Area-Specific Resonance of Excitatory Networks in Neocortex: Control by Outward Currents

Manuel A. Castro-Alamancos and Yoshie Tawara-Hirata

Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, Pennsylvania, U.S.A.

Summary: During disinhibition or low [Mg++]o buffer, 7–14 Hz (∼10 Hz) oscillations are generated by excitatory networks of interconnected pyramidal cells in motor (agranular) cortex but are absent in barrel (granular) cortex. Here we studied if the inability of barrel cortex to produce ∼10 Hz oscillations during these conditions is because barrel cortex networks lack the necessary cellular mechanisms or, alternatively, because those mechanisms are inhibited by outward currents. The results show that blockers of slowly inactivating voltage-dependent K+ currents unmask ∼10 Hz oscillations in barrel cortex, and this occurs in unison with the unmasking of intrinsic inward Ca++ currents that are kept suppressed by the outward currents. Moreover, the ∼10 Hz oscillations unmasked in barrel cortex occur independently in upper and lower layers indicating that the ∼10 Hz oscillation mechanisms are kept suppressed in multiple networks. The results reveal that the propensity of distinct excitatory networks of neocortex to generate epileptiform oscillatory activities is controlled by outward currents.

Key Words: Motor cortex—Barrel cortex—Neocortex—Neural networks—Oscillations—Epilepsy—Afterdischarge—Current source density—Excitation—Inhibition.

The neocortex or six-layer cortex consists of at least 52 cytoarchitectonically distinct areas in humans (Brodmann, 1905), and similar areas can be distinguished in rodents (Zilles, 1985). Each of these areas has a defining set of extrinsic connections; each contains neurons with identifiable sets of functional properties; each has a distinct laminar arrangement; etc. Thus, neocortex is extensively subdivided into areas of anatomical and functional specialization (Brodmann, 1905; Zilles, 1985; Creutzfeldt, 1993; Mountcastle, 1998), but little is known about the specialization of cellular physiology across areas.

Previously, we found that block of GABA_A and GABA_B receptors (disinhibition) produces synchronous and rhythmic ∼10 Hz oscillations in agranular (e.g., motor cortex), but not in granular (e.g., barrel cortex) neocortex (Castro-Alamancos and Rigas, 2002). Interestingly, ∼10 Hz oscillations of motor cortex induced in behaving rats by disinhibition produce overt tremors of the contralateral body that resemble a cortical myoclonus associated with partial epilepsies (Castro-Alamancos, 2006).

The presence of ∼10 Hz oscillations during disinhibition indicates that a pure excitatory network of connected pyramidal cells generates ∼10 Hz oscillations. Here we show that ∼10 Hz oscillations caused by low [Mg++]o buffers are also selectively generated in motor cortex, and not in barrel cortex. The present study further attempts to distinguish between two alternative hypotheses: the barrel cortex is incapable of generating ∼10 Hz oscillations because it lacks the necessary intrinsic or synaptic mechanisms or, alternatively, some current inhibits the ability of barrel cortex to generate ∼10 Hz oscillations.

What current may be responsible for keeping a pure excitatory network in granular neocortex from generating ∼10 Hz oscillations? In the absence of inhibition, intrinsic K+ currents are the counterbalance of excitation. Hence, it is possible that K+ currents in granular cortex impede the generation of ∼10 Hz oscillations. CA1 and layer V pyramidal cells express three major types of voltage-dependent K+ currents in the soma and dendrites (Storm, 1988, 1990; Hoffman et al., 1997; Bekkers, 2000a, 2000b; Kornegren and Sakmann, 2000; Bekkers and Delaney, 2001): a transient current that rapidly activates and inactivates (I_A), a more slowly inactivating current (I_B), and a sustained delayed rectifier (I_K). Importantly, these three voltage-dependent K+ current components have well-known sensitivities to K+ channel blockers; low doses of 4-aminopyridine (4-AP; µM range) block the slowly inactivating K+ current I_B, with little effect on I_K and I_A. Whereas, tetraethylammonium (TEA) at high doses (10–30 mM) blocks the sustained delayed
rectifier \( I_K \) and others (Storm, 1988, 1990; Hoffman et al., 1997; Bekkers and Delaney, 2001).

Here we study if application of \( K^+ \) channel blockers un-masks \( \sim 10 \) Hz oscillations in granular cortex. We found that \( \sim 10 \) Hz oscillations are very robust in granular cortex during low doses of 4-AP. High doses of TEA were also effective at producing \( \sim 10 \) Hz oscillations in granular cortex. The unmasking of \( \sim 10 \) Hz oscillations in barrel cortex, by low doses of 4-AP, was associated with an enhancement of intrinsic inward currents that were suppressed by \( Ca^{++} \) channel blockers. These results indicate that specific outward currents inhibit the ability of barrel cortex to generate \( \sim 10 \) Hz oscillations.

