Fast-spike Interneurons and Feedforward Inhibition in Awake Sensory Neocortex

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'Fast-spike' interneurons of layer 4 mediate thalamocortical feedforward inhibition and can, with some confidence, be identified using extracellular methods. In somatosensory barrel cortex of awake rabbits, these 'suspected inhibitory interneurons' (SINs) have distinct receptive field properties: they respond to vibrissa displacement with very high sensitivity and temporal fidelity. However, they lack the directional specificity that is clearly seen in most of their ventrobasal thalamocortical afferents. Several lines of evidence show that layer-4 SINs receive a potent and highly convergent and divergent functional input from topographically aligned thalamocortical neurons. Whereas the unselective pooling of *convergent* thalamocortical inputs onto SINs generates sensitive and broadly tuned inhibitory receptive fields, the potent *divergence* of single thalamocortical neurons onto many SINs generates sharply synchronous (±1 ms) activity (because of coincident EPSPs). Synchronous discharge of these interneurons following thalamocortical impulses will generate a synchronous feedforward release of GABA within the barrel. Thalamocortical impulses will, therefore, generate only a brief 'window of excitability' during which spikes can occur in the post-synaptic targets of fast-spike interneurons. This fast, synchronous, highly sensitive and broadly tuned feedforward inhibitory network is well suited to suppress spike generation in spiny neurons following all but the most optimal feedforward excitatory inputs.

Introduction

GABAergic inhibition shapes the responses of cortical neurons and constrains runaway excitation (Dykes *et al.*, 1984; Sillito, 1984; Nelson *et al.*, 1994; Vidyasagar *et al.*, 1996; Ferster and Miller, 2000). This inhibitory control is mediated by heterogeneous subpopulations of interneurons that are both morphologically and neurochemically distinct, and which exhibit their influence through both feedforward and feedback circuitry (Fairen *et al.*, 1984; Houser *et al.*, 1984; DeFelipe, 1993; Cauli *et al.*, 1997; Gupta *et al.*, 2000). Feedforward inhibition is the faster of these two mechanisms, being disynaptic, and mediated by direct thalamocortical input to intracortical interneurons (White and Rock, 1981; Agmon and Connors, 1992; Swadlow, 1995; Porter *et al.*, 2001). It is, therefore, very well suited to constrain cortical target neurons, and even to prevent them from ever reaching threshold when stimuli are weak or non-optimal. In contrast, feedback inhibition is slower (trisynaptic), and, unlike feedforward inhibition, requires spike activity in some excitatory cortical neurons before inhibitory neurons can be engaged.

Although GABAergic interneurons are relatively abundant within the cortex [they comprise 15–25% of the neurons in many cortical areas (Hendry *et al.*, 1987; Meinecke and Peters, 1987; Prieto *et al.*, 1994)], these neurons have received little attention in studies of receptive field and other response properties of cortical neurons in intact subjects. This may, in part, be due to the difficulty in identifying these neurons in the extracellular record. Whereas cortical efferent neurons may be unambiguously identified by antidromic activation, the identification of interneurons is more problematic. This chapter will review evidence indicating that one class of inhibitory interneurons can be identified in the extracellular record. The receptive fields and other response properties of these neurons will be described, and the relationship of these receptive fields to a highly divergent/convergent functional network that links these neurons to their thalamocortical afferents will be explored.

The Identification of GABAergic Interneurons in the Extracellular Record

In order to identify a GABAergic interneuron definitively, it is necessary to record intracellularly, label the neuron, and examine its morphological and/or cytochemical properties. This is now readily achieved in slice preparation. However, in sensory cortices of intact subjects, only a small number of such identified interneurons have been subject to detailed receptive field analysis, and these have provided important insights into the properties of these elements (Martin *et al.*, 1983; Azouz *et al.*, 1997; Hirsch *et al.*, 2000). This elegant and difficult strategy, however, limits the range of observations that can be made and the conditions under which these neurons can be studied. If some degree of error can be tolerated in the identification of these neurons, there is considerable evidence that one class of GABAergic interneurons [the 'fast-spike' variety (Connors and Gutnick, 1990; Amitai and Connors, 1995)] can be identified with some confidence using extracellular methods.

It has been recognized for some time that some interneurons have distinctive response properties. A high-frequency burst of spikes elicited by electrical stimulation of convergent inputs was described in 'Renshaw' cells of the spinal cord (Eccles, 1957) and in many other central interneurons (Andersen *et al.*, 1964a,b). In sensory neocortex, Mountcastle *et al.* (Mountcastle *et al.*, 1969) speculated that cells in somatosensory cortex with 'thin spikes' may be interneurons, but added the caveat that such cells could actually be thalamic afferents. Similarly, Simons (Simons, 1978) identified such 'fast-spike' neurons in rat somatosensory barrel cortex, and showed that they have receptive field properties that differ from those of 'regular spike' neurons. The suggestion that these fast-spike cortical elements may be interneurons gained considerable support from intracellular studies showing that a class of cortical neurons with short-duration action potentials had an aspinous or sparsely spinous non-pyramidal morphology (McCormick *et al.*, 1985; Connors and Kriegstein, 1986). In addition, these cells were shown to respond to a depolarizing intracellular current pulse with a very high-frequency, non-adapting discharge of action potentials and, subsequently, to stain positively for GAD. It was soon shown, however, that 'fast-spike' interneurons comprise only a subset of the population of cortical inhibitory interneurons. Other

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identified GABAergic interneurons were shown to lack the physiological signature of the fast-spike population. Moreover, this latter class of inhibitory interneurons did not stain for parvalbumin, a calcium-binding protein that is associated with fast-spike interneurons (Kawaguchi, 1993; Kawaguchi and Kubota, 1993).

