THE ROYAL SOCIETY

Published online 12 November 2002

Thalamocortical control of feed-forward inhibition in awake somatosensory 'barrel' cortex

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Intracortical inhibition plays a role in shaping sensory cortical receptive fields and is mediated by both feed-forward and feedback mechanisms. Feed-forward inhibition is the faster of the two processes, being generated by inhibitory interneurons driven by monosynaptic thalamocortical (TC) input. In principle, feed-forward inhibition can prevent targeted cortical neurons from ever reaching threshold when TC input is weak. To do so, however, inhibitory interneurons must respond to TC input at low thresholds and generate spikes very quickly. A powerful feed-forward inhibition would sharpen the tuning characteristics of targeted cortical neurons, and interneurons with sensitive and broadly tuned receptive fields could mediate this process. Suspected inhibitory interneurons (SINs) with precisely these properties are found in layer 4 of the somatosensory (S1) 'barrel' cortex of rodents and rabbits. These interneurons lack the directional selectivity seen in most cortical spiny neurons and in ventrobasal TC afferents, but are much more sensitive than cortical spiny neurons to low-amplitude whisker displacements. This paper is concerned with the activation of S1 SINs by TC impulses, and with the consequences of this activation. Multiple TC neurons and multiple S1 SINs were simultaneously studied in awake rabbits, and cross-correlation methods were used to examine functional connectivity. The results demonstrate a potent, temporally precise, dynamic and highly convergent/divergent functional input from ventrobasal TC neurons to SINs of the topographically aligned S1 barrel. Whereas the extensive pooling of *convergent* TC inputs onto SINs generates sensitive and broadly tuned inhibitory receptive fields, the potent TC *divergence* onto many SINs generates sharply synchronous activity among these elements. This TC feed-forward inhibitory network is well suited to provide a fast, potent, sensitive and broadly tuned inhibition of targeted spiny neurons that will suppress spike generation following all but the most optimal feed-forward excitatory inputs.

Keywords: fast-spike interneurons; intracortical inhibition; layer 4; receptive field; construction; synchrony

1. INTRODUCTION

Intracortical inhibition is generally acknowledged to play a part in the shaping of sensory cortical receptive fields (Dykes *et al.* 1984; Sillito 1984; Nelson *et al.* 1994). There is, however, little consensus concerning the specific receptive field properties that are under inhibitory control, or the relative contributions of feed-forward versus feedback inhibitory mechanisms. Feed-forward inhibition is the faster of the two processes, being mediated by monosynaptic TC input. In principle, feed-forward inhibition can prevent targeted cortical neurons from reaching threshold to TC input that is weak or sub-optimal. To do so, however, feed-forward inhibitory interneurons must respond to TC input at very low thresholds and generate spikes (and consequent GABAergic inhibition) very quickly. The discovery of TC synapses directly onto the somata of GABAergic interneurons of layer 4 (e.g. Freund *et al.* 1985; White 1986, 1989) is consistent with the generation of such a potent response. Moreover, *in vitro*, fast-spike interneurons of layer 4 generate EPSPs to TC input that are faster rising and of greater amplitude than those seen in 'regular spiking' neurons of this layer (Gibson *et al.* 1999).

A powerful feed-forward inhibitory mechanism could serve to constrain the size of suprathreshold receptive fields and to sharpen the tuning characteristics of targeted cortical neurons. In this vein, Troyer *et al.* (1998) proposed that a sensitive and broadly tuned feed-forward inhibition could account for the contrast-invariant orientation tuning seen in feline visual cortex. Although the existence of such interneurons in cat visual cortex is uncertain (Azouz *et al.* 1997; Hirsch *et al.* 2000), putative inhibitory interneurons with precisely these properties have been described in the somatosensory cortex of rat and rabbit. In the S1 barrel cortex of both of these species, most presumptive spiny neurons are well tuned to the direction of vibrissa displacement, but presumptive inhibitory interneurons are not (Simons 1978; Swadlow 1989). Moreover, in rabbit S1, putative inhibitory interneurons (SINs) are exquisitely sensitive to peripheral stimulation: responding at much lower thresholds and higher stimulus frequencies than any other population studied in S1 (Swadlow 1989). Furthermore considerable physiological evidence indicates that SINs in and near layer 4 receive a powerful TC input: (i) they respond to electrical stimu-

One contribution of 22 to a Discussion Meeting Issue 'The essential role of the thalamus in cortical functioning'.

lation of VB thalamus at very short synaptic latencies (less than 2 ms); (ii) they respond to peripheral sensory stimulation at very short synaptic latencies (6–7 ms); and (iii) cross-correlation analysis shows a sharp peak in SIN spike probability at appropriate intervals following spikes in topographically aligned VB TC neurons (Swadlow 1995; Swadlow & Gusev 2002). Thus, the potent monosynaptic TC drive onto layer-4 SINs, as well as their high sensitivity, and very broad directional tuning to vibrissae deflections suggests that these elements are well-situated to mediate a sensitive and broadly tuned feed-forward inhibition onto targeted cortical excitatory neurons.

The above considerations leave open the question of how the sensitive and broadly tuned receptive fields of inhibitory interneurons are synthesized. The lack of directional selectivity seen in barrel cortex SINs is especially noteworthy because most VB TC afferents to this region show a high degree of directional specificity (Simons & Carvell 1989; Swadlow & Gusev 2002). This paper reviews recent studies that examine the functional connectivity between VB TC neurons and S1 SINs and relate this connectivity to the synthesis of sensitive and broadly tuned inhibitory receptive fields, to the sharp synchrony seen among SINs, and to the consequent wave of feed-forward intracortical inhibition that is generated by TC input. Most of the work reviewed was performed in fully awake rabbits and involved simultaneous recordings from multiple TC neurons, and from multiple cortical interneurons of the topographically aligned cortical barrel. Functional connectivity was examined using cross-correlation methods. These studies show that SINs within layer 4 of an S1 barrel receive a potent, temporally precise, dynamic and highly convergent/divergent functional input from TC neurons of the topographically aligned VB thalamic 'barreloid' (Van Der Loos 1976). Moreover, they support an earlier proposal (Swadlow 1995) that the highly sensitive and broadly tuned inhibitory receptive fields of these neurons are generated by an extensive pooling of convergent functional inputs from topographically aligned TC neurons with very diverse response properties.

