Role of Persistent Sodium Current in Bursting Activity of Mouse Neocortical Networks In Vitro

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Van Drongelen, Wim, Henner Koch, Frank P. Elsen, Hyong C. Lee, Ana Mrejeru, Erin Doren, Charles J. Marcuccilli, Mark Hereld, Rick L. Stevens, and Jan-Marino Ramirez. Role of persistent sodium current in bursting activity of mouse neocortical networks in vitro. J Neurophysiol 96: 2564–2577, 2006. First published July 26, 2006; doi:10.1152/jn.00446.2006. Most types of electrographic epileptiform activity can be characterized by isolated or repetitive bursts in brain electrical activity. This observation is our motivation to determine mechanisms that underlie bursting behavior of neuronal networks. Here we show that the persistent sodium (NaP) current in mouse neocortical slices is associated with cellular bursting and our data suggest that these cells are capable of driving networks into a bursting state. This conclusion is supported by the following observations. 1) Both low concentrations of tetrodotoxin (TTX) and riluzole reduce and eventually stop network bursting while they simultaneously abolish intrinsic bursting properties and sensitivity levels to electrical stimulation in individual intrinsically bursting cells. 2) The sensitivity levels of regular spiking neurons are not significantly affected by riluzole or TTX at the termination of network bursting. 3) Propagation of cellular bursting in a neuronal network depended on excitatory connectivity and disappeared on bath application of CNQX (20 μM) + CPP (10 μM). 4) Voltage-clamp measurements show that riluzole (20 μM) and very low concentrations of TTX (50 nM) attenuate NaP currents in the neural membrane within a 1-min interval after bath application of the drug. 5) Recordings of synaptic activity demonstrate that riluzole at this concentration does not affect synaptic properties. 6) Simulations with a neocortical network model including different types of pyramidal cells, inhibitory interneurons, neurons with and without NaP currents, and recurrent excitation confirm the essence of our experimental observations that NaP conductance can be a critical factor sustaining slow population bursting.

INTRODUCTION

Epilepsy is a common neurological disease characterized by chronic seizures, affecting nearly 1% of the world population. Seizure activity in the neocortex plays a major role in children with intractable epilepsy and a better understanding of neocortical physiology is critical for the development of a rational approach to anticonvulsant therapy. One of the major characteristics of most types of epileptiform behavior is a rhythmic pattern of electrical discharges of the neuronal population. In principle these discharges may be caused by intrinsic neuronal mechanisms, network function, or—most likely—both network and cellular properties. Increasing evidence suggests that epileptiform activity originates within the neocortex (Timofeev and Steriade 2004). When considering network bursting and different cell types known to occur in neocortex, the intrinsically bursting cell type (Connors and Gutnick 1990; Gray and McCormick 1996; Steriade et al. 1998) seems especially interesting. Intrinsically bursting neurons, in particular the so-called fast rapid bursting neurons, have been hypothesized to play an important role in epileptogenesis and various forms of neocortical activity (Blatow et al. 2003; Cunningham et al. 2004; Timofeev and Steriade 2004).

Several studies indicated that intrinsic properties such as the persistent sodium currents may play a role in bursting and oscillatory behavior (e.g., Mantegazza et al. 1998; Timofeev et al. 2000a, 2004; Traub et al. 2003). In animal models it was established that intrinsically bursting neurons are increased in seizure tissue (Jacobs et al. 1999; Sanabria et al. 2002; Steriade et al. 2001; Timofeev et al. 2000b). Indeed, recent evidence indicates that this cell type occurs in neocortex of pediatric patients with epilepsy (Foehring and Wyler 1990; Van Drongelen et al. 2003b). The simplest scenario is a unidirectional path from cell to network, where a bursting neuron paces the activity of a neuronal population. This “pacemaker hypothesis” was first raised in 1949, when Bremer postulated that neurons with intrinsic pacemaker or bursting properties contribute to the generation of neocortical EEG activity (Bremer 1949). However, a better understanding of how neuronal networks generate rhythmic activity led to a major modification of a pure “pacemaker hypothesis” because several studies indicated that bursting and synaptic properties interact in concert (e.g., Steriade et al. 1998, 2001; Traub et al. 2001, 2003). Unfortunately, the close interaction between these properties makes it extremely difficult or even impossible to determine the relative contribution of these properties in functional neuronal networks. The finding that the strength of synaptic and intrinsic bursting properties is not fixed but dynamically modulated by endogenously released neuromodulators or by manipulations such as deafferentation further complicates the question to what extent intrinsic and synaptic properties contribute to rhythm generation (Foehring et al. 2002; Peña and Ramirez 2004; Topolnik et al. 2003).