These latter results showed that not all cortical GABAergic interneurons have action potentials of very short duration. Conversely, it has also become clear that not all short-duration action potentials are generated by fast-spike, parvalbumin-expressing interneurons. Intracellular studies have documented short-duration action potentials in a number of neurons that were subsequently labeled and shown to be of pyramidal morphology (Dykes *et al.*, 1988; Gray and McCormick, 1996) [also see (Takahashi, 1965)], and the spikes of a small but significant number of cortical efferent neurons, recorded extracellularly and identified by antidromic activation, are of very short duration (Swadlow, 1988, 1989, 1990). We can conclude, then, that although a majority of cortical neurons with short-duration spikes are fast-spike GABAergic interneurons, other cortical populations may also have short-duration action potentials.

To reduce this ambiguity, criteria other than spike-duration can be added to the procedure for identifying fast-spike interneurons in the extracellular record. As noted above, these cells can emit very high-frequency bursts of action potentials, and this characteristic has been useful in identifying putative inhibitory interneurons (Swadlow, 1988, 1989, 1990, 1991, 1994, 1995). Thus, suspected inhibitory interneurons (SINs) in visual and somatosensory cortices were identified by a burst of three or more spikes elicited by electrical stimulation of afferent pathways, where peak frequencies were required to exceed 600 Hz. As expected, SINs identified by the above criteria *also* had action potentials that were much briefer (approximately half the duration) than those of efferent populations. In addition, SINs in these cortical regions respond vigorously at short latencies to electrical stimulation of multiple cortical sites, which alleviates concerns that they may be thalamic afferents (above). Figure 1*A1*,*A2* shows the extracellular spikes elicited by ventrobasal (VB) thalamic stimulation in one such neuron in rabbit barrel cortex. Intracellular recordings were obtained from a small number of such neurons (five) in S1 barrel cortex that met the above extracellular criteria for classification as a SIN. Each of these cells, recorded in fully awake rabbits, responded to a depolarizing current pulse with the high-frequency, non-adapting discharge of action potentials that is characteristic of fast-spike, GABAergic interneurons. Figure 1*B* shows results from one of these neurons.

It is significant that, in a wide range of cortical regions (Swadlow, 1988, 1989, 1990, 1991. 1994), only ~0.5% of antidromically identified efferent neurons have responded to electrical stimulation of afferent pathways with a burst of three or more high-frequency (>600 Hz) synaptic spikes (three neurons of >700 corticocortical and corticofugal neurons studied in rabbit sensory and motor cortex). Thus, had antidromic identification of these efferent neurons not been employed in these experiments, <0.5% of these efferent neurons would have been falsely categorized as a SIN using the above identification procedure. The use of spike duration as the sole criterion in these studies would have led to incorrect classi- fication of many more of these neurons (see Figs 15, 7 and 5 of Swadlow, 1988, 1989, and 1990, respectively). It should be noted, however, that at present there is no documented method for identifying spiny stellate neurons using extracellular criteria, so these neurons were not included in the above extracellular analyses of spike

