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Short-Term Dynamics of Thalamocortical and Intracortical Synapses Onto Layer 6 Neurons in Neocortex

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Beierlein, Michael, and Barry W. Connors. Short-term dynamics of thalamocortical and intracortical synapses onto layer 6 neurons in neocortex. *J Neurophysiol* 88: 1924–1932, 2002; 10.1152/jn.00282.2002. Layer 6 is the main source of neocortical connections back to specific thalamic nuclei. Corticothalamic (CT) systems play an important role in shaping sensory input, but little is known about the functional circuitry that generates CT activity. We recorded from the two main types of neurons in layer 6, regular-spiking (RS; pyramidal neurons) and fast-spiking (FS; inhibitory interneurons) cells and compared the physiological properties of different excitatory inputs. Thalamic stimulation evoked two monosynaptic inputs with distinct properties: suspected thalamocortical (TC) synaptic events had short latencies, short-term synaptic depression, and paired-pulse responses that suggested subnormal axonal conduction. A second group of synaptic responses likely originated from intracortical collaterals of CT cells that were antidromically activated from the thalamus. These intracortical responses had longer latencies, short-term synaptic facilitation, and were transmitted by axons with supernormal conduction. Suspected TC inputs to FS cells had significantly larger amplitudes than those onto RS cells. Dual recordings from neighboring neurons in layer 6 revealed both facilitating and depressing synaptic connections; the depressing synapses were probably formed by layer 6 cells that do not project to the thalamus, and thus were not sampled by thalamic stimulation. We conclude that layer 6 neurons integrate a variety of inputs with distinct temporal dynamics that are determined by the presynaptic cell type.

INTRODUCTION

Layer 6 is the main source of axons that project from neocortex back to the thalamus. In sensory thalamic nuclei, corticothalamic (CT) synapses far outnumber those from the ascending sensory systems (Sherman and Guillery 1996). Despite this numerically impressive input, little is known about the functions of this feedback projection. Some studies in the visual system suggest that CT connections help to synchronize the activity of cells in the lateral geniculate nucleus as they respond to a single elongated stimulus (Sillito et al. 1994). More generally, CT feedback might play a role in forming dynamic spatiotemporal maps that encode stimulus information in the thalamus (Krupa et al. 1999). CT connections may also help to trigger and maintain long-range thalamic synchrony, e.g., during sleep spindles (Contreras et al. 1996).

In addition to sending axonal projections to the thalamus, CT cells in layer 6 also project to layer 4 within the same cortical column (Bolz and Gilbert 1986; Callaway and Lieber

1996; Gilbert and Wiesel 1979; Katz 1987; Martin and Whitteridge 1984; Stratford et al. 1996; Zhang and Dechenes 1997). Anatomical (White and Keller 1987) and physiological (Bolz and Gilbert 1986) studies suggest that inhibitory cells in layer 4 are a common target of CT cells (but see Staiger et al. 1996). Layer 6 cells thus control sensory input to the neocortex on at least two levels: in the thalamus and in the cortex within layer 4. In addition, layer 6 contains a large group of neurons that do not project to thalamus but send ascending collaterals to layer 5 as well as to other cortical areas (Zhang and Dechenes 1997).

To understand more clearly the type of information layer 6 is transferring to thalamus or other cortical layers, it is important to characterize the types of inputs that layer 6 receives. Specific sensory thalamic nuclei project directly to layer 6 (Agmon and Connors 1992; Herkenham 1980; LeVay and Gilbert 1976) as well as to layer 4. In the somatosensory system, whisker-evoked activity of layer 6 neurons occurs simultaneously with, and often precedes, activity of layer 4 neurons (Simons 1978; Swadlow 1989). While several studies have investigated the properties and short-term dynamics of thalamocortical (TC) synapses onto layer 4 cells (Gibson et al. 1999; Gil et al. 1999; Stratford et al. 1996), similar studies have not been performed for TC synapses in layer 6. Similarly, little is known about the synaptic properties of local cortical connections within layer 6. Layer 6-to-layer 4 synapses have been shown to display short-term facilitation onto inhibitory (J. R. Gibson and B. W. Connors, unpublished) as well as excitatory cells (Stratford et al. 1996), suggesting that collaterals that remain within layer 6 might facilitate as well (Ferster and Lindstrom 1985).

Here we studied several distinct pathways that converge onto layer 6 cells. Extracellular stimulation in thalamus evoked two types of monosynaptic responses that could be reliably distinguished using cluster analysis; TC synapses had short latencies and displayed synaptic depression, whereas intracortical synapses formed by CT cells had long latencies and displayed short-term synaptic facilitation. Axons of CT cells, but not TC axons, showed strong supernormal conduction. Dual recordings within layer 6 revealed synapses displaying short-term depression, likely formed by cells that do not project to thalamus.

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METHODS

Slice preparation and recording

Thalamocortical slices (400 μm thick) were prepared as described previously (Agmon and Connors 1991; Gibson et al. 1999). Briefly, Sprague-Dawley rats aged P14–P21 were anesthetized with pentobarbital and decapitated, and their brains were quickly immersed in ice-cold, oxygenated artificial cerebrospinal fluid (ACSF). Slices were incubated at 32°C for 1 h, then held at room temperature. The recording chamber was maintained at 32°C. The ACSF contained (in mM) 126 NaCl, 3 KCl, 1.25 NaH_2PO_4 , 2 MgSO_4 , 2 CaCl_2 , 26 NaHCO_3 , and 10 dextrose, saturated with 95% O_2 –5% CO_2 .