The difficulty of determining the relative contribution of synaptic and bursting properties to the generation of a given activity pattern was overcome in some small invertebrate...
neuronal networks that were particularly amenable to a rigorous cellular and systems level analysis (Marder and Calabrese 1996). The increased difficulty of dissecting synaptic and bursting properties in mammalian networks created considerable uncertainty as to how intrinsic and synaptic properties interact to generate rhythmic activity. This is an issue of general interest because bursting neurons exist almost everywhere in the mammalian nervous system (Ramirez et al. 2004) including the thalamus, basal ganglia, spinal cord (Darbon et al. 2004), medulla (Peña and Ramirez 2004), hypothalamus, olfactory bulb, ventral tegmentum, hippocampus (Sanabria et al. 2001), and neocortex (Connors and Gutnick 1990; Dégenètias et al. 2003; Istvan and Zarzecki 1994; Nowak et al. 2003). Although some researchers hypothesize that these neurons are primarily responsible for the generation of rhythmic activity in areas where bursting neurons are found (for a review see Arshavsky 2003), other studies claim that bursting neurons or pacemaker activity plays no obligatory role in the generation of a given rhythmic activity. Prominent examples are the generation of the respiratory rhythm (Del Negro et al. 2005), the slow neocortical oscillations that might underlie sleep (Sanchez-Vives and McCormick 2000), and the slow oscillations generated in the hippocampus (Staley et al. 2001), all of which are believed to be primarily the result of emergent network (i.e., synaptic) properties.

In the present study we examined the role of bursting neurons in neocortical networks by selectively blocking persistent sodium (NaP) currents of pyramidal neurons in brain slices obtained from the neocortex of neonatal mice (P8–P13), while observing whether this blockade affects the generation of spontaneously generated slow network oscillations (0.1–1 Hz) described previously by Van Drongelen et al. (2003a). The spontaneously active isolated neocortical network enabled us to pharmacologically manipulate cellular and network properties, a process that is more difficult under in vivo conditions. Our data suggest that blockade of NaP currents with low concentrations of tetrodotoxin (TTX) or riluzole simultaneously reduces bursting activity of intrinsically bursting pyramidal neurons and spontaneous bursting of neocortical networks. Riluzole is known to have a variety of effects in a variety of systems at higher concentrations (O’Neill et al. 2004), other studies claim that bursting neurons or pacemaker activity plays no obligatory role in the generation of an emergent rhythmic activity close to 0.2 Hz.

**METHODS**

**Neocortical slices**

Neonatal (P8–P13) male or female CD-1 mice (n = 105) were deeply anesthetized and decapitated at the C3/C4 spinal level and the forebrain was isolated in ice-cold artificial cerebrospinal fluid (ACSF). One hemisphere was then glued onto an agar block with its rostral end up and mounted onto a vibrating tissue slicer. Coronal slices (500 μm for current-clamp and 350 μm for voltage-clamp experiments) of the somatosensory cortex were transferred into a recording chamber and submerged under a stream of ACSF (temperature, 30°C; flow rate, 10 ml/min) containing (in mM) 118 NaCl, 3 KCl, 1.5 CaCl2, 1 MgSO4, 25 NaHCO3, 1 NaH2PO4, and 30 d-glucose equilibrated with carbogen (95% O2-5% CO2). In all current-clamp and extracellular experiments, the potassium concentration was routinely raised from 3 to 5 mM over 30 min to obtain spontaneous rhythmic activity close to 0.2 Hz.

**Extracellular and intracellular current-clamp recording**

To obtain a signal containing multiunit action potential (AP) activity, extracellular signals were amplified 10,000-fold and filtered between 0.25 and 1.5 kHz (Fig. 1B, bottom trace). To facilitate detection of bursts, this signal was rectified and integrated by using an electronic integrator with a time constant of 50 ms. By applying this procedure we obtained an index for multiunit AP activity in which bursts were easily distinguished (Fig. 1B, top trace). Both the extracellular recording and the integration were performed with home-built circuitry.

Intracellular current-clamp recordings were obtained from cortical neurons with the blind-patch technique. The patch electrodes were manufactured from filamented borosilicate glass tubes (Clarke GC 150TF), filled with a solution containing (in mM): 140 K-glucocic acid, 1 CaCl2·6H2O, 10 EGTA, 2 MgCl2·6H2O, 4 Na2ATP, and 10 HEPES. The intracellular pipettes contained biocytin (4.5 mg/ml) to allow for identification of neuron location and morphology. Electrodes with a positive pressure of 35–50 mmHg were penetrated deep into the slice in 10-μm steps by using a piezo-driven micromanipulator (Böhm, Neustadt, Germany). As the electrode approaches the cell, the measured electrode resistance increased. To obtain a Giga seal, we removed the positive pressure and applied negative suction. Recordings were low-pass filtered (0–2 kHz, Bessel four-pole filter, –3 dB) (Fig. 1C). Because the extracellular and intracellular electrodes were in close proximity to each other (within 150 μm), the extracellular signal was used as an index for the activity of the surrounding population (network) of neurons. After recording, the slices were placed in a paraformaldehyde solution for subsequent staining procedures.

