Laminar origins of inhibitory synaptic inputs to pyramidal neurons of the rat neocortex

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1. Inhibitory neuron–pyramidal cell interactions were investigated in slices of rat somatosensory cortex in which excitatory synaptic transmission was blocked with bath-applied glutamate receptor antagonists. Local inhibitory neurons were excited by focal pressure ejections of small (~40 pl) volumes of 1–10 mM acetylcholine.

2. The frequency of inhibitory postsynaptic potentials (IPSPs) ('responses per trial' or R/T) declined as the stimulation distance was increased. Inhibitory inputs were most prevalent in layer II/III regular spiking (RS) pyramidal neurons (30 cells) where median R/T was 0·020. In layer V, the median R/T was 0·024 for RS neurons (25 cells), but significantly lower for burst-firing (IB) neurons (17 cells), where median R/T was 0·007 ($P = 0·039$).

3. IPSPs in individual layer V pyramidal cells were recorded with CsCl electrodes. In eight neurons, spontaneous picrotoxin-sensitive IPSPs were recorded and found to display a wide range of 10–90% rise times (1–34 ms), not correlated with amplitude (0·2–18 mV). For a further ten pyramidal neurons, extracellular stimulating electrodes were placed simultaneously in layers II/III and V/VI in order to evoke pairs of IPSPs whose waveforms were averaged and compared. In seven cells, IPSPs evoked from layer II/III (distal location) had longer 10–90% rise times than IPSPs evoked from layer V/VI stimulating electrodes (proximal location). In addition, 'proximal' IPSPs could always be reversed by membrane depolarization whereas 'distal' ones could not ($n = 4/4$).

4. This study showed that pyramid cell–inhibitory neuron interconnections are extensive but their spatial organization varies with cell class and with cortical layer. In addition, pyramidal neurons can receive inhibitory inputs from locations on their apical dendrites.

Inhibitory synaptic connections are an important part of the intrinsic circuitry of the neocortex, serving to modulate the propagation of sensory information. In the rat neocortex, inhibitory neurons comprise less than 10% of the neuronal population and, unlike pyramidal cells, their projections are usually purely intrinsic (Peters, Payne & Josephson, 1990). In anatomical terms, inhibitory neurons are a diverse set of sparsely spiny cell types, including chandelier, basket and bitufted cells (Chmielowska, Stewart, Bourne & Hamori, 1986; Somogyi, 1990). However, not all of these cell types may be represented in every rodent neocortical area so a strict classification of inhibitory neurons cannot necessarily be universally applied (Peters, 1985). Also, the morphologies of individual inhibitory neurons do not correspond to exclusive electrophysiological characteristics (Kawaguchi, 1995). The relationship between cellular morphologies, transmitter types and functional circuitry of neocortical inhibitory neurons is, therefore, far from clear.

Inhibitory cells are intimately associated with pyramidal neurons. Studies in hippocampus suggest that inhibition may act at various levels on individual neurons: viz. chandelier cells synapse onto the initial segments of pyramidal cell axons; basket cells primarily make contact with cell somata and proximal dendrites; and neurogliaform and bitufted inhibitory neurons are thought to contact the more distal dendrites (Connors, 1992; Buhl, Halasy & Somogyi, 1994). Autoradiographical studies show that inhibitory synapses are indeed distributed over the entire dendritic tree of pyramidal neurons (Beaulieu & Somogyi, 1990). The transmitter at these synapses is invariably γ-aminobutyric acid (GABA), which can activate both chloride- ($GABA_A$) and potassium-dependent receptors ($GABA_B$), mediating the 'fast' and 'slow' inhibitory post-synaptic potential (IPSP), respectively (Howe, Sutor & Zieglgänsberger, 1987; Connors, Malenka & Silva, 1988). In hippocampal pyramidal neurons, there is some indication of

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variation in the $GABA_A$ and/or $GABA_B$ mediation of somatic and dendritic synaptic events (Newberry & Nicoll, 1985; Miles, Tóth, Gulyás, Hájos & Freund, 1996). However, a strict segregation of inhibitory cell targets and pharmacology has not been convincingly demonstrated for either hippocampal or neocortical pyramidal cells.

