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CHAPTER 21

GABA-mediated inhibition in the neural networks of visual cortex

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Introduction

The selective responses that are such a familiar feature of cortical neurons, have spurred many imaginative efforts to understand the machinery of neocortex. This program of research has, by any standards, been inordinately successful, particularly for the visual cortex (Hubel and Wiesel, 1977). Certainly more is known about the functional architecture of primary visual cortex, than for any other cortical area. In addition its extensive interconnections have been mapped (see reviews by Orban, 1984; Van Essen, 1985; Zeki and Shipp, 1988) and the component neurons have been studied in great anatomical detail (Lund, 1973; Szentágothai, 1973; Martin, 1984; Peters, 1987; Somogyi, 1989). Nevertheless, many issues remain unresolved.

Of all the components that make up the cortical machinery, the most difficult to place has been the GABA-mediated inhibitory system. That is not to say that inhibition is generally thought to be unimportant. On the contrary, inhibition has been used like leaven in bread for every model that requires selective responses from single neurons. In the visual cortex, for example, directionality is achieved by an inhibitory "veto" operation (Koch and Poggio, 1985), orientation selectivity by "cross-orientation" inhibition (Bishop et al., 1973), hypercomplexity or "end-stopping" by inhibitory end-zones (Hubel and Wiesel, 1965; Orban et al., 1979). The problem is that there is not a coherent or consistent view of the role of inhibition in producing this selectivity. Indeed, there are now so many anomalous findings concerning cortical inhibition, that it seems inevitable that the biologists working in this area should find themselves suffering from a bad case of cognitive dissonance.

Dissonant images of inhibition in visual cortex

In the visual cortex, dissonance has surfaced repeatedly, most notably about the issue of whether inhibition is involved in generating the property of orientation selectivity. Opposing positions have been succinctly and clearly stated. Sillito (1984) declared that "the inhibitory system is seen to be the architect of the orientation selectivity." Contrariwise, Ferster (1987) claimed "that orientation of cortical receptive fields is neither created nor sharpened by inhibition between neurons with different orientation preference." For the issues to be put so clearly, correctly implies that both have data to substantiate their own positions and explanations to account for the other's apparent misperceptions. Such dialectics are, of course, the stuff of science, but what they reveal in this instance, is not that the experimental designs are fatally flawed, nor that the results are incorrectly interpreted, nor that the critical experiments have yet to be carried out. Such fundamental disagreements about cortical inhibition occur, we believe,

because there has been a frank disconnection between the high-level explanations of the phenomena of selectivity, and considerations of the cellular mechanisms that are required to generate this selectivity.

In this chapter we set the issue of cortical inhibition in a different context, that of intra- and intercellular interactions. By doing so, we hoped to reconnect the phenomenology of the selective responses of single units to the rich database of structure and function of cortical neurons and microcircuits, thereby achieving some resolution of the incompatibilities in the experimental literature. At the outset it is worth re-stating three key sets of experimental observations, which are the prerequisites of our thesis. Firstly, cortical inhibition exists. It was found in the earliest single cell recordings from neocortex using intracellular (Phillips, 1959) and extracellular recording (Hubel and Wiesel, 1959). The pioneering pharmacological experiments of Sillito (1975) then established its importance in generating selective responses by demonstrating the deleterious effects of blocking $GABA_A$ receptors. Finally, the structural studies of neocortex, beginning with the Golgi studies of Ramón y Cajal (1911) and continuing with the contemporary technical wizardry of combining light and electron microscopy and immunocytochemistry (Jones, 1984; Somogyi and Freund, 1989; White, 1989), has revealed the rich diversity of the GABAergic components of the cortical circuits. Even taken individually, it is clear that from each of these key observations that there is something important to be understood about the nature and role of the inhibitory system in the neocortex.