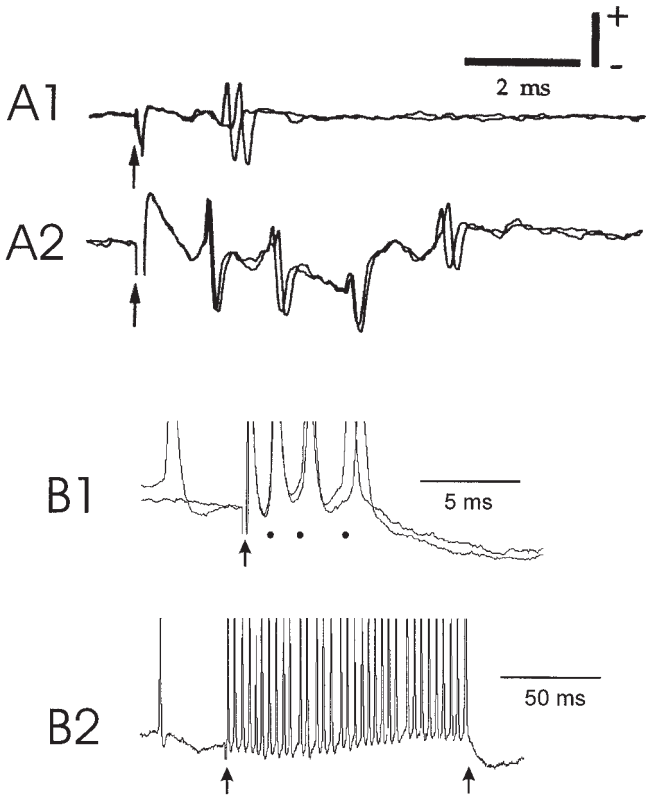

Figure 1. (*A*) Extracellular responses generated in a SIN to electrical stimulation of VB thalamus at 1.2 times threshold (*A*1) and at 5 times threshold (*A*2). (*B*1) Intracellular responses generated in a SIN to electrical stimulation of VB thalamus at 3 times threshold. The cell responds with a burst of three spikes (spikes are truncated; black dots indicate the spikes) at minimal interspike intervals of 1.6 ms (>600 Hz). (*B*2) Non-adapting, high-frequency response of this cell to a depolarizing current pulse. Arrows in *A*1, *A*2 and *B*1 indicate the stimulus artifact. Arrows in *B*2 indicate the onset (left) and offset of the depolarizing current pulse. Source: *A*1, *A*2 (Swadlow, 1995); *B*1, *B*2 (Swadlow *et al.*, 1998).

duration. However, intracellular observations indicate that these neurons are 'regular spiking' [i.e. they do not have short-duration action potentials (Feldmeyer *et al.*, 1999)], as are some classes of GABAergic interneurons (Kawaguchi, 1993, Kawaguchi and Kubota, 1993). The nature of these neurons' responses to electrical stimulation of afferent pathways has not been characterized in any detail.

Response Properties of Fast-spike Interneurons in Sensory Cortex

In awake rabbits, SINs of V1, S1 and S2 (Swadlow, 1988, 1989, 1990, 1991) are highly excitable. *All* of the SINs studied in layers 2–6 of each of these regions were clearly driven by peripheral sensory stimulation. This universal responsivity contrasts markedly with the properties of efferent populations, where 25–50% of corticocortical neurons (found primarily in layers 2–3, but also in deeper layers) and corticothalamic neurons of layer 6 lacked a demonstrable (suprathreshold) receptive field. SINs in the above sensory cortical areas also had much higher levels of *spontaneous* activity than did efferent populations. SINs had spontaneous firing rates of 10.6–19 spikes/s (mean or median values in these areas). In contrast, most corticocortical neurons and corticothalamic neurons of layer 6 were nearly silent, with spontaneous rates of considerably less than 1 spike/s (many of these neurons were *never* observed to fire in the

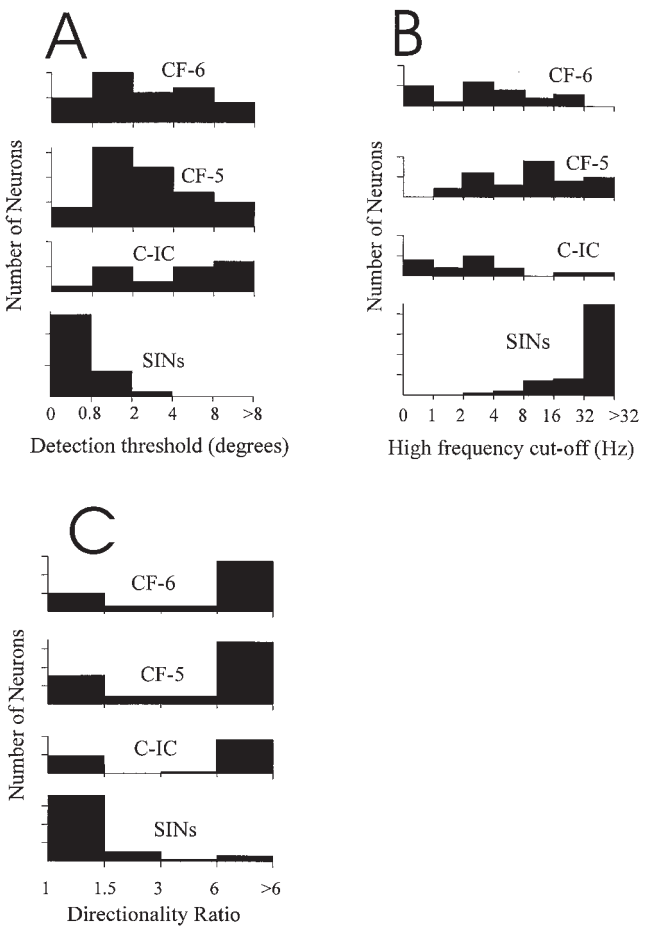

Figure 2. (*A*) Angular threshold for the detection of vibrissa movements of efferent neurons of different classes and SINs located in S1 barrel cortex. Efferent neurons studied included descending corticofugal neurons of layer 6 (CF-6 neurons) and layer-5 (CF-5 neurons) and ipsilateral cortico-cortical neurons projecting to S2 (C-IC neurons). (*B*) The highest frequencies of vibrissa deflections that could be followed by SINs and by efferent neurons of different classes. (*C*) Directionality ratios of efferent neurons of different classes and of SINs. The ratio of the number of spikes elicited in the preferred versus the null direction is shown. Source: (Swadlow, 1989).

absence of antidromic activation). Most descending projection neurons of layer 5 had spontaneous rates of 4–8 spikes/s.