Our knowledge of inhibitory circuitry in neocortex is very incomplete, especially the details of inhibitory–pyramidal cell interconnections. It is still somewhat unclear whether different populations of inhibitory interneurons are responsible for separate $GABA_A$ and $GABA_B$ responses, and the notion of a hierarchy of inhibition on the dendritic tree of individual pyramidal neurons requires further investigation. Furthermore, little is known about the functional organization of lateral inhibitory connections. Horizontal synaptic interactions are important in cortical processing but there have not been many quantitative studies examining functional inhibitory connections. A convenient and profitable procedure for examining the laminar organization of inhibitory circuitry is to record from individual pyramidal neurons while stimulating the cell bodies of small numbers of presynaptic inhibitory cells with acetylcholine (ACh) (McCormick & Prince, 1986). This method was adopted in the present study in order to quantify occurrence and origin of inhibitory synaptic connections onto pyramidal cells in rat neocortex. In addition, proximal and distal GABA responses in individual pyramidal neurons were examined using focal extracellular electrical stimulation. Some of these results have been reported in abstract form (Nicoll, Kim & Connors, 1993).

METHODS

Slices and recording

Sprague–Dawley rats (120–200 g) were anaesthetized with sodium pentobarbitone (Nembutal, 60 mg kg^{-1}, I.P.), decapitated and 400 μm-thick slices of somatosensory cortex were prepared using standard methods (Chagnac-Amitai & Connors, 1989*a*, *b*). The slices were maintained in an interface recording chamber at 34 °C and bathed in standard artificial cerebrospinal fluid containing glutamate antagonists (Tocris Neuramin) at appropriate concentrations: 50 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 50 μM D,L-2-amino-5-phosphonovaleric acid (AP5) (Chagnac-Amitai & Connors, 1989*a*, *b*). Recordings were made from pyramidal neurons in layers II/III and V with whole-cell patch pipettes (10–20 MΩ) filled with (mM): KCH_4O_3S, 120; EGTA, 5; $MgCl_2$, 2; MgATP, 4; $CaCl_2$, 5; and KCl, 10. Data were digitized using pCLAMP software (Axon Instruments).

Responses to ACh

With excitatory synaptic transmission blocked, recordings were made from pyramidal neurons and ACh was used to investigate the pattern of their inhibitory interactions in different cortical layers (McCormick & Prince, 1986). IPSPs were evoked by focal pressure ejection of small drops (40 pl) of 1–10 mM acetylcholine (AChCl, Sigma) from an extracellular pipette. The extracellular pipette was placed sequentially at 100 μm intervals lateral to the postsynaptic pyramidal cell over horizontal distances of 100–1000 μm. In addition, vertical synaptic interactions were examined by the stimulation of layers above and below the postsynaptic neuron. At each distance interval from the postsynaptic cell, ACh drops were ejected at two or three depths within the slice from two to three different entry points close to each other. We called each individual depth at a particular entry point one 'trial', so for each defined distance from the recording site, there were up to nine trials. The order and range of the distances at which trials were made were randomized for each cell. Previous studies have demonstrated that discrete stimulation of neurons can be achieved with minimal spread of transmitter (Williams & Lacaille, 1992; Benardo, 1994). Because the reversal potential of $GABA_A$-mediated IPSPs is close to that of the resting potential of neocortical pyramidal cells, the postsynaptic cells were held at slightly depolarized potentials (~−60 mV) during recording.

Spontaneous and evoked IPSPs

Recordings were also made from layer V pyramidal cells with whole-cell pipettes whose filling solution contained 130 mM CsCl instead of KCH_4O_3S. The quaternary lidocaine (lignocaine) derivative QX-314 (2(triethylamino)-*N*-(2,6-dimethylphenyl)acetamide; Astra, Kings Langley, UK), a Na^+ current blocker, was added to the filling solution to prevent action potential firing. The presence of Cs^+ in the recording pipettes served to increase the input resistance of the cells and to block K^+-mediated conductances, including those coupled to the $GABA_B$ receptor. The limit of our resolution varied but generally IPSPs smaller than ~0·3 mV were lost in the noise. IPSPs were recorded at ~−60 mV and, where possible, their amplitudes and 10–90% rise times were measured to gain an indication of their approximate electrotonic origin. In further experiments, IPSPs were evoked at < 0·03 Hz in layer V pyramidal neurons using extracellular bipolar electrodes simultaneously placed proximally (i.e. layer II/III) and distally (i.e. layer V/VI) to the cell body. In order to facilitate the separation of these inputs, a horizontal cut was made in the slice in layer IV.