GABA-mediated synaptic inhibition: mechanisms

In the neocortex there is no structural evidence for the pre-synaptic inhibitory mechanisms that were described by Eccles (1964) in the spinal cord. Therefore, we assume that cortical inhibition acts primarily through postsynaptic mechanisms (but see Nelson, 1991). The action of the

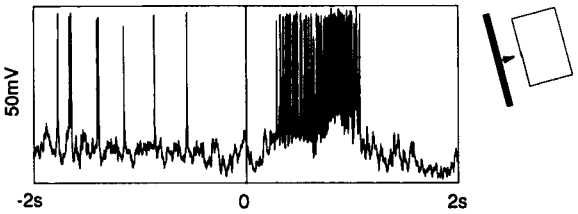


Fig. 1. Intracellular response of layer 3 pyramidal cell with a standard complex receptive field. Control period (−2 sec to 0 sec) is followed by 2 sec test period during which a dark bar appears and moves across the receptive field. (Douglas, Martin and Whitteridge, unpublished.)

inhibitory synapses is to produce an outward current that opposes the inward current produced by the excitatory synapses. In theoretical analyses of postsynaptic inhibition it has been convenient to divide the postynaptic effects into two types: hyperpolarizing inhibition and shunting inhibition. Both act by increasing the permeability or conductance of the membrane to ions whose reversal potentials are near (shunting), or more negative (hyperpolarizing) than the resting membrane potential. This distinction between shunting and hyperpolarizing inhibition is to some extent artificial, particularly in whole animal recordings where the concept of a "resting" membrane potential has to be considerably stretched to be an accurate description of the data (Fig. 1).

Hyperpolarizing inhibition drives an outward current across the membrane by inducing a small increase in conductance to potassium. The magnitude of the current produced depends on the voltage difference between the membrane potential (about −50 to −70 mV, at "rest") and the potassium reversal potential (−90 mV). Shunting inhibition also acts by producing an outward current. In this case the reversal potential of chloride (−70 mV) is closer to the "resting" membrane potential and so the outward current is driven by a relatively small potential difference. The magnitude of the outward current now depends largely on the size of the conductance increase for chloride.

During simultaneous activation of both excitatory and inhibitory synapses, the net inward cur-

rent arriving at the axon hillock will determine whether the membrane reaches threshold. If the net current is sufficient to produce a suprathreshold depolarization, the magnitude of the excitatory current will determine the instantaneous rate at which the neuron will discharge. Theoretical analyses of cortical inhibition usually consider only the inhibitory effects on the sub-threshold membrane potential (Rall, 1964; Blomfield, 1974; Jack et al., 1975; Koch and Poggio, 1985). While it is mathematically convenient to consider only the subthreshold membrane potential, experimentally the effects of inhibition during natural visual stimulation have been assessed by measuring the changes in action potential discharge. The key is then to understand the effects of inhibition on the discharging neuron.

Inhibitory control of cortical pyramidal neurons

We have approached this problem by a combination of computer simulation and experimental data. We developed a general program for simulating neuronal networks (CANON) that permits neurons to be specified as sets of interconnected compartments (Douglas and Martin, 1991a,b). The pyramidal cell, which is really the workhorse of the cortex, was the obvious focus for our analysis. The pyramidal cells used in the simulations were also chosen to straddle the limits of the morphological types seen in the visual cortex. From our catalogue of neurons, recorded intracellularly in vivo and injected with horseradish peroxidase, we selected two. One neuron was from layer 2, because these are the smallest pyra-

