Inhibitory Modulation of Cortical Up States

Maria V. Sanchez-Vives,1,2 Maurizio Mattia,3 Albert Compte,1 Maria Perez-Zabalza,1 Milena Winograd,3 Vanessa F. Descalzo,1 and Ramon Reig1

1Institut d’Investigacions Biomèdiques August Pi i Sunyer; 2Institució Catalana de Recerca i Estudis Avançats, Barcelona; 3Instituto de Neurociencias de Alicante, Universidad Miguel Hernández–Consejo Superior de Investigaciones Científicas, San Juan de Alicante, Spain; and 4Istituto Superiore di Sanità, Rome, Italy

Submitted 16 February 2010; accepted in final form 15 June 2010

INTRODUCTION

The issue of how excitation and inhibition are regulated in the course of physiological network function is highly relevant to understand cortical physiology and its dysfunctions, as for instance in epilepsy. A model in which the engagement of excitation and inhibition in cortical function can be studied is slow oscillatory activity. Basal excitability and recurrent connectivity in the cerebral cortex (Hebb 1949; Lorente de Nó 1938) induces neuronal firing that reverberates in the circuit, resulting in the emergent network activity. During slow-wave sleep and anesthesia, this activity is organized in the cerebral cortex. To understand the influence of inhibitory control on the emergent activity of the cortical network, inhibition was progressively blocked in a slice preparation that generates spontaneous rhythmic up states at a similar frequency to those occurring in vivo during slow-wave sleep or anesthesia. Progressive removal of inhibition induced a parametric shortening of up state duration and elongation of the down states, the frequency of oscillations decaying. Concurrently, a gradual increase in the network firing rate during up states occurred. The slope of transitions between up and down states was quantified for different levels of inhibition. The slope of upward transitions reflects the recruitment of the local network and was progressively increased when inhibition was decreased, whereas the speed of activity propagation became faster. Removal of inhibition eventually resulted in epileptiform activity. Whereas gradual reduction of inhibition induced linear changes in up/down states and their propagation, epileptiform activity was the result of a nonlinear transformation. A computational network model showed that strong recurrence plus activity-dependent hyperpolarizing currents were sufficient to account for the observed up state modulations and predicted an increase in activity-dependent hyperpolarization following up states when inhibition was decreased, which was confirmed experimentally.

METHODS

Ferrets (2–12 mo old, either sex) were anesthetized with sodium pentobarbital (40 mg/kg) and decapitated. The entire forebrain was rapidly removed to oxygenated cold (4–10°C) bathing medium. Coronal slices (0.4 mm thick) from the occipital cortex containing primary and secondary visual cortical areas (areas 17, 18, and 19) (Innocenti et al. 2002) or prefrontal (Krimer and Goldman-Rakic 1998) were used.

A modification of the sucrose-substitution technique developed by Aghajanian and Rasmussen (1989) was used to increase tissue viability. During preparation of slices, the tissue was placed in a solution in which NaCl was replaced with sucrose while maintaining an...
osmolarity of 307 mOsm. After preparation, slices were placed in an interface style recording chamber (Fine Sciences Tools, Foster City, CA). For the first 15 min cortical slices were superfused with an equal mixture in volume of the normal bathing medium and the sucrose-substituted solution. Following this, normal bathing medium was switched into the chamber and superfused the slices for 1–2 h, then modified slice solution was used throughout the rest of the experiment. Bath temperature was maintained at 34.5–36°C.

The normal bathing medium contained (in mM): NaCl, 126; KCl, 2.5; MgSO_4, 2; NaH_2PO_4, 1.25; CaCl_2, 2; NaHCO_3, 26; dextrose, 10, and was aerated with 95% O_2-5% CO_2 to a final pH of 7.4. The modified solution had the same ionic composition except for different levels of (in mM) KCl, 3.5; MgSO_4, 1; and CaCl_2, 1–1.2 (Sanchez-Vives and McCormick 2000). Electrophysiological recordings started after allowing ±2 h recovery.