**METHODS**

Slices were prepared from 57 adult (\( \geq 8 \) weeks) eYFP-H mice, taken from our breeding colony, as previously described (Castro-Alamancos and Rigas, 2002). Mice were deeply anesthetized with sodium pentobarbital (60 mg/kg) or ketamine (150 mg/kg) and upon loosing all responsiveness to a strong tail pinch the brain was rapidly extracted and placed in ice-cold buffer. Slices (400 \( \mu \)m thick) were cut in the thalamocortical plane (Agmon and Connors, 1991) or in the coronal plane in ice-cold buffer using a vibratome. Multiple sequential slices were taken from each animal and identified in the anterior–posterior direction (Castro-Alamancos and Rigas, 2002; Rigas and Castro-Alamancos, 2004). Experiments were performed in an interface chamber at 32\(^\circ\)C. The slices were bathed constantly (1–1.5 ml/min) with artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl (126), KCl (3.5), \( NaH_2PO_4 \) (1.25), \( NaHCO_3 \) (26), MgSO\(_4\) 7H\(_2\)O (1), Dextrose (10), CaCl\(_2\) 2H\(_2\)O (1). The ACSF, which we will refer to as normal ACSF, was bubbled with 95% \( O_2 \) and 5% \( CO_2 \).

During all recordings, slices were bathed in bicuculline methiodide (BMI, 10 \( \mu \)M) to induce spontaneous discharges (termed here, interictal events) that recur spontaneously (Castro-Alamancos and Rigas, 2002; Rigas and Castro-Alamancos, 2004). In addition, two methods were used to induce \( \sim 10 \) Hz oscillations: (1) application of a GABA\(_B\) receptor antagonist (CGP35348, 250–500 \( \mu \)M); or (2) reduction of the \( [Mg^{++}]_o \) in the buffer from 1 mM to 0.1 mM. In some experiments, glutamate receptors were blocked using CNQX and D-AP5 (10 and 50 \( \mu \)M, respectively). \( Na^+ \) channels were blocked using TTX (1 \( \mu \)M). \( K^+ \) currents were suppressed with 4-aminopyridine (4-AP, 25–50 \( \mu \)M) or tetraethylammonium (TEA, 5–10 mM). \( Ca^{++} \) currents were suppressed with cadmium (200 \( \mu \)M), nifedipine (50 \( \mu \)M), NiCl (200 \( \mu \)M), and omega-conotoxin (0.8 \( \mu \)M). The nifedipine stock (50 mM) was dissolved in DMSO and then diluted in buffer. Drugs were purchased from Sigma-Aldrich or Tocris. All procedures were reviewed and approved by the Animal Care Committee of Drexel University College of Medicine.

Extracellular recordings were performed using low-impedance glass pipettes (\( \sim 0.5 \) M\( \Omega \)) filled with ACSF and 16-channel linear array silicon probes with 100-\( \mu \)m inter-site spacing (Neuronexus Technologies, MI). The probes were activated before use to lower and match the impedance of all recording sites to \( \sim 0.5 \) M\( \Omega \). Intracellular recordings were performed using high-impedance glass pipettes (80–120 M\( \Omega \)) filled with K-Acetate (2 M). During recordings, slices in the interface chamber were imaged with a fluorescent M2BIO microscope (Zeiss). This permitted fluorescent imaging of the cortical recording site and of the exact position of the 16-channel silicon probe (i.e., this is the left-most image in each figure). To clearly see the probe in the fluorescent images the gamma correction value of the image was adjusted using Axiovision software (Zeiss). Once an experiment was completed, each slice was transferred into a vial containing 4% formaldehyde in sodium phosphate buffer. Subsequently, the 400-\( \mu \)m slices were immersed in sucrose (30% in PBS) and later sectioned in a cryostat at 80 \( \mu \)m. The 80-\( \mu \)m slices were mounted on slides, embedded with Vectashield (Vector) and cover-slipped. The slides were later transferred to a fluorescent microscope (Zeiss) with a motorized stage and a Neurolucida system (Microbrightfield). Using the Virtual Slice module in Neurolucida, each section was imaged for comparison with the CSD analyses derived from the 16-channel probes. During all stages, a careful calibration was kept of the images so that it would be possible to align the electrophysiological recordings obtained with the 16 channel silicon electrodes with the fluorescent images of the recorded cortical column.

Data analyses were performed using Originlab software. Power spectrum analyses were obtained by calculating fast Fourier transforms (FFT) from the spontaneous activity. Current source density analyses (CSD) were derived from the field potentials recorded from the 16-channel silicon probes using a spatial differentiation grid of 200 \( \mu \)m (Mitzdorf, 1985; Castro-Alamancos and Rigas, 2002). In the CSDs, current sinks are red and current sources are blue. Green is around zero. CSDs of events evoked by electrical stimulation are the average of 10 responses (i.e., those evoked during block of glutamate receptors), while CSDs of spontaneous events are not averaged.

**RESULTS**

**Interictal events**

Neocortex slices were prepared from eYFP-H mice, which express a spectral variant of green fluorescent protein (GFP) at high levels under control of the \( THY1 \) promoter (Feng et al., 2000). In these mice, layer 5 pyramidal cells are labeled with GFP in all neocortical areas. In addition, a few cells also appear labeled in layer III of
granular cortex but not in agranular cortex. This labeling provides several benefits for slice experiments. First, it serves to identify different cytoarchitectonic areas of neocortex based on the fluorescence of layer 5 cells within a slice during recordings. Second, it serves to identify the layers within a neocortical area. Finally, it facilitates the correct alignment of a 16-channel silicon electrode with the apical dendrites of a cortical column in order to derive CSDs to measure current flow. Fig. 1A shows fluorescence photomicrographs of two representative slices used to study granular and agranular areas of neocortex in the present study. The markings in Fig. 1A delineate the areas where recordings were made in granular and agranular cortex; ∼800 µm (i.e., 2 slices) separate the granular and agranular slices in the anterior–posterior dimension.