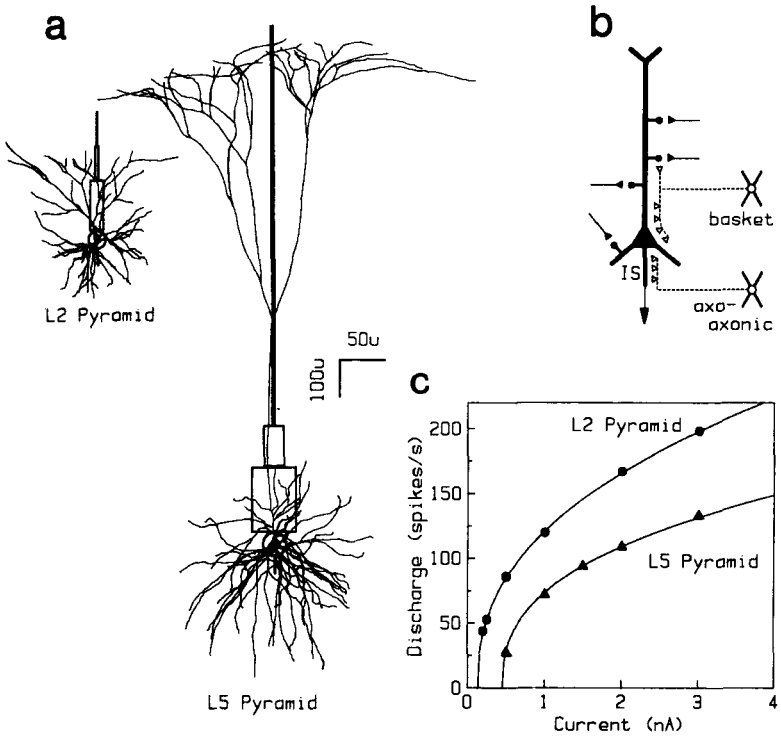


Fig. 2. Simplified compartmental models of cortical neurons. A. Reconstructed cortical pyramidal neurons (dendrites only) from layers 2 and 5. Idealized equivalent cylinder model cells used for simulations are superimposed. Scale bars: 100 μm, reconstructed neurons and vertical axis of model neurons; 50 μm, horizontal axis of model neurons only. B. Schematic of inhibitory inputs from basket and chandelier (axoaconic) cells to a typical pyramidal cell (filled shape). C. Current-discharge curves for the first interspike interval of the model cells shown in B. (See Douglas and Martin, 1990b.)

midal cells found in the cortex, the other neuron was from layer 5, which contains the largest pyramidal cells. The detailed structure of the dendritic arbour and soma was reconstructed in 3-D and then transformed into a simplified compartmental neuron (Fig. 2), which had the same input resistance and time constant as the original neuron recorded in vivo. The method for the simplification is described elsewhere (Douglas and Martin, 1990b; 1991a,b).

The pyramidal cells receive their inhibitory input from a number of sources (see Somogyi, 1989), but for our simulations we considered just two types of inhibitory neurons. One type, the chandelier, or axo-axonic cell, is a specialized GABAergic neuron that forms synapses exclusively on the axon initial segment of the pyramidal cell (Somogyi et al., 1982). Superficial layer pyramidal cells receive about 20–40 synapses from about 5 chandelier cells; deep layer pyramidal cells receive many fewer synapses (Freund et al. 1983; Fariñas and DeFelipe, 1990a,b). The axon initial-segment is a particularly sensitive site, since it is the region where the action potential is initiated, and thus the chandelier cell seems to offer a potent means of inhibiting the pyramidal cell output (for review, see Peters, 1984).

The other type we considered was the basket cell, which is the GABAergic cell most frequently encountered in both Golgi preparations (Ramón y Cajal, 1911) and in our intracellular recording from identified cells (Martin et al., 1983; see Martin, 1988). The basket cells form 85% of their synapses on the soma and dendrites of pramidal cells (Somogyi et al., 1983). Each pyramidal cell seems to receive a convergent input from 10 to 20 basket cells, each contributing about 5–10 synapses to each pyramid. These numbers are estimates based on small samples and there are certainly single instances where a single basket cell may provide many more synapses to an individual pyramidal cell (Kisvárday et al., 1987). However, they provide some limits for the parameters for the simulations.

Unitary synaptic conductances have not been measured for the synapses of identified chandelier or basket cells, so for the simulations we used a range of conductance values. For the soma and proximal dendrites the inhibitory conductances ranged between 0.1 and 1.0 $mS \cdot cm^{-2}$. For the axon initial segment they ranged between 1–10 $mS \cdot cm^{-2}$, because the initial segment has a higher density of GABAergic synapses. In the limiting case, each synapse produced a maximum conductance of about 0.3 nS, which is in excess of that suggested by recent patch-clamp studies (Kriegstein and LoTurco, 1990; Verdoorn et al., 1990). In the simulations, the average inhibitory conductance was held constant over the period of the excitatory current injection. This was done to approximate the sustained inhibition that would be required to prevent a response to non-optimal visual stimulation in vivo (Douglas et al., 1988).