2. METHODOLOGICAL CONSIDERATIONS

S1 cortical barrels receive a topographically precise input from TC neurons of VB thalamic 'barreloids'. In the experiments described here, extracellular recordings were obtained from S1 cortical barrel columns and from VB thalamic barreloids of awake, adult, Dutch-belted rabbits (for details, see Swadlow 1989, 1995; Swadlow & Gusev 2001, 2002). In an early cross-correlation study of this system (Swadlow 1995), only a single recording electrode was placed within a VB barreloid and within the topographically aligned S1 barrel. Generally, only a single TC–SIN pair could be studied at a time, and estimates of divergence/convergence were necessarily inferential. As a means of allowing more direct measures of divergent and convergent functional TC connectivity, procedures were developed that allowed several recording electrodes to be placed within each VB barreloid and S1 barrel (Swadlow & Gusev 2001, 2002). Figure 1 illustrates these methods schematically. Microelectrodes were constructed of fine-diameter (40 μm o.d.) quartz-platinum filaments (Reitboeck 1983) pulled, under high temperature, to a taper and sharpened to a fine tip. A concentric array of seven such electrodes (inter-electrode spacing of 160 μm) was chronically implanted above the VB thalamus, with each electrode independently controlled by one of seven miniature microdrives that were permanently fixed to the skull. Cortical recordings were obtained from topographically aligned S1 barrels, following mapping procedures. Cortical recordings were obtained acutely, using 5–7 of the same type of electrodes described above (electrode spacing less than 160 μm), but positioned using a seven-channel microdrive system (Eckhorn & Thomas 1993). TC neurons were identified by spike-triggered averages of field potentials elicited in the aligned S1 barrel by TC impulses (Swadlow & Gusev 2000).

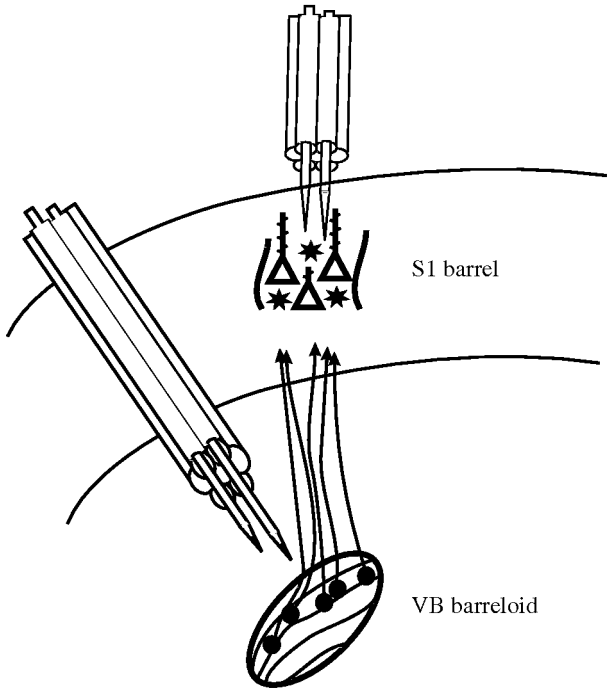

Figure 1. A schematic illustration of the recording method. A concentric array of seven microelectrodes within stainless steel guide tubes (o.d.: 160 μm), were chronically implanted above the VB thalamus. The tips of the guide tubes were lowered to within 3 mm of the thalamus. Each electrode was independently driven by a miniature microdrive that was fixed to the skull. The close spacing of these electrodes (160 μm) often allowed two or more electrodes to record from a single barreloid. A second concentric array of electrodes (up to seven electrodes) is placed acutely within an S1 barrel that is in topographical alignment with one of the thalamic barreloids under study. These electrodes are also independently positioned within guide tubes (o.d.: 160 μm), the tips of which lie above the dura. For clarity, only two microelectrodes are shown in each guide-tube array.

Cortical SINs in rabbit S1 were identified by a high frequency (greater than 600 Hz) burst of three or more spikes elicited by electrical stimulation of the VB thalamus and by their short-duration action potential (Swadlow 1989). A few SINs (five to date; see Swadlow *et al.* (1998, fig. 13)) have been recorded intracellularly following the above identification procedure. All of these neurons responded to a depolarizing current pulse with a high frequency, non-adapting train of action potentials that is characteristic of 'fast-spike' GABAergic interneurons

(Amitai & Connors 1995; McCormick *et al.* 1985). All S1 SINs included in our estimates of TC divergence/convergence (below) responded to brief and intense stimulation of the principle vibrissa (provided by a solenoid controlled air-jet, rise time less than 1 ms) at a latency of less than 7.5 ms. These values are indicative of monosynaptic thalamic input (Swadlow 1995). The great majority (greater than 80%) of SINs in and near layer 4 respond to the air-puff at such latencies.

Topographical alignment of thalamic and cortical recording sites was achieved using receptive field mapping procedures and was confirmed by generating spike-triggered averages of the cortical field potentials elicited by impulses of the thalamic neurons under study (Swadlow & Gusev 2000; also see figure 6). Receptive fields of VB neurons and S1 SINs were tested by deflecting single vibrissae, using hand-held probes and/or a servo-controlled galvanometer (Swadlow 1989). In earlier experiments (Swadlow 1995), spike trains used for the calculation of cross-correlograms were collected while applying periodic stimuli (one per second) to the principal vibrissa and using appropriate measures to eliminate stimulus-induced correlations. However, in more recent experiments (Swadlow & Gusev 2001, 2002) spike trains were collected under conditions of 'spontaneous' impulse activity (after receptive field testing).

The functional connectivity between TC and cortical neurons was assessed by searching for a peak in the correlogram at intervals of 1.1–2.5 ms following the thalamic spike. This peak was defined by the presence of two of three successive bins (located within this 1.5 ms window) that exceeded a probability value of 0.01. The number of SIN spikes expected by chance within each bin was based on the mean number of spikes/bin that occurred between −4 ms and +1 ms of the thalamic spike time. Using this method, the overall probability of finding such a peak by chance was less than 0.005. When a significant peak was detected, we determined values for 'efficacy' and 'contribution' (Levick *et al.* 1972) based on a brief window (± 0.6 ms) on each side of the peak. Efficacy and contribution values were calculated by counting the number of action potentials that occurred in the SIN during this temporal window, subtracting the baseline number of spikes expected by chance during this period (above) and dividing this value by the number of triggering TC spikes (efficacy) or total number of SIN spikes (contribution).