During block of GABA_A receptors with BMI, interictal events occur spontaneously in both granular and agranular cortical areas at ∼0.1 Hz (Castro-Alamancos and Rigas, 2002; Rigas and Castro-Alamancos, 2004). Interictal events correspond electrographically to two phases: a sharp negative wave phase followed by a slower positive wave phase. Fig. 1 shows CSDs and field potentials of typical spontaneous interictal events recorded in an agranular slice (Fig. 1B) and in a granular slice (Fig. 1C) with a 16-channel silicon probe during BMI (10 µM). The panels in Fig. 1B, and in subsequent similar figures, from left to right show: (1) a fluorescent photomicrograph of the position of the 16-channel silicon probe taken during the recording; (2) a fluorescent photomicrograph of the cortical column recorded taken from the fixed slice; (3) a CSD and the corresponding field potential activity of a spontaneous interictal event. Note that the CSD and field potential activity are aligned and scaled with the adjacent fluorescent photomicrograph so that the recorded activities can be compared with the corresponding layers of the cortical column.

**FIG. 1.** CSD analysis of spontaneous interictal events recorded during BMI in granular and agranular neocortex. (A) Fluorescence photomicrographs of two representative slices used to study granular and agranular areas of neocortex in the present study. These are actual slices used in one experiment, which were fixed and sectioned at 80 µm. The markings delineate the areas where recordings were made in granular and agranular cortex in the present study. Two 400 µm slices separate the granular and agranular slices in the anterior–posterior dimension. (B) CSDs and field potentials of typical spontaneous interictal events recorded in an agranular slice during BMI. The panels from left to right show: a fluorescent photomicrograph of the position of the 16-channel silicon probe taken during the recording; (2) a fluorescent photomicrograph of the cortical column recorded taken from the fixed slice; (3) a CSD and the corresponding field potential activity of a typical spontaneous interictal event. Note that the CSD and field potential activity are aligned and scaled with the adjacent fluorescent photomicrograph so that the recorded activities can be compared with the corresponding layers of the cortical column. Thus, the scale of the fluorescent photomicrograph (in mm) is the y-axis of the CSD figure. While the photomicrograph showing the position of the 16-channel electrode (left panel) has its own 1-mm scale. (C) CSDs and field potentials of a typical spontaneous interictal event recorded in a granular slice during BMI. For details about each panel see Fig. 1B legend.
Spontaneous interictal events in each cortical area are quite characteristic. In agranular cortex, each interictal event begins with a large current sink that occurs within layers II-III and upper layer V. A very small (in amplitude and duration) current sink is usually also located in layer VI (Fig. 1B). The large current sink in layers II-III has a corresponding current source around layer I. After ~100 ms from event onset, the large current sink in layers II-III is converted into a current source that lasts ~300 ms and has a corresponding current sink in layer I. Thus, after ~100 ms from the onset of the event, the sink-source relation becomes inverted. This dipolar current flow could be explained by the cells that span between layer V and layer I, such as layer V pyramidal cells. At the electrographic level, the initial sink in layers II-III and upper V corresponds with the negative wave phase of the interictal event, while the following source in the same layers (inverted dipole) corresponds with the positive wave phase of the interictal event. Since inward and outward currents sum in population recordings, the main conclusion from these data is that the negative wave phase of the interictal event (0–100 ms from onset) is dominated by inward currents in layers II-III and upper V, while the positive wave phase (100–500 ms from onset) is dominated by outward currents in layers II-III and upper V.

Typically, in the agranular areas of neocortex, spontaneous interictal events during BMI were followed by several small events ~500 ms after the initial interictal event (see Fig. 1B for an example). These small events occur during the positive wave phase of the interictal event and interrupt the ongoing current source in layers II-III, transiently shifting the current flow toward lower layers. These small events are not observed in granular cortex. As shown later, ~10 Hz oscillations originate in agranular cortex from these small events, although they need not be present (during BMI) for ~10 Hz oscillations to occur.

As shown in Fig. 1C, interictal events are observed in the granular (barrel) neocortex during application of BMI (Castro-Alamancos and Rigas, 2002; Rigas and Castro-Alamancos, 2004). The laminar profile of events in granular cortex were in essence similar to those in agranular cortex in which there was an initial small current sink in layer VI and a larger sink in the upper layers (i.e., IV, III, and II). The upper sink had a corresponding source in layer I-II and coincides with the negative wave phase of the interictal event. Also, as occurs in agranular cortex, the initial sink-source dipole in granular cortex inverts after ~100 ms, and the upper layer sink gives rise to a current source that has a corresponding current sink in layer I-II. This later current flow corresponds with the positive wave phase of the interictal event. A major difference with agranular cortex is that interictal events in granular cortex were never followed by smaller events during the positive wave phase.