The response of the model neurons to a step of excitatory current injected into the soma is shown in Fig. 3. These responses were essentially indistinguishable from those of pyramidal cells recorded in vivo or in vitro. In this study we were only concerned with the peak frequency of the action potential discharge, before the conductances associated with spike adaptation took effect and slowed the discharge rate (Berman et al., 1989a; Douglas and Martin, 1991b). If the first interspike interval was plotted as a function of excitatory current, then the data points were well-fit with a power function (Fig. 2C). The difference between the superficial and the deep layer pyramid is explained by the decreased current load offered by the smaller dendritic arbour of the layer 2 pyramidal cell.

It seems obvious that an excitatory current just sufficient to produce a suprathreshold depolarization of the membrane could be offset by an inhibitory current, which would then keep the membrane potential below threshold and prevent action potentials from being produced. A large excitatory current, however, would overcome the inhibition and produce an action potential discharge. But the frequency of discharge would be reduced in the face of inhibition, because part of

the excitatory current would be used to offset the inhibitory current. Inhibition would seem to have two effects. One is to raise the current threshold for action potential discharge. The second is to reduce the peak discharge rate. Both effects have been predicted in a formal model using idealized neurons (Blomfield, 1974) and apparently confirmed experimentally (Rose, 1977; Burr et al., 1981).

Our simulations confirmed that the principal effect of both types of inhibition, hyperpolarizing and shunting, was to increase the current threshold before action potentials were produced. Once threshold was reached, however, the increase in the frequency of firing for a given increase in excitatory current was greater for the inhibited

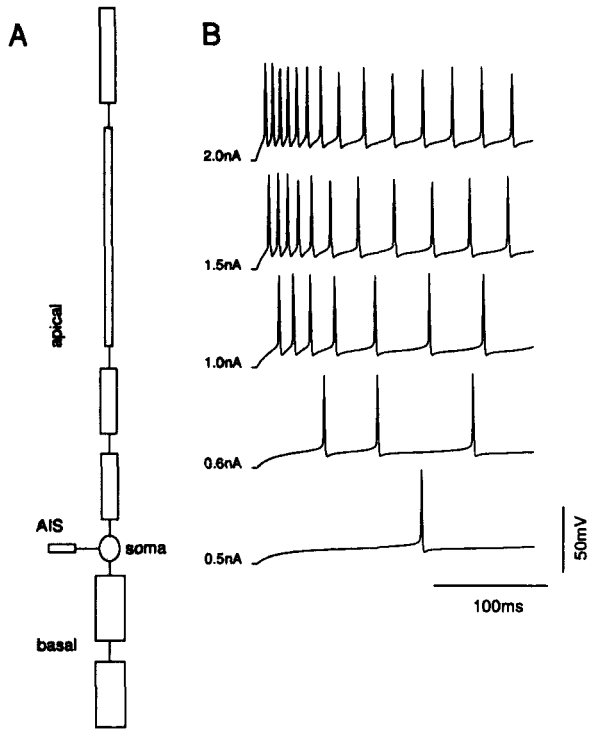


Fig. 3. Model pyramidal cell and its simulated response to excitatory current steps. A. Compartmental model of HRP-filled layer 5 neuron shown in Fig. 13A. Each compartment is allocated appropriate profiles of passive and active conductances based on biophysical data from the recorded cell itself and the literature. B. Response of model cell to simulated depolarizing current step injection. (Details in Douglas and Martin, 1991a,b.)

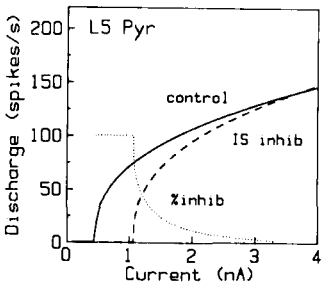


Fig. 4. Simulated effect of axoaxonic cell inhibition on current-discharge relationship of model layer 5 pyramidal neuron (see Fig. 2.). Fitted power curves of current-discharge shown before (continuous line, control) and after (dashed line) initial segment inhibition (5 $mS \cdot cm^{-2}$; E_{rev} −80 mV). Difference between the control and inhibited expressed as percent inhibition (dotted line). (See Douglas and Martin, 1990b.)

than for the non-inhibited case (Fig. 4). This effect was related to the magnitude of the conductance change, and was more marked for shunting than for hyperpolarizing inhibition. For large excitatory currents there was little difference in the discharge rates for the inhibited and non-inhibited case. If the effect of inhibition is expressed as the percentage of the difference between firing rate of the control and the inhibited case ("% inhib" curve in Fig. 4), then it is immediately evident that the effectiveness of the inhibition decreases with increasing excitatory current. Experimentally, sometimes the peak firing rate during inhibition was higher than control, sometimes a little lower, as illustrated in Figs. 5 and 7.