SINs have very low thresholds to sensory stimulation. Figure 2*A* shows that SINs of S1 barrel cortex had much lower angular thresholds for detecting a whisker displacement than did any of the efferent populations of this region (Swadlow, 1989). More than 70% of SINs responded to a deflection of 0.8° in their 'principle vibrissa'. In contrast, most efferent neurons of all classes required a deflection of >2°. Similarly, SINs in the vibrissae and forepaw representations of S1, as well as in S2 and in motor cortex (Swadlow, 1989, 1990, 1991, 1994) responded much more faithfully to high-frequency peripheral stimulation (Fig. 2*B*) than did the efferent populations. Another remarkable difference between SINs and the efferent populations of S1 barrel cortex was in their responses to different directions of whisker deflections. Most efferent neurons of all classes responded selectively to a narrow range of displacement angles (Fig. 2*C*). This is, perhaps, not surprising since most VB thalamocortical neurons also display strong directional selectivity (Simons and Carvell, 1989). However, the great majority of SINs, found in the same microelectrode penetrations as the efferent neurons, showed little or no selectivity for the direction of whisker displacement. This *is* surprising, because most SINs receive strong thalamocortical input, much of which originates in thalamocortical neurons that show strong directional selectivity. I will argue (below) that this results from a non-selective convergence of thalamocortical input onto SINs of layer 4. Similar results are seen in rabbit visual cortex, where most corticocortical and corticofugal (corticotectal and corticothalamic) efferent neurons show both orientation and directional selectivity, but SINs are very widely tuned for both orientation and direction of motion (Swadlow, 1988).

The above results for SINs in rabbit S1, S2 and V1 are very similar to those reported for 'fast-spike' neurons in layer 4 of rat barrel cortex (Simons, 1978). These neurons also demonstrate very high levels of spontaneous activity, and are very responsive to peripheral stimulation, yet they lack the directional selectivity seen in most VB neurons and in 'regular-spike' neurons (presumed pyramidal or spiny stellate neurons) of this cortical region.

It is not yet clear whether the above results in rabbit and rat, showing a *high sensitivity, but low degree of specificity* in the responses of SINs (or fast-spike neurons), hold for these elements in sensory cortex of other species. A small number of GABAergic interneurons have been identified in *in vivo* intracellular studies of cat visual cortex. Both Martin *et al.* (Martin *et al.*, 1983) and Azouz *et al.* (Azouz *et al.*, 1997) report that these neurons are orientation selective, and these results are consistent with results showing orientation selectivity in the IPSPs onto pyramidal neurons of cat V1 (Ferster, 1986). However, recent intracellular studies (Hirsch *et al.*, 2000) have revealed inhibitory interneurons that lack orientation selectivity in layer 4 of cat V1. It would be useful to know whether these latter cells receive significant monosynaptic input from LGNd and could, thereby, mediate a broadly tuned feedforward inhibition onto spiny stellate neurons (Troyer *et al.*, 1998; Ferster and Miller, 2000). Surprisingly, putative fast-spike inhibitory interneurons have not been identified with any regularity in extracellular analyses of feline visual cortex. This is curious, given the great number of studies and intense interest in this cortical region. It may be that these neurons are more difficult to isolate in cat or simply that they have not been sought with sufficient effort.

Thalamocortical Connectivity of Fast-spike Interneurons

VB thalamocortical axons of mouse and rat form synapses with aspinous and sparsely spinous non-pyramidal neurons of S1 layer 4 (White, 1986, 1989). Moreover, some of the synapses onto these neurons occur directly on their somata. This fact may account for the powerful thalamocortical physiological responses observed in these neurons (below). Electrical stimulation of VB thalamus in rat and rabbit elicits short-latency responses in fast-spike interneurons and in SINs, respectively (Agmon and Connors, 1992; Swadlow, 1995). In the intact somatosensory thalamocortical system, latencies to peripheral sensory stimulation are sufficiently short and robust to allow detection of a single synaptic delay (Armstrong-James *et al.*, 1992; Swadlow, 1995). In the rabbit, the median latency of VB thalamocortical neurons to potent air-puff stimulation is ~5 ms (Fig. 3*A*). Based on the conduction time along thalamocortical axons (~1 ms, filled histograms in Fig. 3*A*), these impulses arrive in the cortex at a latencies of ~6 ms following the peripheral stimulus (Fig. 3*B*), and a large population of S1 SINs respond at latencies of 6–7 ms (Fig. 3*C*). Thus, there is little time for more than one synaptic delay between the arrival of VB impulses in S1 and the initial spike elicited in most SINs. Moreover, SINs that responded