Low \([\text{Mg}^{++}]_o\) produces ~10 Hz oscillations in agranular but not in granular neocortex

In a previous study, we found that interictal events caused by block of GABA_A receptors (BMI) could be transformed into ~10 Hz oscillations by additionally blocking GABA_B receptors, but only in agranular neocortex and not in granular neocortex (Castro-Alamancos and Rigas, 2002). Previous studies have shown that similar ~10 Hz oscillations occur in neocortical slices when \([\text{Mg}^{++}]_o\) is lowered (Sutor and Hablitz, 1989; Silva et al., 1991). Thus, the first question we addressed here is if spontaneous interictal events caused by blocking GABA_A receptors could be transformed into ~10 Hz oscillations by lowering \([\text{Mg}^{++}]_o\), and if this occurred selectively in the agranular neocortex. Fig. 2A,B show typical field potential recordings of spontaneous activity recorded from layer III during block of GABA_A receptors (BMI; 10 µM) and subsequent lowering of \([\text{Mg}^{++}]_o\) from 1 mM to 0.1 mM in the agranular (left column) and granular neocortex (right column) of eYFP-H mouse slices. Also shown (Fig. 2C) is the effect of disinhibition in agranular and granular cortices for comparison.

Lowering \([\text{Mg}^{++}]_o\) to 0.1 mM had little effects on the negative wave phase of the interictal events but produced the development of ~10 Hz oscillations during the positive wave phase of the interictal events in agranular neocortex but not in granular cortex. Hence, the effects of lowering \([\text{Mg}^{++}]_o\) are similar to disinhibition (Castro-Alamancos and Rigas, 2002). Fig. 3A overlays the average power spectrum analyses of spontaneous events recorded during disinhibition or during low \([\text{Mg}^{++}]_o\), from pairs of agranular and granular neocortical slices studied side by side under identical conditions (10 randomly selected spontaneous events from 10 pairs of agranular and granular cortex slices are measured; i.e., 100 events per group are compared). The results show that spontaneous events recorded in the agranular cortex, during either disinhibition or low \([\text{Mg}^{++}]_o\), produce a significantly stronger FFT power in the 5–20 Hz frequency range (~10 Hz oscillations) than spontaneous events recorded in the granular cortex (t-test; p < 0.001; see Fig. 3B).

As mentioned above, during our baseline condition (BMI, 10 µM), the negative wave phase of interictal events in agranular cortex is often followed by several small events that occur during the positive wave phase of the interictal events. The numbers of small events during BMI that follow the initial interictal event are variable (between 0–5 events), but only occur during the positive wave phase of the interictal events. This is reflected in the power spectrum analyses shown in Fig. 3C (n = 15 slices, 10 events per slice), which shows that the agranular cortex has a small peak at ~17 Hz during BMI. Lowering \([\text{Mg}^{++}]_o\) in these slices produced overt ~10 Hz oscillations, which resulted in a large increase in the power and a shift to...
CURRENT FLOW ASSOCIATED WITH ~10 Hz OSCILLATIONS IN AGRANULAR NEOCortex

So far, the results show that ~10 Hz oscillations, generated by lowering \([\text{Mg}^{++}]_0\) or by disinhibition, occur in agranular neocortex but not in granular neocortex. We next tested the laminar pattern of current flow during ~10 Hz oscillations caused by low \([\text{Mg}^{++}]_0\) in agranular cortex. Fig. 4 shows CSD analyses derived from spontaneous discharges in a medial (Fig. 4A) and a lateral (Fig. 4B) location of an agranular slice. The ~10 Hz oscillations followed interictal events, and so the current flow at the onset was similar to that described above (see Fig. 1). Approximately 10 Hz oscillations started ~300 ms after the onset of the interictal event and each cycle of the oscillation reflected a large current sink within the upper layers (II-III and upper V), which coincides with the apical dendrites of layer V cells. Each cycle of the ~10-Hz oscillations has two components when measured with a field potential electrode placed in upper layers (II-III): a negative trough component (S) and a positive crest component (w). The large current sink in the upper layers corresponds to the trough component of the oscillation cycle and was associated with current sources in upper and lower layers (see Fig. 4C,D). The initial part of the current sink had a corresponding current source in layers I-II, while the later part of the current sink was associated with a current source in lower layer V. In between each trough component of the oscillation cycle there was a crest component that corresponds to current source centered in layers II-III with a corresponding current sink in lower layer V. These results indicate that each cycle of the ~10 Hz oscillation consists of a strong inward current that spreads from upper layer V into layer I followed by a more localized and smaller outward current in layers II-III. Part of this current flow is likely spreading through the apical dendrites of layer V pyramidal cells, which extend into layer I. However, it is noteworthy that current is also spreading synaptically between lower and upper layers.

In order to determine the intracellular correlates of the current flow observed with the CSDs, we conducted simultaneous intracellular recordings from cells in layers III and V adjacent to the 16-channel electrode. The population of cells recorded (n = 16) had resting membrane potentials more negative than −60 mV, an average input resistance of 47 ± 5 MΩ, overshooting action potentials and responded as regular spiking or bursting cells to current pulses. The activity of these cell types did not differ during the ~10 Hz oscillations. Fig. 5 shows four spontaneous discharges containing ~10 Hz oscillations caused by low \([\text{Mg}^{++}]_0\) in agranular cortex. The locations of the 16-channel electrode and of the intracellular and field potential pipettes are depicted in Fig. 5A,D. All the recordings shown in Fig. 5 were obtained sequentially from the same slice without moving the 16-channel electrode or the field potential recording pipette located in layer III; i.e., only the
intracellular recording electrode moved (see Fig. 5A,D). Each CSD plot in Fig. 5 overlays the membrane potential from an intracellular recording in layer V or III (a different cell is shown in each panel of Fig. 5B,C,E,F). In addition, each CSD plot also overlays a field potential recording obtained from an electrode placed in layer III. The recordings revealed that for either layer III or layer V cells, the trough components of the oscillation cycle (marked as S in Fig. 5B,C) are associated with a sharp depolarization that corresponds to the large sink that propagates between upper layer V and layer I, and has parallel sources in upper and lower layers. This large current sink is, thus, likely an active inward current with corresponding passive sources in upper and lower layers. In contrast, the crest components of the oscillations (marked as w and with arrows in Fig. 5B,C) are associated with hyperpolarizations of the membrane potential of the recorded cells that correspond to the source in layers II-III with a parallel current sink.