Micropipettes were filled with (in mM) 135 K-gluconate, 4 KCl, 2 NaCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Tris, and 0.5–10 phosphocreatine-Tris (pH 7.25, 295 mosM). All recordings (single or dual cell) were made in current-clamp mode (Axoclamp 2B, Axon Instruments), with IR-DIC visualization using a Zeiss Axioskop and a CCD camera (Hamamatsu). Synaptic responses were evoked with extracellular stimuli lasting 200 μs , typically of about 40 μA (range: 5–100 μA), applied through paired microwires located in either the ventrobasal nucleus or the reticular nucleus of the thalamus. Excitatory synaptic responses were measured at postsynaptic membrane potentials of -60 to -70 mV, and 50 μM DL-2-amino-5-phosphopentanoic acid (AP5, Sigma) was included in the bath to block N-methyl-D-aspartate (NMDA) receptors. When inhibitory postsynaptic potentials (IPSPs) were apparent at more depolarized membrane potentials, the recording was discarded.

Threshold synaptic responses were collected with a “minimal stimulus” protocol (Gil et al. 1999; Gibson et al. 1999): stimulus intensity was adjusted to evoke EPSPs in only about 50% of trials. Responses were considered monosynaptic if the latency jitter was less than 1 ms, and synaptic responses had similar rise times from trial to trial.

To measure antidromic latencies of CT neurons, slices were bathed in AP5 and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 20 μM , Sigma), to block both NMDA and AMPA receptors, respectively. Extracellular single-unit recordings were obtained using patch micropipettes filled with ACSF, and signals were band-pass filtered at 0.3–10 kHz.

Analysis

Data acquisition and analysis were performed in Labview (National Instruments) with routines written by Jay R. Gibson. Cluster analysis was carried out with Ward's method (Statistica) and squared Euclidean distances. All data are reported as means \pm SD.

Anatomical labeling

In some recordings biocytin (4 mg/ml) was added to the normal internal solution (Gibson et al. 1999). Slices were fixed in 4% paraformaldehyde (sometimes with 0.2% picric acid) in 0.1 M phosphate buffer, transferred to 30% sucrose, resectioned to a thickness of 80 μm , and reacted with avidin-biotin-peroxidase (Vector).

RESULTS

We recorded from two types of neurons, whose somata were usually in the lower half of layer 6 (Fig. 1A). Regular-spiking (RS) cells had medium- to small-diameter cell bodies as seen under IR-DIC optics. Their action potentials were relatively broad (half-width of 1.0 ± 0.26 ms, $n = 49$), and displayed prominent adaptation of spiking frequency when stimulated with long current steps (Fig. 1B). In contrast, fast-spiking (FS) cells had larger cell bodies and generated nonadapting trains of briefer action potentials (half-width of 0.36 ± 0.11 ms, $n = 18$; compared with RS cells, $P < 0.001$, t -test; Fig. 1C). A second

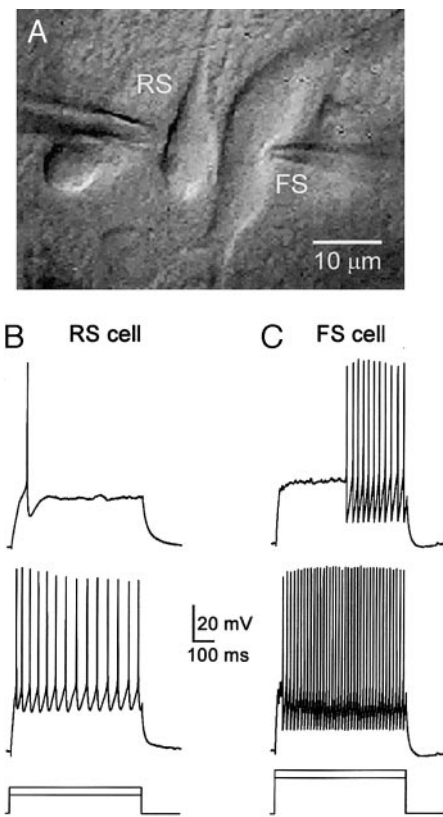


FIG. 1. A, Image of a regular-spiking (RS) neuron and a fast-spiking (FS) neuron in layer 6 under IR-DIC optics during an experiment. Recording micropipettes are also visible. Intrinsic firing properties of RS (B) and FS cell (C). B and C, top: firing at threshold. Current steps applied were 200 and 300 pA for RS, 400 and 500 pA for FS cell.

class of inhibitory interneurons, low-threshold spiking (LTS) cells ($n = 8$, data not shown), displayed broader spikes and stronger spike frequency adaptation, as compared with FS cells. Because few cases of thalamus-evoked synaptic responses onto LTS cells were found (cf. Gibson et al. 1999), data from this cell type are not included in this report. The intrinsic physiological properties of all three cell types were very similar to those previously described for cells in other neocortical layers (cf. Connors and Gutnick 1990; Gibson et al. 1999; McCormick et al. 1985).