Drugs were applied either in the bath or locally, through the delivery of a brief pressure pulse (10–150 ms; 100–350 kPa) to a drug-containing micropipette (volumes of 1–20 pl per pulse). Drugs used were bicuculline methiodide (BMI) and SR95531 (gabazine), both from Sigma.

**Spike recording and analysis**

Extracellular multiunit recordings were obtained with 2–4 MΩ tungsten electrodes (FHC, Bowdoinham, ME). Multiunit activity (MUA) was estimated as the power change in the Fourier components at high frequencies of the recorded local field potentials (LFPs) (Reig et al. 2010). High-frequency components of LFP can be seen as a linear transform of the instantaneous firing rate of the neurons surrounding the electrode tip. We assume then that the normalized LFP spectra provide a good estimate of the population firing rate, given that Fourier components at high frequencies have densities proportional to the spiking activity of the involved neurons (Mattia and Del Giudice 2002). The time-dependent MUs were the average power of the normalized spectra in the frequency band 0.2–1.5 kHz. With respect to a similar approach in Stark and Abeles (2007), the above-cited estimate provides a larger signal-to-noise ratio: using normalized spectra the components at different frequencies have similar orders of magnitude. MUAs were logarithmically scaled to balance the large fluctuations of the nearby spikes. Furthermore, log(MUA) time series were smoothed by a moving average with a sliding window of 80 ms.

Up and down states were singled out by setting a threshold in log(MUA) time series. The histograms of log(MUA) were bimodal and the positions of the high and low peaks were used as reference activity respectively for up and down states. The discriminating threshold was set to 60% of the interval between the peaks. To remove the effects of small activity fluctuations a cutoff in the minimum state duration was set in a range [1/3, 1/2] of the average up state length. This lower limit was chosen case by case to reproduce the up/down oscillation frequency estimated from the log(MUA) autocorrelation. Small periods were recursively removed converting short up (down) states in longer down (up) states. Finally, times of transition between states were better estimated by fitting the log(MUA) in a 100 ms window around the transitions with third-degree polynomials. The adjusted transition was set to the crossing time of the polynomial with the discriminating threshold. The slopes of the upward transitions were measured as the gradients of the linear fits of the average log(MUA) in the time interval (~10, 25 ms) around the detected up state onset. Similarly, the slopes of the downward transitions resulted from the linear fit of log(MUA) centered around the occurrences of the transitions to down states in the time interval (~25, 10 ms).

All the MUA off-line estimates and analyses were implemented in MATLAB (The MathWorks, Natick, MA). We refer to the log(MUA) in the figures and the rest of the study as “relative firing rate,” since this is a relative measure resulting from an average of power spectra ratios (Reig et al. 2010).

**Intracellular recordings**

Sharp intracellular recording electrodes were formed on a Sutter Instrument (Novato, CA) P-97 micropipette puller from medium-walled glass (1B100F-4; WPI, Sarasota, FL) and beveled on a Sutter Instrument beveller to final resistances of 60–100 MΩ. Micropipettes were filled with 2 M KAc. Current-clamp intracellular recordings were obtained using an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA). The transitions between up and down states in the intracellular recordings illustrated in the following text were detected by hand to determine their slopes. Intra- and extracellular recordings were digitized, acquired, and analyzed with a CED interface and Spike2 software (Cambridge Electronic Design [CED], Cambridge, UK). Data are reported as mean ± SD.

