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Two Dynamically Distinct Inhibitory Networks in Layer 4 of the Neocortex

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Beierlein, Michael, Jay R. Gibson, and Barry W. Connors. Two dynamically distinct inhibitory networks in layer 4 of the neocortex. *J Neurophysiol* 90: 2987–3000, 2003. First published June 18, 2003; 10.1152/jn.00283.2003. Normal operations of the neocortex depend critically on several types of inhibitory interneurons, but the specific function of each type is unknown. One possibility is that interneurons are differentially engaged by patterns of activity that vary in frequency and timing. To explore this, we studied the strength and short-term dynamics of chemical synapses interconnecting local excitatory neurons (regular-spiking, or RS, cells) with two types of inhibitory interneurons: fast-spiking (FS) cells, and low-threshold spiking (LTS) cells of layer 4 in the rat barrel cortex. We also tested two other pathways onto the interneurons: thalamocortical connections and recurrent collaterals from corticothalamic projection neurons of layer 6. The excitatory and inhibitory synapses interconnecting RS cells and FS cells were highly reliable in response to single stimuli and displayed strong short-term depression. In contrast, excitatory and inhibitory synapses interconnecting the RS and LTS cells were less reliable when initially activated. Excitatory synapses from RS cells onto LTS cells showed dramatic short-term facilitation, whereas inhibitory synapses made by LTS cells onto RS cells facilitated modestly or slightly depressed. Thalamocortical inputs strongly excited both RS and FS cells but rarely and only weakly contacted LTS cells. Both types of interneurons were strongly excited by facilitating synapses from axon collaterals of corticothalamic neurons. We conclude that there are two parallel but dynamically distinct systems of synaptic inhibition in layer 4 of neocortex, each defined by its intrinsic spiking properties, the short-term plasticity of its chemical synapses, and (as shown previously) an exclusive set of electrical synapses. Because of their unique dynamic properties, each inhibitory network will be recruited by different temporal patterns of cortical activity.

INTRODUCTION

Myriad types of interneurons mediate synaptic inhibition in neocortex, yet models of cortical function often assume that inhibition is a single, homogenous process. This reflects, at least in part, our limited understanding of the physiological variability among interneurons. Interneurons are clearly distinguished by their morphology (DeFelipe 2002; Kawaguchi 1995; Somogyi et al. 1998), the proteins they express (Cauli et al. 1997, 2000; Kawaguchi and Kubota 1997; Porter et al. 2001), the intrinsic firing patterns they generate (Gupta et al. 2000), and their tendency to share electrical synapses only with neurons similar to themselves (Amitai et al. 2002; Chu et al. 2003; Deans et al. 2001; Galarreta and Hestrin 1999, 2001a; Gibson et al. 1999; Szabadics et al. 2001; Tamas et al. 2000). Presumably, each type of interneuron serves a distinct set of functions in the neocortex; unfortunately, those functions are currently unknown (Miles 2000).

Interneurons may also be differentiated by the properties of the synapses they form and receive (Gupta et al. 2000; Reyes et al. 1998; Thomson et al. 1996; Wang et al. 2002; Xiang et al. 1998). This may give clues to their function because the strength and dynamics of cortical synapses vary widely and may be tuned to the type of information they convey (Zador and Dobrunz 1997). For example, thalamocortical inputs onto pyramidal cells (Gil et al. 1997, 1999; Stratford et al. 1996) and the fast-spiking (FS) type of interneuron (Beierlein and Connors 2002; Gibson et al. 1999; Swadlow 2003; Swadlow and Gusev 2001) tend to be strong and reliable, perhaps reflecting the fidelity of information flow from thalamus to cortex. The excitatory synapses between pyramidal cells within the cortex are usually weaker, less reliable (Feldmeyer et al. 1999; Gil et al. 1999; Stratford et al. 1996; Thomson and Deuchars 1997), and seem particularly amenable to long-term plasticity (Bear and Malenka 1994).

The short-term dynamics of neocortical synapses are another dramatically diverse property (Hempel et al. 2000; Reyes et al. 1998; Thomson and Deuchars 1997). When activated repetitively, synapses may display strong depression, robust facilitation, neither, or both (Zucker and Regehr 2002). The relevance of such variable short-term dynamics is still unclear, although it has been suggested that synaptic depression is a mechanism for encoding relative changes in presynaptic firing rates (Abbott et al. 1997; Markram and Tsodyks 1996; O'Donovan and Rinzel 1997).

We have recently studied some of the contrasting characteristics of two types of interneurons in neocortex, the FS cells and the low-threshold-spiking (LTS) cells (Amitai et al. 2002; Beierlein et al. 2000; Gibson et al. 1999). Here we present a quantitative physiological analysis of the synaptic interconnections between the excitatory regular-spiking (RS) cells and the FS and LTS interneurons that inhibit them within layer 4; we also examine the properties of thalamocortical synapses onto FS and LTS cells. Our results show a striking dichotomy: the synapses that form both the inputs and outputs of FS cells are initially strong and reliable but depress during repetitive activation; in contrast, the synaptic inputs and outputs of LTS cells tend to be quite weak and unreliable until facilitated during repetitive activity. The complementary properties of these two systems of inhibition suggest that they serve parallel but dynamically distinct roles in neocortical function.

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METHODS

Slice preparation and recording

Methods were similar to those used in previous studies (Beierlein et al. 2000; Gibson et al. 1999). Thalamocortical slices (350–400 μm) thick were prepared from Sprague-Dawley rats aged P14–P21. Slices were incubated at 32°C for 1 h, maintained in a holding chamber at room temperature until transferred to the recording chamber, and then held at 32°C during measurements. All recordings were done in layer 4 of the barrel region of somatosensory cortex. The bathing solution contained (in mM) 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 26 $NaHCO_3$, 10 dextrose, and 2 $CaCl_2$, saturated with 95% O_2-5% CO_2. Micropipettes of 5–12 MΩ were filled with (in mM) 135 K-gluconate, 4 KCl, 2 NaCl, 10 HEPES, 0–0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Tris, and 5–10 phosphocreatine-Tris (pH 7.25, 290 mosM). Recordings were not corrected for the liquid junction potential. During all recordings 50 μM DL-2-amino-5-phosphopentanoic acid (AP5, Sigma) was present in the bath. Some recordings of inhibitory postsynaptic potentials (IPSPs) were done in the presence of AP5 and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 20 μM, Sigma).

All recordings were made in current-clamp mode (Axopatch 1D, Axoclamp 2B or Axoprobe 1A), with IR-DIC visualization using a Zeiss Axioskop and a CCD camera (Hamamatsu). Where noted, synaptic responses were evoked with extracellular stimuli lasting 200 μs, typically with average amplitude of ~40 μA (range: 5–100 μA), applied through paired microwires (FHC). For local cortical stimulation, electrodes were placed within layers 2/3, ~500 μm lateral from the recording site. To evoke thalamocortical inputs, electrodes were placed into the ventrobasal (VB) nucleus, near the border of the thalamic reticular nucleus (TRN).

Data analysis

Recordings were filtered at 10 kHz, and acquired and analyzed using software written in Labview by J.R.G.

CELL TYPES. Cell types were categorized by their evoked spike-firing pattern. Spike width was measured at half-amplitude, and spike amplitude was measured from the point of inflection on the rising phase. Spike frequency adaptation was quantified as the ratio of the firing frequency at the beginning (1st interspike interval) and the end (last interspike interval) of a spike train evoked by depolarizing current steps of 600-ms duration. The frequency of firing at the end of the smallest current step that generated sustained firing was defined as the "minimum steady-state firing frequency."

SYNAPSES. Excitatory postsynaptic potentials (EPSPs) in interneurons were measured from a holding potential of ~62 mV. IPSPs in excitatory cells were evoked from a holding potential of −55 mV. The measured reversal potential for $GABA_A$-mediated IPSPs was −73 mV. We found that changes in driving force due to temporal summation of IPSPs did not significantly contribute to changes in amplitude. For measurements of EPSP and IPSP shape parameters, multiple trials of postsynaptic recordings were averaged using the peak of the presynaptic spike as a trigger. Amplitude measurements in single trials were done using a fixed baseline-to-peak time window obtained from the averaged response. Response amplitude variability was measured as the coefficient of variation (CV). Noise-corrected values for the CV were computed as: $\sqrt{\text{Variance}_{signal} - \text{Variance}_{noise}}/\text{mean}_{signal}$. Noise was measured from the baseline just before the PSP onset by employing the same fixed baseline-to-peak time window as for the response. Response measurements that were smaller than 1.6 × RMS (root mean square) of the noise were considered synaptic failures (Markram et al. 1997). All data are expressed as means ± SD except as noted.