Seven RS and three FS cells of layer 6 were filled with biocytin, and their morphology was reconstructed (not shown). RS cells often had a distinct apical dendrite that sometimes reached into layer 4 or beyond, and they formed sparse local axonal arbors. It was not routinely possible to trace axons into the internal capsule, thus CT cells could not be distinguished anatomically from corticocortical cells. FS cells had relatively aspiny vertically oriented dendrites and a dense local axonal plexus, suggestive of basket cells (Zhang and Dechenes 1997).

Properties of thalamus-evoked synaptic responses

Extracellular stimulation in the thalamus can potentially activate two distinct monosynaptic pathways that generate excitatory responses in layer 6 cells (Fig. 2A). First, short-latency EPSPs can be evoked by orthodromic activation of TC afferents (Agmon and Connors 1992; Ferster and Lindstrom 1983; Gil and Amitai 1996; Martin and Whitteridge 1984;

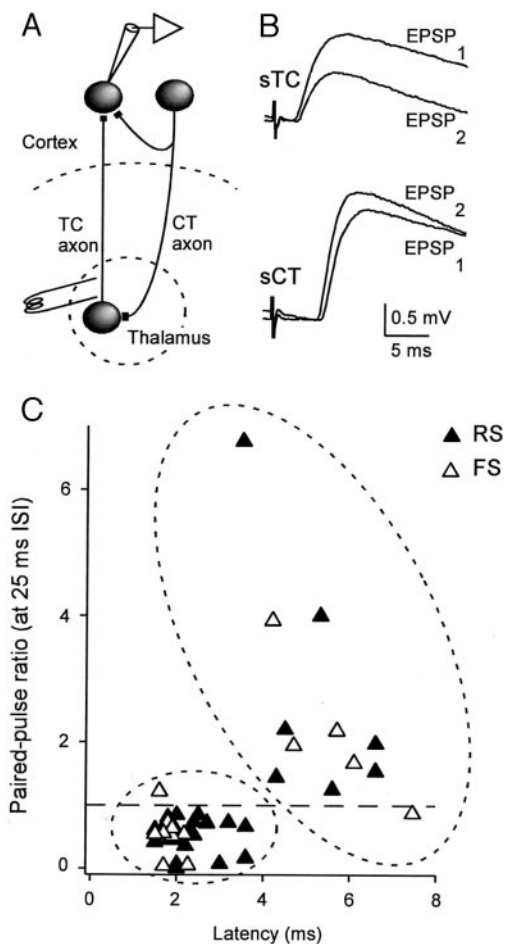


FIG. 2. Thalamic stimulation elicits 2 distinct excitatory postsynaptic potentials (EPSPs). *A*: schematic diagram of pathways leading to monosynaptic excitation of layer 6 cells after extracellular stimulation in the thalamus. *B*: 2 types of EPSPs: recordings shown are from 2 RS cells stimulated with paired-pulses with 25-ms interstimulus interval (ISI). Suspected thalamocortical (sTC, *top*) responses show paired-pulse depression and short latencies, while suspected intracortical (sCT, *bottom*) responses have long latencies and facilitate. *C*: summary graph ($n = 45$ responses). Two data clusters can be distinguished (---).

Swadlow 1995). Second, EPSPs can be evoked by the intracortical collaterals of CT cells in layer 6, whose axons are activated antidromically from the thalamus (Ferster and Lindstrom 1985). These intracortical responses, while still monosynaptic, are expected to have longer latencies due to the generally slower conduction velocity of CT axons (Swadlow 1990). Indeed, we found that stimulation in the thalamus with paired stimulus pulses revealed two types of monosynaptic responses (Table 1). The majority ($n = 33$ EPSPs) had relatively short latencies and almost always (32 of 33 responses) displayed strong paired-pulse depression (Fig. 2*B*). These properties are similar to those of the TC EPSPs in layer 4 (Gil et al. 1999). A smaller number of responses ($n = 12$) had longer latencies, and almost all displayed paired-pulse facilitation (Fig. 2*C*). Plotting the paired-pulse ratio of EPSP amplitude ($\text{EPSP}_2/\text{EPSP}_1$), tested with a 25-ms interstimulus interval versus the EPSP latency for all experiments revealed two distinct groups of data points (Fig. 2*D*), suggesting that the thalamus-evoked responses were mediated by two distinct pathways. Cluster analysis using response latency and paired-

pulse ratio as variables led to the same distinction. In the following, we will refer to the short-latency, depressing synaptic response as the *suspected thalamocortical response* (sTC) and the long-latency, facilitating response as the *suspected intracortical response* of antidromically activated CT neurons (sCT). Both sTC and sCT EPSPs were recorded in RS cells and FS cells, and there were no significant cell type-specific differences in their short-term dynamics or latencies (Table 1). In three cells, both short- and long-latency EPSPs could be evoked from the thalamus (Fig. 3*B*) with the short-latency response always detectable in isolation at a lower stimulus intensity.

CT axons are thinner than TC axons (Jones and Powell 1969). Axon diameter and the spike-threshold amplitude of extracellular current are inversely related (Jack et al. 1975), so we would expect TC axons to have lower thresholds than CT axons (cf. Rose and Methner 2001). Consistent with this hypothesis, there was a significant difference in the threshold current necessary to yield a synaptic response for the two EPSP groups ($50 \pm 37 \mu\text{A}$ for sTC responses vs. $101 \pm 51 \mu\text{A}$ for sCT responses, $P = 0.002$, t -test).