**Computational modeling**

We used the network model of slow oscillatory activity in visual cortical slices of the ferret that has been presented elsewhere (Compte et al. 2003). Briefly, the network model consists of a population of 1,024 pyramidal cells and 256 inhibitory interneurons, equidistantly distributed on a line and interconnected through biologically plausible synaptic dynamics (kinetics of synaptic currents mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), N-methyl-d-aspartate receptors (NMDARs), and γ-aminobutyric acid type A receptors (GABA_A, Rs) are modeled as in Wang (1999). Some of the intrinsic parameters of the cells are randomly distributed, so that the populations are heterogeneous. This and the random connectivity (determined by the synaptic probability distributions) are the only sources of noise in the network. The neurons in the network are sparsely connected to each other through a fixed number of connections that are set at the beginning of the simulation. Neurons make 20 ± 5 contacts (mean ± SD) with their postsynaptic partners (multiple contacts onto the same target, but no autapses are allowed). For each pair of neurons separated by a distance x in the network, the probability that they are connected in each direction is decided by a Gaussian probability distribution P(x) centered at 0 and with a prescribed SD σ_p. For inhibitory connections a Gaussian probability distribution was also used but with a smaller SD σ_i = σ_p/2. Our model pyramidal cells have both a somatic and a dendritic compartment. The somatic compartment hosts the spiking currents, I_Na and I_K, a leak current I_L, a fast A-type K current I_KV, a non-inactivating slow K current I_KS, and a Na–dependent K current I_KNa. The dendrite contains a high-threshold Ca^2+–dependent K current I_Ca^2+ and a Ca^2+–dependent K current I_KCa^2+, a non-inactivating (persistent) Na^+ current I нa,persistent, and an inward rectifier activated by hyperpolarization K current I_KG. For interneurons the model was taken from Wang and Buzsáki (1996). All details about the exact parameter values and model implementations can be found in Compte et al. (2003). The model was implemented in a C++ code and simulated using a fourth-order Runge–Kutta method with a time step of 0.06 ms.

**RESULTS**

Fifty-five slices of ferret visual or prefrontal cortex that generated spontaneous slow rhythmic activity were recorded (<1 Hz; Fig. 1). Spontaneous rhythmic activity consisted of alternating periods of persistent activation or up states interleaved with intervals of relative silence or down states. To understand how inhibition shapes emergent cortical activity, we acted on fast inhibition by decreasing it with BMI or gabazine. The removal of inhibition is known to result in epileptiform activity (Gutnick et al. 1982; Prince and Wilder 1967). Our main objective was to explore how the progressive blockade of GABA_A, Rs would transform cortical rhythmicity before the network became epileptic.
Effect of decreasing inhibition on the frequency and duration of up states

To explore how inhibition controls emerging network activity we applied a low concentration of BMI in the bath (0.1–0.2 μM) and progressively increased the concentration until epileptiform activity would appear (usually >1 μM). Once full-blown epileptiform activity was generated (see following text), a further increase in BMI was not followed by any more gradual changes.

The progressive blockade of inhibition induced different concurrent changes in the physiological up and down states. Some of them are illustrated in Fig. 1 for a particular slice. A progressive removal of inhibition induced a gradual shortening of up states. This can be observed in the raw traces in Fig. 1A, in raster plots in Fig. 1B, and in up state averages for six BMI concentrations in Fig. 1, C and F. The shortening of up states was concomitant with a progressive increase in the firing rate (Fig. 1, C, E, and G). The duration of the subsequent down states was also modified, becoming progressively longer with lesser inhibition (Fig. 1, A–D). This example illustrates that there is a global change in the dynamics of the network activation as a result of inhibition removal. On the other hand, the bistable nature of the network activity remains unchanged and, even at higher BMI concentrations, two preferred activity levels can be recognized (Fig. 1E).

For a detailed quantification of activity transformation during gradual blockade of inhibition only slow oscillations of >0.2 Hz frequency were included (n = 25), to have up/down state dynamics similar to those in vivo. The transformation of the emergent activity described earlier (Fig. 1) following the removal of inhibition was consistent across the population: decrease in the up/down cycle frequency (Fig. 2A), increase in
the firing rate during up states (Fig. 2B), shortening of up states (Fig. 2C), and lengthening of down states (Fig. 2D). Similar results concerning isolated up states were previously described (Mann et al. 2009).

Since BMI is known to have an additional effect to inhibition blockade, which is the blockade of small \(K^+\) channels (Debarbieux et al. 1998; Khawaled et al. 1999), we also used gabazine (SR95531) \((n = 11)\), a blocker of inhibition that does not have such a collateral effect. The specific effects of SR95531 on the spontaneous activity are represented in Supplemental Fig. S1, illustrating that the network effects were equivalent to those obtained for BMI: the frequency of the oscillation decreased, the firing rate during up states increased, up state duration decreased, and down state duration increased. Even when there were no statistically significant differences with BMI-induced changes, we observed that in gabazine both the increase in up state’s firing rate and the elongation of down states were less than those in BMI.