RESULTS

Whole cell recordings were obtained from somata of visually identified cells in layer 4. Among the 968 cells recorded, 960 (99%) could be reliably categorized into one of three groups (Gibson et al. 1999), based on spike and afterpotential shape and repetitive firing characteristics (Fig. 1*A*, Table 1). RS cells always generated adapting trains of spikes, and each spike had a relatively long half-width. FS cells fired at rates of up to 300 Hz, had very high steady-state minimum firing frequencies, and on average displayed no spike-frequency ad-

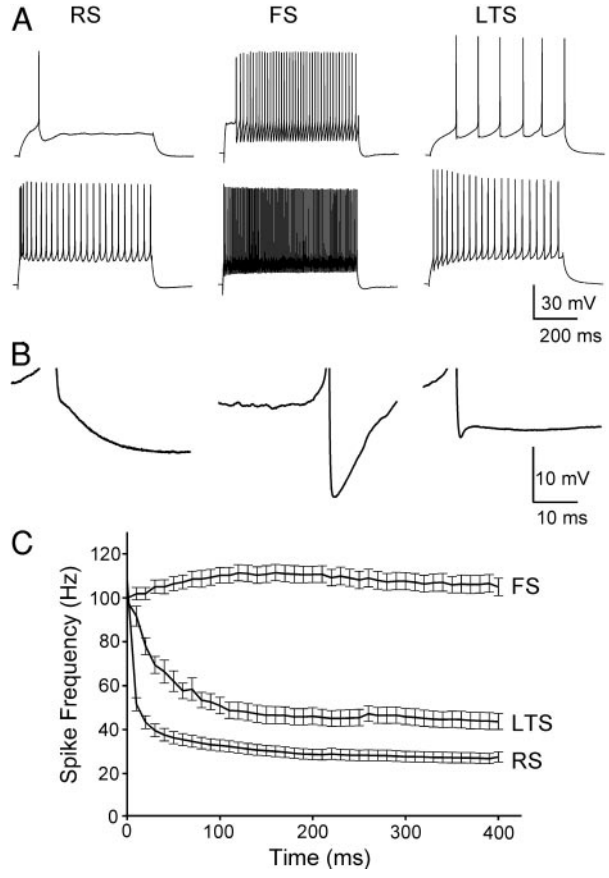

FIG. 1. Intrinsic firing pattern of 3 types of layer 4 neurons. *A*: depolarizing current steps evoked low-frequency firing in regular-spiking (RS) and low-threshold-spiking (LTS) cells at threshold, whereas fast-spiking (FS) neurons displayed high minimum firing frequencies. Current steps at higher amplitudes evoked spike-frequency adaptation in RS and LTS cells but not in FS cells. *B*: close-up of the afterpotential waveforms following the 1st spikes at threshold (*A*, *top*). Notice the monophasic afterhyperpolarization (AHP) in the FS cell, whereas the LTS cell generated both fast and slow AHPs. *C*: spike-frequency adaptation in the 3 cell types. Current step amplitudes were adjusted to evoke an initial firing frequency of 100 Hz in all sampled cells. Data shown are means ± SD for each cell type (FS, $n = 36$ cells; LTS, $n = 34$; RS, $n = 25$).

TABLE 1. *Intrinsic membrane properties of RS, FS, and LTS cells in layer 4*

	RS	FS	LTS
Resting membrane potential, mV	−66 ± 5 (28)	−64 ± 5 (48)	−59 ± 4 (33)
Input resistance, MΩ	133 ± 48 (28)	55 ± 19 (48)	82 ± 42 (33)
Membrane time constant, ms	17.4 ± 4.5 (14)	8.8 ± 2.1 (30)	16.4 ± 7.8 (18)
Action potential half width, ms	0.91 ± 0.18 (28)	0.36 ± 0.07 (48)	0.56 ± 0.12 (33)
Steady-state firing rate at threshold, Hz	12.3 ± 5.5 (28)	56.5 ± 18.6 (48)	17.2 ± 7.9 (33)
Maximal steady-state firing rate, Hz	59.4 ± 13.7 (14)	271.7 ± 51.8 (9)	120.7 ± 41.2 (25)
Spike frequency adaptation	0.23 ± 0.11 (28)	1.13 ± 0.45 (48)	0.39 ± 0.18 (33)

All data are means ± SD with the numbers of tested neurons in parentheses. RS, FS, and LTS, regular, fast, and low-threshold spiking, respectively.

aptation. FS cells also generated brief spikes with fast, deep, monophasic afterhyperpolarizations (AHPs; Fig. 1*B*). Some FS neurons displayed subthreshold oscillations in the range of 20–50 Hz that were strongly voltage-dependent and typically occurred in a narrow voltage range just negative to threshold (not shown) (Llinas et al. 1991). LTS cells had spikes intermediate in duration, with complex AHPs and afterdepolarizations (ADPs), and lower steady-state firing frequencies at threshold than FS cells; LTS cells also showed strong adaptation. The distinct differences in adaptation time course across the three cell types are illustrated by the average data in Fig. 1*C*. Repetitive firing was induced in each cell using step current pulses adjusted so that the initial firing rates were close to 100 Hz. The firing rates for each cell type were significantly different from the others at every time point after the initial one.

To help distinguish LTS from RS neurons more reliably, we also measured the change in amplitude of AHPs during a train of evoked action potentials. Neurons were identified as excitatory or inhibitory by the nature of the PSP they generated in a target postsynaptic cell (see below). In excitatory (RS) cells, the peak of the AHP after the first spike was more depolarized than the peaks of subsequent AHPs, whereas for LTS cells, the peak of the first AHP was more hyperpolarized than that of subsequent AHPs (Fig. 2*A*). Plotting the values of the change in AHP for samples of the two neuronal types yielded nonoverlapping distributions (Fig. 2*B*).

Frequency of synaptic connections in layer 4

Dual whole cell recordings were made from identified neurons in layer 4, with separations of ≤50 μm between cell bodies. Synaptic properties were measured by evoking spikes in presynaptic cells with brief current pulses (Fig. 3*A*). Table 2 summarizes the frequencies of synaptic connections obtained between the various combinations of cell pairs tested in this study. RS cells excited both FS and LTS interneurons with high probability. In turn, both interneuron types made frequent inhibitory synaptic contacts with RS cells. By contrast, excitatory connections between pairs of RS cells were relatively uncommon. The numbers of sampled pairs with reciprocal synaptic connections were consistent with the hypothesis that connection probabilities are independent of one another. Electrical synapses, which are common between inhibitory interneurons of the same type (Gibson et al. 1999), were not observed between the mixed inhibitory-excitatory neuron pairs reported in this study.

Properties of excitatory synapses depend on postsynaptic target type

We tested the strength and reliability of excitatory synapses by evoking single spikes in presynaptic RS cells at low frequencies (<0.1 Hz) while recording postsynaptically from neighboring FS, LTS, or RS cells (Fig. 3*A*, Table 3). EPSPs recorded from FS cells had the largest amplitudes, smallest coefficients of variation (CV), and lowest failure rates (Fig. 3*B*, Table 3). Across FS cells, both the average CV and failure rate were strongly and negatively correlated with the EPSP amplitude (not shown). EPSPs from LTS cells had much smaller amplitudes than those from FS and RS cells, with a large CV and a high failure rate (Fig. 3*B*, Table 3). Only 3 of 23 connections from RS to LTS cells had no failures. EPSPs in RS cells tended to have smaller amplitudes and higher failure rates compared to those in FS cells (Table 3). EPSP rise times and half-widths were shorter in FS cells than in RS and LTS cells in part due to the relatively brief membrane time constant of the FS cells.

Taken together, these data suggest that low-frequency activity in excitatory neurons would preferentially activate local FS cells, leaving LTS cells largely unaffected.

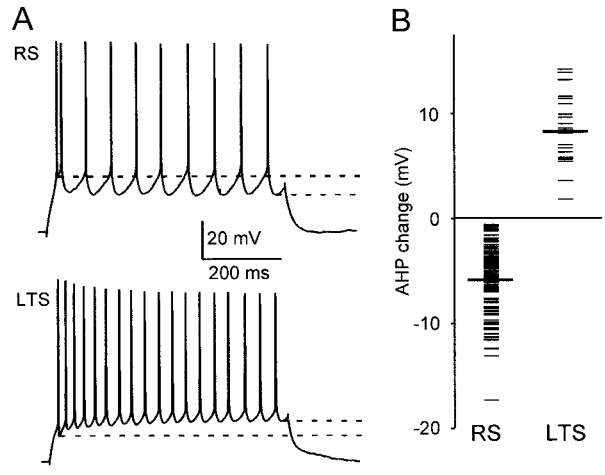

FIG. 2. Change in AHP amplitude during spike train reliably distinguishes RS from LTS cells. *A*: repetitive firing at threshold evoked in RS and LTS cell by depolarizing current steps (600 ms). Dashed lines indicate voltage difference between the peak AHP after the 1st spike and the peak AHP after the final spike. The last AHP is relatively more hyperpolarized in RS cells but relatively more depolarized in LTS cells, compared to the first AHP. *B*: summary data for samples of RS and LTS cells confirmed as excitatory or inhibitory by testing the synaptic responses they evoked in follower cells recorded simultaneously. Each short hash mark is the value from a single neuron; longer hash marks are mean values (mean ± SD for RS cells = −5.9 ± 3.0 mV, n = 120; for LTS cells = 8.3 ± 3.1 mV, n = 24).

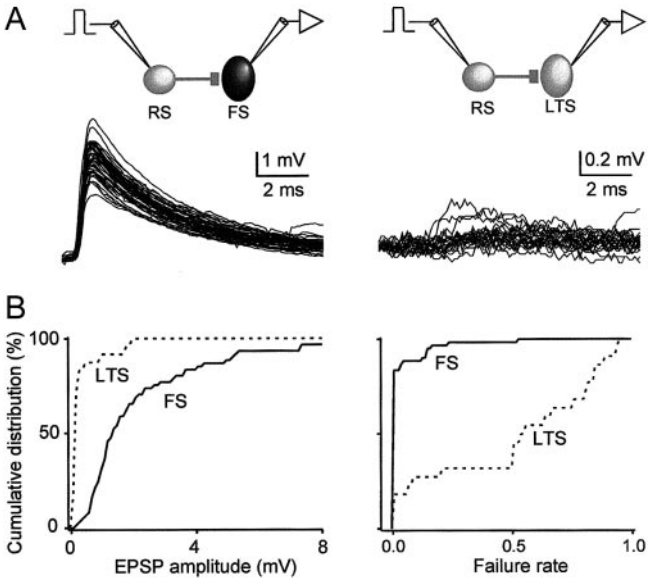

FIG. 3. Excitatory postsynaptic potentials (EPSPs) from FS cells and LTS cells have distinctive properties. *A*: panels show recordings from neuron pairs during multiple trials evoked by low-frequency stimulation (<0.1 Hz) of the presynaptic RS cells. *B*: summary of EPSP properties. Graphs plot cumulative distributions of amplitude and failure rate of EPSPs in LTS ($n = 23$) and FS ($n = 61$) cells. See Table 3 for statistics.