RESULTS

Responses to ACh

Pyramidal neurons were classified according to accepted criteria (Amitai & Connors, 1995) as layer II/III regular spiking (RS) cells ($n = 30$), layer V RS cells (25) and layer V intrinsically burst-firing (IB) cells (17), on the basis of action potential firing characteristics in response to somatically injected depolarizing current pulses (Table 1). Seven presumed inhibitory or fast-spiking (FS) neurons were also encountered, identified on the basis of their high firing rates and large input resistance. Some results of ACh application are shown in Fig. 1. The peak amplitudes of ACh-evoked IPSPs were in the range of 0·8–8·9 mV and were similar for the three pyramidal cell classes. In addition, IPSP amplitude was not correlated to horizontal distance from the stimulus site to the postsynaptic cell (Fig. 1*C*). In most cases, IPSPs decayed smoothly back to baseline by 200 ms; some IPSPs displayed longer decays, perhaps 400 ms. Two IPSPs, both recorded in layer II/III pyramidal neurons, showed a second long and late decay phase not seen in IPSPs observed in the other cell types. When possible, ACh was also ejected directly onto the pyramidal neuron. In all

cases, the cell responded, after a delay, with a gradual depolarization and subsequent firing of action potentials (Fig. 1*D*), as observed by McCormick & Prince (1986).

In an attempt to map the origin of inhibitory inputs onto individual pyramids, ACh was ejected at various distances from the postsynaptic cell. Not all separate sites of application ('trials') resulted in the occurrence of an IPSP ('response') and in order to take account of this, the frequency of IPSPs observed in the different cell classes was expressed in terms of 'responses per trial' (R/T). Most connections evoked were 'horizontal' or intralaminar, where R/T was calculated for stimuli located at ten different distances from the pyramidal neuron (Table 1; Fig. 2). A Friedman test showed that, over the range of separations tested, there were significant differences in the median R/T values in the cell classes ($Fr = 6{\cdot}69$; $P = 0{\cdot}035$). The frequencies of observed inhibition were similar for both classes of regular spiking cell, but intrinsically burst-firing neurons displayed considerably fewer IPSPs. In layer V, median R/T in IB pyramidal neurons (0·007) was significantly lower than in RS neurons (0·024; Wilcoxon matched-pairs test, $P = 0{\cdot}039$). In terms of IPSPs per cell tested, layer V IB neurons also displayed less inhibition than other pyramidal cell classes. For all cell classes, R/T declined as the stimulus distance increased, and this trend could be described by exponential curves (Fig. 2). When plotted graphically, the difference in R/T between cell classes for closer separations was particularly striking, with the layer II/III pyramidal neurons in particular displaying considerable inhibitory interactions for ACh ejection closer than 300 μm (Fig. 2).

Trials were made in a number of pyramidal cells from locations 'vertical' to the cell bodies. In five layer V RS and two layer V IB neurons, local ACh ejections were made around the apical region of the recorded pyramidal neurons and, to a limited extent, laterally from the cell in layers other than the one in which the postsynaptic cell body was located. No IPSPs were observed in layer V IB cells but in the five layer V RS neurons, seven IPSPs (amplitude $2{\cdot}37 \pm 1{\cdot}13$ mV, mean ± S.D.), similar to those in Fig. 1*A*, were recorded in response to eighty trials. In four pyramidal neurons located in the bottom of layer III, two IPSPs were also elicited from 'apical' locations in layer II, but no responses were seen to the sixty ACh trials made in layer V. Although the number of tests was limited, generally speaking, response probabilities in vertical directions increased as trial locations were moved towards the somatic recording site.

Throughout this study, seven cells were recorded from layer V which displayed fast-spiking characteristics considered to be from inhibitory neurons. One of these cells displayed