We used trains of eight stimuli to estimate the steady-state responses for both types of EPSPs. In agreement with findings in layer 4 (Gibson et al. 1999), sTC EPSPs to both RS and FS cells showed strong short-term depression (Fig. 3, *A* and *C*). The normalized steady-state values ($\text{EPSP}_{6-8}/\text{EPSP}_1$) at 10 and 40 Hz were very similar in RS and FS cells (0.38 ± 0.07 , $n = 4$ for RS cells vs. 0.34 ± 0.08 , $n = 4$ for FS cells at 10 Hz; 0.26 ± 0.01 , $n = 5$ for RS cells vs. 0.26 ± 0.02 , $n = 5$ for FS cells at 40 Hz). In contrast, sCT responses consistently facilitated (Fig. 3, *B* and *D*). In FS cells, steady-state values of 40-Hz trains were 1.5 ± 1.0 ($n = 4$) at 10 Hz and 1.7 ± 0.9 ($n = 3$) at 40 Hz. It was not possible to record isolated sCT response trains in RS cells because stimuli late in the train evoked disynaptic IPSPs. However, trains of four stimuli revealed that sCT EPSPs facilitate in RS cells as well ($n = 4$, Fig. 3*D*).

Because the dendrites of many layer 6 cells reach into layer 4, it is possible that TC axons contact layer 6 cells predominantly in layer 4 (Keller and White 1989; White and Hersch 1982). To test whether our sTC responses were generated by

TABLE 1. Properties of suspected TC and CT responses in layer 6 RS and FS cells

	Suspected Synapse Type	RS Cells	FS Cells
Latency, ms	TC	2.4 ± 0.7 (22)	1.9 ± 0.3 (11)
	CT	5.2 ± 1.2 (7)	5.6 ± 1.3 (5)
EPSP amplitude, mV** (range in brackets)	TC	1.2 ± 0.8 (24) [0.4–4.2]	3.9 ± 3.5 (13) [0.5–11]
20–80% rise time, ms*	TC	1.2 ± 0.6 (24)	0.4 ± 0.1 (13)
Time to peak, ms*	TC	5.7 ± 1.6 (24)	3.1 ± 0.5 (13)
Paired-pulse ratio (25-ms interstimulus interval)	TC	0.52 ± 0.25 (22)	0.53 ± 0.33 (11)
	CT	2.7 ± 2.0 (7)	2.2 ± 1.0 (5)

All data are means \pm SD. Numbers of cells are in parentheses. * $P < 0.001$, ** $P = 0.02$, Mann-Whitney rank sum test.

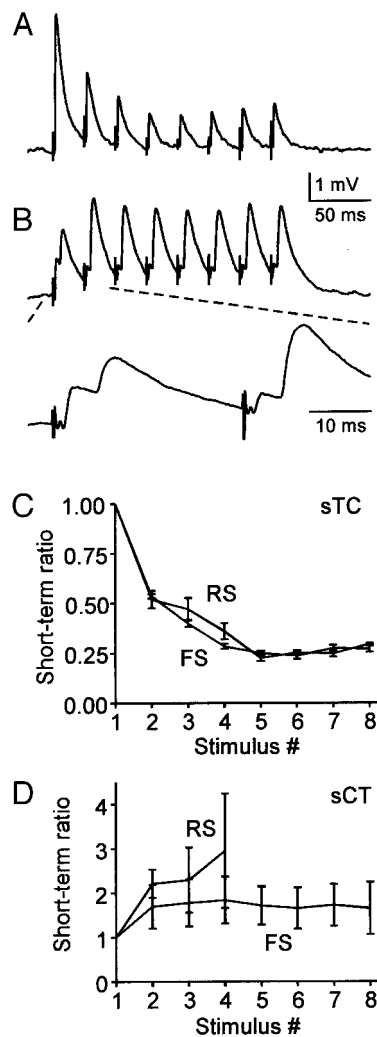


FIG. 3. Short-term dynamics of thalamus-evoked EPSPs. Responses were evoked with short trains of stimuli at 40 Hz. *A*: train of sTC EPSPs in an FS cell evoked by 40-Hz thalamic stimulation. *B*: 2 monosynaptic responses in an FS cell. *Bottom*: blowup of responses to the 1st 2 stimuli. *C*: summary data of sTC response trains, normalized to the 1st response for RS and FS cells. *D*: summary data of sCT responses for RS cells (trains of 4 stimuli) and FS cells (8 stimuli).

synapses within layer 4 or 6, we made approximately 1-mm-long cuts through the slice in upper layer 6, parallel to the pia, and recorded from neurons below the cut. Stimulation in the thalamus still led to depressing sTC EPSPs ($n = 4$) with the same properties as those from uncut slices, suggesting that at least some of the sTC responses originated from synapses within layer 6.

Taken together, these data suggest that thalamus-evoked responses can be reliably categorized into two groups based on their distinct latencies and short-term dynamics.

Minimal TC response is larger in FS than in RS cells

In a previous study of thalamic inputs to layers 4 and 6, we found that single-axon TC responses onto FS cells were twice as large as those onto RS cells (Gibson et al. 1999), suggesting that FS cells play a crucial role in mediating feed forward inhibition. To test whether this circuitry exists specifically in layer 6, we recorded minimal responses from both RS and FS

cells and restricted our sample to sTC responses (Fig. 4*A*). Under these conditions, the amplitudes of EPSPs onto FS cells were three times larger than those onto RS cells on average (Fig. 4, *B* and *C*). Furthermore, EPSP rise times and times to peak were shorter in FS cells than in RS cells (Table 1), suggesting that FS cells will fire action potentials more readily and with higher precision in response to thalamic input (cf. Fricker and Miles 2000).