The pattern was not dependent on the site of the inhibition. The same result was obtained regardless of whether the inhibition was applied by chandelier cells (to the axon initial segment), or basket cells (to the soma), or both together. On a synapse for synapse basis, chandelier inhibition was more efficient than the inhibition applied to the soma by basket cells, simply because the density of inhibitory synapses was so much higher on the initial segment than on the soma (Fariñas and DeFelipe, 1990a,b). The lack of obvious functional differences between the chandelier and the basket cells in the simulation, despite the differences in their postsynaptic targeting, raises the

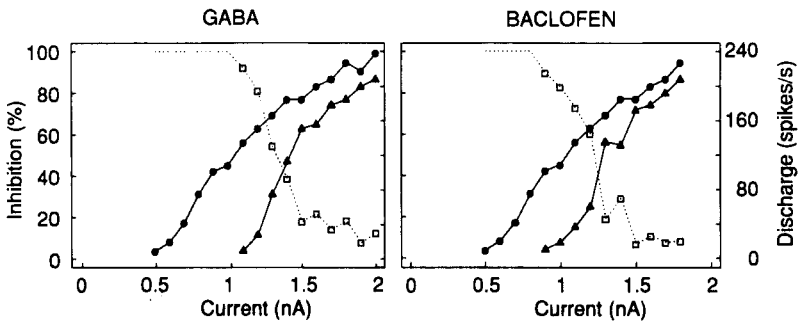


Fig. 5. Inhibition of discharge in a layer 2/3 neuron in rat visual cortex in vitro. Current-discharge curves (right ordinate) for the 1st interspike interval were obtained from current step injections before (closed circles) and during (closed triangles) iontophoretic applications of GABA (19 nA, left graph) and baclofen (20 nA, right graph) to the same neuron. Both GABA and baclofen increased the conductance of the cell by 21% (5 nS) and 74% (18 nS), respectively. The effectiveness of the inhibition is expressed as percentage inhibition (open squares, left ordinate). (Berman, N.J., Douglas, R.J. and Martin, K.A.C., unpublished.)

possibility that the synapses on the initial segment do not act by the conventional mechanisms we modelled.

The simulations showed that there was little difference between inhibition applied by hyperpolarizing or shunting mechanisms. Both increased the current threshold and both had little effect on the instantaneous frequency of firing for large excitatory currents, if only the first action potentials were considered. This result was to us unexpected, but in retrospect the explanation was blindingly simple. The maximum sodium and potassium spike conductances are about 10 times larger than the synaptic and adaptation conductances. Thus, while inhibition tends to prevent the activation of the action-potential current, it does not have much effect on the trajectory of the action potential once it has been initiated, because this phase is dominated by the spike conductances. During the interspike interval the membrane is once more strongly influenced by the synaptic and adaptive conductances. When the discharge rate increases in response to stronger excitatory currents, the spike conductances (particularly the delayed rectifying potassium conductance) do not relax completely. Thus, for strong excitatory currents, the spike conductances dominate at all phases, including the interspike interval, and drive the current-discharge relation into saturation. The theoretical analysis by Blomfield (1974) did not take into account the active action potential conductances and thus he did not predict the reduced effectiveness of inhibition for strong excitatory currents. In the light of our analysis, the apparent experimental support (Rose, 1977; Burr et al., 1981) for the Blomfield theory will need to be reinterpreted.

Our simulation showed that if sufficient excitatory current can be delivered to the axon hillock, the neuron will always be able to fire at near peak discharge rates. The "sufficient current" depends on the input resistance and the membrane time-constant of the neuron. The activity of the GABA synapses reduces the membrane time-constant by increasing the membrane conductance. The shorter time-constant permits the membrane to recharge more rapidly after each action potential, so the neuron fires at a higher rate for a given current. This explains the increased slope of the current-discharge curve once the excitatory current is suprathreshold. Theoretically, the peak discharge rate could be marginally higher in the presence of GABA than in the non-inhibited case, but at high input currents the spike discharge saturates.

