but after addition of dl-2-amino-5-phosphonovaleric acid (APV). A3–C3: same as control, but in bathing solution containing APV and 6-nitro-7-sulfamoyl-benzo(f)quinoxaline-2,3-dione (NBQX).

lar as in the controls. These data suggest that monosynaptic inhibition is intact, but that the excitatory input onto GABAergic interneurons is reduced in microgyric cortex.

**DISCUSSION**

The main results of our study are that 1) focal freeze lesions in the cerebral cortex of newborn rats cause permanent modifications in the cortical architecture that resemble, in many aspects, the malformations described in human microgyric cortex. 2) In correspondence with clinical observations in humans with cortical NMD, the experimentally induced microgyric cortex in rats shows a pronounced hyperexcitability as expressed in stimulus-evoked long-lasting epileptiform responses. 3) This recurrent pathophysiological activity can be reduced by antagonists acting at the NMDA receptor. 4) The presence of monosynaptic APV-sensitive EPSPs at membrane potentials negative to −60 mV indicate that NMDA receptors in microgyric cortex are activated at relatively negative potentials. 5) Polysynaptic GABA<sub>α</sub>-receptor–mediated IPSPs are significantly reduced in microgyric cortex. However, monosynaptic IPSPs recorded in the presence of NBQX and APV show a similar conductance in NMD cortex and controls, suggesting that GABAergic interneurons in microgyric cortex get a decreased glutamatergic input. And 6) intrinsic membrane properties of neurons recorded in NMD-affected cortex differ from those obtained in sham-operated controls, but these changes would rather restrain cortical excitability in the former. Our data indicate, that the pronounced hyperexcitability observed in microgyric cortex is predominantly mediated by a NMD-induced imbalance in excitatory and inhibitory synaptic transmission.

**Intrinsic membrane properties**

Pyramidal neurons recorded in rat microgyric cortex revealed the same resting membrane potential and input resistance as compared with the controls. These data suggest that the sharp microelectrode impalements did not induce any cellular injury in neurons recorded in freeze-lesioned cortex. Significant differences between sham-operated and freeze-lesioned rat cortex could be observed for some active membrane properties. Action potentials recorded from neurons in microgyric cortex were significantly smaller in amplitude and slower in rise time kinetics when compared with the controls. These parameters are typical properties of developing cortical neurons (McCormick and Prince 1987), suggesting that neurons recorded in microgyric cortex may maintain some fea-
works affected by NMD (see INTRODUCTION). This apparent discrepancy suggests that the factors mediating enhanced excitability in microgyric cortex may be rather based on modifications in synaptic interactions than on changes in passive or active intrinsic membrane properties.

Augmentation of NMDA-receptor–mediated excitation

Stimulus-evoked EPSPs recorded from layers II/III regular spiking cells in sham-operated cortex exhibited the typical monophasic appearance, voltage dependence, and NMDA-receptor–mediated late component on depolarization of the membrane (Hwa and Avoli 1992; Thomson 1986). In contrast, EPSPs of regular spiking cells in microgyric cortex were characterized by their long-duration, multiphasic components, burst discharge, and sensitivity to APV. The coincidence of the EPSPs with large extracellular field potentials supports the conclusion that these events represent network-driven epileptiform responses. A very similar activity pattern has been described by Mattia et al. (1995) in human dysplastic cortex analyzed in vitro with extra- and intracellular recording techniques, suggesting that the rat freeze-lesion model replicates not only the structural but to some extent also the functional abnormalities of the human cortex affected by NMD. Our intracellular observations are further in good agreement with previous observations by Jacobs et al. (1996), who reported long-lasting and APV-sensitive epileptiform responses in the same rat model of cortical microgyria. However, our own previous observations with eight extracellular recording electrodes have demonstrated that NMDA receptors play no role in the widespread lateral propagation (>4 mm) of epileptiform activity originating at the site of the microgyrus (Luhmann and Raabe 1996). From these data, we conclude that NMDA receptors are modified in a relatively restricted area at the site of the malformation and not in the remote cortical regions. This area may serve as a trigger zone for the generation of epileptiform activity, which then is transmitted via horizontal connections and AMPA-receptor activation to remote regions. This hypothesis is supported by the results of Jacobs et al. (1996), who demonstrated that APV-sensitive epileptiform activity can be elicited only in a 1- to 2-mm-wide belt surrounding the microgyrus but not by identical stimulation of remote areas. These and our own data suggest that NMDA receptors are modified in this belt region. The NMDA-receptor–mediated responses recorded in this area resemble the EPSPs described in juvenile rat cortex (Burgard and Hablitz 1993a,b; Luhmann and Prince 1990a; Vilagí et al. 1996) and the epileptiform EPSPs observed in different chronic pathophysiological models, such as the aluminum (Prince and Futamachi 1970) and cobalt (Pumain 1981) epilepsy model, the “undercut cortex” (Prince and Tseng 1993), and the cerebral cortex after a focal lesion (Mittmann et al. 1994), stroke (Mittmann et al. 1998), or global forebrain ischemia (Luhmann et al. 1995) (for review, see Luhmann 1996). In these cases and in the freeze-lesion model, a reduced magnesium sensitivity (Ben-Ari et al. 1988; Burgard and Hablitz 1994; Hori and Carpenter 1994; Morrisett et al. 1990), different glycine regulation (Kleckner and Dingledine 1991) or an upregulation in the density of functional...
NMBA receptors (Qü et al. 1998) may have contributed to the augmentation of NMDA-receptor-mediated activity. Changes in the NMDA-receptor subunit composition (Feldmeyer and Cull-Candy 1996; Flint et al. 1997; Monyer et al. 1994; Sheng et al. 1994) may mediate some of these long-term modifications and the unusual voltage dependence of the NMDA component of the EPSPs in microgyric cortex. However, similar prolonged NMDA-receptor-mediated responses also can be observed under conditions of partial disinhibition (Chagnac-Amitai and Connors 1989; Luhmann and Prince 1990b) or in the juvenile cortex with immature GABAergic function (Agmon and O’Dowd 1992; Luhmann and Prince 1991), indicating that inhibition also may be impaired in the microgyric cortex.