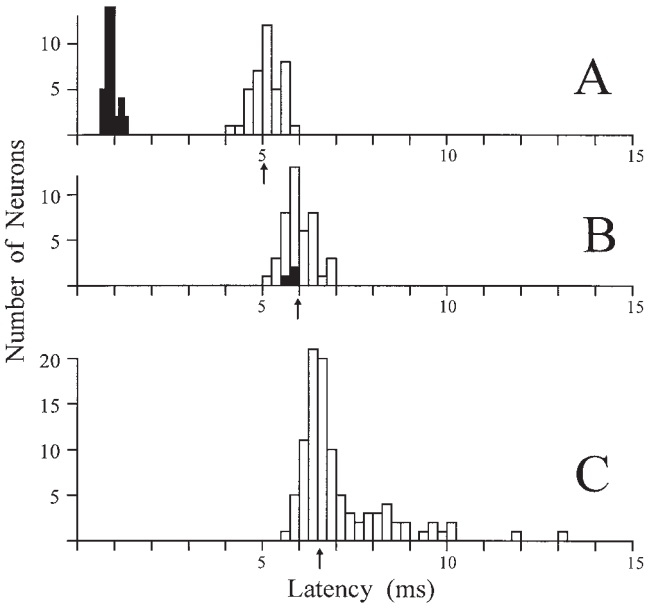

Figure 3. (*A*) Darkened histogram at left shows the antidromic latencies of VB thalamocortical neurons following electrical stimulation of S1; open histogram at right shows the synaptic latencies of these neurons to a high-intensity air-puff stimulus delivered to the dominant whisker. (*B*) Open histogram shows the estimation of the arrival times of the thalamocortical impulses at S1. These values were obtained by adding the antidromic corticothalamic latency to the latency to the air-puff stimulus for each of the thalamocortical neurons shown in (*A*). Solid components of the histogram show the latency to the air-puff stimulation of three thalamocortical axons that were recorded within S1. (*C*) Histogram of the latencies to air-puff stimulation of S1 SINs. The median value of 6.6 ms reflects a strong peak in the distribution that occurs just 0.63 ms after the peak estimated arrival time of the VB impulses in S1. Vertical arrows beneath (*A*)–(*C*) indicate the median values (Swadlow, 1995).

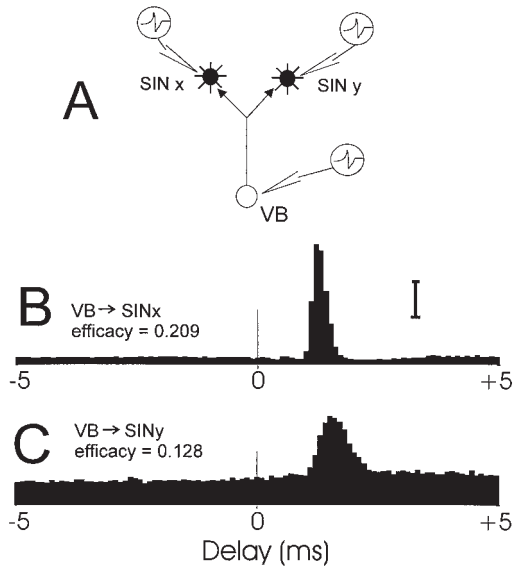

Figure 4. (*A*) Schematic illustration of a single VB thalamocortical neuron making functional contacts with two SINs (SIN*x* and SIN*y*), simultaneously recorded on two microelectrodes located in the topographically aligned S1 barrel. (*B*,*C*) Cross-correlograms of the spike trains of the thalamocortical neuron with those of SIN*x* (*B*) and SIN*y* (*C*). Both SINs receive a powerful functional input from this VB neuron. TC functional connectivity was assessed in the absence of peripheral stimulation, under conditions of 'spontaneous' activity. Cross-correlograms were not filtered or smoothed and bin-widths were 0.1 ms. [Adapted from (Swadlow and Gusev, 2001, 2002)].

at short latencies to the air puff (<7 ms) were largely located in the vicinity of layer 4, and these cells also responded at short latencies (<1.8 ms) to electrical stimulation of VB. Together, these data indicate a powerful monosynaptic input to fast-spike interneurons of layer 4.

Cross-correlation analysis has provided further evidence for strong functional connectivity between VB thalamocortical neurons and S1 SINs (Swadlow, 1995). These studies require precise topographic alignment between thalamic and cortical recording sites. When this was achieved, most SINs within and near to layer 4 showed a sharp increase in spike frequency following a thalamic spike, which is consistent with monosynaptic activation. This response is brief (often <1 ms in duration), and the peak increase in spike rate generally occurs at latencies of 1.2–2 ms following VB action potentials. The 'efficacy' of the functional connectivity between most VB neurons and S1 SINs was 1–2% (Swadlow, 1995). This value reflects the strength of the functional connectivity between the thalamic and cortical neuron, and is given by the ratio of presynaptic (thalamic) spikes to the number of spikes in the peak of the cross-correlogram (minus the number of spikes expected by chance during the period of the peak). In some cases, the efficacy of the thalamocortical input to SINs can be quite high. Figure 4 shows cross-correlograms indicating *very* strong functional connectivity between a single VB thalamocortical neuron and two SINs that were simultaneously studied (on separate cortical microelectrodes) in the aligned S1 barrel. Thalamocortical efficacies were 0.21 and 0.13 in Figure 4*B* and Figure 4*C*, respectively, and peak latencies were 1.3 and 1.6 ms.