**Removal of inhibition and the upward and downward network transitions**

We measured the slopes of the network transition from down to up states (Fig. 3A). The upward transition reflects the recruitment of the local network. Increasing concentrations of BMI (Fig. 3A) or gabazine (Supplemental Fig. S1) resulted in a progressively faster recruitment of the local network and thus a faster upward transition. This was the case at the population level (Fig. 3B). The slope measured at the initiation of epileptiform discharges revealed a still faster recruitment, usually doubling the one reached in the preepileptic state (Fig. 3B). The local network recruitment has its cellular counterpart in the summation of synaptic potentials impinging onto a particular neuron. Eleven intracellular recordings were included: 9 regular spiking, one chattering cell, and one intrinsically bursty neuron, where classification was carried out following Nowak et al. (2003). Intracellular recordings (Fig. 3E) illustrate the accumulation of synaptic events that leads to an up state, accumulation that becomes faster with the progressive removal of inhibition. This faster transition can also be observed in the transformation from slow-wave activity to epileptiform activity in vivo (Steriade and Contreras 1995). These results suggest that in physiological conditions inhibition is slowing down the recruitment of activity in the local network. The slower/faster recruitment of the local network also results in the control of the propagation of activity (Compte et al. 2003) (see following text).

The downward transition from up to down states marks the end of up states. The slope of this transition was also evaluated at the network level by measuring the decay in the local relative firing rate (Fig. 3C). The progressive removal of inhibition also led to a steeper downward transition, reflecting that the transition is occurring more synchronously in the local population. This was also the case when all slices were considered (Fig. 3D). In the case of the downward transition, epileptiform responses did not have a faster downward transition but a slower one. In conclusion, there was a parametric linear relation between removal of inhibition and increase in upward (downward) slope and in both cases this relation became nonlinear when epileptiform activity appeared. The intracellular correlate of the increased downward transition is a faster repolarization of the up states while they became shorter (Fig. 3E). In the following text, we provide a possible mechanistic explanation based on observations in a computational model.

In the course of gradual blockade of inhibition we often (56%) observed what we referred to as “diplets” or even “triplets” (Fig. 4). Diplets (triplets) were up states of shorter duration and higher firing frequency that appeared in pairs (trios) (Fig. 4, B, C, and F), as if they were up states fractioned into two (three) parts. This activity was most often transitory, suggesting a short-lived activity occurring while the network was adapting to a lower level of inhibition (Fig. 4E). No

---

1 The online version of this article contains supplemental data.
relationship was found between the occurrence of diplets and the generation of epileptiform discharges. Even when this may be a phenomenon of inherent interest, to quantify the evolution of up states with changing inhibition as in Fig. 2, diplets (triplets) were excluded.

Inhibition and the wave propagation speed

The speed of propagation of the wave reflects the horizontal propagation of the locally generated up states along the cerebral cortex. By horizontal we refer to the propagation perpendicular to the cortical columns and along cortical layers. We know that the speed of the traveling wave increases around one order of magnitude when inhibition is completely removed (Compte et al. 2003; Sanchez-Vives and McCormick 2000). Here we found that the speed of wave propagation increased linearly with the progressive removal of inhibition. Up states were recorded with two separate electrodes (Fig. 5A) and the time lags between the initiation of every up state recorded from both electrodes were measured for different BMI concentrations (Fig. 5B). The distribution of time lags for each BMI concentration was calculated and the mean time that the activity takes to travel from one electrode to the other was taken as the peak. Sometimes the activity would travel in the opposite direction and then bimodal histograms of time lags occur with almost symmetrical peaks: in these cases we considered the most populated peak to estimate the speed of propagation.