**Voltage-clamp recordings**

Pyramidal neurons in layer 5 of the cortex were visually identified (Zeiss Axioskop 2 FS microscope with IR-DIC). Whole cell patch-clamp recordings (Fig. 1D) were obtained with a sample frequency of 10 kHz and a low-pass filter setting of 2 kHz. Recordings were made with unpolished patch electrodes, manufactured from borosilicate glass pipettes with filament (Warner Instruments G150F-4). The electrodes had a resistance of 3–5 MΩ when filled with the whole cell patch-clamp pipette solution containing (in mM) 110 CsCl, 30 TEA-Cl, 1 CaCl2, 10 EGTA, 2 MgCl2, 4 Na2ATP, and 10 HEPES (pH 7.2). The patch-clamp experiments were performed with a patch-clamp amplifier (AxoPatch 200B), a digitizing interface (Digidata 1322A), and the software program pClamp 9.2 (Axon Instruments).
Neurons located at least three to four cell layers (about 80–150 μm) caudal from the rostral surface of the slice were recorded under visual control. Neurons located directly at the slice surface were not examined because they were more likely to be severely damaged during the preparation than were neurons located deeper within the slice. Current-response traces were recorded with either off- or on-line leak subtraction (P/4 protocol), eliminating the linear leak current and residual capacity currents. The 2-mV liquid junction potential was manually subtracted with the amplifier’s pipette offset regulator immediately before establishing the patch-clamp configuration. The series resistance was always 80% compensated and regularly corrected throughout the experiments. We emphasize that whole cell voltage-clamp recordings from neurons embedded in a functional network are accompanied by difficult space-clamp control. This could lead to incorrect values for current amplitudes. Thus recordings with obvious space-clamp problems (Armstrong and Gilly 1992) were discarded. Poor space clamping was indicated by rebound spikes (rapid, fast inactivating inward currents, which were induced by steps from depolarizing test potentials to the former holding potential) or an increase in the delay to onset of an inward current with increasing magnitude of test pulse. Steps to higher test potentials were typically associated with a reduction in delay to current onset. We also discarded neurons with insufficiently blocked K⁺ currents, evident in outward currents typically commencing at voltage steps to 10 mV.

Using the conventional patch-clamp technique, we pharmacologically isolated both the voltage-activated calcium and the voltage-activated sodium currents by intracellular blockade of voltage-activated potassium currents with 110 mM CsCl and 30 mM tetraethylammonium (TEA) chloride. As shown in a previous study (Elsen and Ramirez 1998), this blockade was sufficient for the investigation of the maximal inward current amplitude. In addition, voltage-activated calcium currents were blocked by extracellular bath application of 200 μM cadmium chloride (CdCl₂) to isolate the Na⁺ current.

**Synaptic input measurements**

To examine potential synaptic effects of riluzole at a concentration of 20 μM, we eliminated AP-evoked synaptic events and added 1 μM TTX to the ACSF. Miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs, respectively) were recorded and digitized for a period of 4 min per data file. Signals were sampled with a frequency of 10 kHz (filtered with 2 kHz) at a holding potential (Vh) of −60 mV and stored for subsequent analysis. The recording electrodes had a resistance of 3–5 MΩ when filled with the whole cell patch-clamp pipette solution containing (in mM) 138 Cs-Me-sulfonate, 2 NaCl, 1 CaCl₂, 10 EGTA, 4 Na₂ATP, 0.3 Na-GTP, and 10 HEPES (pH 7.2). Consulting equilibrium potential to holding potential relationships, all upward deflections had to be chloride-conducting, inhibitory currents, whereas all downward deflection had to be mEPSCs.

To ensure steady drug (riluzole) concentration equilibrium in the recording chamber, a 4-min preapplication of riluzole-containing solution was carried out before the start of the actual 4-min data recording that was used for the later analysis.

**Experiments**

Rhythmically active slices were obtained 30 min after increasing the level of potassium to 5 mM. This preparation allowed us to study both network activity and cellular activity (Fig. 1, B and C). To investigate the role of the Na⁺ current, in the generation of network bursting and cellular activity, we applied either riluzole (20 μM) or a very low concentration TTX (50 nM) to the ACSF. Typically the network bursting decreased and stopped after attenuating the Na⁺ current (Fig. 2). In some of the experiments, the neurons were decoupled from the network by blocking non-N-methyl-D-aspartate (NMDA) and NMDA glutamatergic receptors using 6-cyano-7-nitro-
quinoxaline-2,3-dione (CNQX, 20 μM) and (+/-)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 10 μM); adding this combination of drugs in the ACSF stopped network activity within a few minutes.

To explore the effectiveness of riluzole and a low concentration of TTX and the role of persistent sodium in cellular and network activity, we carried out the following experiments.

1) Riluzole (n = 13) or TTX (n = 8) was added to the ACSF and network bursting activity was observed and quantified for 10 min after drug application.

2) The neuronal response to current injection (50–150 pA) was determined before and after bath application of riluzole (n = 12) or low-concentration TTX (n = 8) and every 30 s thereafter for 10 min. Responses of regular spiking (RS)-type neurons were evaluated only during absence of overt network activity. The intrinsically bursting (IB)-type neurons were isolated from the network to confirm that the bursting was intrinsic and not network driven. The response evoked by the current injection was quantified by the number of action potentials in 1 s after onset of injection.

3) By means of the voltage-clamp technique, the persistent sodium conductivity before and after addition of riluzole (n = 5) or a low concentration of TTX (n = 7) was determined in pyramidal neurons after they were isolated from the network.

4) In five experiments we also determined the effect of riluzole on calcium currents in the presence of TTX (1 μM).

5) The effect of riluzole on postsynaptic activity was recorded in voltage-clamp mode (n = 9).

The extracellular and intracellular current-clamp recordings were performed in a different setup from that for the voltage-clamp measurements. Because of differences in the tubing of each setup, there was a 60-s difference in latency between drug delivery to the ACSF system and arrival in the bath. The drug delivery difference is compensated for in the graphs of Fig. 5 by shifting the graphs relative to each other.