Convergence and Divergence of Thalamocortical Input to S1 SINs

In an earlier study of this system (Swadlow, 1995), single thalamocortical neurons were studied simultaneously with only a single cortical SIN. One surprising result of that analysis was that *most* of the S1 SINs under study showed evidence of functional connectivity with *most* of the thalamocortical neurons that were identified within the aligned VB barreloid. Unlike the high degree of specificity seen in the functional connections between geniculocortical neurons and simple cells of VI (Reid and Alonso, 1995), and between auditory thalamocortical neurons and their cortical recipients (Miller *et al.*, 2001), the thalamocortical input to S1 SINs showed little evidence of specificity, other than the requirement for precise topographic alignment. VB thalamocortical neurons show a wide variation in receptive field properties [sustained vs. transient responses to maintained whisker deflection, directionally selective responses (which can be sharply tuned) versus multi-directional responses]. However, correspondence in these characteristics was not required for thalamocortical connectivity with S1 SINs. These results imply a very high degree of convergence and divergence in the thalamocortical connectivity of this feedforward inhibitory system (Swadlow, 1995).

Recently, we provided a more direct demonstration of this high degree of functional convergent and divergent connectivity by obtaining simultaneous recordings of multiple VB neurons and multiple, topographically aligned S1 SINs (Swadlow and Gusev, 2002). This strategy allowed us to compare the impact of several different VB neurons on the same SIN and, conversely, to examine the influence of a single thalamocortical neuron on multiple post-synaptic targets. Our results confirmed the highly convergent/divergent functional thalamocortical input to S1 SINs. SINs that lacked any directionality were shown to receive a strong convergent functional input from multiple VB neurons that each showed a strong directional selectivity, but in widely

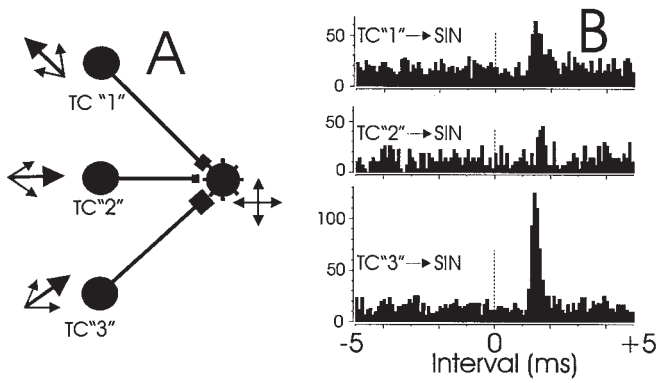

Figure 5. Convergent input from three VB neurons to a single SIN. (*A*) Schematic illustration of the functional connectivity between the three thalamocortical (TC) neurons and the SIN. Arrows indicate the very different directional preferences (range = 135°) of the TC neurons and the multidirectional responses of the SIN. (*B*) Cross-correlograms of each of these TC neurons and the SIN under study. TC functional connectivity was assessed in the absence of peripheral stimulation (following the receptive field analysis), under conditions of 'spontaneous' activity. Values on the *y*-axis are given in spikes/s. Cross-correlograms were not filtered or smoothed and bin-widths were 0.1 ms. Source: (Swadlow and Gusev, 2002).

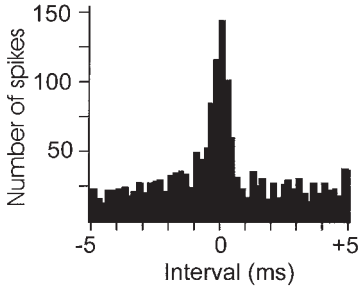

Figure 6. Cross-correlogram of the spike trains of two SINs recorded within the same S1 barrel. Data were obtained in the absence of peripheral stimulation, under conditions of 'spontaneous' activity. Source: (Swadlow *et al.*, 1998).

varying directions. Figure 5 shows one such case, in which three VB neurons with widely varying directional selectivities (range = 135°) were shown to provide input to an S1 SIN that lacked any directional preference.

Remarkable divergence from single VB neurons to multiple SINs of a barrel was also demonstrated (Swadlow and Gusev, 2002). One VB neuron (dubbed 'Hercules' because of potent functional contacts with many SINs) was shown to make a functional contact with each of nine SINs that were studied over a 4 day period in the aligned S1 barrel. The VB–SIN pairs shown in Figure 4 show two of the contacts made by Hercules on one of these days.

