Cerebral Cortex May 2006;16:688-695
doi:10.1093/cercor/bhj014
Advance Access publication August 17, 2005

Impact of Cortical Network Activity on Short-term Synaptic Depression

Ramon Reig[1], Roberto Gallego[1], Lionel G. Nowak[2] and Maria V. Sanchez-Vives[1]

[1]Instituto de Neurociencias de Alicante, Universidad Miguel Hernandez-CSIC, Apartado 18, 03550 San Juan de Alicante, Spain and [2]Centre de recherche 'Cerveau et Cognition', CNRS-Université Paul Sabatier.133, route de Narbonne. 31062 Toulouse Cedex, France

Repetitive stimulation of synaptic connections in the cerebral cortex often induces short-term synaptic depression (STD), a property directly related to the probability of transmitter release and critical for the computational properties of the network. In order to explore how spontaneous activity in the network affects this property, we first studied STD in cortical slices that were either silent or that displayed spontaneous rhythmic slow oscillations resembling those recorded during slow wave sleep *in vivo*. STD was considerably reduced by the occurrence of spontaneous rhythmic activity in the cortical network. Once the rhythmic activity started, depression decreased over time in parallel with the duration and intensity of the ongoing activity until a plateau was reached. Thalamocortical and intracortical synaptic potentials studied *in vivo* also showed stronger depression in a silent than in an active cortical network, and the depression values in the active cortical network *in vivo* were indistinguishable from those found in active slices *in vitro*. We suggest that this phenomenon is due to the different steady states of the synapses in active and in silent networks.

Keywords: cortex, *in vivo*, plasticity, rhythmic activity, slow oscillations, spontaneous activity

Introduction

Synaptic transmission between neocortical neurons often shows activity-dependent synaptic depression. Short-term synaptic depression (STD) is mainly attributed to depletion of a readily releasable vesicle pool that occurs with repetitive presynaptic activation (Zucker, 1989; Dobrunz and Stevens, 1997) resulting in a decline in neurotransmitter release. Other mechanisms, such as feedback presynaptic inhibition and desensitization of postsynaptic receptors, may also participate (for a review, see Jones and Westbrook, 1996; see also Zucker and Regehr, 2002). A variety of STD rates (tens of milliseconds to seconds) have been described in the cerebral cortex *in vitro* (Thomson and Deuchars, 1994; Finlayson and Cynader, 1995; Gil *et al.*, 1997; Tsodyks and Markram, 1997; Varela *et al.*, 1997; Galarreta and Hestrin, 1998; Thomson *et al.*, 2002). The decay of synaptic potential amplitude dependent on presynaptic firing frequency introduces nonlinearities between the input and ouput functions, deeply influencing cortical processing. By acting as a dynamic, input-specific gain control mechanism, STD may represent a crucial mechanism in neural coding (Abbott *et al.*, 1997; Tsodyks and Markram, 1997). For these reasons, this particular form of cortical synaptic plasticity has been incorporated as a key mechanism in numerous computational models of cortical function. In particular, these model studies proposed that both thalamocortical and intracortical STD play a role in the adaptation of cortical responses to repetitive sensory stimulation (Todorov *et al.*, 1997; Carandini *et al.*, 1998; Chance *et al.*, 1998; Adorjan *et al.*, 1999a; Chung *et al.*, 2002).

STD *in vivo* has also been reported in several studies (Castro-Alamancos and Connors, 1996; Chung *et al.*, 2002; Fuentealba *et al.*, 2004), and some indicate that it may be less pronounced than *in vitro* (Kang *et al.*, 1991; Sanchez-Vives *et al.*, 1998; Boudreau and Ferster, 2003). This difference between *in vivo* and *in vitro* values of synaptic depression may be due to several factors, among them differences in ionic environment or on the existence of spontaneous activity in the brain *in situ*.

Here we have explored how an inherent property of the cortical circuit *in vivo*, ongoing spontaneous activity, affects synaptic depression. To do this we used both silent and rhythmically active cortical slices, which generate activity closely resembling the one during slow wave sleep (Sanchez-Vives and McCormick, 2000). Furthermore, we examined STD in thalamocortical and intracortical synaptic connections *in vivo* in relationship to the spontaneous activity level, that was eventually manipulated by varying the depth of anesthesia. We conclude that ongoing activity in the network reduces synaptic depression.

Materials and Methods

Slices Preparation

The methods for preparing cortical slices were similar to those described previously (Sanchez-Vives and McCormick, 2000). Briefly, cortical slices were prepared from 2- to 6-month-old ferrets of either sex that were deeply anesthetized with sodium pentobarbital (40 mg/kg) and decapitated. Four hundred-micrometer-thick coronal slices of the visual cortex were cut on a vibratome. A modification of the technique developed by (Aghajanian and Rasmussen, 1989) was used to increase tissue viability. After preparation, slices were placed in an interface-style recording chamber (Fine Sciences Tools, Foster City, CA) and bathed in what we refer to as 'classical' artificial cerebrospinal fluid (ACSF), containing (in mM): NaCl, 124; KCl, 2.5; $MgSO_4$, 2; $NaHPO_4$, 1.25; $CaCl_2$, 2; $NaHCO_3$, 26; and dextrose, 10, and aerated with 95% O_2, 5% CO_2 to a final pH of 7.4. Bath temperature was maintained at 34–35°C. Intracellular recordings were initiated after 2 h of recovery. In order to induce spontaneous rhythmic activity, the solution was switched to 'modified' ACSF containing (in mM): NaCl, 124; KCl, 3.5; $MgSO_4$, 1; $NaHPO_4$, 1.25; $CaCl_2$, 1–1.2; $NaHCO_3$, 26; and dextrose, 10.