Isolated CT fibers have relatively long latencies

The latency of a synaptic response depends on axonal conduction velocity and length as well as the kinetics of synaptic transmission. We obtained estimates of CT axonal conduction time alone by blocking excitatory synaptic transmission with DNQX and AP5, stimulating the thalamus, and activating layer 6 CT neurons antidromically (Fig. 5*A*). To sample a large number of neurons, we recorded extracellularly in layer 6 from neurons that generated antidromic all-or-none spikes (i.e., single units). Similar to our findings when excitatory transmission

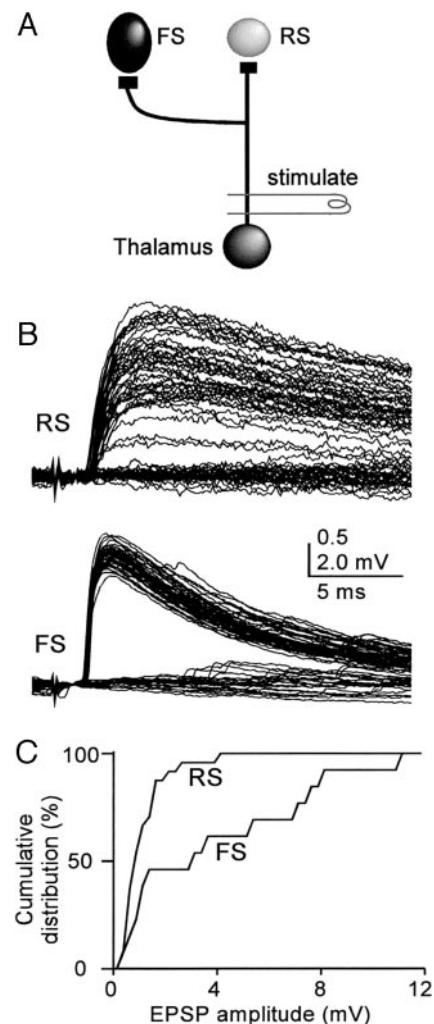


FIG. 4. sTC EPSPs are stronger in FS than in RS cells. *A*: schematic diagram of experimental setup. Thalamus was activated extracellularly, and recordings from FS or RS cells were made in cortex. *B*: threshold sTC responses in RS (top) and FS cell (bottom) evoked by minimal thalamic stimulation. *C*: cumulative distribution of sTC response amplitudes in RS ($n = 24$) and FS ($n = 13$) cells.

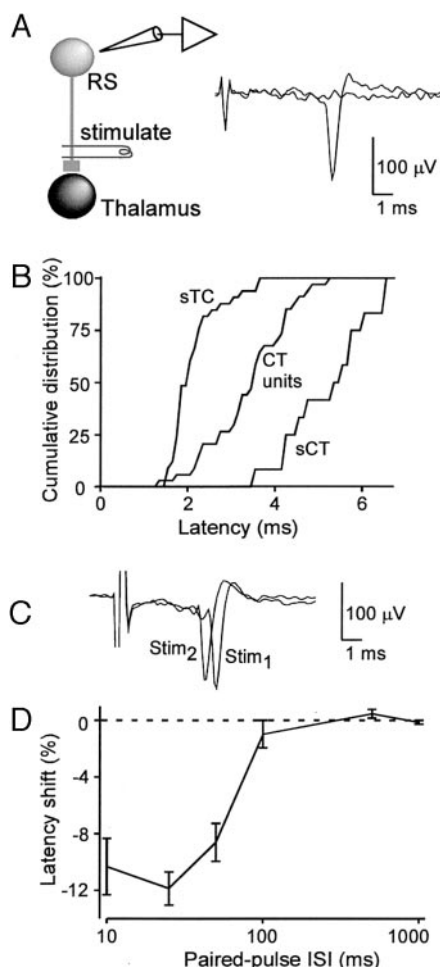


FIG. 5. Antidromically activated corticothalamic cells have longer latencies than sTC responses and display supernormality. *A*: extracellular single-unit recordings were made in layer 6 in the presence of APV and 6,7-dinitroquinoxaline-2,3-dione (DNQX) while stimulating axons of CT cells in thalamus (*left*). *Right*: recording shows a layer 6 cell antidromically activated by thalamic stimulation (2 successive trials at the same threshold stimulation intensity). *B*: summary data of latencies of CT unit spikes, compared with sTC and sCT EPSPs (all statistically different, $P < 0.05$, ANOVA on ranks, pairwise multiple comparisons). *C*: CT unit with decreased latency evoked by 2nd stimulus at 25 ms ISI. *D*: summary data of conduction shifts ($(\text{latency}_2 - \text{latency}_1)/\text{latency}_1$) for CT units ($n = 34$) for paired stimuli with ISI ranging from 10 to 1000 ms.

was intact, the threshold stimulus intensities necessary to evoke antidromic CT responses were relatively high ($98 \pm 45 \mu\text{A}$, $n = 34$).

Antidromic spikes in CT cells had an average latency of 3.4 ± 0.9 ms, significantly longer than the EPSP latencies measured for the sTC responses (2.1 ± 0.55 ms) but also, on average, significantly shorter than the EPSP latencies of the sCT responses (5.2 ± 1.0 ms; Fig. 5*B*). Differences between the synaptic latencies of sCT EPSPs and spike latencies of CT cells are expected because spike propagation through the intracortical axon collaterals and synaptic transmission lead to additional delays.