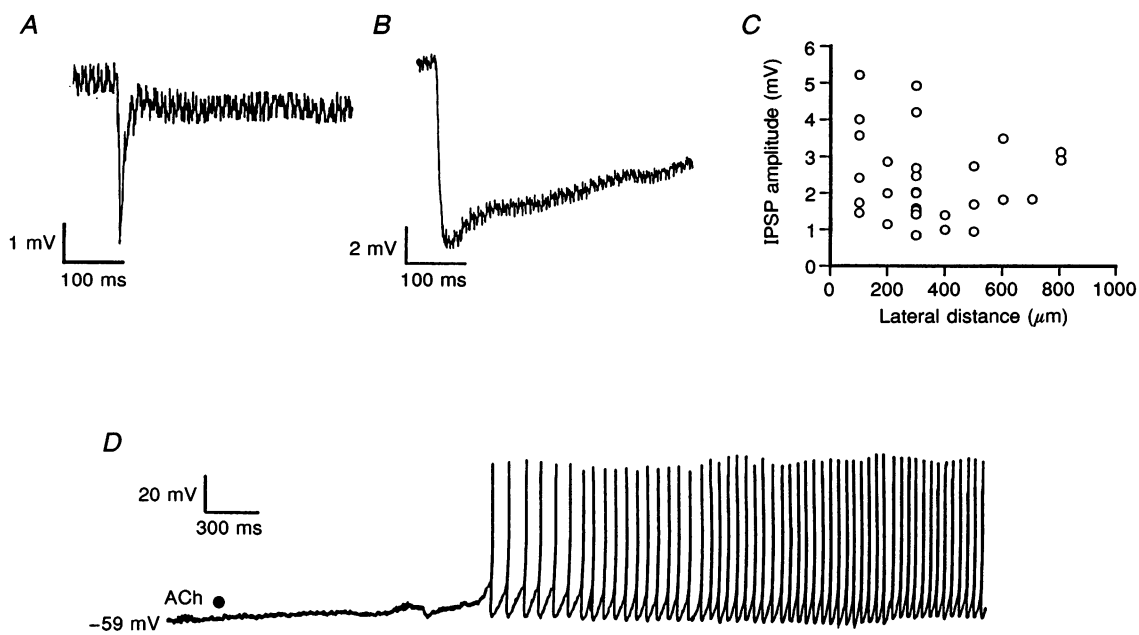

Figure 1. Pyramidal cell responses to acetylcholine (ACh)

A, an ACh-evoked IPSP from a layer V regular spiking (RS) cell. The IPSP shown here is similar to those observed in all cell classes. *B*, an apparently biphasic IPSP recorded in a layer II/III RS pyramidal neuron, evoked from a lateral distance of 300 μm. Of all trials made, only 2 IPSPs with late, slow components were seen in 2 layer II/III pyramidal neurons. Biphasic IPSPs were not seen in layer V cells. *C*, IPSP amplitude *versus* horizontal distance of trial for 25 layer V RS cells. There was no relationship between the amplitude of ACh-evoked IPSPs and the lateral distance of ACh ejection ($r^2 = 0{\cdot}118$; $P = 0{\cdot}08$). *D*, direct effect of applying ACh (●) to a layer II/III pyramidal neuron.

Table 1. ACh-evoked IPSPs in neocortical pyramidal neurons

	R_{in} (MΩ)	Number of trials at each distance	IPSP amplitude (mV)	D (μm)	Mean R/T up to D	Median R/T (range) (up to 1000 μm)
Layer II/III RS	47 ± 17 (30)	39 ± 19	3·5 ± 2·8 (26)	500	0·131 ± 0·136	0·020 (0·370)
Layer V RS	83 ± 46 (25)	64 ± 37	2·4 ± 1·2 (31)	800	0·066 ± 0·077	0·024 (0·233)
Layer V IB	47 ± 28 (17)	54 ± 12	2·1 ± 1·1 (10)	500	0·043 ± 0·032	0·007 (0·090)

Responses of regular spiking (RS) and burst-firing (IB) pyramidal neurons to focal ejections of acetylcholine (ACh). Each ejection of ACh made at a separate location/depth in the slice was called a 'trial' and the occurrence of inhibitory postsynaptic potentials (IPSPs) in response to ACh excitation of presynaptic inhibitory neurons was expressed in terms of 'responses per trial' (R/T). R/T was signifcantly lower in layer V IB neurons than in other cell classes. In addition, IPSPs in layer V RS neurons could be achieved even when ACh was ejected 800 μm laterally to the postsynaptic cell (see Fig. 2). R_{in}, input resistance; D, maximum horizontal distance of response. Values are means ± S.D. Numbers in parentheses in R_{in} and IPSP amplitude columns are the number of cells (n) and number of IPSPs, respectively.

spontaneous IPSPs and in three others where it was possible to conduct limited ACh trials, IPSPs as in Fig. 1*A* were observed, suggesting direct functional interactions between inhibitory neurons (Deuchars & Thomson, 1995). Direct application of ACh on two FS neurons resulted in depolarization and subsequent action potential firing, similar to that encountered in pyramidal neurons.