Animal Preparation for In Vivo Recording

Intracellular recordings *in vivo* from the primary visual cortex of cats were obtained following the methodology that we have previously described (Sanchez-Vives *et al.*, 2000). In short, adult cats were anesthetized with ketamine (12–15 mg/kg, i.m.) and xylazine (1 mg/kg, i.m.), and then mounted in a stereotaxic frame. A craniotomy (3–4 mm wide) was made overlying the representation of the area centralis of area 17. To minimize pulsation arising from the heartbeat and respiration, a cisternal drainage and a bilateral pneumothorax were

performed, and the animal was suspended by the rib cage to the stereotaxic frame. During recording, anesthesia was maintained with continuous i.v. infusion of propofol (5 mg/kg/h) and sufentanyl (4 μg/kg/h). The animal was paralyzed with norcuron (induction 0.3 mg/kg; maintenance 60 μg/kg/h) and artificially ventilated. The heart rate, expiratory CO_2 concentration, rectal temperature and blood O_2 concentration were monitored throughout the experiment and maintained at 140–180 bpm, 3–4%, 37–38°C and >95%, respectively. The EEG and the absence of reaction to noxious stimuli were regularly checked to insure an adequate depth of anesthesia. After the recording session, the animal was given a lethal injection of sodium pentobarbital.

Ferrets and cats were cared for and used in accordance with the Spanish regulatory laws (BOE 256; October 25, 1990), which comply with the EU guidelines on protection of vertebrates used for experimentation (Strasbourg, March 18, 1986).

Recordings and Stimulation

Sharp intracellular recording electrodes were formed on a Sutter Instruments (Novato, CA) P-97 micropipette puller from medium-walled glass and beveled to final resistances of 50–100 MΩ. Micropipettes were filled with 2 M KAc. Recordings were digitized, acquired and analyzed using a data acquisition interface and software from Cambridge Electronic Design (Cambridge, UK). Electrical stimulation (0.1 ms, 10–300 μA) was delivered by means of a WPI A-360 stimulus isolation unit (Sarasota, FL) that prevents electrode polarization. *In vitro*, a concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) was placed in layer 4 and the postsynaptic neurons recorded in layer 2/3. *In vivo* thalamocortical (t.c.) or intracortical (i.c.) fibers were stimulated with bipolar electrodes made of sharpened tungsten wires. The eyes were focused onto a tangent screen at 114 cm using corrective, gas permeable contact lenses. The position of the area centralis and optic discs was localized by retroprojection. Receptive fields location was determined with a handheld projector. The electrode placed in the LGN was first used as a recording electrode and re-located following the coordinates in (Sanderson, 1971) until the local receptive fields were centered on the fovea and overlapped with the ones from the recorded cortical neurons. Once in place, it was used for t.c. stimulation. I.c. stimulation was delivered at 500–1500 μm from the intracellularly recorded neuron. Both *in vivo* and *in vitro*, and both in t.c. and i.c., the intensity of the stimulation was adjusted to achieve a stable PSP amplitude, which at the population level ranged between 2 and 7 mV. Only monosynaptic connections were included, the criteria being: reliably evoked synaptic potentials (no failures) of constant amplitude and shape and with a constant latency (jitter < 1 ms) of 1.5–3 ms. The latency to peak was between 2.8 and 6.7 ms. To confirm that the PSPs were excitatory, their amplitude were often examined at different membrane potentials. However, since we cannot rule out a possible participation of reversed IPSPs, we refer to the synaptic response to as PSPs. During stimulation protocols aimed at examining STD, neurons were hyperpolarized to –80 ± 2 mV to prevent action potentials firing. Repetitive stimulation was delivered at 5, 10 and 20 Hz for 20 s.

Analysis

Amplitude of PSPs was measured at the peak. For 18 neurons recorded *in vitro* we confirmed that the measurements of PSP's slope and the amplitude were significantly correlated ($P < 0.0001$; 200 measurements). The control PSP amplitude was the result of averaging 12 PSPs given at 0.2 Hz prior each period of high frequency stimulation. *In vitro,* the absolute values of control PSPs' amplitudes were: 4.4 ± 1.3 mV for 'classical' ACSF; 4.4 ± 2.1 mV for 'modified' solution, silent; and 4.2 ± 1.3 mV in 'modified' solution, active. These values should not be used to evaluate amplitude of PSPs in different ionic solutions, since the intensity of stimulation was adjusted at the beginning of each intracellular recording and then kept constant throughout the recording. However, in five neurons that underwent through the three conditions (silent cortex in 'classical' and 'modified' ACSF) and active, oscillatory cortex, no significant ($P < 0.05$) differences were observed between amplitude of the PSPs evoked in the different conditions, and this observation has been discussed (see below). No statistically significant correlation (correlation coefficient $R = 0.09$; $P = 0.5$) was found between the amplitude of PSPs and the degree of STD. In the results, all the values that are presented have been normalized to the control PSP amplitude. In all the cases 2–6 trials (repetitions of the same stimulation protocol) were averaged for each frequency of stimulation, to account for the distortions imposed by the existence of spontaneous activity.

In Figures 2, 3*A,C*, 4*G* and 5*B* we have represented a connecting curve between points called a B-spline, with the purpose of improving visualization of the data. The B-spline curve can be described by parametric equations $P(t) = (x(t), y(t))$ as t varies over a given range. The B-spline curve approximates the original data points without necessarily passing through them.

Differences in STD over time and under different conditions (Fig. 2; t.c. versus i.c. in Fig. 4) were estimated by analysis of covariance (ANCOVAR) after transforming the amplitude to a log scale. ANCOVAR was therefore used to carry out a regression analysis of the logarithm of the amplitude versus time for each condition, therefore allowing for the possibility that the slopes and intercepts vary between the different conditions. The null hypothesis is that they are all the same under the different conditions. A slope not different from 0 indicates absence of change in the amplitude over time.