FIG. 3. Population power spectrum analysis of spontaneous events recorded during disinhibition or during low \([\text{Mg}^{2+}]_o\). (A) Comparison of fast Fourier transforms (FFT) calculated from events in granular and agranular cortex slices (10 events \(\times\) 10 slice per group) during low \([\text{Mg}^{2+}]_o\) buffer (left panel) and during disinhibition (right panel). (B) Comparison of FFT power in the 5–20 Hz range between granular and agranular cortex during low \([\text{Mg}^{2+}]_o\) buffer and during disinhibition (p < 0.001 granular vs. agranular cortex). (C) Comparison of FFT power between events in the same agranular cortex slices (10 events \(\times\) 15 slices) during BMI and after lowering \([\text{Mg}^{2+}]_o\) to 0.1 mM. Note that during BMI the agranular events display a small peak at \(\sim 17\) Hz, which shifts to \(\sim 10\) Hz during low \([\text{Mg}^{2+}]_o\) buffer.

FIG. 4. CSDs and field potentials of typical \(\sim 10\) Hz oscillations recorded in a medial (A) and a lateral (B) location of an agranular slice during low \([\text{Mg}^{2+}]_o\) buffer. C and D. Close-up of the events shown in A and B. For details about each panel see Fig. 1B legend.
in lower layer V. Thus, the lower layer V sinks during the oscillations are associated with the crest component of the oscillation cycle (this is most clear in the CSDs in Fig. 5E,F) and with the hyperpolarizing phase of the \( \sim 10 \) Hz oscillation rhythm in layer III and V cells, suggesting that these are passive sinks corresponding to the active sources observed in layer II-III during the crest component of the oscillation cycle. These results indicate that the source in layers II-III likely reflects an active outward current sink of the trough component of the oscillation cycle. These results indicate that these are passive sinks corresponding to the active sources observed in layer II-III during the crest component of the oscillation cycle. These results indicate that these are passive sinks corresponding to the active sources observed in layer II-III during the crest component of the oscillation cycle. These results indicate that these are passive sinks corresponding to the active sources observed in layer II-III during the crest component of the oscillation cycle.

**4-AP unmasks \( \sim 10 \) Hz oscillations in granular cortex**

Disinhibition or low \( [\text{Mg}^{+ +}]_0 \) buffer did not produce \( \sim 10 \) Hz oscillations in granular cortex. There are several possibilities to explain this result. One possibility is that a membrane current, such as an outward \( K^+ \) current, may impede the generation of \( \sim 10 \) Hz oscillations in granular cortex. In this case, suppressing such a current would result in the generation of \( \sim 10 \) Hz oscillations in granular cortex. To test this possibility, we tested the effects of \( K^+ \) channel blockers in granular cortex.

Low doses of 4-AP (in the \( \mu \)M range) are well known to block \( I_D \), the slowly inactivating \( K^+ \) current, while higher doses (in the mM range) block \( I_A \), the rapidly inactivating \( K^+ \) current (Storm, 1988, 1990; Hoffman et al., 1997; Coetzee et al., 1999). High doses of TEA (10–30 mM) significantly block a good portion of the sustained potassium current \( I_K \), will little effect on \( I_D \) and \( I_A \) (Storm, 1988; 1990; Hoffman et al., 1997; Bekkers and Delaney, 2001). To test the effect of \( K^+ \) channel blockers, granular slices were first placed in low \( [\text{Mg}^{+ +}]_0 \) buffer to demonstrate that \( \sim 10 \) Hz oscillations were not generated in the recorded column. Interestingly, in every experiment, subsequent application of a low dose of 4-AP (25–50 \( \mu \)M) resulted in the generation of \( \sim 10 \) Hz oscillations in granular cortex. Fig. 6A shows population data (\( n = 10 \) slices) comparing the power spectrum of events recorded in the granular cortex during three consecutive conditions: BMI, low \( [\text{Mg}^{+ +}]_0 \) buffer, and 4-AP application. The results reveal that the FFT power in the 5–20 Hz range was significantly larger for events during 4-AP than during low \( [\text{Mg}^{+ +}]_0 \) buffer or BMI alone (t-test; \( p < 0.001 \), \( n = 10 \)).

In addition, we tested the effects of TEA (5 and 10 mM). TEA did not induce \( \sim 10 \) Hz oscillations at 5 mM. This is reflected in the lack of increase in the 5–20 Hz range FFT power (\( n = 8 \) slices; t-test, n.s.; Fig. 6A). However, TEA produced \( \sim 10 \) Hz oscillations when applied at 10 mM (n = 8; t-test \( p < 0.001 \); Fig. 6A), but these oscillations were not as strong as those caused by 4-AP. The effectiveness of high doses of TEA (10 mM) in generating \( \sim 10 \) Hz oscillations in granular cortex. This is not surprising.
considering the slow inactivation kinetics of both $I_K$ and $I_D$.