Data analysis

Data were analyzed by using MiniAnalysis 5.41 (Synaptosoft) and statistical analysis was performed with Prism 4.03 (Graphpad). Significance between data values was assessed with the Student’s t-test and assumed when P ≤ 0.05. Voltage-clamp data were analyzed off-line with the software program ClampFit 9.2 (Axon Instruments). Quantitative data are given as means ± SE.

A computerized preanalysis of each 4-min recording was performed to tag all events that qualified as miniature inhibitory postsynaptic currents (mEPSCs) according to the user-defined, programspecific detection parameters. However, an additional manual user-performed screen through each data trace was necessary to eliminate computer errors. During this eyeballed-screening process, wrongly tagged artificial events were unmarked and missed real mEPSC events were tagged. To determine the representative amplitude and decay time of the recorded mEPSCs in each data trace, an average mEPSC from 50 single mEPSCs was created as follows: For each 4-min data trace, the computer randomly picked 75 single events. Those events were superimposed and aligned at 50% rise time. Single events that did not line up within a rise-time window of 4 ms were manually eliminated. In addition, the decay phase of each event had to steadily decay without any interruption by additional events or noise for 50 ms. This procedure was followed until 50 events were included into the average. Computerized single-exponential fit through the decay phase (10–90%) of the average mEPSC (Fig. 9B) delivered a representative amplitude and decay time constant (τ). This analysis was not performed on the inhibitory component because of a low frequency of occurrence of mIPSCs before and after adding riluzole.

Model

The purpose of the computational approach in this study was to explain the relationship between persistent sodium conductivity and emergent network bursting behavior. The neural network in the computational model representing neocortex includes excitatory and inhibitory cell populations with a multicompartmental representation of each cell type. The network connectivity is based on histological and physiological work (DeFelipe et al. 2002; Feldmeyer and Sakmann 2000; Hellwig 2000; Krimer and Goldman-Rakic 2001; Mountcastle 1997; Nieuwenhuys 1994) and includes the essence of neocortical microcircuitry.

1) A large fraction of excitatory cell types relative to inhibitory interneurons (a ratio of approximately 4:1).

2) Excitatory connectivity ending on the dendritic portion, whereas important components of the inhibition are located on the initial segment, soma, and dendrite (DeFelipe et al. 2002).

3) Recurrent excitation and reciprocal connection between inhibitory cells. A unique neocortical feature is the presence of axo-axonic interneurons (the chandelier cells) that do not make synaptic interconnections between themselves (DeFelipe et al. 2002; Somogyi et al. 1998).

4) Direct electrical contact between inhibitory interneurons (Amirai et al. 2002).

The spatiotemporal relationship between the neuronal elements is determined by the geometry of the network, that is, realistic cell dimensions and spacing, associated with realistic conduction speeds. Details of the model were published previously (Van Drongelen et al. 2004, 2005); further parameters are summarized in the APPENDIX.

In short, the excitatory network (Fig. 3A) consists of superficial pyramidal cells from layers 2/3 (five compartments, Table 1) and deep pyramidal cells from layers 5/6 (seven compartments, Table 1). The inhibitory cells (I, Fig. 3A) receive input from both types of pyramidal neurons. Gap junctions (R, Fig. 3A) connect inhibitory neurons to each other with connectivity rules derived from connections in the mammalian cortex. In a short range, the synaptic connectivity decreases with distance between source and target elements (e.g., Feldmeyer and Sakmann 2000; Hellwig 2000; Krimer and Goldman-Rakic 2001; Nieuwenhuys 1994). Excitatory synaptic connections were simulated by an alpha function with a time constant of 1–3 ms for excitatory connectivity; inhibitory synaptic activity was represented by a dual-exponential function with time constants of 1 and 7 ms.

For fast sodium and potassium currents, implemented on the soma and initial segment of the model cells, we used conductivity param-
A Excitatory Connectivity

B Inhibitory Connectivity

FIG. 3. Diagram of the computational model of neocortex. Superficial pyramidal cells (S) and deep pyramidal cells (D) are located in different layers. Interneurons (I) are inhibitory cells located in a 3rd layer. A: excitatory connections between pyramidal cells and between pyramidal cells and interneurons. Interneurons of each type are interconnected with gap junctions (R). Parameters associated with the excitatory connections indicated by 1–14 can be found in Table A1 in the APPENDIX. B: inhibitory connectivity between basket cells (B) and the soma of the pyramidal neurons and between chandelier cells (C) and the initial segment of the pyramidal neurons. Inhibitory connections indicated by a–h and disinhibition I–IX correspond to Table A1 in the APPENDIX.