Short-term dynamics of excitatory synapses onto two types of interneurons

We investigated the short-term dynamics of layer 4 excitatory synapses by triggering brief trains of action potentials in the presynaptic cell at frequencies of 10 and 40 Hz. Figure 4*A* shows an example of postsynaptic responses from an FS cell evoked by a 40-Hz train of RS cell action potentials. EPSPs from FS cells showed similar strong depression at both 10 and 40 Hz (Fig. 4*B*). Across cell pairs there was no correlation between initial EPSP amplitude and short-term dynamics at 40 Hz ($n = 24$, $r = -0.03$, $P = 0.89$, Pearson product moment correlation), suggesting that differences in EPSP amplitude are primarily due to differences in the number of release sites rather than variations in release probability.

In contrast to those of FS cells, the EPSPs of LTS cells displayed dramatic short-term facilitation (Fig. 4*A*) whose magnitude depended strongly on the stimulation frequency (Fig. 4*B*). Low-frequency stimuli ≤10 Hz often generated little or no postsynaptic response, whereas higher frequencies evoked strongly facilitating EPSPs (Fig. 5*A*). However, even at high frequencies facilitation in LTS cells often developed slowly: at 40 Hz, the first spike in the train elicited EPSPs that, on average, were only 0.18 ± 0.2 of the maximum amplitude, and it took an average of 4.3 spikes to reach half-maximal amplitude ($n = 18$ cell pairs). Synaptic depression in LTS cells was rarely observed in short trains and only became apparent during long-lasting, high-frequency stimulation (Fig. 5*A*). Closer inspection of individual trials in LTS cells revealed that high-frequency spike trains evoked a 100 to 300 ms barrage of asynchronous synaptic events (Fig. 5*B*) in addition to spike-locked EPSPs. Interestingly, some of the asynchronous events were many times larger than the spontaneous EPSPs under control conditions. It is unlikely that asynchronous release was mediated by polysynaptic activity because it was never seen at any other synapse type tested, it was not observed at lower stimulation frequencies, and recordings were made in the presence of AP5.

To ensure that facilitation and asynchronous release were not artifacts induced by the presynaptic whole cell pipette, we also evoked excitatory inputs in LTS cells using extracellular stimulation electrodes, with essentially the same results (see following text).

Mechanisms of short-term dynamics

To further explore the mechanisms underlying the distinctive dynamics of the excitatory synapses contacting FS and LTS interneurons, we measured changes in failure rates during trains of presynaptic stimuli. For excitatory inputs onto FS cells, reduction in response amplitude was accompanied by an increase in the probability of synaptic failures (Fig. 6*A*). This is consistent with the idea that short-term depression arises from a progressive reduction in the probability of transmitter release. In contrast, EPSPs onto LTS cells displayed a progressive reduction in failure rate (Fig. 6*A*), suggesting that short-term facilitation at these synapses was mediated by an activity-dependent increase in the probability of transmitter release.

Further evidence for a presynaptic mechanism mediating short-term depression at synapses onto FS cells was obtained by a CV analysis of amplitude fluctuations between successive EPSPs in a 40-Hz train (Fig. 6*B*). At synapses with multiple release sites, changes in the inverse of the square of CV (CV^{-2}) can be used as an indicator of quantal content, although certain assumptions have to be made (Faber and Korn 1991). Plotting CV^{-2} against the mean amplitudes of the second EPSP, both normalized to the respective values of the preceding EPSP, revealed an average comparable decrease in CV^{-2} and in EPSP amplitude. These findings are most consistent with a use-dependent decrease of quantal content.

TABLE 2. *Frequencies of synaptic connections between neuron pairs*

Connection Type	Pairs Recorded	No. of Connections	Percent	No. of Reciprocal Connections Observed	No. of Reciprocal Connections Predicted
RS ↔ RS	89	11	6	1	0
FS → RS	190	83	44	34	32
RS → FS	172	74	43	34	32
RS → LTS	63	36	57	13	13
LTS → RS	74	26	35	13	13

Summary of all cell pairs (intersomatic distance < 50 μm) recorded in this study. All recordings made in the presence of AP5. In some experiments DL-2-amino-5-phosphopentanoic acid (AP5) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) were present, so the excitatory connection could not be tested. The expected numbers of reciprocal connections reflect the product of the sampled incidences of connections in each direction.

TABLE 3. *Properties of EPSPs evoked by RS cells onto three postsynaptic targets*

	RS	FS	LTS
Amplitude, mV*	1.1 ± 1.1	2.2 ± 2.2	0.3 ± 0.5
Range, mV	0.2–4.1 (11)	0.2–10.1 (61)	0.02–1.9 (24)
Rise time, ms† (20–80% amplitude)	0.88 ± 0.26 (11)	0.37 ± 0.11 (61)	0.86 ± 0.48 (8)
Half-width, ms†	12.3 ± 2.2 (11)	4.9 ± 1.9 (61)	8.9 ± 2.9 (19)
Failure rate*	0.11 ± 0.18 (11)	0.03 ± 0.08 (61)	0.57 ± 0.35 (23)
CV of EPSP*	0.30 ± 0.19 (11)	0.27 ± 0.13 (24)	1.04 ± 0.54 (18)

All data are means ± SD with the numbers of tested cell pairs in parentheses. EPSP, excitatory postsynaptic potential. Significance: (*) FS vs. LTS, RS vs. LTS, (†) FS vs. LTS, FS vs. RS. $P < 0.001$, ANOVA on ranks, pairwise multiple comparison.

EPSPs onto FS cells are likely to be mediated by multiple synaptic contacts (Angulo et al. 1999; Buhl et al. 1997). To test this hypothesis, we stimulated synapses with paired pulses separated by a 25-ms interstimulus interval and compared the amplitude distributions of $EPSP_1$ and $EPSP_2$. Assuming a connection with only one release site, depression would be expressed solely as an increase in failure rate with no significant change in the amplitude distribution. However, for all connections examined, the average amplitude of the first EPSP, excluding failures, was significantly larger than the amplitude of the second EPSP (mean $EPSP_1/EPSP_2 = 1.7 \pm 0.4$, $P < 0.001$, paired *t*-test, $n = 11$), suggesting that the majority of connections consisted of multiple release sites. Across individual trials, there was no significant correlation between the amplitude of $EPSP_2$ and $EPSP_1$ for any of the synapses tested ($n = 7$, data not shown), suggesting that paired-pulse depression did not depend on previous release (Xiang et al. 2002).

Properties of IPSPs depend on presynaptic cell type

FS and LTS cells have distinct anatomical and biochemical phenotypes (Gibson et al. 1999; Gupta et al. 2000; Reyes et al.

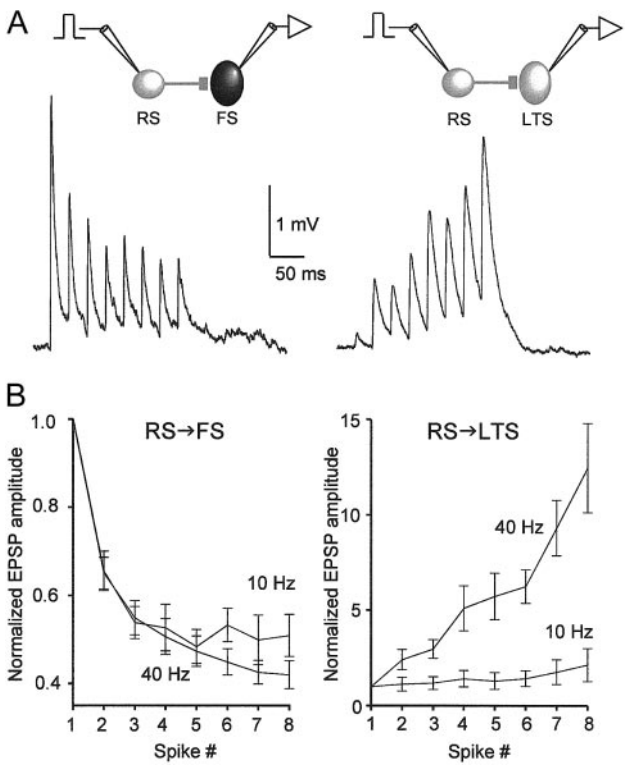

FIG. 4. EPSPs recorded from FS and LTS cells have distinctive short-term dynamics. *A*: EPSPs (averaged) recorded from FS and LTS neurons, evoked by stimulation of presynaptic RS cells (8 spikes at 40 Hz). *B*: summary data (means ± SE) of EPSPs from FS cells (24 pairs) and LTS cells (18 pairs) evoked by 10- and 40-Hz trains in RS cells. Data are normalized to 1st response in each train. FS (*left*): ratio of average amplitudes of $EPSP_{7-8}$ to $EPSP_1 = 0.50$ for 10 Hz, 0.42 for 40 Hz. LTS (*right*): ratio of average amplitudes of $EPSP_{7-8}$ to $EPSP_1 = 1.9$ for 10 Hz, 10.9 for 40 Hz.