3. TC ACTIVATION OF S1 SUSPECTED INHIBITORY INTERNEURONS IS TEMPORALLY AND TOPOGRAPHICALLY PRECISE

The activation of S1 SINs by TC impulses occurs within a very narrow temporal window. Two recent studies of TC–SIN functional connectivity (Swadlow & Gusev 2001, 2002), were based on more than 60 functionally connected TC–SIN pairs. Of these, 10 pairs generated peaks in the cross-correlograms at appropriate intervals following the TC action potentials that were more than 20 standard deviations above the baseline activity. These very well isolated peaks, although biased towards the more potent TC–SIN connections, are especially instructive about the time-course of the impact of TC impulses on S1 SINs. Figure 2 presents three of these cases. The cor-

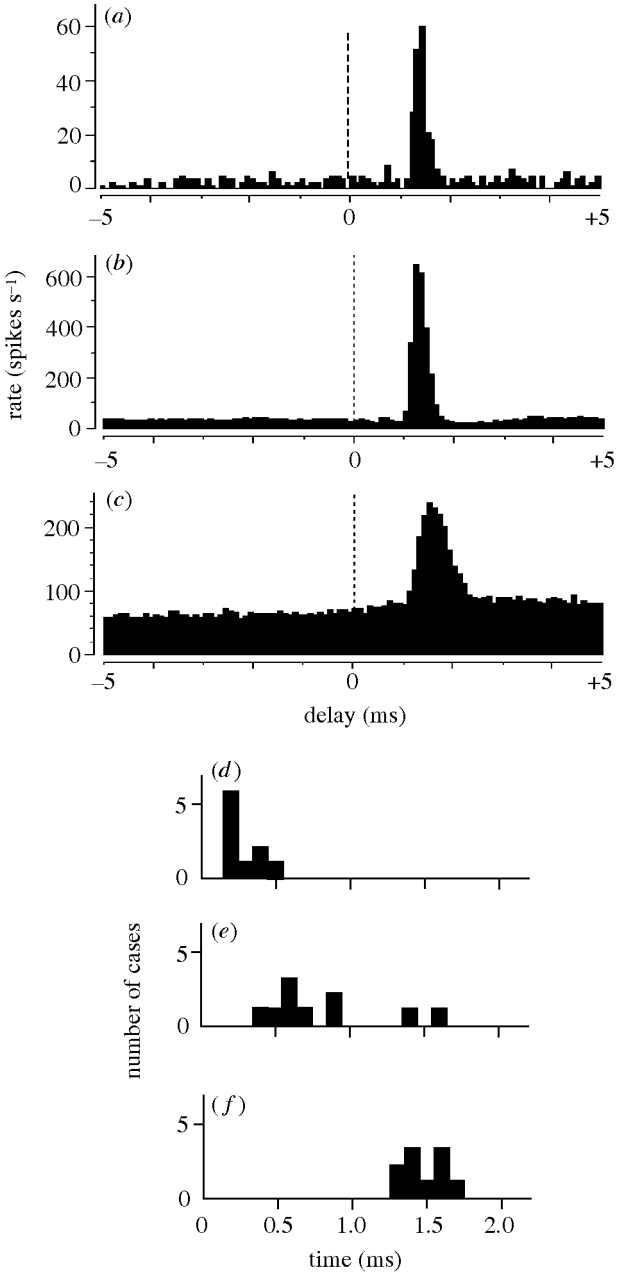

Figure 2. TC impulses generate action potentials in SINs with high temporal precision. (*a*,*b*) Cross-correlograms from two TC–SIN pairs, from two different rabbits, showing very brief and well isolated peaks in spike-rate at intervals indicative of monosynaptic TC activation. Note that the rise time of this response was 0.2–0.3 ms and that the entire duration of the response was less than 0.7 ms. (*c*) The TC neuron that generated the correlograms shown in (*b*) also made contact with a second SIN, simultaneously recorded within the same barrel (This TC neuron and its cortical targets are further described below and in figures 3 and 5.) The resulting increase in spike rate was of somewhat longer duration than that seen in (*a*) or (*b*). (*d*,*e*) The rise time (from 10% of maximal amplitude to the peak) and the total duration (from 10% of the maximal amplitude to the peak and down to 10% of the amplitude) of the cross-correlogram peaks generated by 10 well-connected TC–SIN pairs. (*f*) For these same pairs, the latency of the peak in the cross-correlogram following the TC spike.

relograms presented in figure 2*a*,*b* were generated by TC–SIN pairs studied in two different rabbits. A remarkable feature of these correlograms is the temporal precision of the elicited response. In both of these cases, a dramatic increase in spike rate begins 1.1 ms after the onset of the VB spike and this reaches a peak only 0.2–0.3 ms later. For these cases, the entire response generated by the TC neuron lasts only 0.5–0.7 ms. It is especially interesting to compare the correlogram shown in figure 2*b* with that shown in figure 2*c*. These correlograms were generated simultaneously by a single TC neuron that contacted two S1 SINs recorded within the same barrel (via closely spaced microelectrodes). For the SIN presented in figure 2*c*, the increase in spike frequency begins at the same time after the TC spike as seen in figure 2*b* (1.1 ms). However, the rise time is considerably slower, and the entire peak has a duration of *ca*. 1.3 ms. In cross-correlational studies of many systems a peak duration of 1.3 ms would be considered very brief (e.g. Alonso *et al.* 2001; Miller *et al.* 2001). However, when contrasted with the peak generated by the same TC neuron in another SIN (figure 2*b*), this peak seems quite prolonged. Figure 2*d*,*e* shows, for the 10 TC–SIN pairs with very well-isolated peaks (greater than 20 s.d. above baseline), the rise time and total duration of the peak, as well as the time of the peak following the TC action potential. It is clear that most of these peaks are very brief.

EPSPs generated by TC synapses onto S1 fast-spike interneurons are more potent, of shorter duration and have faster rise times than those seen in 'regular spike' neurons (Gibson *et al.* 1999; B. W. Connors, personal communication). Since the rise time of the EPSP (or its derivative) may be closely related to spike generation (Kirkwood & Sears 1982; Cope *et al.* 1987), it could be expected that TC–SIN cross-correlograms display very brief peaks. Moreover, GABAergic interneurons in layer 4 of S1 receive TC synapses directly on their somata (White 1986, 1989), and it is reasonable to speculate that the briefest peaks among these cross-correlograms (such as those seen in figure 2*a*,*b*) may be generated by such synapses. Direct axosomatic synapses could also account for the extremely potent functional connectivity between many TC neurons and S1 SINs. Such powerful functional connectivity is well suited to generate a potent feed-forward inhibition onto the targets of these cortical elements.