The computational model is implemented in the parallel GENESIS simulator (Bower and Beeman 1998) to facilitate a high degree of detail, large-scale simulations, and multiple parameter searches. A scalable version of the model runs on the Jazz computing cluster (Argonne National Laboratory), which enabled us to perform large sets of parameter searches within a reasonable time period. [For those interested, the current version of the pGENESIS scripts are available; please contact the first author for details.] Neuronal parameters were determined with a brute-force parameter search (see the APPENDIX) in which intrinsic and evoked cellular behaviors were used as a target. A subsequent brute-force parameter search was used to determine network behavior. The compartmental and cellular activity consists of complete knowledge of the local membrane potential and current. The extracellular activity was obtained as a weighted sum of currents generated by the model neurons (Nunez 1981). Where needed, network bursting (NB) was quantified by using the procedure described below. To obtain an estimate of the power, a 150-s epoch of the extracellular signal was first squared; this signal was then integrated with the same filter used for the recorded network activity (a filter with a 50-ms time constant). In these integrated traces, bursts were clearly visible and a threshold separating ongoing activity and bursts was visually determined (Fig. 4–H). Finally the total area above the threshold served as an index for the bursting power in the trace. Although selection of the threshold was not critical for the qualitative result (reduced bursting on reduction of NaP current), a threshold at 0.01 arbitrary units (AUs; Fig. 4–H) for all traces was used to generate the NB burst index shown in Fig. 10, A and B.

TABLE 1. Overview of cell compartments, compartment size, associated voltage-sensitive ion channels, synaptic channels, and gap junctions

<table>
<thead>
<tr>
<th>Cell—Compartment</th>
<th>Size, µm</th>
<th>V-Channels</th>
<th>Synapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>S_PYRAMIDAL—soma</td>
<td>22, 16.1</td>
<td>Na, K, NaP</td>
<td>i</td>
</tr>
<tr>
<td>sd1</td>
<td>140, 2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>sd2</td>
<td>190, 3.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>bd</td>
<td>200, 2.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>is</td>
<td>50, 2.2</td>
<td>Na, K</td>
<td>i</td>
</tr>
<tr>
<td>D_PYRAMIDAL—soma</td>
<td>22, 16.1</td>
<td>Na, K</td>
<td>i</td>
</tr>
<tr>
<td>dd1</td>
<td>250, 2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>dd2</td>
<td>400, 2.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>dd3</td>
<td>400, 4.4</td>
<td>—</td>
<td>e</td>
</tr>
<tr>
<td>dd4</td>
<td>400, 4.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>bd</td>
<td>200, 6.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>is</td>
<td>50, 2.2</td>
<td>Na, K</td>
<td>i</td>
</tr>
<tr>
<td>BASKET1-3—soma</td>
<td>5.5–22, 4–16.1</td>
<td>Na, K</td>
<td>e, i</td>
</tr>
<tr>
<td>d</td>
<td>300–900, 2</td>
<td>—</td>
<td>gj</td>
</tr>
<tr>
<td>CHANDELIER—soma</td>
<td>5.5, 4</td>
<td>Na, K</td>
<td>e, i</td>
</tr>
<tr>
<td>d</td>
<td>150, 2</td>
<td>—</td>
<td>gj</td>
</tr>
</tbody>
</table>

sd1, sd2, and dd1–dd4 are the apical dendritic compartments for the superficial and deep pyramidal neurons; bd, the basal dendritic compartment; is, the initial segment; d, dendrite of the inhibitory cell types. Excitatory and inhibitory synaptic channels are indicated be e and i, and the gap junctions between the inhibitory cells by gj.
TTX and 403.8 ± 40.2 s after 20 μM riluzole was added to the ACSF. In both TTX and riluzole application, the overall reduction in network bursting was significantly reduced for latencies ≥150 s (P < 0.05).

**Cellular activity**

Simultaneous extracellular recordings from population bursts and intracellular recordings from individual neurons revealed that all neurons were either excited or inhibited in phase with population activity; that is, neurons received synaptic inputs from the network. Typical patterns (Van Drongelen et al. 2003a) were excitation, excitation followed by inhibition, and inhibition as shown for RS-type cells in Fig. 6A and for IB-type neurons in Fig. 6B. The membrane potentials were 61.0 ± 2.7 and 66.4 ± 5.1 mV for the RS- and IB-type neurons, respectively.

Attenuation of the NaP current affects the intrinsic excitability of neocortical pyramidal neurons; however, the effect depended on cell type (Fig. 7). Examples of the effect of riluzole on RS and IB cells are shown in Fig. 7, A1 and A2. When network bursting stopped, the RS-type neurons were not significantly affected by riluzole (n = 8, P = 0.26) (Fig. 7C1); the average number of action potentials per second (AP/s) was 10.7 ± 0.8 before and 9.4 ± 1.3 after riluzole application. However, 10 min after network cessation, the neural response evoked by current injection into RS neurons became significantly reduced (P < 0.05, 3.0 ± 1.2 AP/s). By contrast, bursting activity in IB neurons completely disappeared and became regular spiking within 5–6 min after riluzole application (Fig. 7, A2 and B2). As a consequence of the loss of bursts the number of APs generated by the current injection was significantly decreased (n = 4; P < 0.05, AP/s 9.2 ± 1.0 before adding riluzole and 4.4 ± 0.8 after the application of riluzole, Fig. 7C2). The difference between activity levels of the RS- and IB-type neurons under control conditions was not significant. The time course of the activity levels of both RS- and IB-type neurons in the presence of riluzole is depicted in Fig. 5B.