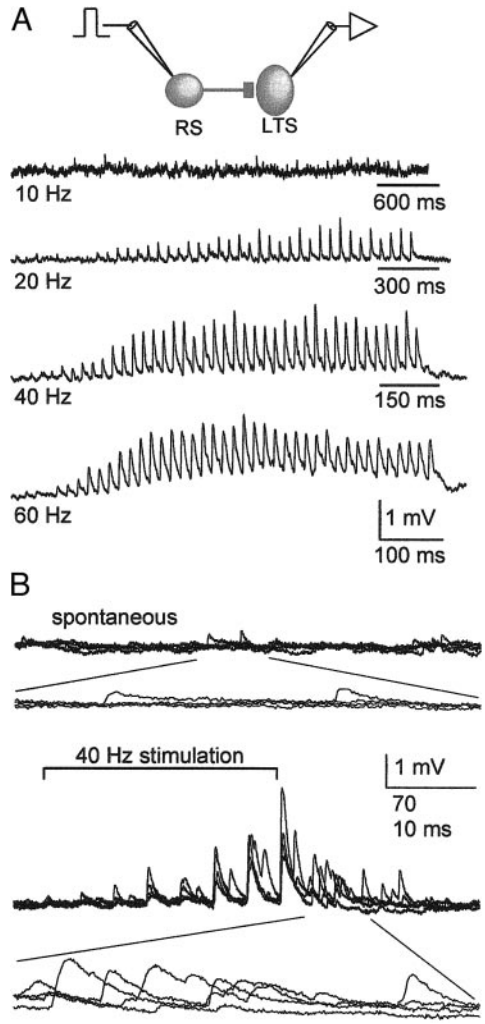

FIG. 5. Frequency-dependent short-term behavior of RS-LTS synapses. *A*: trains of 40 action potentials at frequencies between 10 and 60 Hz evoked facilitating EPSPs. *B*: facilitating excitatory synapses onto LTS cells display asynchronous release: *top*: 4 superimposed trials of spontaneous activity in an LTS cell, with expanded traces below. *Bottom*: EPSPs evoked by 8 stimuli at 40 Hz. Notice period of asynchronous release during and after evoked response (bottom expanded traces).

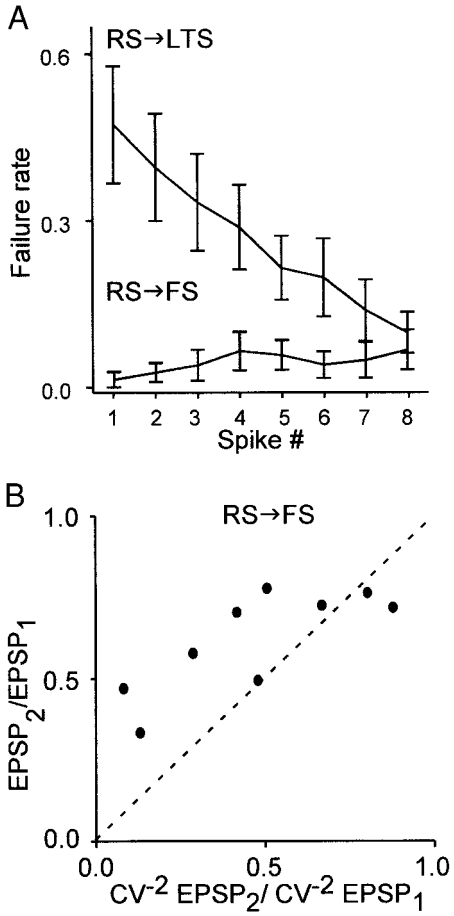

FIG. 6. Differential changes in failure rates at excitatory synapses onto FS and LTS cells. *A*: summary data for failure rate (means ± SE) for EPSPs during 40-Hz presynaptic trains. In FS cells, failure rate was 0.01 for $EPSP_1$ and increased to 0.07 for $EPSP_8$ ($n = 9$, no statistical change, $P = 0.25$). In 4 of 9 FS synapses, no failures occurred during the train. In LTS cells, initial failure rate was 0.47 and decreased to 0.10 ($n = 14$, $P < 0.001$, paired *t*-test). *B*: correlated changes in CV^{-2} and EPSP amplitude indicate decrease in release probability. Graph plots ratio of CV^{-2} against ratio of EPSP amplitude, for responses measured during paired stimuli at 25-ms interval ($n = 9$ RS-FS synapses).

1998), and each cell type contacts its postsynaptic targets at different sites (Somogyi et al. 1998; Xiang et al. 1998, 2002). IPSPs evoked by FS and LTS cells in layer 2/3 have different properties and short-term dynamics that are correlated with the postsynaptic cell type (Reyes et al. 1998). Here we tested the properties and short-term dynamics of IPSPs evoked by FS and LTS cells in neighboring RS cells. Under our recording conditions, the estimated reversal potential for IPSPs was −73 mV. To measure IPSPs, the resting potential of postsynaptic RS neurons was held close to −55 mV. Table 4 summarizes the properties of the two types of unitary IPSPs during low-frequency stimulation (<0.1 Hz). FS-evoked IPSPs had moderate to large amplitudes, (Fig. 7, *A* and *B*) with low CVs, and very low failure rates. LTS-evoked IPSPs, however, were smaller, had longer rise times, larger CVs, and higher failure rates.

When stimulated with short trains at 10 and 40 Hz, IPSPs evoked by FS cells always depressed (Fig. 8, *A* and *B*), and the degree of depression was slightly dependent on frequency between 10 and 40 Hz. In contrast, the IPSPs evoked by LTS

TABLE 4. *Properties of IPSPs evoked by FS and LTS cells onto RS cells*

	FS-Evoked IPSPs	LTS-Evoked IPSPs
Amplitude, mV*	1.1 ± 0.8	0.48 ± 0.45
Range, mV	0.3–4.0 (69)	0.1–1.8 (25)
Rise time, ms† (20–80% amplitude)	1.5 ± 0.7 (68)	2.1 ± 1.0 (24)
Half-width, ms	24 ± 10.8 (66)	22.6 ± 13.7 (24)
Failure rate†	0.03 ± 0.07 (69)	0.29 ± 0.26 (25)
CV of IPSP‡	0.25 ± 0.11 (19)	0.41 ± 0.21 (12)

All data are means ± SD with the numbers of tested pairs in parentheses. IPSP, inhibitory postsynaptic potential. Significance: (*) $P < 0.001$, Mann-Whitney rank sum test. (†) $P = 0.02$, (‡) $P = 0.01$.

cells tended to facilitate or moderately depress at both 10 Hz (not shown) and 40 Hz (Fig. 8*C*). This suggests that the short-term dynamics of IPSPs correlate with the presynaptic interneuron type (Gupta et al. 2000; Thomson et al. 1996).

Pairs of cells are interconnected by synapses with similar dynamics and amplitudes

Significant numbers of FS-RS and LTS-RS cell pairs we sampled were reciprocally connected (Table 1). Figure 9 summarizes data from 24 such pairs and plots the short-term dynamics of the EPSP as a function of the short-term dynamics of the IPSP. Two well-separated clusters of cell pairs are apparent, corresponding to the type of interneuron involved. RS-LTS pairs were interconnected by facilitating excitatory synapses, and inhibitory synapses that were, on average, slightly depressing. RS-FS pairs were interconnected exclusively by depressing synapses.

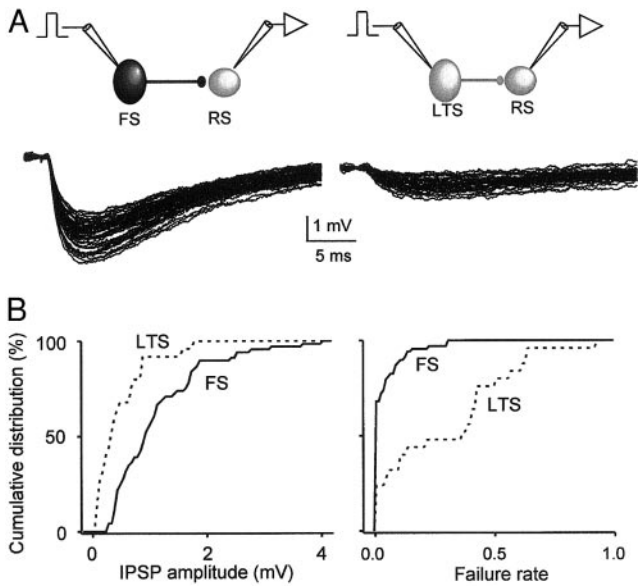

FIG. 7. IPSP properties correlate with presynaptic interneuron type. *A*: IPSPs evoked by FS or LTS cells recorded from RS cells. Responses from multiple trials evoked by low-frequency stimulation (<0.1 Hz) of presynaptic neuron. *B*: graphs plot cumulative distributions of amplitude and failure rate of FS and LTS cell-evoked IPSPs recorded from RS cells. See Table 4 for statistics.