For comparisons involving two different conditions or populations, a *t*-test was used. For both type of tests (ANCOVAR and *t*-test), differences are considered 'significant' at a P level of <0.05. Population data are given as mean ± SD.

Results

A first set of experiments was performed in adult ferret visual cortical slices prepared as previously described (Sanchez-Vives and McCormick, 2000). Layer 2/3 neurons from the visual cortex were intracellularly recorded and monosynaptic potentials were evoked by electrical stimulation of layer 4 below the recording site. The occurrence of spontaneous slow rhythmic activity was controlled by the ionic composition of the ACSF. Cortical slices were silent (no spontaneous ongoing activity) when maintained in 'classical' ACSF (for composition see Materials and Methods). However, when the slice was bathed in a 'modified' ACSF that mimics the ionic concentrations *in situ* (Hansen, 1985; the same composition as the 'classical' ACSF except for 3.5 mM KCl, 1 mM $MgSO_4$, 1–1.2 mM $CaCl_2$) a slow rhythmic activity was spontaneously generated (Sanchez-Vives and McCormick, 2000; Fig. 1*C*). In order to study the effect that network activity had on STD, we measured the changes in the amplitude of synaptic potentials (PSPs) induced by high-frequency stimulation (5, 10, 20 Hz) of presynaptic axons in three different experimental conditions: (i) silent slices maintained in 'classical ACSF'; (ii) silent slices maintained in 'modified' ACSF prior to the development of organized rhythmic activity; and (iii) slices with rhythmic oscillatory activity maintained in 'modified' ACSF for different periods of time.

Short-term Depression is Smaller in Active Than in Silent Cortical Slices

Figure 1 shows recordings from one neuron that underwent the three experimental conditions mentioned above. PSPs were evoked by repetitive stimulation at 10 Hz for a period of 20 s, and the first five and last two PSPs evoked by the train are displayed. The figure also shows the intracellular recordings (middle traces) and the concurrent multiunit activity in the vicinity of the intracellularly recorded neuron (bottom traces) when no stimulation was applied. It is noticeable that the same stimulus train produced less STD when there was ongoing activity in the network (Fig. 1*C*; depressed to 0.87 of the control amplitude) than when the network was silent (Fig. 1*A,B*; 0.33 and 0.54 of the control amplitude respectively). Slices that did

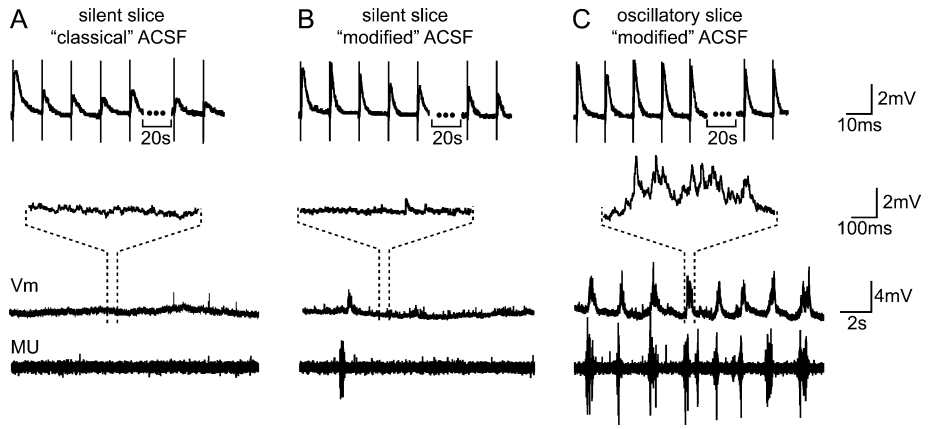

Figure 1. Intracellular (same neuron) and multiunit recordings *in vitro* during short-term depression (STD) protocols in three experimental conditions. (*A*) Monosynaptic potentials recorded from a layer 2/3 neuron as evoked by 10 Hz repetitive electrical stimulation of the underlying layer 4 (upper trace) while in 'classical ACSF'. The first five and the last two postsynaptic potential (PSPs) of a 20 s train are plotted. The membrane voltage recorded in the same neuron between trials shows no spontaneous activity (middle trace and inset). Lack of spontaneous activity is also revealed in a multiunit recording (MU) performed in the vicinity of the neuron (lower trace). (*B*) Same as in (*A*) but after 21 min in 'modified ACSF'. The synaptic depression induced by the stimulus train is smaller than in (*A*). The intracellular and multiunit recordings reveal the absence of organized spontaneous activity, although there are sporadic bursts of PSPs (middle trace) and spikes (lower trace). (*C*) Following 35 min of organized spontaneous rhythmic activity, the last two PSPs of the train show only a very moderate depression. The intracellular and extracellular recordings show rhythmic activity consisting on bursts of PSPs (middle trace and inset) and spikes (lower trace) at a frequency of 0.4 Hz. During recordings the membrane potential was maintained at −80 mV.