If the chandelier and basket cells were required to inhibit the neuron completely via the mechanisms we have modelled, they could approximate complete blockade only by forcing the threshold to the upper end of the operational

range of excitatory current (approx. 1.5–2.0 nA). The conductance required to produce such an enormous increase in threshold would have to be of the same size as the spike conductances. Even so, if the excitatory current did exceed threshold during the period of inhibition, there would be a catastrophic failure of inhibition: the neuron would respond immediately at a high discharge rate. Most models of cortical selectivity unsuspectingly run the gauntlet between complete suppression of maximal excitation and a catastrophic break-through discharge. In these models, the absence of response to a stimulus arises through inhibition of a strong excitatory discharge. Our theoretical work shows that strong excitatory transients are very difficult to suppress, unless the magnitude of the inhibition is very large indeed. We have tested our hypothesis experimentally, and the results will be discussed below.

GABA-mediated post-synaptic inhibition: in the dish

The simulations emphasised that the difficulty of GABA-mediated postsynaptic inhibition to contain strong excitation was not dependent on the location of the inhibitory synapses. This allowed us to test our theory by iontophoresing GABA directly onto the postsynaptic membrane of cortical neurons to assess its effect on the current-discharge relationship. The experiments were performed on slices of rat visual cortex, maintained in vitro (Berman, N.J., Douglas, R.J. and Martin, K.A.C., unpublished). Neurons in various layers were impaled and excited by injections of current through the recording pipette. Most of the neurons responded to a step of constant current by discharging initially at a high rate and then rapidly adapting. This pattern is typical of pyramidal neurons (Connors et al., 1982; McCormick, 1990). When GABA was then iontophoresed onto the neurons, the input conductance of the neuron increased and the membrane potential showed a complex sequence of hyperpolarization and depolarization before settling to a constant value. The change in the current-discharge curve was as our theory predicted (Fig. 5). The current threshold was raised, but once the threshold was crossed, the neurons rapidly reached near-control rates of firing. This effect is reflected in the rapid decrease of the percent inhibition curve. Very similar results were obtained if the $GABA_B$ receptor agonist, baclofen, was iontophoresed onto the same neurons (Fig. 5).

Our results using baclofen agree well with a similar study by Connors et al. (1988). They also found that iontophoresis of baclofen increased the current threshold, steepened the initial slope of the current-discharge curve, but had little effect on the discharge rates produced by large currents. The increased threshold is explained by the additional outward currents, which result from activation of the $GABA_B$ conductances, and which offset the inward excitatory currents. The steepened slope of the current-discharge curve is due to the reduction in the membrane time-constant induced by the $GABA_B$ agonist.

Our results using GABA, which of course acts on both the $GABA_A$ and the $GABA_B$ receptors, appear to contradict those of Connors et al. (1988), who were unable to elicit two sequential spikes during GABA iontophoresis. The difference between their results and ours is simply that we carefully titrated the GABA iontophoresis so that the increase in the current threshold and input conductance remained within physiological ranges. With larger iontophoretic currents it was possible to prevent the membrane from reaching threshold with the maximum excitatory currents used here (3 nA). Similar results have been observed in extracellular recordings that have used iontophoretically-applied GABA to inhibit visual responses (Rose, 1977; Hess and Murata, 1974). However, the membrane conductance was not measured in those studies. In our in vitro study, blocking discharge was only achieved by applying amounts of GABA that drove the membrane conductance to levels far in excess of anything seen during the peak of an electrically-evoked IPSP, or during visually-evoked inhibition (Ber-

man et al., 1989b, 1991). The fact that conductance changes outside the normal physiological range can be obtained by iontophoresis, does raise the further important issue of what receptors are actually being activated during the extracellular GABA application.