Such a high degree of divergence/convergence is suggestive of the 'complete transmission line' between successive nodes of a network that was described by Griffith (Griffith, 1963) [also (Abeles, 1991)]. These networks are characterized by very reliable transmission between the input and output nodes, but the cost of this reliability is a sacrifice in 'complexity of task' (Griffith, 1963). This description is very consistent with the response properties of S1 SINs. As described above, these neurons are exquisitely sensitive to very low amplitude peripheral stimulation and they respond faithfully to high stimulus frequencies. Enhanced sensitivity is an expected consequence of a high degree of convergence because spikes in a large number of input neurons each have an opportunity to elicit a response in the target neuron. SINs have sacrificed 'complexity of task', however, in that they lack the directional selectivity that is seen in most of their thalamocortical inputs. In this view, SINs obtain their multidirectional receptive fields by pooling the input from a large number of thalamocortical neurons with widely differing directional preferences.

Sharp Synchrony Among Fast-spike Interneurons of S1

The recipient neurons of such a highly divergent/convergent network would be expected to demonstrate another property: *sharply synchronous activity*. This would be expected because of the near-simultaneous EPSPs generated by the common, *diverging* presynaptic input (Moore *et al.*, 1970). Based on such reasoning, Sears and Stagg (Sears and Stagg, 1976) predicted and documented a 'short-term' synchrony between homonymous motorneurons [which receive a highly divergent input from muscle spindle 1a afferents (Mendell and Henneman, 1971)]. Similarly, potent coincident input from diverging optic tract fibers is believed responsible for the observed sharp synchrony between LGNd neurons with overlapping receptive fields and common response properties (Alonso *et al.*, 1996). If SINs of an S1 barrel are, indeed, the recipients of a highly divergent input from many thalamocortical neurons, then they should also demonstrate such sharply synchronous activity. Moreover, because the VB thalamocortical terminal arborization is largely limited to a single barrel (Jensen and Killackey, 1987), the synchrony should also be so limited.

These predictions were tested by recording from pairs of SINs within an S1 barrel (Swadlow *et al.*, 1998). The cross-correlogram presented in Figure 6 shows results generated by two SINs of the same barrel, each of which gave strong evidence of receiving monosynaptic thalamic input (latencies of <1.7 ms to electrical stimulation of VB *and* <7.5 ms to air-puff stimulation, above). Here, and in nearly all pairs of such thalamocortically driven SINs that were found within the same barrel, a sharp increase in spike frequency occurred in each SIN nearly simultaneously (±1 ms) with a spike in the other SIN. This effect did not depend upon peripheral stimulation, as it occurred whether action potentials were 'spontaneous' or stimulus driven. Moreover, it was seen at horizontal inter-electrode distances of up to 350 μm, as long as the two SINs were found within the same barrel. For pairs of SINs recorded within a single barrel, ~4% of the spikes of each SIN were sharply synchronous with the spikes of the other. As expected, sharp synchrony between SINs of neighboring barrels was minimal or absent, even when inter-electrode distances were <300 μm. We found that sharply synchronous activity in layer-4 SINs was not oscillatory. Autocorrelograms generated by the action potentials of individual SINs and by the synchronous events occurring between two SINs showed no signs of 'side-bands' that are indicative of oscillatory activity. Moreover, sharp synchrony between SINs was present in both fully awake and anesthetized states. Sharp synchrony was not seen between SINs and other populations of the same barrel that showed no evidence of monosynaptic thalamic input. Recent preliminary evidence based on recordings of triads of SINs within a barrel (unpublished) indicate 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 that can 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 thalamocortical impulse generates an action potential in

one SIN, a significant population of the SINs in the same barrel discharge in sharp synchrony (~4% according to the above results). This coincident discharge of GABAergic interneurons will generate a synchronous feedforward release of GABA onto post-synaptic targets. We have recently argued that the compound IPSPs generated by this synchronous release of GABA can be detected in the extracellular record (using methods of spike-triggered averaging) as a positive field potential within layer 4 that follows action potentials of individual thalamocortical neurons (Swadlow and Gusev, 2000).

Functional Thalamocortical Connectivity, Sharp Synchrony and Electrical Coupling Among Fast-spike, GABAergic Interneurons

The brief and powerful peaks in spike probability occurring in SINs at intervals of 1.2–2 ms following thalamocortical impulses (Fig. 4) are strongly suggestive of monosynaptic excitatory input, and the sharply synchronous activity seen between pairs of SINs within an S1 barrel (Fig. 6) are consistent with this idea. However, recent findings of electrical coupling among fast-spike interneurons in rat and mouse (Galarreta and Hestrin, 1999; Gibson *et al.*, 1999) suggest that S1 SINs may be functionally linked by both divergent thalamocortical axons *and* electrically coupled dendrites. Although electrical coupling has been studied primarily in immature tissue, such coupling has also been seen in slices taken from 26-day-old pups (Gibson *et al.*, 1999). Moreover, connexin-36, which is believed to underlie this electrical coupling, is present in the cortex of mature brains (Deans *et al.*, 2001). If electrical coupling is, indeed, present among fast-spike interneurons in adult brains, then it could contribute to the observed sharply synchronous activity among these elements and, to some extent, to the observed *functional* divergent/convergent thalamocortical connectivity. Divergent thalamocortical synaptic input and electrical dendritic coupling both could serve to couple the behavior of GABAergic interneurons, and these two mechanisms of could act together, in a cooperative, synergistic manner, to facilitate widespread, synchronous discharge of fast-spike interneurons following thalamocortical impulses. Indeed, it is possible that some SINs with little or no direct (synaptic) thalamocortical connectivity could show an increase in spike probability following thalamic spikes due to electrical coupling with other SINs that do receive direct thalamocortical input.