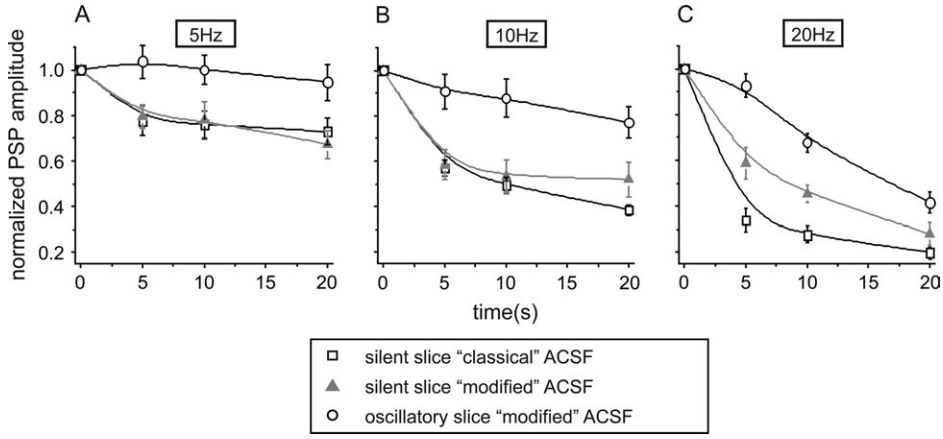

Figure 2. STD induced by repetitive stimulation at 5 (*A*), 10 (*B*) and 20 Hz (*C*) in the three *in vitro* experimental conditions: silent slice in 'classical ACSF' (open squares), silent slice in 'modified solution' (filled triangles) and ongoing activity in 'modified ACSF' (open circles). All repetitive stimulation series lasted 20 s. Each point corresponds to the average of several cells (between $n = 5$ and 17) and the error bar represents ± SEM. The values plotted correspond to the normalized values: control value (0 s) and following 5, 10 and 20 s of stimulation. The connecting line between points is a B-spline (see Materials and Methods). All the recordings included in this graph are from slices that have been kept at least 20 min in the aforementioned solutions.

not develop oscillatory activity in 'modified' ACSF — or during periods of recording before the oscillatory activity developed — were useful to differentiate the effect of changes in ionic concentrations from that of the spontaneous activity itself. As shown in Figure 1*B*, bathing the slice in the ionically 'modified' solution (in the absence of activity) also resulted in a decrease in STD but to a lesser extent. This is an expected effect due to the diminished Ca^{2+} in the solution and the subsequent decrease in the probability of release that characteristically reduces STD (Tsodyks and Markram, 1997; Varela *et al.*, 1997; Zucker and Regehr, 2002; Crochet *et al.*, 2005).

Less STD in the presence of spontaneous rhythmic activity in the network was a major finding in the population of neurons recorded *in vitro*. The study *in vitro* included a total of 44 neurons. The mean STD they exhibited was calculated for repetitive stimulation at 5, 10 and 20 Hz under the three aforementioned conditions (Fig. 2). All the changes in amplitude with repetitive stimulation were normalized, and this is how they are reported in this section and represented in the graphics.

For all stimulation frequencies there was less STD in active, oscillating slices than in silent slices ($P < 0.02$; ANCOVAR). During 5 Hz stimulation (Fig. 2*A*) there was no depression, and therefore no significant decay in the amplitude of the evoked synaptic potentials in oscillatory, active slices (ANCOVAR test; see Materials and Methods). In contrast, 5 Hz induced-STD in silent slices was significant, where it reached a final value (after 20 s of stimulation) of 0.71 ± 0.16 (Fig. 2*A*). Stimulation at 10 Hz (Fig. 2*B*) also induced a significantly larger STD in silent (0.43 ± 0.16 at 20 s) than in active slices (0.77 ± 0.27 at 20 s). STD induced by 5 and 10 Hz in silent slices ('classical' and 'modified' ACSF) (Fig. 2*A*,*B*), were not significantly different

(t-test; $P < 0.05$). Finally, during 20 Hz presynaptic stimulation, the decay of the PSPs amplitude over time was significantly different in the three conditions (ANCOVAR; $P < 0.05$), with a faster-developing and more prominent depression in silent slices than in active ones; the exact values for each condition are represented in Figure 2*C*.

Synaptic Depression In Vitro Correlates Inversely with the Degree of Ongoing Rhythmic Activity in the Network

The results presented above suggest that spontaneous ongoing activity in the network may induce a decrease in STD. In order to further explore this possibility, we first correlated the time course of the decrease in STD in active slices to the length of time during which the network had been active with oscillations. We averaged STD across time (Fig. 3*A*), whereby each value of STD corresponded to the depression at the end of 20 s of repetitive stimulation at 10 Hz. A total of 11 neurons were included, having been recorded in one, two or all three conditions (silent slice in 'classical' ACSF, silent slice in 'modified' ACSF and active slice in 'modified' ACSF) for different periods of time. The average STD in silent slices in 'classical' ACSF remained stationary in time and the mean was 0.37 ($n = 7$) of the normalized control amplitude. STD in neurons that were recorded in silent slices but in 'modified' ACSF ($n = 4$) also remained constant, regardless of the time (up to 110 min) after the solution change (Fig. 3*A*). The mean value of STD in this condition was 0.43 of the normalized amplitude, which was significantly larger than the one in 'classical' ACSF (t-test; $P < 0.05$) and therefore represents less STD. This observation demonstrates that there was a decrease in STD due to the ionic modification ('modified' ACSF), that remained constant once the new situation was stable. The next measures of STD were obtained following different periods of oscillatory activity in the slice, taking as zero the beginning of the organized rhythmic activity (Fig. 3*A*). Following 10 min of rhythmic activity, the normalized amplitude after the train was already increased to 0.59. There was a progressive decrease in STD over longer periods of activity in the network. For example, at 25 min the normalized amplitude was 0.64, and was 0.73 at 40 min (Fig. 3*A*). After 40 min of ongoing activity, the degree of depression reached a plateau value that remained constant up to 125 min of activity. Fifteen minutes after silencing the activity by returning to the 'classical' solution the original STD value was recovered (Fig. 3*A*). This result demonstrates that there was a close relation between the onset and development of rhythmic activity and the time course of the decrease in STD.