The average speed of propagation determined in that way was calculated in five slices for five concentrations of BMI (Fig. 5C). Before reaching epileptiform activity, the speed of propagation of activity had approximately doubled (from 6 to 12 mm/s). However the increase in speed once full-blown epileptiform activity occurred was nonlinear with the previous ones, reaching an average speed of 43 mm/s. During activity propagation, up states are locally regenerated along the network, such that the initiation of up states appears intracellularly as a summation of synaptic potentials (Fig. 3E). The local recruitment of network activity is also represented by the transition from down to up states of the firing rate (Fig. 3, A and B). We observed that the faster the down to up state transition is, the faster the speed of wave propagation (Fig. 5D), thus the local buildup of the upward transition influences the speed of the wave.
Transition towards a epileptiform discharges

We further investigated the changes at higher levels of disinhibition capable of eliciting the onset of epileptic-like activity. Full-blown epileptiform discharges occurred unexpectedly as a dramatic and sustained increase of activity simultaneously observable from distant electrodes as in Fig. 6A (at ~460 s). Dynamics of probed populations of neurons had an abrupt and qualitative change, as witnessed by the time course of the latency between the onset of up states detected by distant electrodes (Fig. 6A, bottom), showing a discontinuity in the speed of propagation that induced an almost simultaneous state transition in different points of the slice (see also Fig. 5C).

When inhibition is progressively removed, there is a transformation of the emergent pattern of activity until it eventually becomes epileptiform. This transformation is illustrated in Fig. 6D. Stage 0 represents up and down states during physiological slow oscillations. As we have characterized in this study, up states become progressively shorter with inhibition blockade and this is illustrated in Stage 1: shorter up states and elongated down states. The parametric changes in up/down states induced by a progressive removal of inhibition described earlier correspond to the transformation between Stage 0 and Stage 1 (Figs. 1 and 2). Before full-blown epileptiform discharges appear (Stage 3), there are short bursts of activity of steep rise characterized by their triangular or cuneiform shape (Stage 2), instead of the fusiform shape of up states in Stages 0 and 1. The triangular (or cuneiform) shape corresponds to an abnormally fast recruitment of the local network, inducing a heavy spike discharge and that has a progressive decay. This pattern of activity may be local (Fig. 6B) or propagate between electrodes.

Computational modeling and predictions

To test the consistence of our findings with our mechanistic interpretation, we used a previously defined computational network model of the slow oscillation (Compte et al. 2003), which relies on strong recurrent coupling and activity-dependent potassium channels for the generation of the slow rhythm. This model consists of 1,024 excitatory cells and 256 inhibitory cells modeled with detailed Hodgkin–Huxley-type channels and interconnected through realistic synaptic dynamics (METHODS). Critical for the model behavior is strong recurrent excitation and inhibition and Ca\(^{2+}\) - and Na\(^{+}\)-dependent potassium channels in pyramidal cells, which are responsible for the transitions between up and down states. The model network displays activity organized in recurring up states that propagate across the network, closely resembling the experimental data (Compte et al. 2003, 2008, 2009).

We wanted to study what effects progressive inhibition blockade induced in the network activity of the computer model in Compte et al. (2003). Because we know in detail the mechanisms that generate the network activity in the model (Compte et al. 2003), this specifically tested our mechanistic
understanding of the experimental results. The removal of inhibition was simulated by the reduction of GABA$_A$ channel conductances in inhibitory synapses to pyramidal neurons of the network. This manipulation resulted in increased excitability, as measured by the firing rate of neurons during the up state (Fig. 7C), but also induced significant reductions in the duration of the up states (Fig. 7, D and E) and an increase in the interval between oscillations (Fig. 7, C and E), in agreement with the experimental results. In addition, the model also reproduced the augmented slope of down-to-up activity buildup after inhibition blockade (Fig. 7D) and the progressive increase in wave propagation speed as inhibition was gradually blocked (see Fig. 10B in Compte et al. 2003). We explored what model mechanisms were underpinning these network activity changes. The membrane potential traces of excitatory neurons in the network revealed a larger afterhyperpolarization (AHP) following up states after inhibition was decreased (Fig. 7F). This observation led us to suggest that the activation of K$^+$ channels with the neuronal firing during up states could be a relevant mechanism in the termination of up states (Compte et al. 2003; Cunningham et al. 2006), some of which could be associated with GABA$_B$ receptors (Mann et al. 2009; Parga and Abbott 2007). The outcome of our investigations of the computational network model thus proves that the combination of strong recurrent feedback and activity-dependent potassium currents is sufficient to explain the typical modulations of network activity patterns observed on inhibition manipulation.