In the studies described above (Swadlow & Gusev 2001, 2002) and in a previous analysis of the somatosensory TC system (Swadlow 1995), all of the cases of functional connectivity between TC neurons and SINs were found under conditions of precise topographical alignment. No instance of functional connectivity was seen in these experiments when cortical and thalamic recording sites were misaligned by even a single S1 barrel. This is not surprising, given the dense and restricted arbourization pattern of most VB TC neurons (Jensen & Killackey 1987) to the aligned barrel. A similar requirement for precise topographical alignment is also seen in the visual TC system (Alonso *et al.* 2001). However, in the visual system, the receptor periphery is represented in a continuous manner in both thalamus and primary visual cortex. For this reason, various 'degrees' of alignment are possible (full overlap or various degrees of partial overlap of receptive field centres). By contrast, the representation of the mystacial vibrissae in thalamic barreloids and in layer-4 barrels is discrete, and neurons within these structures are dominated by a single whisker. Except for a small zone of overlap at the borders of S1 barrels, thalamic and cortical recording sites are either in or out of alignment. In practice, this discrete topography allows for a rapid and reliable characterization of the alignment of thalamic and cortical recording sites.

4. TC ACTIVATION OF SUSPECTED INHIBITORY INTERNEURONS IS DYNAMIC: ENHANCED SYNAPTIC EFFICACY FOR 'SPECIAL' TC IMPULSES

TC synapses *in vitro* exhibit paired-pulse depression that lasts for hundreds of milliseconds (Gil *et al.* 1997), and similar effects have been observed *in vivo* (Swadlow & Gusev 2001; Castro-Alamancos & Oldford 2002; Chung *et al.* 2002; Swadlow *et al.* 2002). This fact, together with the high levels of spontaneous activity seen in most TC neurons in the awake state, suggests that TC synapses may be in a chronic state of depression in intact subjects. If this is the case, then the long interspike intervals, such as those that precede TC 'bursts', should allow recovery from this depression and subsequent impulses should have an enhanced efficacy in eliciting postsynaptic action potentials (because of enhanced EPSP amplitude; Ramcharan *et al.* (2000)). This, indeed, is the case, and such enhancement is readily demonstrated in TC–SIN pairs that are strongly connected. One such case is shown in figure 3, where a single TC neuron makes a strong functional contact with two S1 SINs (from Swadlow & Gusev 2001). Here, the efficacy with which the TC neuron elicits spikes in these two cortical neurons was strongly enhanced for the initial impulses in a TC 'burst' (figure 3*c*) and for TC impulses that do not initiate a burst, but were selected for equivalently long preceding interspike intervals (median values: 278 ms in this case, figure 3*d*). At longer preceding interspike intervals (figure 3*e*), efficacy values more than doubled for these neurons, reaching more than 0.5 for the connection with SINg.

5. TC CONVERGENCE ONTO SINGLE SUSPECTED INHIBITORY INTERNEURONS: THE SYNTHESIS OF SENSITIVE AND BROADLY TUNED INHIBITORY RECEPTIVE FIELDS

A highly convergent TC input could account for the very high sensitivity and broad directional tuning observed in S1 SINs. Evidence in support of this notion was provided by the high proportion of topographically aligned TC–SIN pairs that were shown to be functionally connected (Swadlow 1995). This result implies a similarly high degree of divergence and convergence in the functional connectivity of topographically aligned TC neurons and S1 SINs. In the above study, however, only single TC neurons could be generally studied with single SINs. To examine this more directly, simultaneous recordings were obtained from multiple TC neurons of the same VB barreloid, and from multiple SINs of the aligned barrel (Swadlow & Gusev 2002; figure 1). To examine TC convergence, 29 SINs were identified, each studied simultaneously with 2–4 aligned TC neurons. Most of these