To address the possibility that these results were caused by the known unspecific effects of riluzole, we repeated these experiments with TTX at low concentrations. The response in RS-type neurons (n = 4) was not significantly affected (P = 0.55) within 5–6 min of bath-applied TTX (Fig. 7C1). By contrast, the bursting in IB-type neurons was blocked within 5–6 min (Fig. 7, A2 and B2), significantly decreasing the frequency of APs (n = 4; P < 0.01, AP/s 11.1 ± 2.8 before adding TTX and 4.7 ± 2.2 after the network stopped bursting, Fig. 7C2). The time course of the activity levels of both RS- and IB-type neurons is depicted in Fig. 5E.

**Voltage-clamp data**

We recorded in the voltage-clamp mode from 51 neurons in layer 5 of the neocortex. The average cell capacity was 107 ± 14 pF and cell resistance was 365 ± 92 MΩ. After blockade of voltage-activated calcium (bath: 200 μM CdCl2) and potassium (intracellular: 110 mM CsCl, 30 mM TEA-Cl) currents, we evoke voltage-activated NaP currents with specific voltage-step protocols (Fig. 8, A and D, insets). The NaP amplitudes were measured at the steady-state level toward the end of the voltage steps (● in Fig. 8A) and were plotted against the respective test potentials (average I–V curve, Fig. 8B; normalized curves, Fig. 8C). The peak voltage of the NaP currents was found at −10 mV, with an amplitude of 333 ± 34 pA.

To determine the activation properties, we calculated the NaP conductance (g) to compensate for the changing driving force at respective test potentials [g = I/(Vt - Eq)], where Vt is test potential and Eq is equilibrium potential. The NaP conductance values were normalized and the average was plotted.
against the respective test potentials (Fig. 8F). A sigmoidal Boltzmann-curve fit $1/[1 + e^{-(V-V_{50})/\text{slope}}]$ for the normalized Na\textsubscript{p} amplitudes as a function of the test potential V delivered the activation parameters: $V_{50} = -46.2 \pm 1.4$ mV and slope $= 9.9 \pm 1.3$. Both low TTX and riluzole significantly reduced the Na\textsubscript{p} current (Fig. 8B) and resulted in a lower level of activation (Fig. 8F).

To determine the inactivation properties of the Na\textsubscript{p} currents, we measured the Na\textsubscript{p} current amplitudes at the peak voltage ($-15$ mV) immediately after different prolonged (10-s) test potentials (Fig. 8D). The remaining, activatable Na\textsubscript{p} amplitudes were normalized ($I/I_{\text{max}}$) and the average was plotted against the respective test potentials (Fig. 8E). A sigmoidal Boltzmann-fit delivered the inactivation parameters: $V_{50} = -65.4 \pm 2.3$ mV and slope $= -19.0 \pm 2.5$. No significant effects of riluzole on Ca\textsuperscript{2+} currents were measured ($n = 5$).

**Synaptic properties**

To investigate possible synaptic effects of riluzole, we compared miniature excitatory postsynaptic currents (mEPSCs) under control conditions [with TTX (1 $\mu$M) to disable spike activity] with mEPSCs after additional application of 20 $\mu$M riluzole (Fig. 9). The difference of the frequency of mEPSCs,
0.9 ± 0.5 Hz under control conditions and 1.3 ± 0.6 Hz after adding riluzole, was not significant. For the mIPSCs the frequency was very low both under control conditions (0.05 Hz) and after adding riluzole (0.04 Hz). In five cells using 50 single events of a 4-min recording for each neuron, we found that neither the mEPSC amplitudes (TTX: 19.8 ± 1.1 pA, TTX + riluzole: 20.4 ± 1.2 pA), nor the mEPSC decay time constants (TTX: 4.6 ± 0.4 ms, TTX + riluzole: 4.2 ± 0.2 ms) were significantly different from each other (Fig. 9, B and C). Because of these low numbers for the inhibitory events, a meaningful averaging procedure and subsequent quantitative analysis was not feasible.

Simulated activity

The purpose of the simulations is to relate the persistent sodium (NaP) current to bursting both at the cellular and network levels; for details of the modeling procedure see METHODS and the APPENDIX. Although we found that the NaP current is not strictly a persistent current because it does inactivate, its voltage-clamp characteristic shows a fairly large window in which these channels can be activated (Fig. 8E).

This property indicates that a small, “persistent” sodium current is present during the resting state of the neurons, causing the membrane to slowly depolarize up to the threshold for generating action potentials. This small depolarizing current causes neurons to burst at regular intervals. At small offset depolarizations (or current injections) the neurons burst more frequently, whereas at higher levels of depolarization the neurons show beating (continuous spiking) behavior identical to the activity pattern described by Butera et al. (1999).