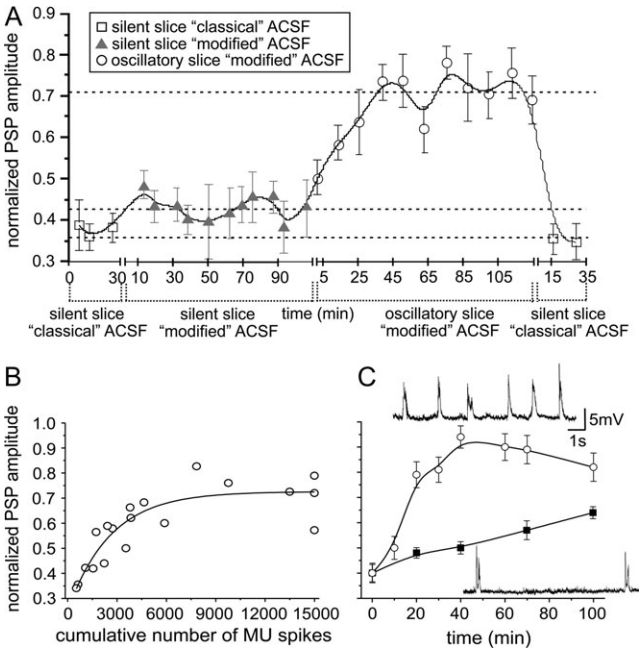

Figure 3. Ongoing activity reduces short-term depression. (*A*) Normalized STD induced by a 10 Hz train of 20 s duration as a function of the time spent in the different experimental conditions shown in the inset (notice that the time scale starts at 0 for each condition). Each point is the mean ± SEM of the normalized PSP amplitude at the end of the train of stimuli. The dotted lines are the average values for the five trains recorded in silent slices in control solution (before and after perfusing with the modified solution), the 11 trains recorded in silent slices in modified solution and the eight trains recorded after at least 45 min of organized oscillatory activity, when a plateau of STD was reached. Data from 11 cells that have spent different periods of time under one or more conditions have been included. Five of the included neurons went through at least three of the four conditions, including recovery. (*B*) Relationship between activity in the slice and changes in STD. The cumulative number of extracellularly recorded multiunit activity has been used to quantify the rhythmic activity occurring in a slice from the start of organized activity. There is a decrease in STD directly related to activity in the slice that ends up reaching a plateau. This graph corresponds to 10 Hz stimulation and includes data from six cells. An exponential has been fittet to the data ($R^2 = 0.79$; $P < 0.0001$). (*C*) Evolution of STD as a function of time for two cells in slices with high rhythmic activity (circles and intracellular recording in upper trace; frequency 0.1 Hz) and for two neurons in slices with low rhythmic activity (squares and intracellular recording in lower trace; frequency 0.5 Hz). Data points (± SEM) correspond to the average of three trains applied at the given times after the initiation of the organized activity (see Materials and Methods). The connecting line between points in (*A*) and (*C*) is a B-spline (see Materials and Methods).

The relation between cortical network activity and STD was further tested by correlating the STD variation over time with the amount of network activity that occurred in the slice from the moment when spontaneous activity started. Network activity was quantified as the cumulative number of spikes (above a threshold of 2SD of the noise) recorded by a multiunit recording electrode placed in the close vicinity of the intracellularly recorded cell (Fig. 3*B*). There was a significant relationship (ANCOVAR, $P < 0.0001$) between amount of activity and decrease in STD until a plateau was reached, meaning that a further increase in activity did not induce a further decrease in STD, which remained constant.

Finally, to substantiate the relationship between decreases in STD and build up of spontaneous activity, we selected four cells: two of them were in slices with very low frequency oscillations (0.05–0.1 Hz) and the other two were in slices with higher (and more common) oscillatory frequencies (0.3–0.5 Hz), in all cases with upstates of similar durations (600 ms; Fig. 3*C*). Slices with higher frequency of oscillation had therefore more global activity for a given length of time. Time 0 represents the start of oscillatory activity in the slice. Over a period of 100 min, the rate of decrease of STD over time was faster for those synapses in more active slices with respect to those in less active slices. These results confirm that the amount of decrease in synaptic depression is related to the amount of ongoing spontaneous activity in the network.

Synaptic Depression in Thalamocortical and Intracortical Connections In Vivo

Forty-two *in vivo* intracellular recordings from neurons in the primary visual cortex of the cat were included in this study. Neurons were recorded through all layers and were electrophysiologically characterized according to criteria in

(Nowak *et al.*, 2003) as regular spiking (n = 29), chattering (n = 6), intrinsically bursting (n = 4) and fast spiking (n = 1), plus two unclassified cells. In general, the membrane potential of neurons recorded *in vivo* showed vigorous spontaneous synaptic activity (Figs 4*C,D* and 5*A*) that was often organized in slow (<1 Hz) oscillations, as previously reported (Steriade *et al.*, 1993a,b; Pare *et al.*, 1998; Lampl *et al.*, 1999; Sanchez-Vives and McCormick, 2000; Steriade and Timofeev, 2003). Monosynaptic potentials were evoked by electrical stimulation of either thalamocortical (Fig. 4*C*) or intracortical (Fig. 4*D*) connections (see Materials and Methods). Thalamocortical (t.c.) connections were activated by electrical stimulation of the LGN (n = 21; latencies, 1.9 ± 0.4 ms; amplitudes, 5.2 ± 1.5 mV; Fig. 4*A,C*). The stimulating electrode was placed in an area of the LGN with neuronal receptive fields overlapping with the one from the recorded cortical neuron, therefore increasing the probability for monosynaptic connections (Reid and Alonso, 1995). Intracortical (i.c.) connections were activated by electrical stimulation applied in the vicinity (0.5–1.5 mm) of the intracellularly recorded neuron (n = 21; latencies, 1.9 ± 0.3 ms; amplitudes, 4.4 ± 1.9 mV; Fig. 4*B,D*). Repetitive electrical stimulation at frequencies of 5, 10 or 20 Hz for a period of 20 s induced decreases in the amplitude of the evoked thalamocortical (Fig. 4*E*) and intracortical (Fig. 4*F*) synaptic potentials that were larger for higher stimulation frequencies. No significant (P > 0.05) differences were found between the STD exhibited by the thalamocortical and intracortical connections (Fig. 4*G*).