Spontaneous and evoked IPSPs

Spontaneous activity was analysed in eight layer V pyramidal neurons (input resistance, 118 ± 30 MΩ; mean ± S.D.) at membrane potentials of ~−60 mV in the presence of glutamate receptor antagonists. IPSPs showed a wide range of amplitudes from 0·5 to 19 mV (Fig. 3*B*). In three cells, IPSPs reversed at ~−30 mV and could be blocked by bath application of 50 μM picrotoxin, consistent with $GABA_A$-mediated synaptic transmission. Even within the same cell, IPSP 10–90% rise times also displayed a wide range of values (1–40 ms; Fig. 3*C*), suggesting that the inhibitory synapses were distributed across different dendritic sites. IPSP peak amplitude was not correlated with rise time (Fig. 3*D*), suggesting that there was little relationship

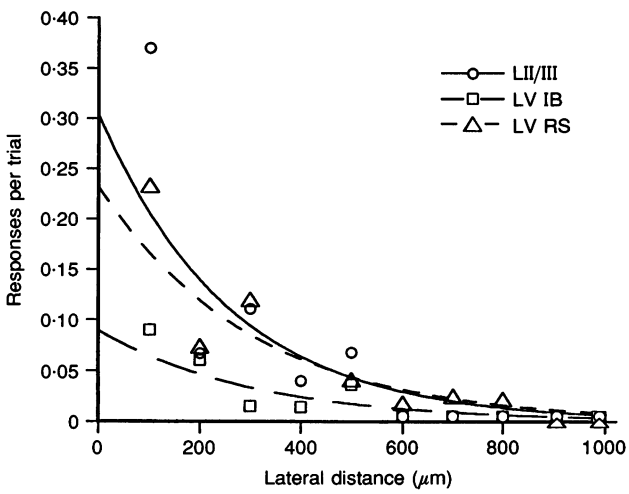

Figure 2. Relationship between the frequency of occurrence of IPSPs in pyramidal cells and the separation of the stimulus

Pyramidal neurons in layers (L) II/III and V were classified as regular spiking (RS) or intrinsically burst-firing (IB), and acetylcholine was ejected in the same layer as the postsynaptic cell. IPSP occurrence was measured in terms of IPSPs observed (responses) per separate ejections of acetylcholine made (trials). For all cell classes, levels of inhibition declined exponentially ($r = 0·84$–$0·90$) as the stimulation site was moved away from the postsynaptic cell (one outlying point removed). At all distances, there was markedly less inhibition associated with burst-firing neurons than with the other 2 cell classes. In addition, inhibitory responses could be evoked in layer V RS cells from longer horizontal distances than for other cell types. Thus, pyramidal–inhibitory cell interactions vary according to cortical layer and neuronal class. (See also Table 1.)

between the size of the events and their likely electrotonic origin on the dendritic tree.

Picrotoxin-sensitive, proximal and distal IPSPs evoked by extracellular electrical stimulation were recorded with CsCl electrodes in nine layer V pyramidal neurons (Fig. 4). We concluded that it was possible to achieve distinguishably separate proximal and distal inhibitory synaptic inputs for several reasons. Stimulation of afferent fibres whose target was not substantially horizontal to the site of stimulation was reduced by the cut made in the slice between the proximal and distal 'pathways'. In addition, for four cells where it was possible to reverse the proximal IPSP by injecting continuous depolarizing current, the distal IPSP never reversed or reversed at much more depolarizing potentials (Fig. 4*B*). This was a strong indication that the distal input was, indeed, further from the site of current injection (the soma) than the proximal one. Furthermore, in two cases, initial stimulation resulted in a depolarizing proximal IPSP and a hyperpolarizing distal IPSP, which took some minutes to reverse polarity following the onset of recording (data not shown). This appeared to be a result of the slower washing in of CsCl in these two cells and hence the initial lack of chloride further away from the cell body. Lastly, the 10–90% rise times of the majority of distal IPSPs were generally slower than proximal ones, although this effect failed to be significant overall (Table 2). Taken together, these results suggest that $GABA_A$-mediated IPSPs can be evoked in the apical dendrites of neocortical pyramidal neurons.

DISCUSSION

Effect of ACh on pyramidal neurons

By blocking excitatory glutamatergic synaptic transmission, we were able to use ACh to excite interneurons and evoke IPSPs recorded in pyramidal neurons (McCormick & Prince, 1986). IPSP characteristics and the pattern of inhibitory–pyramidal neuron interaction varied with cortical layer.