Fig. 6B shows a representative example of the effect of 4-AP in a granular cortex slice. Shown are CSDs of events during low [Mg$^{++}$]o buffer and during subsequent application of 4-AP (50 µM), which includes a $\sim$10 Hz oscillation. The largest current sinks associated with these oscillations are in the upper layers (layer IV-I). Also, different sets of sinks and sources were confined to the lower layers (V-VI). This suggested that $\sim$10 Hz oscillations caused by 4-AP may be generated independently by different networks of cells in a cortical column. Perhaps, there was a lower layer and an upper layer network capable of generating $\sim$10 Hz oscillations independently. If this is the case, a cut separating the lower layers from the upper layers should reveal oscillations occurring independently within those layers.

In several experiments, we made a cut along the border between layer IV and V to separate upper and lower layers in granular cortex slices displaying $\sim$10 Hz oscillations caused by 4-AP application. These slices were also isolated through vertical cuts from adjacent cortical areas. The results from every experiment (n = 6) revealed that a horizontal cut separating layers V and IV led to the independent generation of $\sim$10 Hz oscillations in both the lower and the upper layers. An example of such an experiment is shown in Fig. 7A–C. Moreover, Fig. 7D shows population data of power spectrum analysis for $\sim$10 Hz oscillatory events caused by 4-AP in upper and lower layers before and after the horizontal cut. The results revealed that the FFT power in the 5–20 Hz range in either upper or lower layers was not significantly different before and after the cut (t-test; n = 6 slices). In these experiments we also tested the effect of electrical stimulation in the different portions of the slice before and after the horizontal cut. Electrical stimulation in layer V or in layers II-III of an intact slice during 4-AP produced a $\sim$10 Hz oscillation that occurs synchronously in upper and lower layers (like in Fig. 7A). When the slice is cut between layers V and IV, then stimulation in layer V produces only a $\sim$10 Hz oscillation in the lower portion of the slice (like in Fig. 7B, left panel) and no effect in the upper portion, while stimulation in layer II-III produces only a $\sim$10 Hz oscillation in the upper portion of the slice (like in Fig. 7B, right panel) and no effect in the lower portion.

These results show that a low dose of 4-AP readily unmasks $\sim$10 Hz oscillations in granular cortex slices that do not oscillate during low [Mg$^{++}$]o buffer, indicating that granular cortex pyramidal cells are kept from generating $\sim$10 Hz oscillations by slowly inactivating K$^+$ channels. Moreover, the $\sim$10 Hz oscillations produced in granular cortex by 4-AP are generated completely independently by upper layer (IV-I) and lower layer networks (V-VI), suggesting that the enhanced excitability yielded by 4-AP is sufficient to drive the generation of $\sim$10 Hz oscillations in different populations of pyramidal cells.

**4-AP application unmasks intrinsic Ca$^{++}$ currents**

Application of 4-AP results in the generation of $\sim$10 Hz oscillations in the granular neocortex, implying that the outward currents blocked by 4-AP are masking inward currents that are necessary for the generation of
A ∼10 Hz oscillations. If this is the case, 4-AP should unmask intrinsic inward currents in granular cortex. To test this possibility, granular cortex slices were placed in a low [Mg^{++}]_o buffer with BMI (10 µM) and glutamatergic synaptic transmission was blocked using CNQX and D-AP5 (20 and 50 µM, respectively). A stimulating electrode was positioned in the lower part of layer V (see Fig. 8A), and an electrical stimulus (200 µs pulse, 100–200 µA) was applied to trigger intrinsic membrane population currents in pyramidal cells within the stimulated cortical column.

Fig. 8A–D shows CSD analyses of activity in a granular cortex column comparing spontaneous and stimulus-evoked interictal events during low [Mg^{++}]_o buffer (control) and during block of glutamate transmission (CNQX + AP5). As described above, spontaneous interictal events consisted of an initial large current sink localized in layers II-III that lasted ∼100 ms and had a corresponding source in layer I (Fig. 8B; note that the layer I source is not seen in this particular figure, but was apparent when the probe moved higher; not shown). Similar events are evoked by electrical stimulation in lower layer V (Fig. 8C). In addition, an electrical stimulus evokes a fast current sink that propagates from layer V (1–2 ms peak after stimulus onset) through layer IV into layers II-III (2–4 ms peak after stimulus onset) where it merges with the layer II-III current sink (∼100 ms duration) typical of spontaneous interictal events (Fig. 8B). Thus, the current flow evoked by electrical stimulation is similar to a spontaneous interictal event, the major difference being the large fast sink propagating from layer V to II-III within the initial 5 ms after stimulus onset.