Synaptic Depression In Vivo in a Silent versus an Active Cortical Network

Although synaptic and suprathreshold spontaneous activity *in vivo* was usually intense under propofol/sufentanil anesthesia there was a certain variation among recordings (Fig. 5*A*), probably related to the depth of the anesthesia. In order to silence the cortical network, we induced an even deeper anesthesia by administrating 1–2 ml of a barbiturate (thiopental, 8 mg/ml i.v.). We quantified the amount of ongoing synaptic activity by calculating the SD of the distribution of membrane potential values (Pare *et al.*, 1998; Fig. 5*A*; see Materials and Methods). The resulting values of SD ranged between 0.15 and

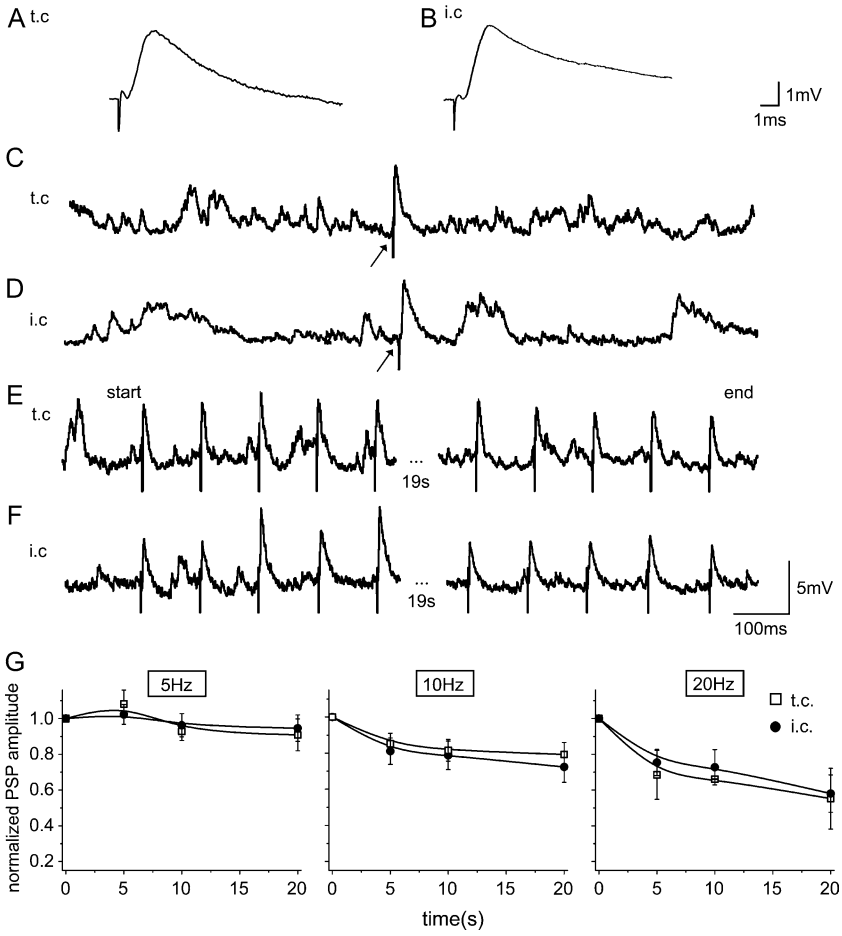

Figure 4. Spontaneous and evoked synaptic activity recorded *in vivo* from cortical neurons. (A,B) Examples of intracellularly recorded thalamocortical (*A*) and intracortical (*B*) PSPs. Each trace is the average of 12 stimuli at 0.2 Hz. (*C*, *D*) Spontaneous and evoked (see arrow) synaptic activity in a neuron in which depression of thalamocortical PSPs was analyzed (*C*) and in another neuron in which depression of intracortical PSPs was analyzed (*D*). (*E*) First and last five PSPs evoked in a cortical neuron by a train of electrical stimuli (10 Hz; 20 sec) delivered at the LGN. (*F*) First and last five PSPs evoked in a cortical neuron by electrical stimulation (10 Hz, 20 s) of the neighboring cortex. (*A*, *C*, *E*) and (*B*, *D*, *F*) correspond to recordings from the same neuron respectively. (*G*) STD induced by repetitive stimulation at 5, 10 and 20 Hz of either thalamocortical (t.c.; n = 9) or intracortical (i.c.; n = 12) connections. The plotted values are normalized to the pre-train amplitude (0 s) and correspond to 5, 10 and 20 sec of stimulation. The connecting line between points is a B-spline (see Materials and Methods). All the recordings included in this graph were obtained from intracellular recordings in the *in vivo* active (SD > 0.62; see Results) cortex.