The model network that included the bursting pyramidal cell type immediately settled into a pattern of bursting network activity. This activity was used to relate neuronal and network bursting. In Fig. 10A, the burst index CB of the neuronal response to a 220-pA current injection and the burst index of the network NB (see METHODS for the definition of these indices) are both plotted against the maximum conductance of persistent sodium ($G_{\text{NaP}}$). In the absence of sufficient NaP conductance, the cellular response disappeared and spontaneous network bursts were absent as well. The relationship between the cellular and network bursting indices is depicted in Fig. 10B, where the line represents linear regression. The

FIG. 6. Neuronal activity of the population (top traces in each panel) usually showed a relationship with the individual neuron (bottom traces). In RS neurons (A panels) and IB neurons (B panels), we found 3 types of activity associated with the population burst: excitation (A1, B1), a combination of excitation followed by a phase of inhibition (A2, B2), or inhibition (A3, B3). A subclass of bursting cells remained active after the network effect was removed by bath application of CPP and CNQX.
correlation coefficient \( r = 0.89 \) between both the cellular and network bursting indices was significant (\( P < 0.01 \)). The trace shown in Fig. 10C was obtained by reducing the maximum conductance of \( N_a \) from 65 to 0.005 S/m^2 over a time interval of 12 min. This decrease was done in a logarithmic fashion to mimic the penetration of riluzole or TTX into the slice. By following this procedure we gradually attenuated the effect of \( N_a \) similar to the riluzole or TTX experiments shown in Fig. 5. A typical example of a recorded trace of network activity in a slice is shown in Fig. 2; in this experiment riluzole was used to attenuate bursting activity. Both in the recorded (Fig. 2) and simulated traces (Fig. 10C) network activity initially decreases and stops within 10 min.

Our data suggest that the \( N_a \) current is important both for the generation bursting activity in a subset of neocortical neurons and for the generation of bursting at the network level. In the presence of low concentrations of TTX or the neuroprotective drug riluzole, the spontaneous network bursting activity in neocortical slices of neonatal mice was suppressed (Fig. 5, A and D). To our knowledge this is the first study in which the role of riluzole is quantified and modeled at the level of

1) network activity (Fig. 5)
2) single-neuron activity (Figs. 5 and 7)
3) intrinsic (persistent sodium) membrane currents (Fig. 8)
Although we emphasize that our modeling was not an exact-fitting procedure to the recorded data, the experimental findings were essentially confirmed in the computational model (Fig. 10).

Our study also confirmed previous experimental and computational studies that indicate that the NaP conductance plays a major role in providing intrinsic excitation to cortical networks (Agrawal et al. 2001; Bazhenov et al. 2002, 2004). Combined in vivo recordings and computational models indicate that the interplay between the hyperpolarization-activated depolarizing current ($I_{h}$), Ca$^{2+}$-sensitive potassium current, and a NaP current could organize paroxysmal oscillations (Timofeev et al. 2004). The NaP current plays an important role in activating intrinsic bursting in neocortical neurons (Guatteo et al. 1996) and a number of studies demonstrated that the number of intrinsically bursting neurons is significantly increased after neocortical trauma or conditions that enhance the propensity of epilepsy (Sanabria et al. 2002; Topolnik et al. 2003). Steriade and coworkers demonstrated that intrinsically bursting neurons are frequently found in vivo (20–25% of the...
total cortical neuronal population) and these authors proposed that these bursting neurons play a critical role in the generation of seizure activity (Timofeev and Steriade 2004; Timofeev et al. 2000b). Indeed, intrinsically bursting neurons are rhythmically active during Lennox–Gastaut-type seizures, specifically during spike-wave (SW) discharges, polyspike-wave (PSW) complexes, and fast runs (Timofeev and Steriade 2004). Mechanistically, bursting in these neurons could have a substantial impact on local networks that initiate seizures focally and in related structures as proposed by Steriade and coworkers (Steriade et al. 1998; Timofeev and Steriade 2004; Traub et al. 2001). The cellular bursting property together with a modest level of synchrony and recurrent excitation can lead to a seizurelike pattern (Van Drongelen et al. 2003a, 2005). Our current result, suggesting that intrinsic cellular bursting properties associated with the NaP current are a critical component of synchronous activity in cortical neuronal populations, agrees well with the reported anticonvulsant action of persistent sodium blockers in some patients with epilepsy (Köhling 2002).

In addition to the potential role of NaP in epilepsy, the bursting properties associated with this persistent current may very well drive physiological oscillations observed in sleep and memory formation (e.g., Agrawal et al. 2001; Bazhenov et al. 2002; Timofeev et al. 2000b). The rhythmic network bursting as described in the present study could represent an in vitro form of sleep slow oscillation. Such oscillations are known to develop into SW/PSW seizures in vivo (Steriade and Amzica 2003; Timofeev and Steriade 2004). We emphasize, however, that in a functional network, the persistent sodium channels not only function in form of intrinsic bursting but also could amplify currents flowing through glutamate-activated dendritic channels (Crill 1999; Schwindt and Crill 1999; Timofeev et al. 2000b). The involvement of the NaP current in the generation of intrinsic bursting could also explain the finding that the propensity to burst is a “flexible” property (Steriade 2004). The NaP current and bursting neurons are a target of various neuromodulators (Peña and Ramirez 2002; Staiger et al. 2002).