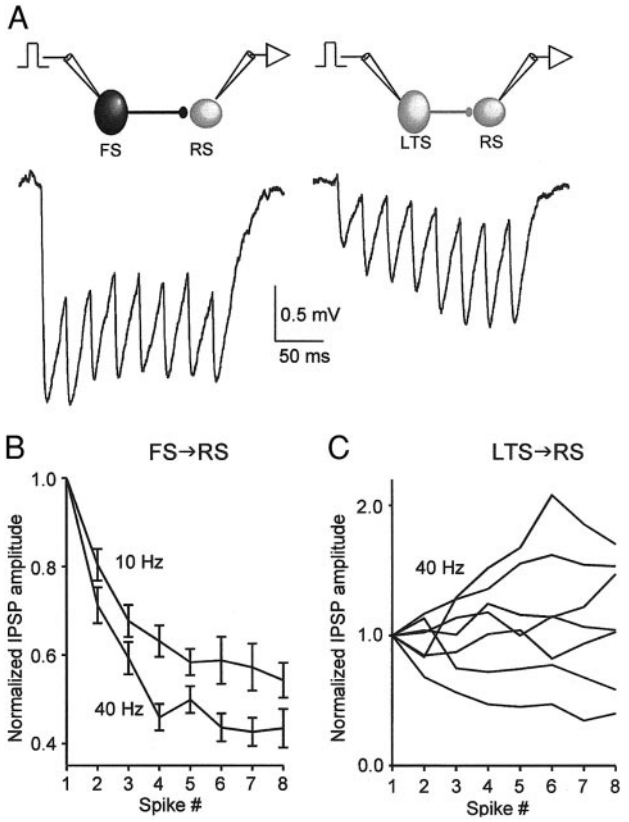

FIG. 8. IPSPs evoked by FS cells and LTS cells onto RS cells have distinctive short-term dynamics. *A*: IPSPs in RS cells evoked by 40-Hz trains of spikes in each presynaptic interneuron type. Postsynaptic membrane potentials were held at −55 mV. *B*: summary data of response amplitudes (means ± SE) at 10 and 40 Hz for FS-evoked IPSPs (24 pairs). Data are normalized to first response in each train. Ratio of average amplitude of $IPSP_{7-8}$ to $IPSP_1$ was 0.56 at 10 Hz and 0.43 at 40 Hz. *C*: short-term dynamics of LTS-evoked IPSPs. Graph plots response amplitudes of individual synaptic connections (7 pairs) at 40 Hz normalized to the 1st response.

Thalamocortical inputs to layer 4 neurons

Many types of neurons in layer 4 receive monosynaptic input from specific relay nuclei of the thalamus. We studied the monosynaptic thalamic input to the three cell types in layer 4 by stimulating thalamic afferents with an extracellular stimulating electrode placed in the VB nucleus. First, a minimal stimulation paradigm was used to assess the unitary response. This method involved adjusting the extracellular stimulation intensity until clear all-or-none events occurred (Fig. 10*A*) (Gil et al. 1999). Most response failures under these conditions were probably due to failures of axon stimulation because slightly increasing the stimulation intensity dramatically decreased the number of failures while the average response amplitude (excluding failures) and shape remained the same. Unitary responses from each cell were averaged. The average unitary responses from samples of cells of the same type were aligned by baseline and latency and then averaged (Fig. 10*B*). The statistics for unitary responses by each cell sample are shown in Table 5. As reported previously, FS cells had the largest unitary responses and LTS cells had the smallest (Gibson et al. 1999). Furthermore, we were often unable to evoke any measurable EPSP in LTS cells even when RS and FS neurons generated reliable responses in the same cortical column. Because we relied on extracellular stimulation, however, we could not further quantify these differences in connectivity rate.

The early time courses of the thalamocortical EPSPs from each cell type are illustrated in Fig. 10*C*). EPSPs of FS cells had the fastest rise time and latency to peak (Table 5). The speed of the FS thalamic response is at least partly attributable to its fast membrane time constant compared to the other two cell types (see Table 1), but differences in the time course of the synaptic currents may also contribute (Angulo et al. 1999; Fricker and Miles 2000; Hestrin 1993). Measurements of thalamocortical EPSPs from LTS cells are more variable due to their relatively small amplitude and the small sample size. On average, all cell types had the same response latencies, and there was very little variation among individual responses at a given synapse [0.077 ± 0.007 (SD) ms, $n = 17$ synapses].

Thalamocortical inputs to RS cells show depression (Gil et al. 1997). Here we found, using suprathreshold stimulation, that thalamocortical inputs onto both FS and RS cells were strongly depressing at all tested frequencies from 0.5 to 40 Hz (Fig. 11, *A* and *B*). The magnitude of depression was generally stronger for inputs to FS cells than RS cells (Fig. 11, *B* and *C*). Thalamocortical inputs onto LTS cells displayed short-term depression (not shown); however, the small size of the inputs did not allow us to quantify the degree of depression at different frequencies.

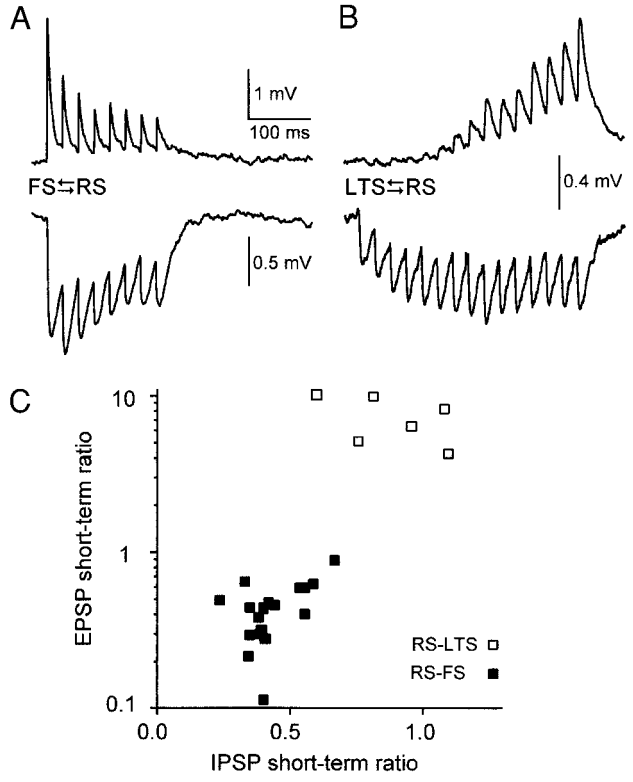

FIG. 9. Reciprocally connected neuron pairs are interconnected by synapses with matching short-term dynamics. Data were obtained from pairs of interneuron-RS cells that had reciprocal synapses. *A*: recordings from an FS-RS pair, showing responses to 40-Hz trains applied to each cell in turn. *B*: recordings from an LTS-RS pair, showing responses to 40-Hz trains applied to each cell in turn. *C*: graph plots short-term ratios (amplitudes of PSP_{7-8}/PSP_1), measured at 40 or 100 Hz of the EPSPs against IPSPs from 24 pairs (□ from 6 RS-LTS pairs; ■ from 18 RS-FS pairs). Correlation coefficient $r = 0.73$ with $P = 0.0002$ calculated over all pairs.

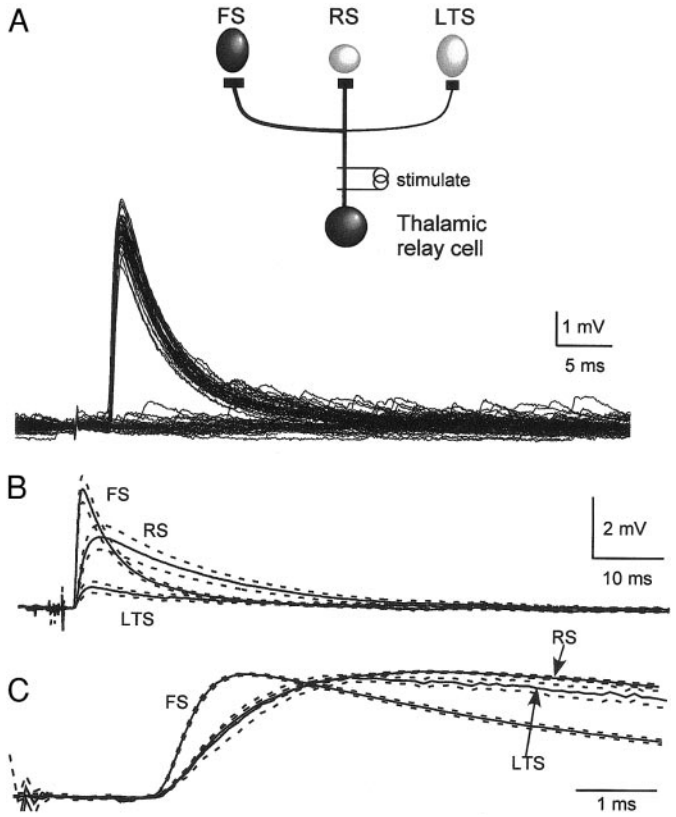

FIG. 10. Unitary thalamocortical EPSPs. *A*: an example of a unitary thalamocortical response in an FS cell observed with minimal stimulation applied to the thalamus. Multiple superimposed trials illustrate the all-or-none nature of responses. *B*: average unitary responses from the 3 cell types (FS, $n = 54$ cells; LTS, $n = 8$; RS, $n = 23$). Average responses from each cell were first aligned to the onset of the responses; – – –, the SE. *C*: same data from *B* shown at faster time scale, with peaks normalized, to highlight differences in EPSP time course.

Long-latency facilitating inputs from collaterals of corticothalamic axons

An important excitatory input to neurons of layer 4 comes from ascending collaterals of corticothalamic neurons in layer 6 (e.g. Stratford et al. 1996; Zhang and Deschenes 1997) (Fig. 12*A*). Thalamic stimulation can activate thalamocortical fibers, orthodromically, as well as intracortical branches of corticothalamic fibers, antidromically (Ferster and Lindstrom 1985). Both types of EPSPs are monosynaptic and thus could in principle be mistaken for one another. However, in a recent study of neurons in layer 6 (Beierlein and Connors 2002), we showed that EPSPs evoked by thalamic stimulation could be reliably segregated into two functional groups. Suspected

TABLE 5. *Properties of thalamocortical EPSPs onto FS, LTS, and RS cells*

	RS	FS	LTS
Amplitude, mV	2.4 ± 2.0 (23)	4.1 ± 3.2 (54)	0.7 ± 0.5 (8)
Latency, ms	3.0 ± 1.1 (23)	2.9 ± 0.8 (54)	3.1 ± 0.5 (8)
Rise time, ms (20–80%)	1.16 ± 0.27 (23)	0.41 ± 0.15 (54)	1.12 ± 0.48 (8)
Peak latency, ms*	6.4 ± 1.2 (23)	4.1 ± 0.9 (54)	5.5 ± 1.4 (8)

All data are means ± SD with the numbers of tested pairs in parentheses.
* Time between stimulation and peak of thalamocortical EPSP.