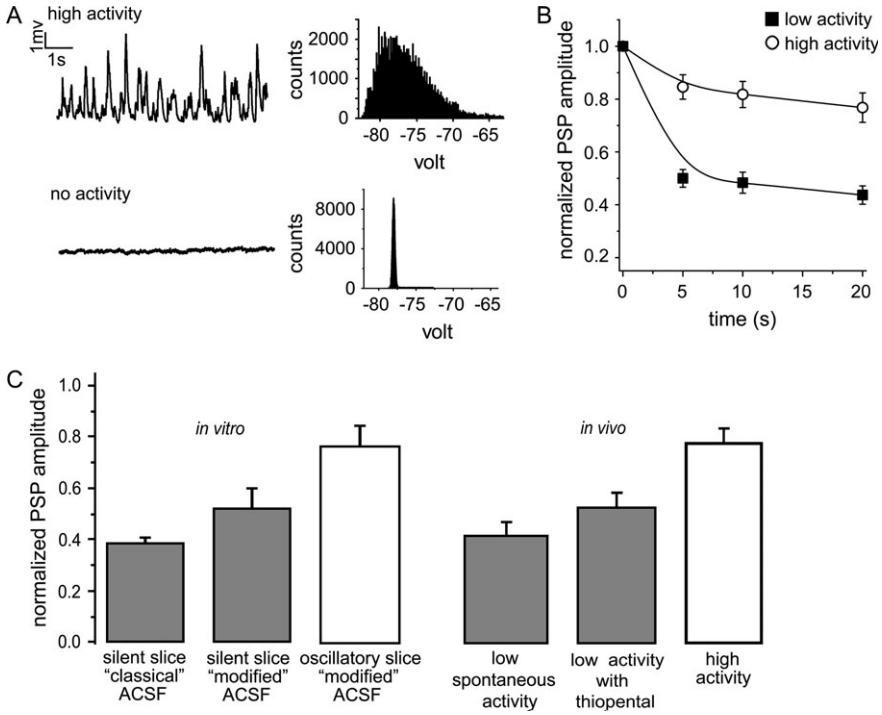

Figure 5. Synaptic depression depends on the level of spontaneous synaptic activity. (*A*) Membrane potential fluctuation in two neurons, one with spontaneous synaptic activity (top) and one without (bottom). Distributions of the membrane potential values measured in a time window of 30 s and with a sampling rate of 10 KHz are shown on the right panel. (*B*) Normalized synaptic depression following 20 s of 10 Hz repetitive electrical stimulation for neurons with levels of spontaneous synaptic activity segregated in two groups on the basis of the standard deviation of their membrane potential values. The number of included neurons were $n = 21$ for the high activity condition (SD $>$ 0.62) and $n = 21$ for the low activity condition (SD $<$ 0.62). The connecting line between points is a B-spline (see Materials and Methods). Neurons with higher spontaneous activity had less synaptic depression. (*C*) Synaptic depression is related to spontaneous activity both *in vivo* and *in vitro*. Bar diagram showing STD values at the end of 20 s of 10 Hz stimulation *in vitro* (silent slices — 'classic' ACSF; silent slices — 'modified' ACSF and active slices) and *in vivo* (low activity, thiopental induced low activity and high activity). Since no difference in synaptic depression was observed between thalamocortical and intracortical teminals, values from both groups have been pooled together in this graph.

3.75 (n = 42 recordings). We considered activity as low when the SD was below the median of the SD distribution (0.62; n = 21) and as high when it was above (n = 21). Out of the 21 cases with low activity, 11 were recordings in the presence of added barbiturate and 10 corresponded to low spontaneous activity with the usual anesthetic (see Materials and Methods). In some cases, another form of short-term synaptic plasticity, facilitation, occurred during the first 3 s of the high frequency stimulation. No difference related to the activity level was found in the number of neurons that showed facilitation. Thus, for an stimulation of 10 Hz, 6 out of 20 neurons recorded in a silent cortex showed facilitation, while 7 out of 21 recorded in the active cortex did. It is interesting that the time course of facilitation was different ($P < 0.05$) between both (silent and active) conditions, such that it peaked earlier in the silent cortex (1.8 s) than in the active cortex (2.8 s). The facilitation in both groups was to an amplitude of 121%. Facilitation was found more often in the intracortical (42.8%) than in the thalamocortical (20%) connections.

Short-term depression was found to differ considerably depending on the level of ongoing activity in the cortex *in vivo*. Population data for repetitive electrical stimulation at 10 Hz in high versus low activity are shown in Figure 5*B*. STD for thalamocortical and intracortical did not differ significantly, neither in the active (Fig. 4*G*), nor in the silent (not shown) cortical network. For this reason results from both connections were pooled in one single group. Analysis of covariance then revealed that there was significantly more STD in the silent than in the active cortex ($P < 0.001$; Fig 5*B*).

The impact of ongoing synaptic activity on STD is summarized in Figure 5*C*. At the end of 20 s of 10 Hz stimulation, short-term depression was very similar in silent or low activity cortical networks both *in vitro* and *in vivo* (gray columns). The level of depression was also indistinguishable in the oscillating cortical slice and in the active *in vivo* cortex. Altogether, we can conclude that short-term depression is consistently stronger in a silent than in an active network.

Discussion

Here we have demonstrated that STD of synaptic potentials in the cerebral cortex varies as a function of the spontaneous ongoing activity in the network. We found an inverse correlation over time between the development of slow rhythmic activity in a previously silent cortical slice and the degree of synaptic depression induced by repetitive stimulation, until a plateau of minimal depression was reached. These variation in STD depending on network activity results in less depression in an active than in a silent *in vitro* cortical network. These findings are in agreement with our results in the anesthetized preparation *in vivo*, where both thalamocortical and intracortical connections display lesser depression in an active than in a silent cortical or, rather, thalamocortical network.