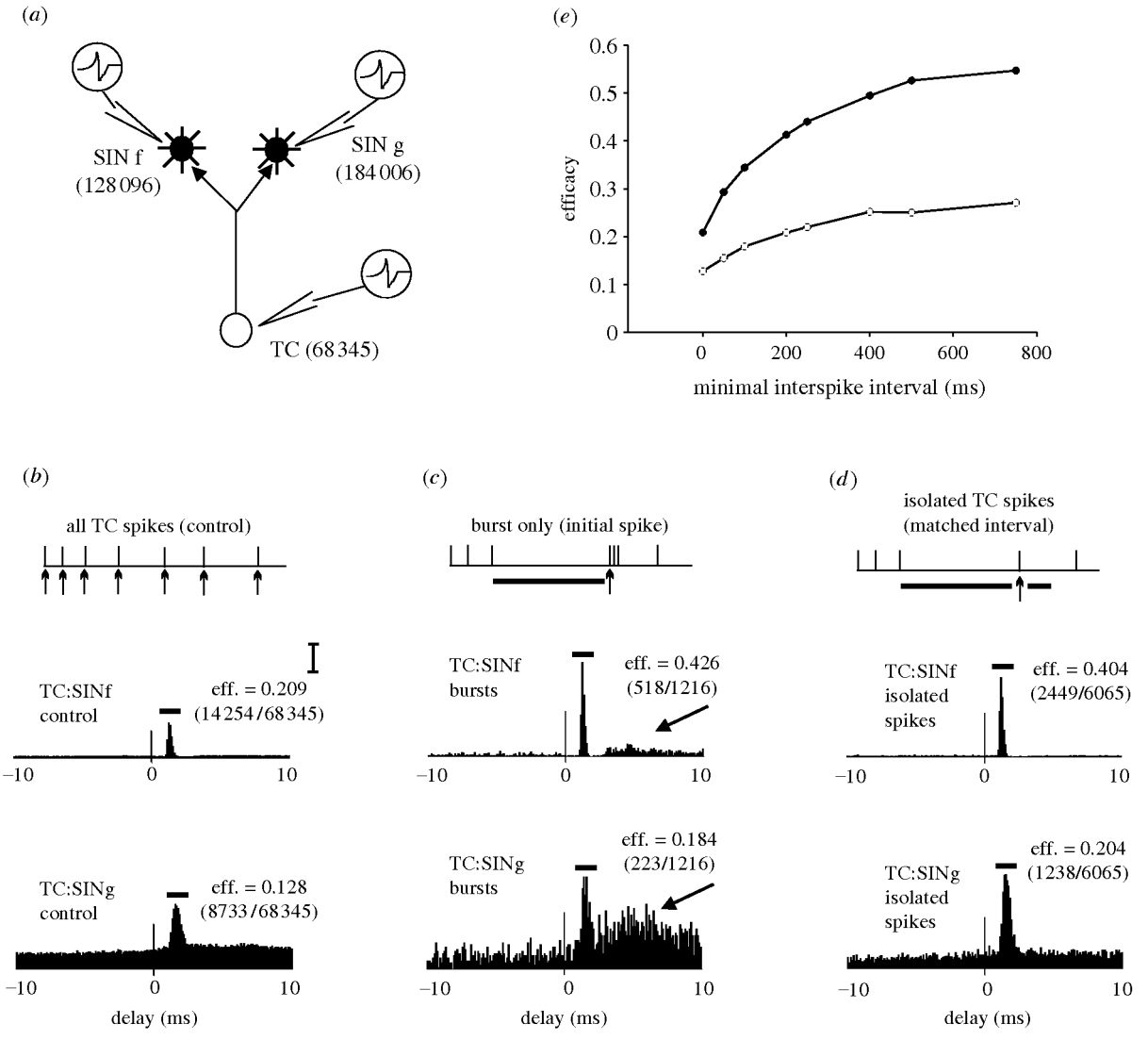

Figure 3. Enhanced synaptic efficacy for isolated TC impulses and TC 'bursts'. (*a*) The experimental situation: an extracellular microelectrode recorded the spontaneous action potentials of a TC neuron, while two electrodes recorded the spikes of two SINs (f, g). Data from this triad of neurons are also presented in figure 2(*b*,*c*) and in figure 5. All correlograms were constructed using a bin width of 0.1 ms and were calculated in the absence of peripheral stimulation. (*b*) Cross-correlograms were initially calculated using the entire spike trains of the TC neuron (vertical arrows, 68 345 spikes), SINf (128 096 spikes) and SINg (184 006 spikes). The 'efficacy' of the connections was computed for a period of 1.2 ms that was centred on the peak in the cross-correlogram (indicated by horizontal lines above the peaks in the cross-correlograms). The computed efficacies of 0.209 and 0.128 indicate extremely potent synaptic contacts with these two SINs. The ratio of the numbers of SIN spikes in the peak of each correlogram (minus the baseline) and the number of TC spikes used in the calculations is given besides each peak. (*c*) Method for selecting only initial spikes in a TC burst and computing the cross-correlograms based on these spikes. Only the first spike in each burst was selected (vertical arrow) and the cross-correlogram with each SIN was computed. This resulted in an enhanced efficacy for these selected TC spikes. (*d*) Method for selecting only isolated TC spikes and the computed cross-correlograms based on these spikes. The duration of the interval preceding such spikes was chosen to match those seen in the burst condition. These isolated TC spikes were highly potent in activating the cortical SINs. (*e*) For these same neurons, the duration of the required silent interval that preceding a TC spike was varied from 0 to 750 ms. Efficacy values were clearly related to the duration of this interval. The vertical bar in (*b*) applies to all of the correlograms and represents 500 and 100 spikes s^{-1} in the TC-SINf (filled circles) and TC-SINg (open circles) correlograms, respectively. From Swadlow & Gusev (2001).

SINs (24/29) received functional input from at least half of the topographically aligned TC neurons that were studied (mean: 65% of the TC neurons tested). Figure 4 illustrates such convergent input, where one SIN was studied with three aligned TC neurons. All three TC neurons showed clear functional connectivity with the SIN, generating significant peaks in SIN spike frequency at intervals of 1.4–1.8 ms after TC spikes. This is an especially interesting case because each of the TC neurons showed strong directional selectivity, but these preferred directions differed over a range of 135°. The SIN responded equally to all directions of vibrissa displacement. We thoroughly tested nine SINs in this manner, each showing a minimal directional preference, and each receiving functional input from two to three TC neurons. For six of these SINs each of the TC input neurons was directionally selective, and the preferred directions varied by 80–135°. Two other SINs each received input from two non-directional TC

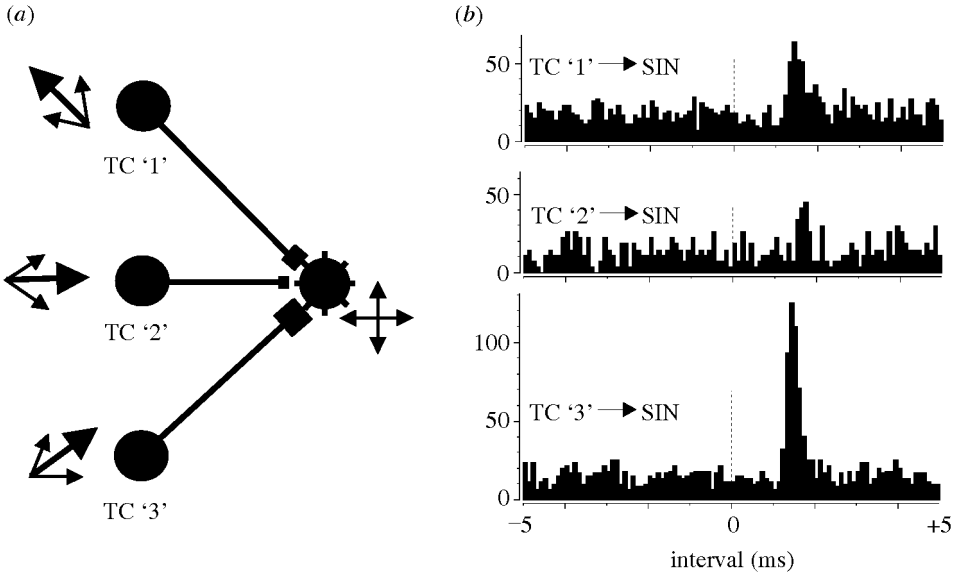

Figure 4. Convergent input from three TC neurons to a cortical SIN. (*a*) Schematic of the functional connectivity. Arrows show that the directional preferences of the TC neurons differed over a range of 135°. The SIN responded well to all directions. (*b*) Cross-correlograms between the TC neurons and the SIN. Functional connectivity was assessed in the absence of peripheral stimulation, during 'spontaneous' activity. 'Y' axis values are given in spikes s^{-1}. From Swadlow & Gusev (2002).

neurons, and in the final case two TC neurons provided input, one being strongly directional and the other lacking any directional preference.

Evidence from cross-correlation studies in visual and auditory systems indicates that TC connectivity of presumptive spiny neurons is highly selective, being controlled by many factors other than precise topographical alignment. In the visual system of the cat, for example, Alonso *et al.* (2001) proposed a set of 'rules' governing the functional connectivity between LGNd neurons and 'simple' cells in layer 4 of the visual cortex. In addition to a requirement for precise topographical alignment, functional connectivity was seen when receptive fields were matched in: (i) time-course; (ii) sign (spatial position of on- or off-region); (iii) sub-region strength; and (iv) receptive field size. A related set of 'rules' applies to connection specificity in the auditory system, where only 29 functionally connected pairs were seen from a total of 741 pairs studied (Miller *et al.* 2001). Thus, although topographical precision is a necessary condition for functional connectivity, it is, by no means, sufficient. These 'connectivity rules' imply a high degree of specificity in the functional connectivity between individual TC neurons and their cortical targets. We do not know how the receptive fields of spiny (excitatory) neurons in rabbit or rodent barrel cortex are synthesized but, as most are well tuned to stimulus direction (Simons & Carvell 1989; Swadlow 1989; Swadlow & Gusev 2002) it is likely that they receive selective input from similarly tuned TC neurons.