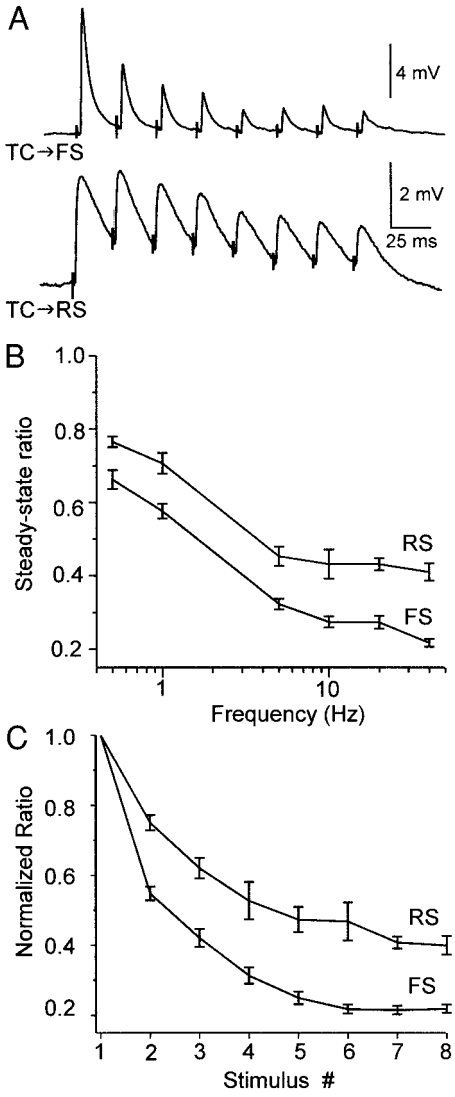

FIG. 11. Thalamocortical EPSPs from FS and RS cells have different short-term dynamics. *A*: average traces from individual cells showing thalamocortical (TC) EPSPs during a 40-Hz stimulus train. Stimulus artifacts are blanked. *B*: frequency-dependence of short-term depression. Graph plots the steady-state amplitude ratio ($EPSP_{7-8}/EPSP_1$, mean ± SE) as a function of stimulus frequency for RS and FS cells. Thalamocortical EPSPs from FS cells ($n = 8$) displayed stronger synaptic depression at all frequencies compared to EPSPs from RS cells ($n = 5$). *C*: the time course of synaptic depression evoked by 40-Hz trains was similar for EPSPs from FS cells ($n = 17$) and RS cells ($n = 11$).

thalamocortical EPSPs had relatively fast latencies, robust short-term depression, and axons with subnormal conduction properties. Subnormal conduction is characterized by a decrease in axonal conduction velocity during repetitive activation (Swadlow et al. 1980). In contrast, suspected corticothalamic EPSPs had longer latencies and displayed short-term synaptic facilitation and supernormal axonal conduction (i.e. conduction velocity increased during repetitive activation). Here we found that the same segregation of thalamically evoked response properties occurred in neurons of layer 4. Figure 12*B* shows a facilitating EPSP recorded in an FS cell during thalamic stimulation. Interestingly, almost all facilitating responses were observed in FS and LTS cells. Among all thalamically evoked responses in which short-term plasticity

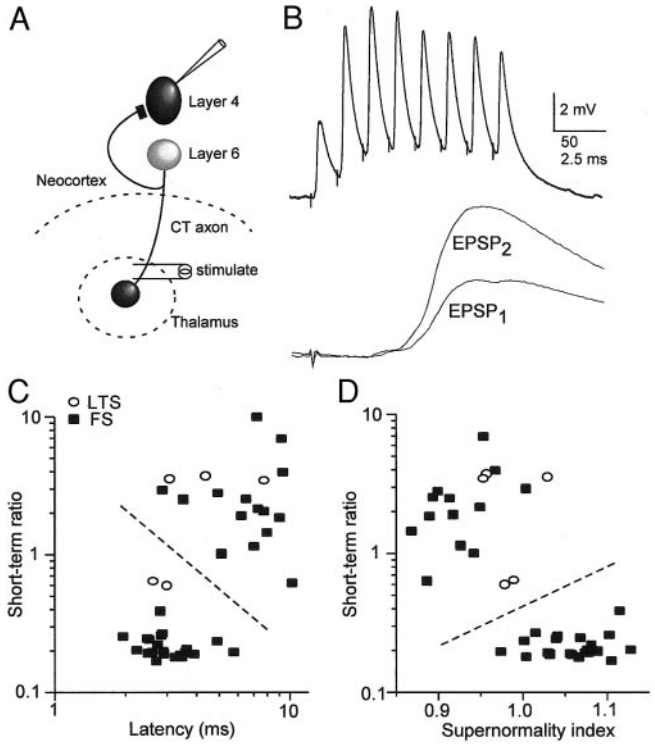

FIG. 12. Thalamic stimulation can trigger facilitating EPSPs mediated by corticothalamic (CT) axon collaterals. *A*: schematic circuit involved in generating monosynaptic CT-EPSPs in layer 4. *B*: thalamus-evoked facilitating EPSPs recorded from an FS cell (8 stimuli at 40 Hz). Close-up (*bottom*) shows overlay of EPSPs evoked by the 1st 2 stimuli. Note slight decrease in latency for 2nd EPSP in the train. *C* and *D*: thalamus-evoked EPSPs can be separated into 2 clusters, based on latency, short-term amplitude ratio ($EPSP_{7-8}/EPSP_1$, 40 Hz), and supernormality of presynaptic conduction (measured with 40-Hz stimulation).

was tested, 37% (26/70) of FS cells, 57% (4/7) of LTS cells, and only 4% (1/23) of RS cells had facilitating responses. This is consistent with anatomical studies suggesting that the layer 4 terminations of layer 6 corticothalamic neurons contact only inhibitory interneurons (White and Keller 1987; but see Staiger et al. 1996). Plotting the short-term synaptic ratios (from 40-Hz stimulation) against conduction latency for each thalamically evoked response indicates that the more facilitating synaptic responses had the longest latencies (Fig. 12*C*). Using a K-means cluster analysis, and assuming two groups a priori, the data are divided (Fig. 12*C*, – – –) into two distinct clusters. Comparing the two groups across this dividing line, the latencies of facilitating EPSPs were very significantly longer than the latencies of depressing EPSPs (6.65 ± 0.53 ms for facilitating and 3.12 ± 0.18 ms for depressing, $n = 16$ and $n = 23$, respectively).

A supernormality index for axonal conduction was defined as the ratio of the average latency of the last two responses to the latency of the first response in a 40-Hz train. A value <1.0 indicates supernormality. Plotting the short-term synaptic ratio against the supernormality index (Fig. 12*D*) shows that the facilitating synaptic responses tended to be generated by axons that displayed supernormality and depressing synaptic responses were generated by subnormally conducting axons. As before, a K-means cluster analysis with a two group a priori assumption divided the points (– – –) into two distinct clusters. All points, except for two representing LTS cell responses, fell into the same clusters derived from the analysis of the same data set in Fig. 12*C*. Using the clusters generated here, we calculated that facilitating synaptic responses had an average supernormality index significantly less than the index of depressing synaptic responses (0.93 ± 0.01 for facilitating and 1.05 ± 0.01 for depressing, $n = 16$ and $n = 23$, respectively).

Overall, these data suggest that suspected thalamocortical axons have relatively fast conduction velocity, subnormal conduction during repetitive firing, and synapses that display short-term depression; in contrast, axons of suspected corticothalamic projection cells tend to conduct more slowly, display supernormal conduction, and terminate in synapses that facilitate.

Feedforward inhibition mediated by FS and LTS cells

Based on the properties described in the preceding text, it seems likely that FS neurons mediate rapid feedforward inhibition of sensory evoked activity, particularly at low stimulus frequencies. In many experiments, thalamically evoked disynaptic inhibition has been observed (cf. Gil and Amitai 1996). These IPSPs had a relatively short latency (3.99 ± 0.16 ms, $n = 23$), i.e., only ~1.0 ms longer than the monosynaptic thalamocortical EPSP. Thalamically evoked disynaptic inhibition was strongly frequency-dependent: IPSPs occurred reliably during stimulus trains of 0.5, 1, and 5 Hz but typically disappeared by the second or third stimulus in a 40-Hz train ($n = 12$, Fig. 13*A*).