From a theoretical perspective, network bursting activity can be caused by cellular oscillations arising from intrinsic membrane conductance, network properties, or a combination of both. Current modeling results and earlier simulation studies demonstrate that networks of RS-type pyramidal cells can oscillate at an appropriate strength of connectivity but that intrinsic bursting neuronal activity is critical for sustaining slow bursting network activity (Traub et al. 2003; Van Drongelen et al. 2004, 2005). In our study, low concentrations of TTX or riluzole inhibited the NaP current (Fig. 8), while leaving synaptic function intact (Fig. 9). At the cellular level, we found that reducing the NaP current through low TTX or riluzole can dramatically change the firing properties of IB neurons (Fig. 7). The bursting behavior of these neurons was altered and they converted to a pattern similar to that of regular firing, with a reduced output of action potentials per injected current pulse (Fig. 7C2). Similar experiments with regular firing (RS-type) neurons showed that their firing pattern was unchanged at the cessation of network bursting (Fig. 7, A1, B1, and C1). However, current injections ≥10 min after cessation of network bursting showed that all cell types had a significantly decreased activity level. Our findings suggest that the NaP current plays a role in enhancing excitability of the majority of neocortical neurons but that the intrinsic bursting property is more sensitive to the blockade of NaP than the generation of regular spiking activity. Such a differential sensitivity could explain why intrinsic bursting was blocked first. However, we have not attempted to investigate the reason for the observed differential effect; the focus of the present study was to use this differential effect as a pharmacological tool to determine factors involved in reducing network activity that finally leads to the cessation of network bursting.

The computational approach confirmed the essence of our experimental observation and showed that NaP caused periodic bursting at the cellular level (Fig. 4A), identical to the pattern reported in Butera et al. (1999). At the network level this feature was associated with a bursting pattern, although without the simple periodicity (Fig. 10C). This finding together with the reduced network bursting at attenuated NaP currents suggests that the bursting neurons in a network recruit surrounding neurons at irregular intervals. In this state of the network the neurons are not acting as simple pacemakers that synchronize other units into their intrinsic periodic rhythm; rather, they function merely as units that occasionally can drive or recruit a critical mass of neurons resulting in a burst of activity, whereas the network in its turn codetermines the state of these bursters—clearly a bidirectional process, as similarly discussed by Ramírez et al. (2004). Therefore we propose that the intrinsic (NaP current) and network property (excitatory connectivity) together sustain the rhythmic network behavior that we observed in the neocortical slices.

**Appendix**

The overall approach to our modeling effort in this study was to determine acceptable cellular behavior first and then to obtain emergent network behavior. To determine the part of parameter space that would create the desired cellular behavior, we first determined the voltage sensitivity of the sodium and potassium channels by using the literature values originally reported by Hodgkin and Huxley (1952) and our recorded data for NaP (Fig. 8). The compartment sizes (Table 1) were based on cellular dimensions and a procedure to collapse cellular parts into single cylindrical compartments (Bush and Sjénowski 1993; Van Drongelen et al. 2004, 2005). Because the model’s surface areas represent an estimate, the maximum levels of conductivity were determined in a brute-force parameter search of 58,644 simulations. For regular and fast spiking cells, the target was to obtain spiking at different levels of current injection (Fig. 4) and no spontaneous activity at a normal resting potential. The cells, including NaP current, were adjusted to show bursting behavior during rest and moderate current injection levels. At higher levels of current injection the behavior transitioned into beating behavior (Butera et al. 1999). We evaluated the following parameter ranges; the values we selected for the simulations reported here are indicated in parentheses.

1) Membrane resistance: 0.1–5.0 Ωm$^2$ (5 and 0.1 for the axon/initial segment)
2) Axial intracellular resistance: 0.3–10 Ωm (2 and 1 for the axon/initial segment)
3) Maximum fast sodium conductance in the soma: 300–3,000 S/m$^2$ (2000)
4) Maximum persistent sodium conductance in the soma: 0.3–350 S/m² (65)
5) Maximum potassium conductance in the soma: 6–1,000 S/m² (650)
6) Maximum fast sodium conductance in the initial segment/axon: 30–100,000 S/m² (6000)
7) Maximum potassium conductance in the initial segment/axon: 6–100,000 S/m² (2000)

Membrane capacitance was 0.01 F/m². Gap junctions had a resistance of 200 MΩ, a value representing five gap junctions with a 1-nS conductance for each (Traub et al. 2005). In spite of the common belief that one may easily simulate any type of activity pattern with the rich parameter set of biophysically realistic models, the large number of parameters is more an obstruction than a facilitator for finding the part of parameter space associated with acceptable behavior; in our example above we found about 0.05% of the simulations generated the desired, realistic behavior. This set was not distributed over the parameter space but encompassed a small area showing desired spontaneous bursting; this area was located around the parameter choice indicated in parentheses in the list above.

As the next step in defining the network, we connected the cells according to the diagrams in Fig. 3. By evaluating a wide range of synaptic strength parameters for the individual synapse model in a brute-force parameter search, we adjusted the synaptic strength to obtain desynchronized network bursting behavior (Van Drongelen et al. 2005). The synaptic connectivity parameters based on values reported for mammalian cortex (Van Drongelen et al. 2004) are summarized in Table A1. The reference numbers of the different connections in column I of Table A1 correspond with those in Fig. 3. The area of connectivity for each connection type was determined by the destination area around the cell and the hole in the destination area that was excluded from making synaptic contacts (columns 2–4, Table A1). In this destination area, the probability for a synapse to occur declined exponentially with horizontal distance between source (presynaptic) and target (postsynaptic) cells (Probability and Exponential column 1). In this destination area, the probability for a synapse to occur declined exponentially with horizontal distance between source (presynaptic) and target (postsynaptic) cells (Probability and Exponential column 1).

A C K N O W L E D G M E N T S

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