**Experimental testing of model predictions**

Intracellular recordings from neurons in a network with reduced inhibition revealed that AHPs following up states showed increased amplitude relative to control conditions with intact inhibition (Fig. 8), as predicted by the model (Fig. 7F).

An increase in the AHP following up states with the removal of inhibition is suggestive of a more efficiently recruited mechanism to terminate up states. This is compatible with the increased firing rate during up states with lesser inhibition (Figs. 1 and 2). Consistent with this, the increase in firing rate during up states was significantly correlated with the slope of the subsequent downward transition (Supplemental Fig. S2).

**DISCUSSION**

We studied the functional contribution of inhibition to the slow oscillatory patterns generated by the cortical network in vitro. It is agreed that excitation and inhibition balance each other both during spontaneous and during sensory activated cortical activity (Anderson et al. 2000; Compte et al. 2009; Haider et al. 2006; Monier et al. 2008; Okun and Lampl 2008; Shadlen and Newsome 1998; Shu et al. 2003a; Wehr and Zador 2003). When this balance is altered, pathological patterns of activity such as epilepsy are generated (Prince and Wilder 1967; Steriade et al. 1998; Timofeev et al. 2002). Our objective was to analyze from a network perspective how inhibition regulates cortical emergent activity by inducing a progressive excitatory/inhibitory imbalance.

Progressive reduction of inhibition resulted in a parametric decrease in the duration and frequency of up states. As a result, less inhibition led to an apparently decreased activity in the network (see Fig. 1B), a rather counterintuitive effect. We suggest here that the link between reduction of inhibition and shorter and less frequent up states is the concurrent, parametric increase in firing rate during up states. Our investigations with a computational model of the slow oscillation (Compte et al. 2003) show that activity-dependent K$^+$ channels together with strong intracortical recurrence are sufficient to account for our typical experimental results (Fig. 7). In this reduced model of
a cortical network, less inhibition during up states leads to higher firing rates, that recruit more efficiently activity-dependent $K^+$ channels, shortening up states, and elongating down states. In support of the proposed mechanism there are various experimental results showing, on the one hand, that the higher the firing rate during up states, the longer down states become (Fig. 2, B and D). On the other hand, the firing rate during up states was significantly correlated with the up to down state slope that reflects how fast network activity decreases at the end of up states (Supplemental Fig. S2). Thus the larger the firing rate during the up state, the faster the silencing of the network. Furthermore, the increased AHP following up states when there is less inhibition also supports this mechanism (Compte et al. 2003; Cunningham et al. 2006; Sanchez-Vives and McCormick 2000) and the main results presented here concur with this working model, although other activity-dependent mechanisms such as synaptic depression (Holcman and Tsodyks 2006) or GABA$_B$ activation (Mann et al. 2009; Parga and Abbott 2007) may also participate.

These results suggest that the transformation of the emergent activity when inhibition is decreased requires a dynamic interplay between up and down states. For that to occur is necessary that the oscillatory frequency is close to the physiological one. The frequency of slow oscillations was originally described as being $<1$ Hz (Steriade et al. 1993), specifically 0.3–0.4 Hz in association (areas 5 and 7) with motor and visual cortical areas of the anesthetized cat. The same frequency (0.3 Hz) was described in the ferret visual and prefrontal cortex in vitro and cat visual cortex in vivo (Sanchez-Vives and McCormick 2000). Rat neocortex in vivo generates higher frequencies, in the range 0.3–1.5 Hz (Cowan and Wilson 1994). The frequency of up states varies in other areas or preparations, including: 1.8 Hz in ferret piriform cortex in vitro (Sanchez-Vives et al. 2008); lower frequencies in rat entorhinal cortex in vitro, varying between 0.17 Hz (Cunningham et al. 2006) and 0.02 Hz (Mann et al. 2009); and still lower in cortical slabs (0.002–0.008 Hz) (Timofeev et al. 2000). This diversity of frequencies implies that down states vary between 0.5 s and 11 min and thus must respond to the participation of different network mechanisms. In cases where the average duration of the down states lasts tens of seconds, $K^+$ channel activation is probably not the main mechanism that maintains the complete duration of down states. Afterhyperpolarizations (AHPs) are often observed following up states (Sanchez-Vives and McCormick 2000) and they increased...
for higher preceding firing rates (Fig. 8). However, the discharge in an up state is not enough to induce AHPs that would last for as long as a tens of seconds. We propose that the mechanism determining the duration of down states in these cases is not an AHP but the time it takes for the mechanisms that give rise to a new up state to build up. For example, summation of randomly occurring miniature synaptic potentials is a mechanism proposed for up state initiation in cortical slabs, where up states occur at frequencies well below 0.2 Hz, with down states of up to 60 s (Timofeev et al. 2000).