There was no correlation between IPSP amplitude and the distance of ACh ejection, a finding consistent with stimulation of somata and/or dendrites of inhibitory

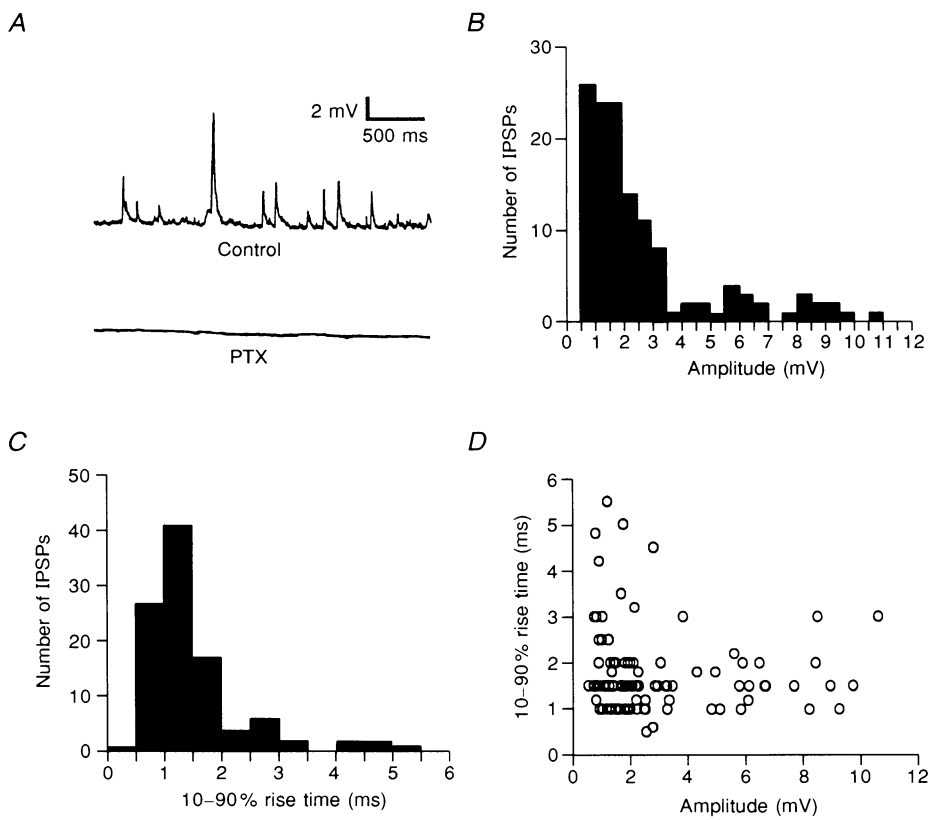

Figure 3. Typical characteristics of spontaneous IPSPs recorded in one layer V pyramidal neuron

All results are from the same cell. *A*, records showing spontaneous IPSPs in control artificial cerebrospinal fluid and in artificial cerebrospinal fluid containing 50 μM picrotoxin (PTX). *B*, distribution of spontaneous IPSP amplitudes at −73 mV, showing the wide range of amplitudes recorded. *C*, distribution of 10–90% rise time of spontaneous IPSPs at −73 mV (one outlying point excluded). *D*, relationship between spontaneous IPSP amplitude and 10–90% rise time for this cell. Although there was no correlation between the two parameters in this or any other cell, the largest IPSPs all tended to have relatively fast rise times, consistent with their being proximally placed; the longest rise times, on the other hand, were all among the smallest amplitudes.

Table 2. Proximal and distal IPSPs in layer V pyramidal cells

	Amplitude (mV)	10–90% rise time (ms)
Proximal	1·1 ± 0·4	10·0 ± 7·80
Distal	0·8 ± 0·4	14·5 ± 8·44
P	< 0·05	n.s.

The amplitudes (means ± S.D.) and 10–90% rise times of 9 pairs of IPSPs recorded from the same number of layer V pyramidal neurons are shown. Most distally evoked IPSPs displayed slower rise times than proximally evoked ones, as would be expected from passive neuronal models. However, the difference in rise times overall was not significant (n.s.). Significance was determined using Student's paired *t* tests.

neurons rather than their axons. In layer II/III, there were two cases where a biphasic response was recorded, i.e. an early fast IPSP followed by a slow, late IPSP. These responses were reminiscent of $GABA_A$- and $GABA_B$-mediated events, respectively, although this cannot be stated with certainty without pharmacological testing. Benardo (1994) has also examined GABA-mediated responses of neocortical pyramidal neurons to focal ACh application and showed that most layer V pyramidal cells displayed only $GABA_A$ IPSPs, but a small number exhibited both $GABA_A$- and $GABA_B$-mediated responses. Similar results have been reported using electrical stimulation in cat motor cortex (van Brederode & Spain, 1995). In neither the present nor the above studies were slow, late, putative $GABA_B$ events seen in isolation; it is not known whether this is a result of non-specific stimulation or because pure $GABA_B$-utilizing cells do not exist. In only one study in the hippocampus, Williams & Lacaille (1992) were able to evoke isolated, slow, late IPSPs using glutamate pressure ejection, but there were differences between those responses and the ones evoked by electrical stimulation. The fact that IPSPs could be evoked in inhibitory cells by ACh confirms the likelihood of specific connections between inhibitory cells in neocortex. Very little is known about the nature of synaptic connections between inhibitory neurons but a relatively high degree of connectivity has been proposed in the hippocampus (Whittington, Traub & Jefferys, 1995). In an *in vitro* study of human cerebral cortex, it has been observed that there is an apparent increase in the amplitude of $GABA_B$-mediated IPSPs after $GABA_A$ synaptic transmission was blocked (McCormick, 1989). In that case, inhibition of $GABA_B$ inhibitory neurons by separate $GABA_A$-utilizing cells would explain the observations.