In contrast to the specificity of TC connectivity described above for presumptive cortical spiny neurons, our results suggest a very different model for receptive field construction in feed-forward inhibitory interneurons. Here, an unselective, highly divergent and convergent functional network links TC neurons with fast-spike cortical interneurons. No evidence of specificity was seen in the functional connectivity between TC neurons and targeted SINs, other than the requirement for precise topographical alignment. Instead, TC neurons with very different preferred stimulus directions functionally impact the same cortical SIN. This high degree of convergence and lack of specificity in TC connectivity is consistent with the exquisitely low thresholds of SINs to sensory stimulation (because many TC neurons have an opportunity to activate each SIN) and with their very broad directional tuning. In feline visual cortex, neurons with such properties could account for contrast-invariant orientation tuning (Troyer *et al.* 1998) and in rabbit (or rodent) barrel cortex, such neurons may serve a similar function for directional tuning and/or may serve to constrain the spatial extent of the receptive field. The present results support the notion that SINs obtain their receptive field properties by extensive pooling of inputs from TC neurons that display a broad range of very different response properties (Swadlow 1995).

6. TC DIVERGENCE: SINGLE TC NEURONS DIVERGE TO ACTIVATE MULTIPLE S1 SUSPECTED INHIBITORY INTERNEURONS

To analyse the functional divergence of TC neurons onto SINs, 28 TC neurons were identified that were each studied simultaneously with 2–9 topographically aligned SINs (mean: 2.89 SINs; Swadlow & Gusev (2002)). Nearly all of the TC neurons (26/28) showed functional connectivity with at least half of the SINs studied in the topographically aligned S1 barrel (mean: 64.7% of the SINs tested). A remarkable instance of TC divergence is shown in figure 5. Here, a single TC neuron (dubbed 'Hercules', because of the potent TC connectivity) was studied with nine different SINs over a 4 day period. Hercules had two 'signature' features that ensured that the same neuron was under study on the successive recording days: (i) the spike train was unusually 'bursty', yielding

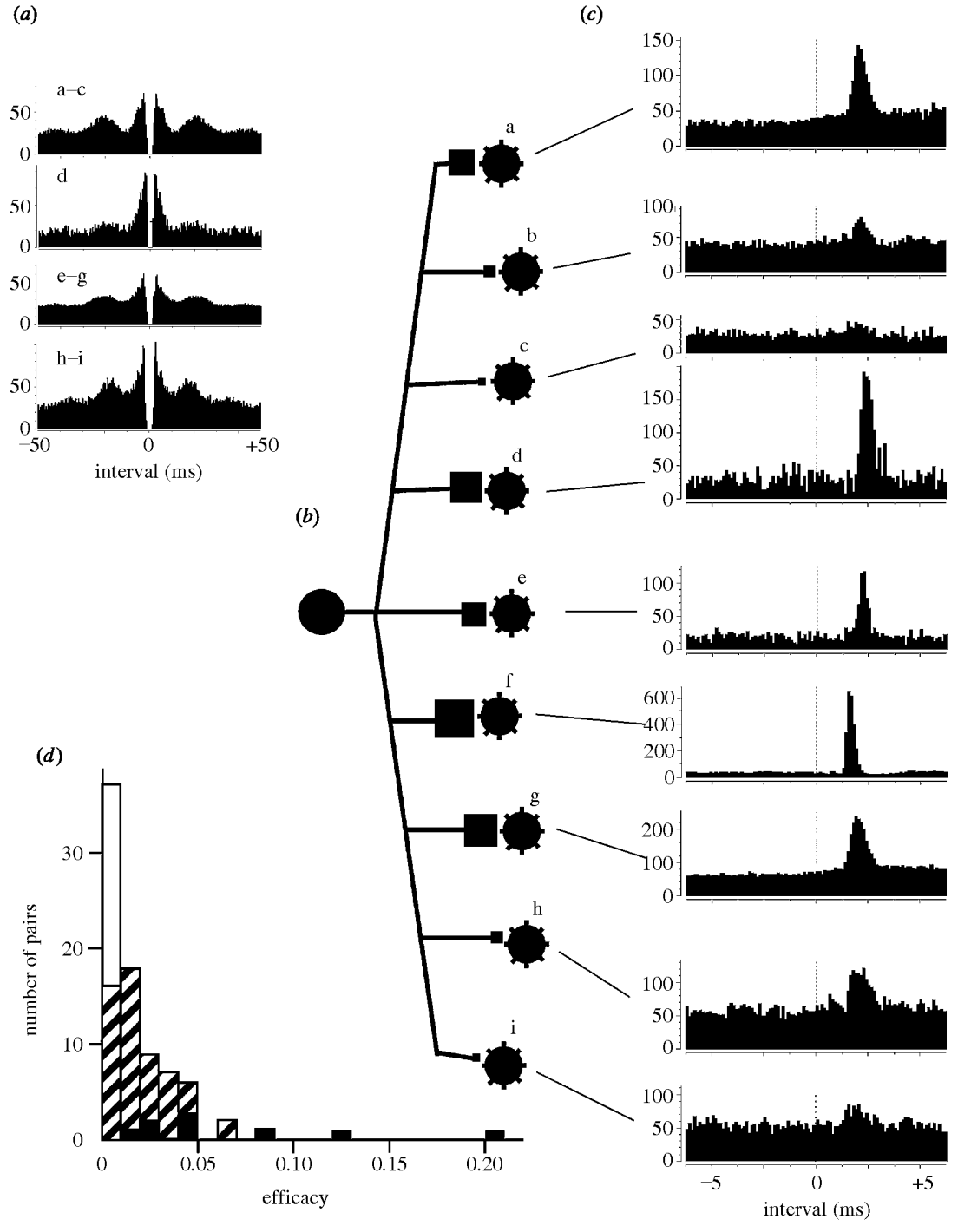

Figure 5. A powerful case of TC divergence: TC neuron 'Hercules', studied with nine different SINs (a–i) during four recording sessions. (*a*) Autocorrelograms of Hercules, obtained during the four recording sessions when the indicated SINs were studied. (*b*,*c*) Functional connectivity between this TC neuron and these SINs (*b*) and associated cross-correlograms (*c*). Although the efficacy of the functional contacts varied considerably, each correlogram showed a significant peak in discharge rate at intervals of 1.2–1.9 ms after TC spikes. (*d*) Efficacy values for TC contacts made by Hercules (black), and for other TC–SIN pairs that either reached (stripes) or failed to reach (open histograms) statistical significance. From Swadlow & Gusev (2002).