In every experiment (n = 8), block of glutamate receptors abolished spontaneous interictal events and all current flow evoked by the electrical stimulation except for the fast sink (and associated sources) propagating from layer V (1–2 ms) to layers II-III (2–4 ms) and a small amplitude longer latency sink localized in layers II-III between 5–50 ms after stimulus onset (see Figs. 8D,F, and 9A for examples from different experiments). In fact, there was no additional significant current flow 50 ms after stimulus onset. In every experiment, block of Na^+ channels with
FIG. 8. Intrinsic membrane population currents evoked in granular cortex by an electrical stimulus during block of glutamate receptors and the effects of 4-AP. (A) Fluorescent photomicrographs showing the location of the stimulating electrode and 16-channel electrode in granular cortex (left panel) and the recorded cortical column (right panel). (B) CSD of a spontaneous interictal event in granular cortex during low [Mg$^{++}$]$_o$ buffer. This plot only shows the initial current sink of the interictal event that occurs in layers II-III and lasts $\sim$100 ms. (C) Same as B but the response shown is evoked by an electrical stimulus delivered in lower layer V by the stimulating electrode (100 $\mu$A). In all figures, stimulus-evoked CSD responses are the average of 10 stimulus trials. The left and right panels show the same event with different x-axis durations. (D) Same as C but the response was evoked after block of glutamate receptors (CNQX + AP5). (E) Fluorescent photomicrographs showing the location of the stimulating electrode and 16-channel electrode in granular cortex (left panel) and the recorded cortical column (right panel). (F) CSD of stimulus evoked activity during block of glutamate receptors in granular cortex. In all cases, stimulus-evoked CSD responses are the average of 10 stimulus trials. The left and right panels show the same event with different x-axis durations. (G) Effect of 4-AP (50 $\mu$M) on the stimulus evoked activity shown in F. (H) Effect of cadmium (200 $\mu$M) on the stimulus evoked activity shown in G.

TTX (1 $\mu$M) abolished these sinks and their associated sources (not shown).

Figs. 8E–H and 9 show examples and population data of the effect of 4-AP during block of glutamate receptors in low [Mg$^{++}$]$_o$ buffer on current flow evoked by electrical stimuli to lower layer V. Application of 4-AP (50 $\mu$M; n = 5) had a particularly strong effect on long-latency current flow. The major effects were: (1) the fast current sink (1–2 ms) in layer V was slightly enhanced in amplitude ($t$-test; $p < 0.05$), while the fast sink (2–4 ms) in layers II-III was also slightly increased in amplitude but this was not significant across experiments ($t$-test; n.s.); (2) the longer latency sink (5–15 ms) in layers II-III showed a slight but significant increase in peak amplitude ($t$-test; $p < 0.05$); (3) a subsequent current sink (15–50 ms) that spreads into layer I showed a more significant increase ($t$-test; $p < 0.01$); (4) the strongest increase in current flow occurred at latencies above 50 ms, where new current flow was unmasked. In particular, a layer II-III sink (50–150 ms) with an associated source in layer I appeared showing a more than 10-fold increase in current amplitude ($t$-test; $p < 0.001$). This sink was followed by a large current source in the same layers that had a current sink (150–500 ms) in layer I ($t$-test; $p < 0.001$).

These results demonstrate that 4-AP unmasks intrinsic inward currents in granular cortex. One possibility is that Ca$^{++}$ currents are being unmasked. To test this possibility, we applied cadmium (200 $\mu$M; n = 5) to block high-threshold calcium currents during 4-AP application. Cadmium did not affect the increase of the short latency current sink (1–2 ms) in layer V produced by 4-AP. However, cadmium completely abolished the longer latency sinks in layers II-III and layer I that had been unmasked by 4-AP (Fig. 9). In addition to cadmium, we also tested other calcium channel blockers. We found that NiCl (200 $\mu$M; n = 5), omega-conotoxin (0.8 $\mu$M; n = 3), and nifedipine (50 $\mu$M; n = 4) were all effective at suppressing the long latency current sinks unmasked by 4-AP (Fig. 9C). However, there were considerable differences in their efficacies. The most effective at suppressing the unmasked currents were cadmium and NiCl, and the least effective were omega-conotoxin and nifedipine. The effectiveness
of all these drugs indicate that different types of calcium channels (L, N, P/Q, R, T) are unmasked by 4-AP.

These results demonstrate that the K\(^+\) channels that are blocked by low doses of 4-AP in granular cortex are suppressing Ca\(^{2+}\) currents that may be required for the generation of \(~10\) Hz oscillations. These results are compatible with the hypothesis that a K\(^+\) current is interfering with the ability of granular cortex pyramidal cells to generate \(~10\) Hz oscillations during disinhibition and low [Mg\(^{2+}\)]\(_o\) buffer.

**DISCUSSION**

The granular neocortex (e.g., barrel cortex) does not generate \(~10\) Hz oscillations during disinhibition or low [Mg\(^{2+}\)]\(_o\) buffers, while agranular neocortex (e.g., motor cortex) readily generates \(~10\) Hz oscillations. The results presented here indicate that the reason excitatory networks of granular neocortex do not generate \(~10\) Hz oscillations is because an outward current that is blocked by 4-AP (most likely, slowly inactivating K\(^+\) currents), impedes the generation of these oscillations. Thus, block of this outward current unmasks \(~10\) Hz oscillations in granular cortex and this occurs in unison with the unmasking of intrinsic Ca\(^{2+}\) currents, which are kept suppressed by the outward current. In conclusion, generation of \(~10\) Hz oscillations in excitatory networks of granular cortex is controlled by outward currents. Granular cortex is capable of generating \(~10\) Hz oscillations, just like agranular cortex, as long as those outward currents are suppressed.