Intracortical extracellular stimulation sometimes evoked disynaptic inhibition whose dynamics were inconsistent with FS cell involvement. Weak, low-frequency stimulation caused monosynaptic excitation of RS cells, but increasing the stim-

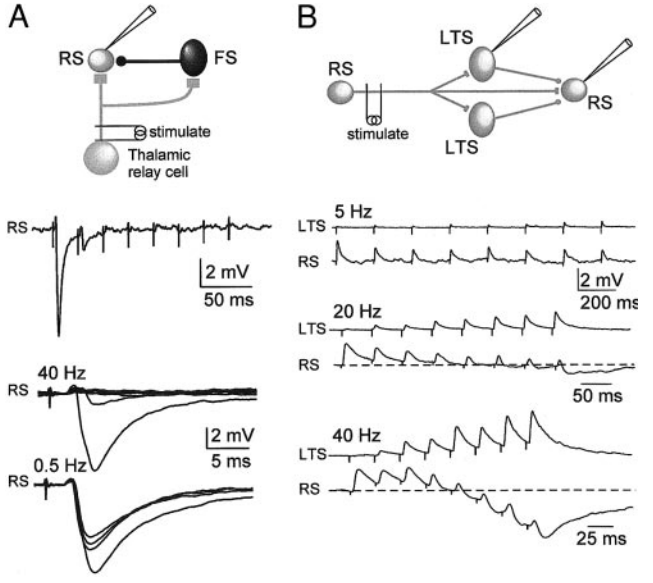

FIG. 13. FS and LTS cells mediate feedforward inhibition with distinctly different dynamics. *A*: example of thalamocortically triggered inhibition recorded in an RS cell. A 40-Hz train evoked small EPSPs followed by powerful disynaptic IPSPs; the IPSPs rapidly attenuated during the train. *B*: high-frequency intracortical stimulation could recruit LTS-mediated disynaptic inhibition. Traces show progressive facilitation of EPSPs from LTS cells activated by stimulus trains of 5, 20, and 40 Hz. The 5-Hz train evoked mainly depressing EPSPs in an RS cell, whereas 20- and 40-Hz trains initially excited the RS but ultimately inhibited it strongly. Schematic diagrams illustrate postulated circuits for the responses illustrated below them.

ulus frequency to ≥20 Hz led to a delayed recruitment of powerful disynaptic IPSPs (Fig. 13*B*). The depressing dynamics of both the excitatory inputs (Fig. 4) and the inhibitory outputs of FS cells (Fig. 8) make them very poor candidates for the type of facilitating inhibition illustrated in Fig. 13*B*. However the frequency and time dependence of this facilitating inhibition closely resembled the characteristics of the facilitating excitatory synapses onto LTS interneurons (Fig. 13*B;* see also Figs. 4 and 5), as well as the IPSPs generated by LTS cells (Fig. 8). We conclude that LTS cells mediate a form of local inhibition that is only engaged when local activity reaches high frequencies for a few tens of milliseconds.

DISCUSSION

One of the most salient features of neocortical architecture is its wide variety of inhibitory interneurons (Peters and Jones 1984; Mountcastle 1998). The presence of so many types of interneurons suggests that each type may have a specialized role and that there are diverse functions of interneurons in the cortex. Deducing the nature of these functions requires information about the physiological properties of the interneurons themselves and the synaptic circuitry they participate in. Here we show that the synaptic dynamics of two common types of inhibitory interneurons in layer 4 are dramatically different. Both the inputs and outputs of FS cells are relatively strong and reliable and display short-term depression. In contrast, synaptic inputs and outputs of LTS cells tend to be weak and unreliable at low frequencies, show relatively little short-term depression, and often facilitate at high frequencies. Thus FS and LTS cells differ sharply in the properties of their chemical synapses and their intrinsic membrane properties (Gupta et al. 2000; Kawaguchi 1995; Kawaguchi and Kubota 1997), and each makes cell-type-specific electrical synapses (Gibson et al. 1999). These differences strongly imply that FS and LTS cells serve distinctly different functions in layer 4.

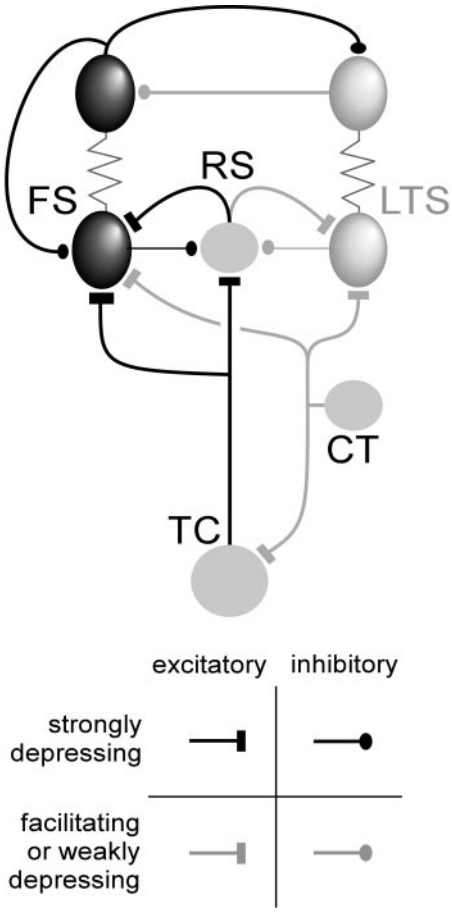

FIG. 14. Two dynamically distinct systems of inhibition in layer 4. Black pathways have synapses with relatively reliable transmission and short-term depression. Gray pathways have synapses that are relatively unreliable initially but generate short-term facilitation (LTS-to-RS connections range from facilitating to weakly depressing). Excitatory synapses are rectangular, inhibitory synapses are round. Details are described in the text.

Functional patterns of inhibitory circuitry in layer 4

By sampling paired combinations of RS, FS, and LTS neurons, we were able to provide a functional anatomy of the neural circuitry within layer 4 (Table 2). Figure 14 schematizes this circuitry together with some connections observed in other studies. Judging from their connection probabilities, both FS and LTS cells seemed to be targeted monosynaptically by a large percentage of their neighboring excitatory neurons. Input from thalamocortical relay cells was more selective; it frequently and strongly excited FS cells (Agmon and Connors 1991; Beierlein et al. 2002; Porter et al. 2001) but only rarely and weakly excited LTS cells (Gibson et al. 1999). As stated in the preceding text, we cannot quantify the connection probabilities from thalamic axons because we used extracellular stimulation. Nevertheless studies in the somatosensory barrel cortex in vivo suggest that each FS cell receives monosynaptic input from a majority of the relay cells in its corresponding thalamic barreloid (Swadlow 2003; Swadlow and Gusev 2001; Swadlow et al. 1998). LTS cells have not been studied in vivo because there is no unambiguous way to distinguish their action potentials extracellularly. In general, the data suggest that individual FS cells are the frequent recipients of both intrinsic and extrinsic sources of excitation. By contrast, LTS interneurons appear to be more exclusively dedicated to the neural activity of the local cortical circuit. It remains to be seen how much input each interneuron receives from more distant neocortical cells.

Both FS and LTS cells made frequent inhibitory synapses onto local excitatory neurons (Fig. 14). Thus we can infer that both cell types contribute to feedback inhibitory pathways onto RS cells. In addition, FS cells also mediate a fast and strong feedforward inhibitory pathway triggered by thalamic input. The amplitude of FS-mediated IPSPs was, on average, twice the size of the IPSPs mediated by LTS cells. This may reflect differences in release probabilities to some extent (see following text), but it is also consistent with different sites of termination on the postsynaptic cells. In other cortical areas and layers, axons of FS cells project primarily to perisomatic areas of postsynaptic pyramidal cells neurons, whereas LTS cells have more terminations in distal dendritic regions than do FS cells (Kawaguchi and Kubota 1997; Xiang et al. 1998). Thus smaller amplitudes and slower rise times of LTS-evoked IPSPs are consistent with the expected electrotonic attenuation of more distal synaptic events (Xiang et al. 2002).

In this study, we focused on the inhibitory connections that FS and LTS cells make onto excitatory RS neurons. However, it is important to point out that inhibitory interneurons com-

monly inhibit one another; in neocortical layer 4, FS cells frequently inhibit LTS cells, and vice versa, and FS cells frequently inhibit other FS cells (Fig. 14) (Gibson et al. 1999). Interestingly, inhibition among LTS cells is relatively rare. The presence of reciprocal inhibition between the FS and LTS networks reinforces the idea that each inhibitory system serves a distinct and complementary role—activity in one network would tend to control activity in the other network.

In a study of the mouse barrel cortex in vitro, Porter et al. (2001) found that a variety of inhibitory interneuron types, including both FS cells and "regular-spiking nonpyramidal" cells (RSNP cells, which have interneuron morphology and spike-frequency adaptation), were strongly and monosynaptically excited by thalamic synapses. Beierlein et al. (2002) also found that both FS and RSNP neurons in mouse layer 4 received strong excitation from thalamocortical synapses. Our studies of rat layer 4 (Gibson et al. 1999; this study) suggest, on the other hand, that thalamocortical axons tend to avoid most LTS cells. If RSNP cells and LTS cells are homologous cell types, then comparison of these studies suggests that rats and mice differ in the circuits they use to mediate thalamocortical feedforward inhibition. Alternatively, it may be that the neurons we call LTS cells in the rat are not the equivalent of the RSNP cells studied in the mouse. In both mouse studies (Beierlein et al. 2002; Porter et al. 2001), the RSNP cells had very short spike durations, similar to those of FS cells, whereas rat LTS cells generate spikes much broader than FS cell spikes (Table 1). However, in another study of the mouse from our laboratory, FS and LTS cells were clearly distinguished in layer 4 (Deans et al. 2001). Finally, we cannot rule out the possibility of a technical problem. If the axons innervating FS, RS, and LTS cells take different trajectories as they course between thalamus and cortex, it could be that the slicing procedure cut one set of axons—those to LTS cells—preferentially. This seems unlikely, as there is no evidence for the segregation of thalamocortical axons by postsynaptic target.