It is important to note that we do not yet know the extent to which fast-spike interneurons are electrically coupled in intact adults, and how general this phenomenon is among various mammalian lines. Given outstanding questions concerning the orientation tuning of GABAergic interneurons in layer 4 of cat (Azouz *et al.*, 1997; Hirsch *et al.*, 2000) and the putative role of such neurons in controlling the receptive fields of excitatory neurons (Troyer *et al.*, 1998), it would be very useful to know whether these elements are electrically coupled in adult cats, the spatial extent of any such coupling, and the relationship of this coupling to orientation tuning and other receptive field properties of these neurons. If fast-spike GABAergic interneurons of feline visual cortex are electrically coupled over distances similar to those seen in rodents [±~200 μm (Amitai *et al.*, 2001)], one would expect this to broaden any orientation preference in these neurons that was directly generated by feedforward (thalamocortical) connectivity. This effect could be especially prominent near 'pinwheel' centers, where the distance between orientation columns is reduced (Maldonado *et al.*, 1997). Clearly, further experimental work and computational studies are required to unravel the relative contributions of diverging/converging thalamocortical *synaptic* input and electrical dendritic coupling to (i) the observed sharp synchrony among these interneurons, (ii) their highly divergent/convergent *functional* thalamocortical connectivity and (iii) their receptive field properties.

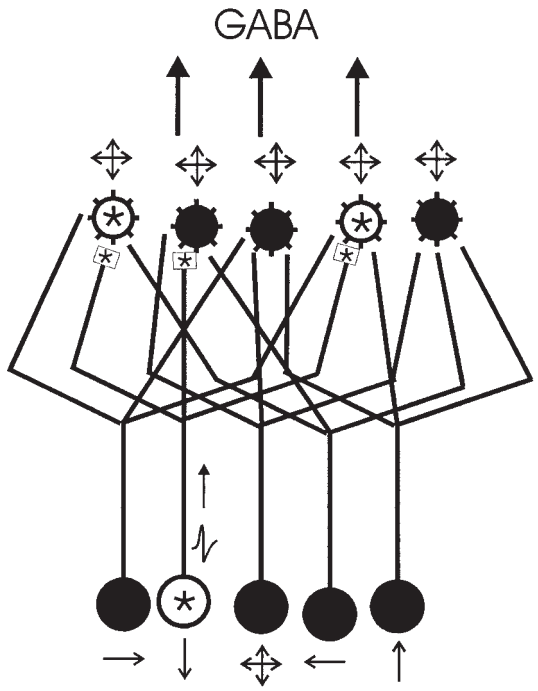

Figure 7. Model relating the proposed highly divergent/convergent functional linkage between VB thalamocortical neurons and S1 fast-spike interneurons to the sharply synchronous activity of these neurons and to their receptive field properties. Here, an impulse is generated in one VB neuron (asterisk), which makes synaptic contact with a large proportion (asterisks) of the SINs within the aligned barrel. Sharply synchronous spikes are generated in a subset of these neurons (the unfilled neurons). The convergence of multiple VB thalamocortical neurons with diverse directional preferences (denoted by the fine arrows) onto SINs generates a response in the SINs that is highly sensitive, but which lacks directional selectivity. The coincident spiking of multiple interneurons within the barrel generates a potent wave of feed-forward inhibition within the barrel.

Fast-spike Interneurons of Layer 4: a Substrate for Fast, Potent Feedforward Inhibition

Figure 7 schematically illustrates many of the above results. Thalamocortical impulses functionally diverge and converge to generate a rapid, widespread and synchronous activation of layer-4 fast-spike interneurons of the topographically aligned S1 barrel (Swadlow, 1995; Swadlow and Gusev, 2002). One result of this *convergent* circuitry is a receptive field transformation: VB thalamocortical neurons with widely differing directional preferences converge non-selectively onto fast-spike interneurons of layer 4, generating highly sensitive but broadly tuned receptive fields. A result of the *divergent* thalamocortical circuitry is a sharp synchrony among these interneurons following thalamocortical impulses (Swadlow *et al.*, 1998). This will, in turn, generate a sharply synchronous release of GABA, and a consequent compound IPSP in recipient cortical spiny neurons that limits the duration of the excitation generated by the thalamocortical EPSPs (Swadlow and Gusev, 2000). Thus, single thalamocortical impulses will generate only a brief 'window of excitability' during which cortical spikes can occur and contribute to feedforward and recurrent excitatory processes. This fast, synchronous, highly sensitive and broadly tuned

feedforward inhibitory network [cf. (Troyer *et al.*, 1998)] is well suited to suppress spike generation in spiny target neurons following all but the most optimal feedforward excitatory inputs.

Notes

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