Here, as in the hippocampus, connection probabilities between inhibitory and pyramidal cells appear to be low (Miles, 1990; Deuchars & Thomson, 1995). Over a horizontal

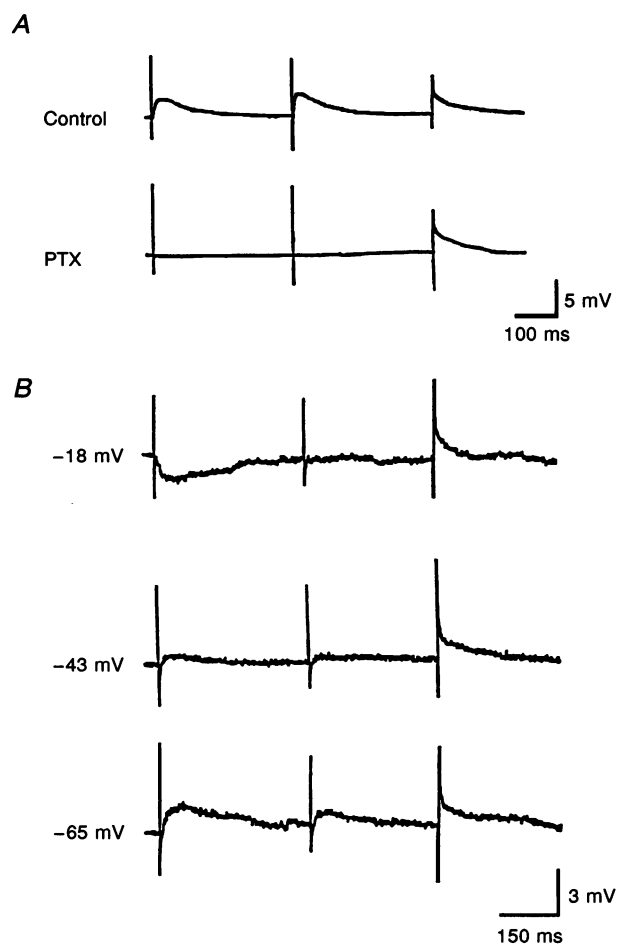

Figure 4. Proximal and distal electrically evoked IPSPs in layer V pyramidal neurons

A, averaged voltage records in a layer V pyramidal neuron recorded with a Cs^+ pipette (R_{in} = 125 MΩ). The proximal (left responses) and distal (middle responses) IPSPs are shown interleaved with the membrane response to an injected short, somatic current pulse (right responses), both in control and in 50 μM picrotoxin (PTX). *B*, individual voltage records in a Cs^+-loaded layer V pyramidal neuron. On depolarizing the cell, the proximal IPSP was reversed but the distal IPSP was not, confirming that the two inputs were, in fact, separate.

distance of 0·8 mm, inhibitory responses could be evoked in pyramidal neurons from an average of about 5% of trials using focal ACh application. Overall, our results did not show any 'patchiness' in connectivities (Weliky, Kandler, Fitzpatrick & Katz, 1995) but the strength and occurrence of inhibitory inputs onto individual pyramidal neurons seems to vary according to cortical layer and cell class. Our results support those of Silva & Connors (1986) who found that inhibitory responses were more prevalent in layer II/III than in layer V of rat somatosensory cortex. On the other hand, Douglas & Martin (1991) suggested that inhibition, especially that mediated by $GABA_A$, may be stronger in the deeper layers of cat visual cortex. In contrast, morphological studies in rat visual cortex have demonstrated a substantially higher density of non-pyramidal neurons in superficial layers than in deep ones (e.g. Peters & Kara, 1985). Indeed, superficial neocortical layers may be more 'connective' generally, with a greater scope for intracortical amplification of afferent sensory data, than deeper layers (Nicoll & Blakemore, 1993). Whatever the significance of prevalent inhibitory connections onto superficial layer pyramidal neurons, our results clearly demonstrate the effect, with response probabilities being greatest for layer II/III cells at every lateral distance tested. Interestingly, Salin & Prince (1996) recently reported that levels of inhibition in superficial and deep layers of rat somatosensory cortex were similar. The differences in their results could be ascribed to the differences in the stimulus and analysis procedures employed.