autocorrelograms with unusual and distinctive side-bands that were stable over days (figure 5*a*); and (ii) the receptive field was plotted several times each day, and this field was characteristic of only a small subset of TC neurons (transient response to only a single whisker, strongly directionally selective in a downward and back direction). Figure 5*b* illustrates the functional contacts made by Hercules with each of the SINs studied, and associated cross-correlograms are shown in figure 5*c*. Each correlogram showed a significant peak in SIN spike discharge rate at intervals of 1.2–1.9 ms following the TC spike. The functional input provided by Hercules to SINs of the aligned S1 barrel was not only remarkably widespread (9/9 SINs studied) but it was also extremely potent. Figure 5*d* shows efficacy values for all of the TC–SIN pairs studied in this experiment. Efficacy values generated by Hercules are

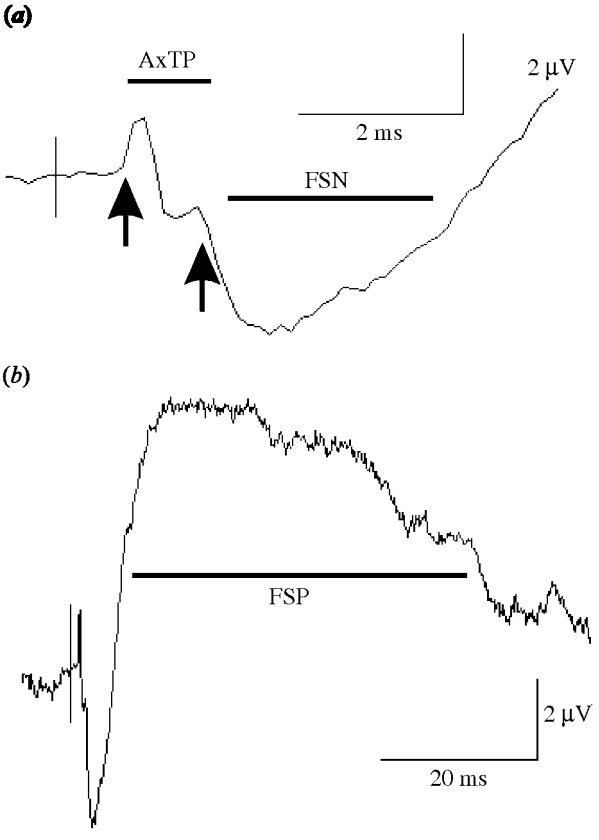

Figure 6. (*a*) Spike-triggered average waveform elicited in a cortical barrel by spontaneous action potentials of a TC neuron located in the topographically aligned VB thalamic barreloid. The left and right arrows indicate the onset of the axon terminal potential (AxTP) and the focal synaptic negativity (FSN, reflecting monosynaptic excitatory events), respectively. (*b*) the same spike-triggered average, taken at a slower sweep speed, showing the time course of the focal synaptic positive potential (FSP). This potential reverses in upper cortical layers and is thought to reflect disynaptic (feed-forward) inhibition. From Swadlow & Gusev (2000).

shown in black. Remarkably, Hercules was responsible for the three most potent TC contacts made (with SINs d, f and g, where efficacy values were 0.08, 0.21 and 0.13, respectively). The median efficacy of this TC neuron and these nine SINs was 0.048, which was significantly higher than values generated by the other 27 TC neurons (median value: 0.012, Mann–Whitney *U*-test, $p = 0.0002$).

The above results from TC neuron 'Hercules' indicates that some TC neurons are especially effective in driving multiple cortical interneurons. Thus, Hercules elicited spikes in all nine of the S1 SINs with which he was studied, and the efficacy values of these connections were much higher than those of other VB neurons. It is noteworthy that the SINs receiving input from Hercules were studied in separate microelectrode penetrations on four separate recording days. This assured that a reasonably large portion of the S1 barrel was sampled. Thus, it is likely that Hercules exerted a powerful influence throughout his targeted cortical barrel, not just on a small subsector of this structure. The notion that TC neurons differ considerably in their overall impact upon the cortex receives further support from large differences (greater than 10 : 1) observed in the amplitude of spike-triggered cortical field potentials that are elicited by different TC neurons. These results imply that a relatively small subset of TC neurons may effectively dominate the thalamic contribution to a cortical column.

7. TC DIVERGENCE AND THE GENERATION OF 'SHARP' SYNCHRONY AMONG SUSPECTED INHIBITORY INTERNEURONS

Neurons receiving a potent and divergent input from single afferent fibres would, because of simultaneously generated EPSPs, be expected to display a degree of sharply synchronous activity (Moore *et al.* 1970). Accordingly, coincident, potent input from diverging optic tract axons is believed to be responsible for the observed precise synchrony seen among LGNd neurons with overlapping receptive fields and common response properties (Alonso *et al.* 1996) and among homonymous spinal or brainstem motoneurons that receive a profusely divergent input from 1a afferent fibers (Kirkwood *et al.* 1982; Sears & Stagg 1976). In a similar vein, SINs of the same S1 barrel should show sharply synchronous activity because of potent and divergent TC input. Moreover, because the terminal arbourization of VB TC neurons is largely limited to a single barrel (Jensen & Killackey 1987), the synchrony should also be so limited. These predictions of a sharp, intra-barrel synchrony among SINs have been confirmed. Simultaneous recordings obtained from pairs of such SINs located within the same barrel show a sharp increase in spike frequency in each SIN that is coincident (± 1 ms) with a spike in the other SIN (Swadlow *et al.* 1998). This effect did not depend upon peripheral stimulation, occurring whether action potentials were 'spontaneous' or stimulus driven. Moreover, it was seen at horizontal inter-electrode distances of up to 350 μm, provided that the two SINs were found within the same barrel. For pairs of SINs recorded within a single barrel, *ca.* 4% (mean value) of the spikes of each SIN were sharply synchronous with the spikes of the other. As expected, sharp synchrony between SINs of neighbouring barrels was minimal or absent, even when inter-electrode distances were less than 300 μm. This sharply synchronous activity between layer-4 SINs is not oscillatory, is present in both fully awake and anaesthetized states, and is not seen between SINs and other populations of the same barrel that show no evidence of monosynaptic thalamic input. Preliminary evidence based on recordings of triads of SINs within a barrel (H. A. Swadlow, unpublished data) indicates that sharply synchronous activity between two SINs does not reflect a communal synchrony among all SINs of the barrel. Instead, synchronous events among the SINs of a barrel are roughly independent.