To test the possibility that layer 5 CT neurons (via their axonal projections to layer 6) contributed to some of the synaptic responses recorded in this study, we tested whether these cells could be activated antidromically. In studies *in vivo*, layer 5 neurons had the fastest conduction velocities of corti-

cofugal axons (Swadlow 1990). However, we failed to detect antidromically activated CT cells in layer 5 even when layer 6 cells in the same cortical column could be reliably activated (0/8 slices).

In summary, these data suggest that the relatively long-latency synaptic responses in layer 6 originated from antidromically evoked CT neurons with somata in layer 6.

CT fibers display supernormality

To further distinguish sTC from sCT responses, we measured the recovery properties of axonal conduction. When an axon is activated, the relative refractory period is sometimes followed by a period during which a second action potential shows a small increase in conduction velocity and a decrease in threshold; this is the "supernormal" period (Swadlow and Waxman 1975; Swadlow et al. 1980). The magnitude and time course of supernormality varies widely in different axonal pathways. Supernormality is prominent in CT fibers (Kelly et al. 2001; Swadlow 1990) but appears to be absent in TC axons *in vivo* (H. A. Swadlow, personal communication). Although the cellular mechanisms of supernormality are not well understood, it offers a second independent test (along with conduction velocity itself), to distinguish the two types of thalamus-evoked synaptic responses.

Extracellular single-unit recordings in layer 6 were obtained by antidromic activation in the thalamus, as described in the preceding text. Indeed, CT axons displayed clear supernormal conduction during paired-pulse stimulation (Fig. 5*C*). At an interstimulus interval (ISI) of 25 ms, the average reduction in spike latency was about 12%. Paired stimuli at various intervals revealed that supernormality lasted about 100 ms and peaked at short ISIs (10–20 ms; Fig. 5*D*).

We carefully examined latencies of thalamus-evoked EPSPs from whole cell recordings in response to paired-pulse stimuli (Fig. 6*A*). Indeed, 11 of 12 sCT responses displayed supernormality, the magnitude of which depended on the ISI (average decrease in latency at 25 ms ISI was 8%). In contrast, most of the sTC responses displayed subnormal conduction at comparable intervals (Fig. 6*B*; average increase in latency at 25 ms ISI was 5%, $n = 33$).

We carried out a cluster analysis using the paired-pulse ratio, response latency, and percent change in latency as variables. Using the same data pool described in the preceding text we found two clusters of responses (Fig. 7) that were identical to those defined previously (Fig. 2*C*). Thus it appears that sTC and sCT responses can be reliably distinguished based on their latency, short-term synaptic dynamics, as well as the presence of axonal supernormality.

Intracortical excitatory synapses can display short-term depression

Our data suggest that the intracortical synapses of CT cells displayed short-term facilitation, while TC EPSPs were strongly depressing. A third excitatory connection originates locally from layer 6 cells that do not project to thalamus but target other cortical areas. In somatosensory cortex, these corticocortical neurons make up roughly 50% of the excitatory cells in layer 6 (Zhang and Deschenes 1997). To probe the properties and dynamics of intracortical synapses more di-

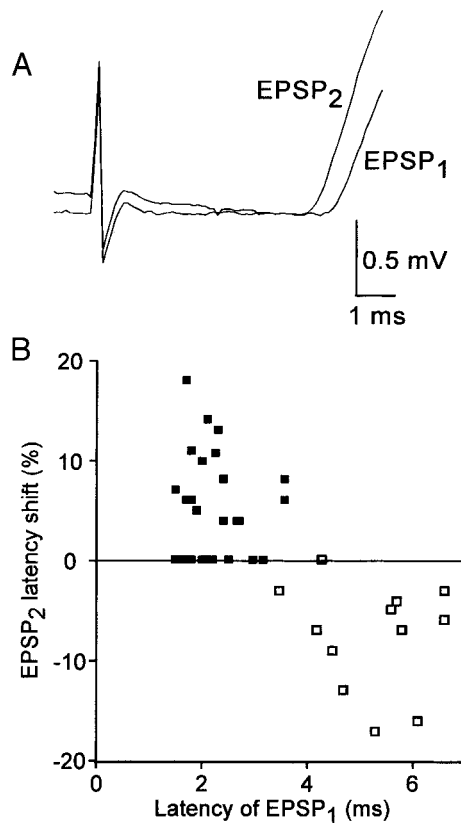


FIG. 6. A: close-up of facilitating sCT EPSP to show supernormal latency shift. Responses evoked by paired pulses at 25 ms ISI are superimposed. Note decrease in latency for EPSP₂. B: supernormality in sCT responses: graph plots supernormality (decrease in latency, negative values) or subnormality (increase in latency, positive values) against first-response latencies for 45 thalamus-evoked EPSPs. Same responses as in Fig. 2C. ■, sTCs; □, sCTs.

rectly, we recorded from pairs of neighboring layer 6 cells (somatic spacing $<50 \mu\text{m}$). Connectivity was relatively sparse. Among 102 RS-RS cell pairs, only four synaptic connections were found (a probability of about 2% because each pair has two possible connections). Three pairs showed short-term depression (Fig. 8A), and one showed short-term facilitation. All depressing connections showed a modest rate of EPSP failures ($P = 0.18$, $n = 3$). Excitatory connections onto FS cells were