Transitions between up and down states and wave propagation

The transition from down to up state corresponds to the recruitment of the local network. A network that is recruited faster has a steeper slope in the transition and vice versa and we have shown this here both for the population firing rate and for the accumulation of synaptic potentials while recorded intracellularly. Excitation and inhibition accumulate at a similar rate during the down to up transition in cortical slices (Compte et al. 2003).

**FIG. 7.** Reducing inhibition in a cortical network model. Manipulation of inhibition in a computational network model that relies on recurrent coupling and activity-dependent adaptation mechanisms (Compte et al. 2003) produces the same effects on the duration and interval between up states and on mean firing rate as in experiments. A: sample network rastergram in control. B: sample network rastergram after partial inhibition blockade to a remaining 25% of inhibition relative to control (A). C: the activity profiles for model neurons aligned at the beginning of the up state show that reducing inhibition (from control to 50% and further to 25% inhibition) increases firing rates in the up state and the interval between successive up states (down state). D: normalized activity profiles for one up state show how inhibition blockade reduces up state duration and increases down-to-up activation slope. E: summary graph of up state duration (measured as time duration for which curves in D exceed 10% of maximum value; black trace) and interval of oscillation (measured as interval between maxima in C; red trace) for intermediate values of inhibition in the network (100, 90, 80, 70, 60, 50, 40, 30, and 25%) shows progressive gradual changes as in experiments. F: membrane potential traces from 2 neurons (left and right panels) in the cortical network model during slow oscillations. In both examples, control traces are shown in the left column (traces expanded around resting voltage in the bottom panel). Right column: membrane potential traces in the same neurons after network inhibition was decreased. Notice the shortening of the up states and the increased afterhyperpolarization (AHP) following the up states.

**FIG. 8.** AHPs following up states are increased when there is less inhibition. A: intracellular recordings of a neuron in an oscillating slice in control (left) and during the partial blockade of inhibition with BMI (right). The bottom trace is expanded to better see the increased AHP that followed up states when inhibition was partially blocked. B: multiunit recording of network activity in the close vicinity of the intracellular recording shown in A. C and D represent another neuron example equivalent to A and B in a different slice. Vertical scale bar corresponds to 10 mV and horizontal scale bar to 1 s.
The excitatory postsynaptic potentials impinging onto cortical neurons in cortical slices may originate in distant excitatory neurons through horizontal connections or in local ones, whereas inhibitory postsynaptic potentials are originated in local interneurons. Long horizontal connections also have inhibitory interneurons as a target (20%), although in lesser proportion than excitatory target neurons (80%) (McGuire et al. 1991). Therefore excitatory neurons that are firing during an up state would contribute to the build up of excitatory and inhibitory activity in the distant regions they project to. This initial depolarization still requires the local reverberation of activity to generate an up state, which appears to activate local neurons in a stereotypical order (Luczak et al. 2007). The local reverberation necessary for wave propagation is probably the reason that there is a relationship between the speed of wave propagation and the slope of the transition from down to up states (Fig. 5D). Long-range connectivity in the cortical model had relevant influence in the speed of wave propagation, decreasing with the length of horizontal connections (Compte et al. 2003). Progressive elimination of network inhibition accelerates the transition from down to up states, during which synaptic events accumulate faster. Not only is distant excitation then balanced toward excitation, but the local circuit is also not slowed down by inhibitory summation. Still, the progressively faster propagation with the removal of inhibition is nonlinear towards the still faster propagation of epileptiform activity (Figs. 5C and 6A). The speed of wave propagation approximately doubles with progressive inhibition blockade, but then it leaps toward speeds almost one order of magnitude higher when epileptiform activity appears, as in Sanchez-Vives and McCormick (2000). Epileptiform bursts should thus not be seen as the last step of a progressive transformation of up states when inhibition is removed, since all aspects of these bursts are the result of a nonlinear change. Radically different network mechanisms are at play.