Impairment of GABAergic inhibition

Stimulus-evoked hyperpolarizing IPSPs in freeze-lesioned cortex were either absent or smaller in conductance when compared with the sham-operated controls. GABA_B-receptor-mediated inhibition was reduced significantly by 27% in microgyric cortex. Previous studies have shown that a bicuculline-induced disinhibition by 10–20% is already sufficient to induce propagating epileptiform activity in cortical slices prepared from normal adult rats (Chagnac-Amitai and Connors 1989). A hyperexcitability in the rat freeze-lesion cortex also has been reported by Jacobs et al. (1996) and partly been attributed to a loss of parvalbumin-containing, presumably inhibitory interneurons in the aberrant cortex. A reduction in the density of parvalbumin-immunoreactive neurons also has been observed in different types of experimentally induced cortical malformations in the rat, including the focal freeze-lesion model (Ferrer et al. 1993). The same group also reported a reduction of parvalbumin-, calbindin D_28k- and somato-statin-immunoreactive cells and fibers in dysplastic cortical specimen obtained during neurosurgical procedures from patients showing focal NMD and suffering from pharmaco-resistant epilepsy (Ferrer et al. 1992, 1994). These anatomic results would further support the hypothesis that cortical malformations due to NMD are associated with modifications in the GABAergic system. Our electrophysiological observations on the rat freeze-lesion model may give some further insights into the mechanisms underlying this disinhibition. Intracortical inhibition in microgyric cortex is clearly reduced and our intracellular recordings under blockade of glutamatergic transmission indicate that this functional disinhibition partially results from a decrease in excitatory drive of GABAergic neurons. A similar mechanism has been described for the CA1 hippocampal region during short-term hypoxia (Congar et al. 1995; Khazipov et al. 1993). Under this condition, inhibitory interneurons are disconnected transiently from their glutamatergic input (Congar et al. 1995). Whether additional mechanisms, e.g., a decrease in GABA-stimulated chloride uptake (Lewin et al. 1989), a reduction in GABA_A-receptor phosphorylation (Chen et al. 1990; Stelzer et al. 1988), or a decreased potency and/or efficacy of GABA in activating chloride channels (Kapur and Coulter 1995), also contribute to the hyperexcitability in NMD cortex remains to be studied. Our own studies on the

FIG. 8. Voltage-dependence and pharmacology of inhibitory postsynaptic potentials in sham-operated cortex (A) and in cortex from 2 rats with neuronal migration disorder (B and C). A1–C1: responses to suprathreshold orthodromic stimulation in normal bathing solution. A2–C2: same as control but after addition of APV. A3–C3: responses to identical stimulus intensities in APV and NBQX containing bathing solution.
GABA \textsubscript{A} receptor distribution in rat microgyric cortex revealed a significant reduction in muscimol binding (Zilles et al. 1998), suggesting that postsynaptic mechanisms also are involved in the loss of GABAergic function in NMDA-affected cortex.

**Clinical relevance**

Cortical malformations due to NMD often are associated with severe or drug-resistant epilepsy (Aicardi 1994; Palmini et al. 1991b) and a reduction or control of seizure activity can be only obtained by removal of the structural malformations (Palmini et al. 1991a). The question arises of why NMD-related epilepsy shows this pronounced insensitivity to currently available anticonvulsants. Our observations in the rat freeze-lesion model suggest that the hyperexcitability is caused by an increase in NMDA-receptor–mediated excitation and a simultaneous decrease in GABA \textsubscript{A} receptor–mediated inhibition. However, other possibly nonsynaptic mechanisms have to be considered as additional factors contributing to the resistance to conventional drug therapy. These factors include abnormal cellular morphology and connections as they have been described in human dysplastic cortex (Battaglia et al. 1996; Belichenko et al. 1994; Marco and DeFelipe 1997).

We thank P. Schwarz for excellent technical assistance. This work was supported by Deutsche Forschungsgemeinschaft Grants SFB 194/B4 to H. J. Luhmann and SFB 194/A6 to K. Zilles. Address for reprint requests: H. J. Luhmann, Institute of Neurophysiology, University of Düsseldorf, PO Box 101007, D-40001 Düsseldorf, Germany.

Received 28 January 1998; accepted in final form 17 March 1998.

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