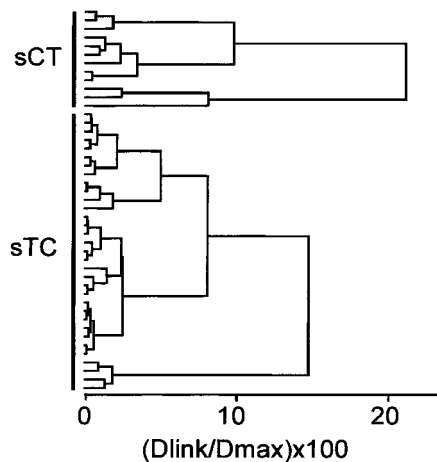


FIG. 7. Cluster analysis of thalamus-evoked responses reveals 2 distinct groups. Variables were latency of EPSP, supernormality shift, and paired-pulse ratio at 25 ms ISI. Dendrogram for same data set shown in Figs. 2C and 6B.

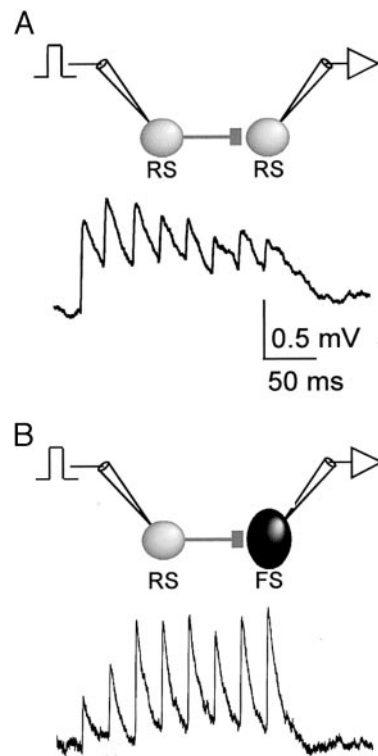


FIG. 8. Two types of intracortical EPSPs in layer 6 neurons. A: short-term depression in an RS-RS cell pair. Dual recordings were made from 2 RS cells with $<50 \mu\text{m}$ separating somata. Presynaptic cell was triggered to fire trains of 8 action potentials at 40 Hz. B: short-term facilitation in an RS-FS cell pair. Presynaptic cell was triggered to fire trains of 8 action potentials at 40 Hz.

found more frequently. Among 41 RS-FS pairs, four excitatory connections were found (10% probability because excitation goes only from RS to FS). Two such pairs displayed short-term facilitation (Fig. 8B) and two pairs displayed short-term depression.

While preliminary, these data suggest that there are at least two types of local excitatory connections within layer 6, whose dynamic properties are determined by the presynaptic cell type. Synapses displaying short-term facilitation likely originate from CT cells, and they can be probed by thalamic stimulation as described above. In contrast, depressing synapses likely originate from corticocortical cells, as they can only be found using intracortical stimulation. Thus the short-term dynamics of excitatory synapses in layer 6 are apparently determined by the presynaptic cell type.

DISCUSSION

In this study, we used extracellular thalamic stimulation to evoke two distinct types of monosynaptic responses in layer 6 neurons. Each synaptic response was distinguished by its latency, synaptic dynamics, and axonal conduction properties. TC inputs generated short-latency EPSPs with strong short-term depression and subnormal conduction. In contrast, inputs originating from intracortical CT collaterals generated long-latency facilitating EPSPs carried by axons with supernormal conduction. These physiological differences between TC and CT systems may be specializations related to their particular functions within the thalamocortical system.

Identification of TC and CT pathways

Extracellular stimulation techniques can ultimately offer only indirect evidence about the identity of the synaptic pathways stimulated. Thus most studies of thalamocortical slices have devoted considerable effort to separating specific, identified synaptic responses from one another (e.g., Agmon and Connors 1991, 1992; Crair and Malenka 1995; Cruikshank et al. 2002; Gil et al. 1996; Isaac et al. 1997; Laaris et al. 2000; Rhoades et al. 1994; Rose and Metherate 2001). Nevertheless, the physiology of the relevant pathways had not been explicitly compared. Using cluster analysis of three variables—latency, axon conduction recovery, and short-term synaptic dynamics—we were able to separate two distinct pathways. This study therefore provides the first objective separation of thalamically evoked responses from other responses in an *in vitro* slice preparation.

The fast, depressing EPSPs probably arose from thalamocortical synapses. *In vivo* studies have shown that the most rapidly conducting axons between cortex and thalamus are typically thalamocortical fibers (Swadlow 1995). It is also very likely that the long-latency facilitating EPSPs evoked by thalamic stimulation originated from layer 6 CT neurons. While extracellular recordings indeed revealed antidromically activated layer 6 cells, we never observed antidromic spikes during whole cell recordings. It is possible that extracellular recordings allowed the sampling of a larger number of neurons. Rose and Metherate (2001), recording with whole cell electrodes in an auditory thalamocortical slice, found that fewer than 3% of infragranular neurons could be activated antidromically by strong stimulation of the medial geniculate nucleus. Dual whole-cell recording from neurons in thalamus and neocortex (Golshani et al. 2001), will ultimately provide the most direct test of the properties of the TC and CT pathways.