The transition from up to down states also increases its rate with the removal of inhibition. The more efficient activation of K+ channels following a higher firing rate during up states would induce neuronal AHPs (Compte et al. 2003). Since maintenance of the up state requires reverberation of activity in the network, this sudden hyperpolarization would stop this process and induce a transition toward the down state. A faster network transition would therefore reflect a more synchronized end of the reverberatory activity, associated with lesser inhibition. A similar mechanism based on K+ -channel recruitment terminates epileptiform bursts in hippocampus (Alger and Nicoll 1980). Even when the mechanisms that determine the down to up and the up to down transitions are totally different, they seem to covary. We have found the same covariation in the transformation of slow oscillations with temperature (Reig et al. 2010). The link between both slopes is the firing rate during up states. A fast upward transition is usually associated with a high-frequency rate once the up state occurs, which will normally induce a fast transition toward the next down or silent state.

Sufficient mechanisms: recurrent coupling and adaptation channels

We used a computational network model of the slow oscillation (Compte et al. 2003) and prove that the combination of strong recurrent coupling and activity-dependent hyperpolarizing currents was sufficient to explain the modulations of network activity patterns induced by inhibition manipulations in our experiments. Investigations with the model network showed trends in up state firing rate, up state duration, down state duration, down-to-up transition slope, and wave propagation speed similar to those in experiments on gradual inhibitory transmission manipulations. At least qualitatively, the control of network activity by inhibition during the slow oscillation in vitro can be understood primarily as the interplay of excitation, inhibition, and slow adaptation currents. This underscores the importance of adaptation currents in the control of physiological network activity and suggests its possible compensatory role on failing inhibitory regulation.

References


Mann EO, Kohl MM, Paulsen O. Distinct roles of GABA(A) and GABA(B) receptors in balancing and terminating persistent cortical activity. J Neurosci 29: 7513–7518, 2009.


Supplemental Figures and text

Inhibitory modulation of cortical up states

Maria V. Sanchez-Vives,1,2* Mauzio Mattia,3 Albert Compte,1 Maria Perez-Zabalza,1 Milena Winograd,4 Vanessa F. Descalzo,1 and Ramon Reig 1

Supplemental text and Figure S1. Effect of blockade of inhibition with gabazine on the slow oscillations.

Since BMI is has an additional effect to inhibition blockade which is the blockade of small K+ channels (10, 11), we also used gabazine (SR95531) (n=11). The specific effects of SF95531 on the spontaneous activity are represented in Fig. S1, showing that the network effects were equivalent to those in bicuculline. Thus the frequency of the oscillation decreased, the firing rate during up states increased, up state duration decreased and down state duration increased. Even when there were no statistically significant differences with bicuculline induced changes (except for the slope of down states increase), in gabazine both the increase in up state’s firing rate and the elongation of down states were smaller than in BMI, an observation that could be explained in the frame of our model (see below).

Figure legend S1. Variation in 6 different parameters of the slow oscillations with gradual increasing of gabazine concentration. Averages from 11 cases, the error bars represent the s.e.m. (A) Up/down cycle frequency (B) Relative firing rate in up states. (C) Down state duration (D) Up state duration. (E) Transition slope from down to up state. (F) Transition slope from up to down state.

Supplemental Figure S2. Firing rate in up states and the slope of the up to down transition.

Relation between relative firing rate in up states and downward transition slope in the population (n=117 points corresponding to measurements in 25 slices; R=0.78; P<0.0001).