FS inhibitory cell system

FS cells are the most prevalent type of interneuron in the neocortex to judge from the distribution of parvalbumin-expressing neurons as compared to other immunocytochemically identified inhibitory interneuron types (Amitai et al. 2002; Gonchar and Burkhalter 1997; Kawaguchi and Kubota 1997). If we also consider their abundant connections with input axons and output targets (Table 2), and the strength and high reliability of their input and output synapses, it seems clear that FS cells comprise the most dominant inhibitory system in the neocortex.

The kinetics of EPSPs onto FS cells were faster than those of EPSPs onto RS and LTS cells. Previous studies have shown that the EPSPs of FS cell have relatively short rise times and half-widths, primarily due to the fast kinetics of AMPA channels that lack the GluR-B subunit (Geiger et al. 1995) and negligible contributions of *N*-methyl-D-aspartate (NMDA) currents (Angulo et al. 1999). The decay rate of the EPSP is also a function of the short membrane time constant in FS cells. As suggested before (Angulo et al. 1999), these properties make FS cells very sensitive to the temporal correlations between EPSPs.

The input and the output synapses of the FS inhibitory system had similar short-term dynamics. Excitatory synapses onto FS cells, regardless of source, generated EPSPs that were generally large in amplitude and had small trial-to-trial fluctuations and few failures. All excitatory connections onto FS cells showed strong short-term depression with the exception of corticothalamic collaterals (Fig. 14; cf. black vs. gray pathways). It is likely that the depression displayed by these synapses is mediated by a use-dependent decrease in transmitter release because it was also accompanied by an increase in CV and, where measurable, an increase in failure rate. The output synapses of FS cells also displayed short-term depression with a mean magnitude similar to that of the input synapses to FS cells (cf. Figs. 4*B* and 8*B*, as well as the reciprocal synapses of RS-FS pairs shown in Fig. 9). Thus the synapses of the FS inhibitory system are tuned for reliability in response to transient activation, and depression during high-frequency activation.

LTS inhibitory cell system

LTS cells are, at first glance, anomalously sparse and ineffectual. There are significantly fewer LTS cells than FS cells as determined by both electrophysiological sampling and immuno-cytochemical markers (Amitai et al. 2002; Gibson et al. 1999; Kawaguchi and Kubota 1997). We found that EPSPs onto LTS cells were, on average, much smaller and less reliable than the EPSPs onto FS cells, when activated at low frequency.

The most dramatic feature of the RS-to-LTS connection was its short-term facilitation, which was evident at frequencies $>$10–20 Hz. Facilitation at synapses between excitatory neurons and somatostatin-containing neurons has previously been described in layers 2/3 (Reyes et al. 1998) and layer 4 (Thomson and Deuchars 1997), although the degree of facilitation reported in those studies was more modest. The mechanism of RS-to-LTS facilitation is likely to reside presynaptically. We found that the failure rate decreased during stimulus trains that evoked facilitation, suggesting a progressively increasing probability of transmitter release. Consistent with this, as RS spike-evoked EPSPs facilitated, many synapses also generated delayed, asynchronous transmitter release. Facilitation and delayed release depend on residual calcium buildup within the presynaptic terminal (Atluri and Regehr 1996; Cummings et al. 1996; Kamiya and Zucker 1994), although the calcium dependence, magnitude, and time course are distinct for the two processes (Atluri and Regehr 1998), suggesting that they do not share a common final mechanism. The functional consequences of asynchronous release are not known. Its strong sensitivity to frequency and pulse number (Atluri and Regehr 1998) could serve as a type of short-term synaptic memory.

Within the LTS inhibitory system, as in the FS system, both the input and output synapses tended to have similar short-term dynamics (Fig. 14, gray pathways). Whereas IPSPs evoked by FS cells inevitably depressed, those generated by LTS cells were, on average, quite stable at frequencies $\leq$40 Hz. Thus the facilitating excitatory synapses onto LTS cells were matched with relatively frequency-insensitive output synapses (cf. Figs. 8*B* and 9), yielding an inhibitory system quite unresponsive to transient stimuli, but exquisitely responsive to sustained, high-frequency activity in the local cortical circuit.

Control of synaptic dynamics by interneuron type

The short-term dynamics of cortical synapses usually depend on presynaptic mechanisms (Zucker and Regehr 2002). However, the induction of synapse dynamics during development is likely the result of a complex, synapse-specific interplay of signals from both the pre- and postsynaptic cells. The short-term dynamics of excitatory synapses onto pyramidal cells depend primarily on the identity of the presynaptic neuron (Gil et al. 1997; Reyes et al. 1998; Stratford et al. 1996). In contrast, studies characterizing synapses onto and from interneurons have concluded that the postsynaptic cell type determines the short-term properties (Markram et al. 1998; Reyes et al. 1998). In a study of short-term synapse dynamics within layer 5 of the rat visual cortex, Xiang et al. (2002) found that inhibitory synapses from LTS cells onto pyramidal cells showed more depression than those of inhibitory synapses from FS cells onto pyramidal cells. The determination of short-term synapse dynamics may vary, to a surprising degree, with cortical area, layer, neuronal subtype, and perhaps species.

In addition to the results presented here, we have recently analyzed the short-term dynamics of synapses among and between FS and LTS cells (unpublished data). The inhibitory synapses formed by FS cells onto both LTS cells and other FS cells were depressing, whereas LTS cells formed facilitating inhibitory synapses onto FS cells (Fig. 14). Thus at least within layer 4, all synapses to and from FS cells were strongly depressing, whereas all synapses to and from LTS cells were only weakly depressing or facilitating. It therefore seems likely that the developmental processes determining synapse dynamics in inhibitory neural networks differ from the ones at synapses between excitatory cells.

Two dynamically distinct inhibitory systems in layer 4

There is now overwhelming evidence that FS neurons help to control sensory activity in the neocortex. In vivo studies in rabbit and rat somatosensory cortex have shown that single relay neurons can powerfully drive the activity of several FS neurons in individual barrels (Bruno and Simons 2002; Swadlow 2003; Swadlow and Gusev 2001). FS neurons within a barrel often generate spikes with an exceptional degree of synchrony (Swadlow et al. 1998). Several cellular mechanisms mediate activity within this high-fidelity circuit: thalamocortical synapses onto FS neurons are powerful, mediated by a large number of release sites with high release probability. Precision in spike initiation arises from the fast kinetics of the synaptic currents (Angulo et al. 1997; Geiger et al. 1995) and the intrinsic conductances of FS cells (Fricker and Miles 2000; Galarreta and Hestrin 2001b). Furthermore FS cells are interconnected by both electrical synapses (Fig. 14) (Galarreta and Hestrin 1999; Gibson et al. 1999; Tamás et al. 2000) and chemical synapses (Galarreta and Hestrin 1999; Gibson et al. 1999; Tamás et al. 1998), which might promote synchronous spiking by selectively inhibiting asynchronous activity (Galarreta and Hestrin 2001b).

While the role of FS neurons in feedforward inhibition seems well supported, a more sophisticated role for FS cells in information processing remains to be established. Feedforward inhibition seems to be critical in sharpening the tuning of layer 4 neurons in both the somatosensory and visual systems (Miller 2003; Miller et al. 2001), and it is very likely that FS cells mediate such effects. Recent theoretical and experimental work suggests that synaptic inhibition can increase the spike variability in postsynaptic targets (Shadlen and Newsome 1998), a feature crucial to some models of cortical information processing. Networks of FS neurons have also been implicated in synchronizing the activity of postsynaptic pyramidal neurons (Bush and Sejnowski 1996; Cobb et al. 1995; Whittington et al. 1995). It is not clear whether these phenomena are relevant to cortical information processing.

The short-term behavior of different inhibitory systems will contribute strongly to the balance of excitation and inhibition during different activity regimes. Recent studies suggested that IPSPs depress less than EPSPs during short (Varela et al. 1999)- or long-lasting (Galarreta and Hestrin 1998) stimulation in the neocortex. However, these studies made no distinction between the synapse dynamics evoked by different types of interneurons (Varela et al. 1999), or they focused exclusively on FS cells (Galarreta and Hestrin 1998). While some studies have found no significant differences (Tamas et al. 1997; Tarczy-Hornoch et al. 1998; Thomson et al. 1996; Xiang et al. 2002), other recent work has shown that synapse dynamics vary dramatically with interneuron type (Gupta et al. 2000; Wang et al. 2002). Furthermore, the diverse dynamics of excitatory synapses onto interneurons will also contribute to the differential recruitment of interneurons under specific circumstances.

The role of LTS neurons in cortical circuits is likely to be quite different from that of FS neurons. Given their strong sensitivity to a variety of neuromodulators (Beierlein et al. 2000; Kawaguchi 1997; Kawaguchi and Shindou 1998; Xiang et al. 1998) and sustained high-frequency synaptic input, we can predict that LTS cells are most strongly engaged during behavioral states associated with cortical activation and enhanced input from ascending modulatory systems. It is also possible that LTS cells provide a fail-safe function, providing progressively more inhibition whenever cortical activity rises to dangerously high frequencies that might otherwise trigger seizures. In general, LTS cells may provide a time-averaged gain control in neocortex.

The highly unreliable excitatory inputs and outputs of LTS cells might suggest that these cells do not play a role in controlling the exact timing of action potential generation. However, LTS neurons are electrically coupled to one another (Deans et al. 2001; Gibson et al. 1999), and they generate synchronous and rhythmic spiking in response to activation of postsynaptic metabotropic glutamate and muscarinic receptors (Beierlein et al. 2000). Under these conditions, convergent input from several synchronously active LTS neurons can reliably entrain the spiking of postsynaptic RS neurons (M. A. Long and B. W. Connors, unpublished data).

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DISCLOSURES

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