In vitro studies often report a large STD in most of the connections examined, thalamocortical (Gil *et al.*, 1997; Beierlein and Connors, 2002), as well as for most of the intracortical connections between excitatory neurons (Finlayson and Cynader, 1995; Thomson, 1997; Tsodyks and Markram, 1997;

Varela *et al.*, 1997; Galarreta and Hestrin, 1998). Although there are reports indicating synaptic depression of the thalamocortical connection *in vivo* (Chung *et al.*, 2002), several *in vivo* studies have reported weak STD, and eventually a replacement of STD by facilitation (Kang *et al.*, 1991; Sanchez-Vives *et al.*, 1998; Boudreau and Ferster, 2003). There are several explanations for these differences between *in vivo* and *in vitro* synaptic depression (Sanchez-Vives *et al.*, 1999), including neuronal ionic environment, with lower Ca^{2+} and Mg^{2+} and higher K^+ *in vivo* (Hansen, 1985) than those typically used in *in vitro* studies. Other factors are animal age, since younger animals (commonly used in *in vitro* studies) have stronger STD (Varela *et al.*, 1997; Angulo *et al.*, 1999; Reyes and Sakmann, 1999), and the existence of neuromodulators *in vivo* (see below). In the current study we have explored the effect of the presence of ongoing activity in the network, a factor that seems to be important in determining differences in STD in different preparations. We cannot rule out that interactions between postsynaptic neurons could play some role in the reported STD. Although reduced $[Ca^{2+}]_o$ should have resulted in a reduced PSP amplitude (Katz and Miledi, 1967; Tsodyks and Markram, 1997), we found no significant difference between the PSPs in the neurons that went through the three conditions (silent cortex in 'classical' and 'modified' ACSF, and spontaneously active slices). A possible explanation is that the decrease of the PSP amplitude expected from the lowered $[Ca^{2+}]_o$ was compensated for by the concomitant lowering of $[Mg^{2+}]_o$ (Volgushev *et al.*, 1995). In addition, increasing $[K^+]_o$ and lowering $[Ca^{2+}]_o$ may have resulted in higher excitability of the network, with more axons recruited at the same stimulus intensity. Therefore our results do not imply that unitary PSPs would not have a smaller amplitude in the '*in vivo* like' or 'modified' ACSF. For the rest of the neurons included in the study, possible changes in amplitude of PSPs due to ionic differences could not be evaluated since the intensity of stimulation was adjusted at the beginning of each intracellular recording (see Materials and Methods). However, we did not observe sistematic differences in the intensities that were required to evoke PSPs of similar amplitude.

In this study, we found similar levels of synaptic depression in both thalamocortical and intracortical connections *in vivo* that varied depending on network activity (Fig. 4*G*). *In vitro* studies, on the other hand, describe thalamocortical inputs as showing more STD and paired-pulse depression than intracortical connections (Stratford *et al.*, 1996; Gil *et al.*, 1997). The larger STD exhibited by thalamocortical connections could be attributed to the larger number of release sites and the greater probability of release exhibited by these synapses (Gil *et al.*, 1999). Several differences between the reported *in vitro* studies and our own could contribute to the discrepancy of this result, such as the cortical area, the animal species and age, the ionic environment or the definition of depression, since we compared depression after 5–20 s of stimulation at high frequencies, while others (Gil *et al.*, 1997) referred to paired pulse depression. On the other hand, the existence of ongoing activity in the *in vivo* brain has probably affected the steady state of the synaptic connections and altered their plasticity. Finally, *in vivo* there are neuromodulators that vary with brain states, and these can regulate glutamatergic and gabaergic synaptic transmission (McCormick, 1992; Gil *et al.*, 1997).

One functional consequence of the results presented here is that ongoing spontaneous activity *in vivo* would permanently maintain synapses in a state of relative depression. Supporting this, there is evidence showing that an increased tonic firing of thalamocortical neurons during activated states decreases the efficacy of thalamocortical bursts to activate the cortex (Swadlow and Gusev, 2001) and that sensory responses are modulated by behavioural states (Fanselow and Nicolelis, 1999). Depending on the level of spontaneous activity, synaptic depression might contribute in different degrees to the dynamic adjustment of the cortical responses to sensory stimulation. One characteristic of sensory responses is adaptation with repetitive stimulation. An example is adaptation to contrast, which has been examined extensively in visual cortex and has been hypothesized to result from synaptic depression of thalamocortical and/or intracortical pathways (Finlayson and Cynader, 1995; Todorov *et al.*, 1997; Carandini *et al.*, 1998; Chance *et al.*, 1998; Adorjan *et al.*, 1999b). According to these results, the role of synaptic depression on adaptation would vary depending on the previous history of firing on the network. Tonically depressed synapses in activated brain states could have other roles such as to allow a more accurate spatial (Castro-Alamancos and Oldford, 2002; Chung *et al.*, 2002) and temporal (Castro-Alamancos, 2002) representations of the sensory inputs.

Synaptic strength has been found to be modulated in the long term depending on the ongoing activity in the network by homeostatic synaptic scaling (Turrigiano *et al.*, 1998; Gil and Amitai, 2000; Desai *et al.*, 2002). Recent studies (Wang *et al.*, 2004) find that in low $[Ca^{2+}]_o$, low activity increases the probability of release, therefore increasing short-term depression in the neuromuscular junction. Through synaptic scaling, network activity may also be regulating information processing in the adult cortex *in vivo*. Our data in the active slices and in the anesthetized animal suggest that activity causes short-term tuning of synaptic plasticity. Higher rates of activity in the alert state should induce a different steady state in cortical synapses, and therefore affect cortical processing.

Notes

We thank M. Slater for his help in the data analysis, D.A. McCormick and J. Bullier for their participation on *in vivo* preliminary experiments, and A. Compte for helpful discussions. This work has been sponsored by Human Frontiers Science Program Organization and MCYT (BFI2002-03643) to M.V.S.-V. L.G.N. was supported by the CNRS and by the Ministry of Foreign Affairs (Egide, 'Picasso' travel grant). R.R. was supported in part by a fellowship CSIC-Bancaja.

Correspondence should be addressed to M.V. Sanchez-Vives, Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-CSIC, Campus de San Juan, Apartado 18, 03550 San Juan de Alicante, Spain. Email: mavi.sanchez@umh.es.

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