Layer V RS neurons displayed inhibitory interactions extending over longer horizontal distances than other cell classes. Long-range inhibition has been shown previously in the visual cortex (McGuire, Gilbert, Rivlin & Wiesel, 1991), and perhaps the layer V lateral inhibitory connections shown morphologically by McDonald & Burkhalter (1993) provide the anatomical basis for such results. The lateral extent of inhibitory interactions was much shorter for layer V IB neurons, which also showed lower inhibitory response probabilities for every lateral distance investigated. In another study, Silva, Chagnac-Amitai & Connors (1988) also found that layer V IB cells appeared to display much less inhibition than RS pyramidal neurons. Hence, it has been postulated that layer V IB neurons form a strongly, yet sparsely, connected subnetwork of pyramidal cells which may be responsible for initiation of synchronous firing behaviour in the neocortex (Chagnac-Amitai & Connors, 1989*a*, *b*). Our results would be consistent with the notion of a unique position for IB neurons, reflected in the reduced efficacy of inhibitory inputs onto those cells, which would favour them in their role as synchronous oscillators (White, Amitai & Gutnick, 1994). However, the exact function of these horizontal connections in neocortical integration remains unclear and only further experiments will reveal the significance of these variations in inhibition between different cortical layers and cell classes.

As well as horizontal inhibitory connections, application of ACh applied 'vertically' to pyramidal cell bodies also resulted in inhibitory responses, some from trials several hundred micrometres away. Although present results are limited, one can speculate on the cellular substrate of these events. A likely candidate responsible for the IPSPs evoked along the 'axis' of the apical dendrite of pyramidal neurons is the double-bouquet type of inhibitory cell, since its axons collateralize vertically with respect to pyramidal neurons. On the other hand, basket cells would be more likely to mediate horizontal inhibition as the axon branching of these cells tends to be more laterally displaced (Somogyi *et al.* 1993).

Distal inhibitory synaptic responses

In this study, evidence has been provided to demonstrate that there are $GABA_A$ electrophysiological responses in the apical dendrites of neocortical pyramidal neurons. Apical inhibitory inputs presumably modify distal synaptic information, interacting with intrinsic membrane conductances present on pyramidal cell apical dendrites (Kim & Connors, 1993; Kim, Beierlein & Connors, 1995). The characteristics of the spontaneous IPSPs recorded here are similar to other studies (Luhmann & Prince, 1991), with many $GABA_A$-mediated events displaying a range of amplitudes. The amplitude of a synaptic potential is thought to depend on both pre- and postsynaptic factors, whereas the rise time depends only on postsynaptic factors (Jack, Redman & Wong, 1981). It might have been expected that events of larger amplitudes would display slower rise times, but we found no correlation between the rise times of the spontaneous IPSPs and their amplitude. Therefore, the variation in the rise times resulted only from differences in the electrotonic origins of the events recorded at the soma, regardless of their amplitude. Hence, slower IPSPs were presumably more distal than the faster ones (Jack, Redman & Wong, 1981).

GABA synapses are distributed throughout the somatodendritic structure of pyramidal neurons but most are found on dendrites (Beaulieu & Somogyi, 1990). Electrophysiological studies of hippocampal CA3 pyramidal neurons have suggested that $GABA_A$ responses could be prominent in the soma, whereas $GABA_B$-mediated events may be more prevalent in the dendrites (Miles *et al.* 1996). In contrast, using pressure ejections of GABA, Connors *et al.* (1988) observed $GABA_A$ responses from both apical and basal regions of neocortical pyramidal neurons, but $GABA_B$-mediated responses were predominantly somatic. This does not rule out the possibility that the inhibitory input to pyramidal cells is segregated, with a different class of inhibitory cell only synapsing with either type of GABA receptor (Müller & Misgeld, 1990; Segal, 1990; Otis & Mody, 1992; Benardo, 1994; Deuchars & Thomson, 1995); while some progress has been made in classifying inhibitory neocortical cells (Kawaguchi, 1995), this segregation has yet to be conclusively shown. Work in the hippocampus suggests

that there is a high degree of selectivity of inhibitory neuron–pyramidal cell interaction (Buhl *et al.* 1994) but even in that region, the functional roles of different inhibitory inputs are unknown.

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