Are there alternative explanations for these results? Some layer 5 neurons send an axonal branch to the thalamus and also have collaterals terminating in layer 6. Thus thalamic stimulation could lead to monosynaptic activation of layer 5 to layer 6 synapses. However, in our slice preparation we were unable to activate layer 5 cells antidromically. It is possible that the CT projection from layer 5 is mostly severed in the thalamocortical slice.

Our results provide the strongest evidence yet that thalamocortical slice preparations can be used reliably to study the physiological properties of monosynaptic thalamocortical synapses (Agmon and Connors 1991; Cruikshank et al. 2002). There are caveats, however. Strong extracellular stimulation of the thalamus, or the pathways between thalamus and cortex, can antidromically activate corticothalamic axons, and their intracortical collaterals and synapses. However, by using low stimulation strength and careful assessment of axonal and synaptic response properties, the afferent or efferent origins of most thalamically evoked cortical responses can be identified.

Axonal conduction properties of TC and CT pathways

A variety of axonal systems display supernormality, defined as an increase in conduction velocity and a decrease in threshold after the relative refractory period (Swadlow and Waxman 1975). While the cellular mechanisms of supernormal conduction remain poorly understood, it has been used to reliably

distinguish different pathways *in vivo* by antidromic activation of neurons from their respective projection targets. However, no *in vitro* study has made use of this phenomenon to distinguish pathways within the thalamocortical system. Our finding that corticothalamic units as well as long-latency synaptic responses display supernormal axonal conduction agrees with studies in rabbits (H. A. Swadlow, personal communication) and rats (Kelly et al. 2001). In contrast, all thalamocortical responses show either no change in axonal velocity or a slight decrease.

It is likely that these properties contribute to the functional differences between the two systems. Only minimal jitter in thalamocortical propagation is introduced by the variability in axonal conduction. In contrast, spike propagation in corticothalamic axons might be more variable, suggesting that precise timing of activity is not a requirement in this system.

Synaptic properties of TC and CT pathways

Recent studies have suggested that short-term synapse dynamics are important for the functions of circuits in the cerebral cortex (Markram et al. 1998; Thomson 2000; Zucker and Regehr 2002). Synapse dynamics can vary widely, and the pathways between and within neocortex and thalamus provide a dramatic example of this diversity. TC EPSPs in layer 6 showed strong short-term depression together with relatively small variations in amplitude during minimal stimulation. Both features suggest a high release probability from TC synaptic terminals. When tested by dual recordings, intra-layer 6 synapses also showed depression but to a lesser degree than TC synapses. While the small number of pairs recorded does not allow definite conclusions, our results agree with those seen previously in layer 4, where TC synapses are more effective than intracortical excitatory synapses because the former have more release sites per axon and a higher probability of transmitter release (Gil et al. 1999).

Similar to synapses in layer 4 (Gibson et al. 1999; Porter et al. 2001), TC EPSPs onto FS cells in layer 6 were distinctly larger than those onto RS cells. Both anatomical and physiological specializations may contribute to this difference. FS cells might be contacted by TC synapses with more release sites as well as a higher probability of release and quantal size. Our data, obtained using minimal stimulation, do not help to distinguish between these possibilities. Regardless, our results do support the idea that feed-forward inhibition of afferent input is a general principle in a variety of brain regions (Shepherd 1988).

Intracortical synapses of CT cells in layer 6 targeted both FS and RS cells and showed short-term facilitation. CT cells also form facilitating synapses on cells of the thalamic relay nuclei (Turner and Salt 1998; von Krosigk et al. 1999). Furthermore, CT cells project into layer 4, where they form facilitating synapses onto both FS and LTS cells but not RS cells (J. R. Gibson and B. W. Connors, unpublished data). This suggests that the presynaptic CT neuron determines the short-term properties of all its synaptic terminals.

Synaptic properties play an important role in controlling which aspects of the presynaptic activity pattern are extracted by the postsynaptic cell, although few studies have addressed this issue directly (Chance et al. 1998). Thus layer 6 neurons might preferentially respond to transient changes in thalamo-

cortical activity (cf. Abbott et al. 1997). In contrast, local inputs mediated by facilitating synapses formed by CT cells might provide information about the ongoing tonic activity pattern, which is relayed back to thalamus.

Local and global connectivity in the thalamocortical system

While layer 6 and layer 4 show similarities in the properties of thalamocortical synapses, sensory activity is likely to be processed differently by these two layers. Several studies have shown that excitatory neurons within individual layer 4 barrels in somatosensory cortex are highly interconnected (Feldmeyer et al. 1999; Petersen and Sakmann 2000), thus forming the substrate for amplification and redistribution of sensory inputs to other layers. In contrast, layer 6 appears to be much more sparsely connected as suggested by the low percentage of synaptically connected cell pairs observed in our sample.

One reason for this sparse connectivity might be the unique excitatory loop that exists between cortical layer 6 and the ventrobasal nuclei of the thalamus (Deschenes et al. 1998). Due to the precise topographic architecture of the rodent somatosensory system, layer 6 CT cells project to the same thalamic barreloid from which they receive thalamocortical inputs. In fact, this synaptic reciprocity might exist for individual pairs of neurons in thalamus and layer 6, although that has never been shown directly. Restricted interconnected networks of layer 6 and thalamic neurons might form an important substructure of cortical processing (Jones 2001; Steriade 2001).

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