One consequence to be inferred from the above findings is that fast-spike interneurons of layer 4 rarely discharge in isolation, even in the absence of peripheral stimulation. Instead, when a TC impulse generates an action potential in one SIN, a significant population of the SINs in the same barrel discharge in sharp synchrony (*ca.* 4%, on average; Swadlow *et al.* (1998)). This coincident discharge of GABAergic interneurons will generate a synchronous feed-forward release of GABA onto postsynaptic targets. We have recently argued that compound IPSPs generated by this synchronous release of GABA are manifested in the extracellular record as a positive field potential (figure

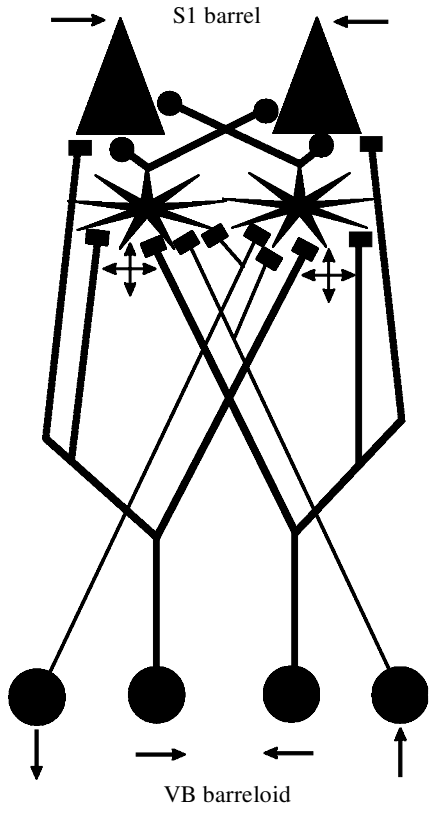

Figure 7. A schematic illustration of proposed feed-forward inhibitory mechanisms. TC neurons with a wide range of receptive field properties (varying directional preferences are indicated by arrows) converge/diverge onto topographically aligned SINs of an S1 barrel. This extensive but unselective pooling of TC inputs results in the construction of sensitive and broadly tuned receptive fields in recipient SINs. Divergent TC input to multiple SINs results in sharply synchronous activation of these elements, and this results in a synchronous feed-forward release of GABA onto postsynaptic targets. TC input to spiny neurons of a barrel is proposed to be more selective than is the input to SINs. Electrical coupling among SINs, if present in intact adults, may enhance both synchronous activity and the pooling of receptive field properties.

6*b*) within layer 4 that follows action potentials of individual TC neurons (detected using methods of spike-triggered averaging; Swadlow & Gusev (2000)).

Recent findings of electrical coupling among fast-spike interneurons in rat and mouse (Galarreta & Hestrin 1999; Gibson *et al.* 1999) suggest that S1 SINs may be functionally linked by both divergent TC axons *and* electrically coupled dendrites. Electrical coupling has been seen in slices taken from rat pups almost one month old (Gibson *et al.* 1999), and connexin-36, which is believed to underlie this electrical coupling, is present in the cortex of mature brains (cf. Deans *et al.* 2001). Clearly, electrical coupling, if present in intact adults, could contribute to the observed sharply synchronous activity among SINs within a barrel and, to some extent, to the observed functional divergent TC connectivity. Indeed, divergent TC synaptic input *and* electrical dendritic coupling could both serve to couple the behaviour of GABAergic interneurons, and these two mechanisms could act together, in a cooperative, synergistic manner to facilitate sharply synchronous discharge of fast-spike interneurons following TC impulses.

8. CONCLUSIONS

Figure 7 provides a schematic summary of many of the above results:

(i) *Receptive field synthesis in S1 inhibitory interneurons*. SINs of an S1 barrel receive a potent and highly convergent functional input from many TC neurons of the topographically aligned VB thalamic barreloid. Notably, TC neurons contacting a single SIN demonstrate a broad range of receptive field properties (Swadlow 1995; Swadlow & Gusev 2002), and this extensive pooling of TC inputs results in the synthesis of highly sensitive and broadly tuned SIN receptive fields. This manner of receptive field construction contrasts sharply with that proposed for putative spiny neurons of layer 4. There is considerable evidence that receptive fields of presumptive first-order excitatory neurons in layer 4 of the visual and auditory cortex are generated by a highly selective convergence of TC inputs (Reid & Alonso 1995; Alonso *et al.* 2001; Miller *et al.* 2001). We do not know how the receptive fields of spiny (excitatory) neurons in rabbit or rodent barrel cortex are synthesized but, since most are well-tuned to stimulus direction, they probably receive selective input from similarly tuned TC neurons.

(ii) *Sharp synchrony among SINs*. Sharp synchrony (± 1 ms) among SINs is generated by potent and highly *divergent* TC input, and this may be potentiated by electrically coupling among the dendrites of these elements (Galarreta & Hestrin 1999; Gibson *et al.* 1999). Even in the absence of any peripheral stimulation, *ca*. 4% of the spikes of each SIN occur in sharp synchrony (± 1 ms) with spikes in each of the other SINs within an S1 barrel (Swadlow *et al.* 1998).

(iii) *Feed-forward inhibition onto spiny neurons*. This broadly tuned and synchronous inhibitory network is engaged with high temporal precision and potency by single topographically aligned TC neurons. The potency of this activation is enhanced considerably for TC impulses with long preceding inter-spike intervals, such as those preceding a TC 'burst' (Swadlow & Gusev 2001). Because the axons of GABAergic interneurons in S1 barrels are short, and remain within the barrel (Harris & Woolsey 1983) axonal conduction time is short, and synchronous activity among fast-spike interneurons results in a corresponding synchronous feed-forward release of GABA onto postsynaptic targets. The consequent compound IPSP that is elicited in recipient cortical spiny neurons will limit the duration of the excitation generated by the thalamocortical EPSPs (Swadlow & Gusev 2000; cf. Pouille & Scanziani 2001). Thus, thalamocortical impulses will generate only a brief 'window of excitability' during which cortical spikes can occur and contribute to feed-forward and recurrent excitatory processes.

Supported by grants from NIMH (MH-64024) and NSF (IBN-0077694).

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GLOSSARY

EPSP: excitatory postsynaptic potential
GABA: γ-aminobutyric acid
IPSP: inhibitory postsynaptic potential
LGNd: dorsal lateral geniculate nucleus
o.d.: outside diameter
SIN: suspected inhibitory interneuron
TC: thalamocortical
VB: ventrobasal