The results distinguish between two alternative hypotheses to explain why agranular cortex but not granular cortex produce \(~10\) Hz oscillations during disinhibition or low [Mg\(^{2+}\)]\(_o\) buffers. One hypothesis is that the granular cortex does not have the intrinsic currents or connectivity essential for \(~10\) Hz oscillations to occur. An alternative hypothesis, supported by our results, is that the essential currents or connectivity are kept suppressed by an outward current. It is important to note that the results presented here do not address what currents or connectivity may be important for the generation of \(~10\) Hz oscillations. The mechanisms may involve Ca\(^{2+}\) currents that are unmasked either at presynaptic terminals to increase neurotransmitter release or in dendrites of pyramidal cells to...
increase dendritic excitability, or both. Interestingly, computational modeling has indicated that Ca\(^{++}\) currents in dendrites of pyramidal cells are important for generating ∼10 Hz network afterdischarges in hippocampus (Traub et al., 1993). Moreover, 4-AP sensitive K\(^{+}\) channels, likely of the I\(_D\) type, play a major role in inhibiting dendritic calcium spikes in CA1 pyramidal cells (Golding et al., 1999).

The observation that a low dose of 4-AP generates ∼10 Hz oscillations in granular cortex is in agreement with previous studies in cortical slices, which have shown that 4-AP application induces repetitive bursting in neocortex (Hoffman and Prince, 1995; Yang and Benardo, 2002). As discussed above, low doses of 4-AP blocks a slowly inactivating component of the voltage-dependent K\(^{+}\) current, I\(_D\) (Storm, 1988; Hoffman et al., 1997). Such a current appears to be perfectly suited to interfere with ∼10 Hz oscillations because it would activate as a consequence of the interictal event and slowly inactivate within the period (0.5–2 sec) when the oscillation occurs. In contrast, the fast inactivating component, I\(_A\), would inactivate too rapidly (∼50 ms) to be able to control the oscillation, which usually originates 300 ms after the onset of an interictal event.

The results also showed that high doses of TEA produce ∼10 Hz oscillations in granular cortex, which suggests a role for I\(_K\) in inhibiting these oscillations. Like I\(_D\), the time course of I\(_K\) appears well suited to dampen ∼10 Hz oscillations. Another possibility is that the protein subunits that form the relevant voltage-dependent K\(^{+}\) channel involved in inhibiting ∼10 Hz oscillations in granular cortex, such as perhaps Kv1.1 or Kv1.2 subunits, are sensitive to both low doses of 4-AP and high doses of TEA, as described in heterologous expression systems (Coetzee et al., 1999).

Regardless of the exact K\(^{+}\) channel involved, the present results demonstrate that voltage-dependent K\(^{+}\) channels inhibit granular cortex networks from generating ∼10 Hz oscillations.

In a recent study, we determined that ∼10 Hz oscillations of agranular cortex caused by disinhibition or low [Mg\(^{++}\)]\(_o\) are abolished by blockers of persistent sodium- and M-currents suggesting that these channels are critically involved in their generation (Castro-Alamancos et al., 2006). This finding also agrees with recent modeling work (Golomb et al., 2006). Future work will have to determine if the ∼10 Hz oscillations unmasked in granular cortex by K\(^{+}\) channel blockers are mechanistically similar to those of the agranular cortex.

**Why may motor cortex generate ∼10 Hz oscillations without the need to suppress intrinsic outward currents?** This important question is not addressed in the present study but the results suggest an intriguing hypothesis. One possibility is that the motor cortex has a population of layer V pyramidal cells that are devoid of the slowly inactivating K\(^{+}\) current and are absent in barrel cortex. Thus, these cells would readily generate ∼10 Hz oscillations simply by lowering [Mg\(^{++}\)]\(_o\) or by disinhibition because a slowly inactivating outward current would not interfere. Major differences in the expression of voltage-dependent K\(^{+}\) currents have already been reported between pyramidal cell dendrites of cortex and hippocampus when different studies are compared (Hoffman et al., 1997; Johnston et al., 2000; Korngreen and Sakmann, 2000; Bekkers, 2000a, 2000b; Bekkers and Delaney, 2001). Also, the motor cortex is known to contain a unique layer V cell population, called Betz cells or giant pyramidal cells (Betz, 1874), which are generally encountered in layer Vb (Sherwood et al., 2003). Further work will need to clarify if a unique population of pyramidal cells localized in agranular cortex is responsible for the selective generation of ∼10 Hz oscillations in this area during disinhibition and low [Mg\(^{++}\)]\(_o\) buffer.

**What is the functional significance of these results?** It has been recognized for several decades that certain seizures may develop from rhythmical afterdischarges that are similar to ∼10 Hz oscillations described here (Ralston, 1958). In behaving rats, ∼10-Hz oscillations induced by disinhibition in the primary motor cortex produce rhythmical jerks of the affected body part that recur continuously at regular intervals, resembling the cortical myoclonus of a continuous partial epilepsy (Castro-Alamancos, 2006). Interestingly, deletion of the Kv1.1 subunit in mice, a K\(^{+}\) channel subunit that in combination with other Kv1 subunits may be responsible for I\(_D\) (Coetzee et al., 1999), leads to frequent generalized spontaneous tonic–clonic seizures electrophysiologically and behaviorally (Smart et al., 1998). The present results indicate that the frontal agranular cortex is particularly susceptible to generate afterdischarges associated with cortical myoclonus, while parietal granular areas are much less susceptible due to suppression by slowly inactivating K\(^{+}\) currents.

**REFERENCES**