With the development of specific antibodies to subunits of the GABA receptor, it has now become possible to map out the distribution of the GABA receptor at the subcellular level. This has been done for the cortical pyramidal cells using antibodies directed against the alpha-subunit of the benzodiazepine/$GABA_A$/chloride channel complex (Somogyi, 1989). The results were clear-cut, but rather disturbing for physiologists, because they showed that there were a large number of extrasynaptic sites that were immunoreactive. If the immunoreactivity does indeed indicate the sites of active receptors, the observation raises the possibility that many of the receptors activated during iontophoretic application of GABA are not those involved in normal synaptic transmission (but see Somogyi, 1989). In fact it seems conceivable that the receptors lying beneath the synaptic boutons may be relatively protected from the GABA delivered by extracellular application. Thus, the results of iontophoretic experiments, including our own, should be interpreted with a degree of caution. They may not give much insight into the mechanisms that operate during normal function.

In view of the ambiguity in the interpretation of these results, we took the analysis one step further and studied the effects of synaptically-applied neurotransmitter. We stimulated the white matter underlying the cortex to produce IPSPs in the recorded neurons. We could then show that excitatory currents delivered through the recording pipette could break through a synaptically-evoked IPSP to produce a discharge (Fig. 6). Using this protocol we were not able to construct a current-discharge curve because the inhibition was not in steady state, but varied both in the magnitude of the conductance and in the membrane potential over the duration of the IPSP.

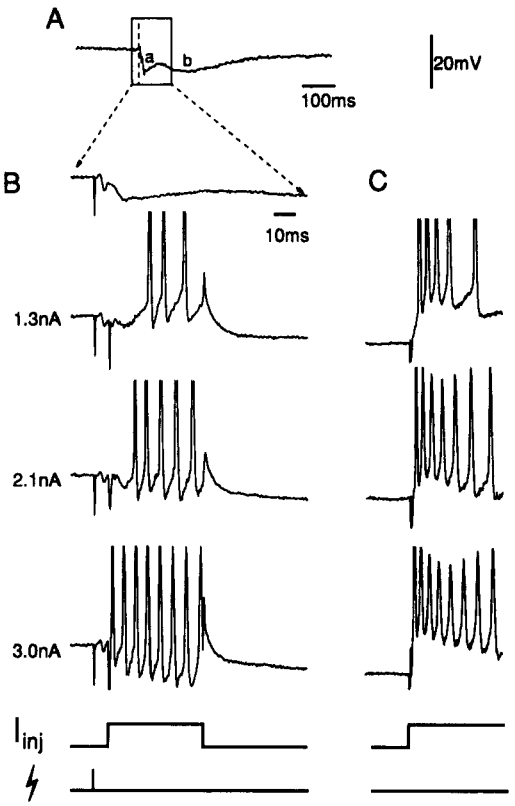


Fig. 6. Breakthrough discharge during synaptically evoked IPSP. A. Typical response of layer 5 neuron in rat visual cortex in vitro to electrical stimulation of white matter (vertical dashed line). Short duration EPSPs are followed by short $GABA_A$ (a) and long $GABA_B$ (b) IPSPs. B. Region dominated by $GABA_A$ IPSP in A shown on expanded timebase in first trace. Subsequent traces show the response to injection of current steps (I_{inj}) timed to coincide with the $GABA_A$ IPSP (8–50 msec) following white matter stimulation (bolt). C. Control responses to identical current injections without white matter stimulation. (Berman, N.J., Douglas, R.J. and Martin, K.A.C., unpublished.)

Nevertheless, in the face of strong transient excitation, the inhibitory synaptic mechanisms failed to prevent the excitatory current from reaching threshold and producing action potentials. This observation clearly raises problems for any model that requires inhibition to quench strong excitation.

We should emphasize that these experiments, like most laboratory models, are concerned with the limits of the behaviour of the system, not the normal dynamics. Although we have kept the

discharge rates within physiological ranges, it is clear that simply looking at the first interspike interval of a current-discharge curve gives only one part of the picture. The same neurons in vivo are required to respond to a stimulus for durations of hundreds of milliseconds, even seconds. While inhibitory control of the transient excitatory response is clearly necessary, inhibition is also required to act on sustained discharges. We have thus examined in some detail the action of GABA on neurons in the adapted state.

Adaptation of discharge is achieved by turning on intrinsic potassium conductances, which produce outward currents (Connors et al., 1982; Rudy, 1988; Schwindt et al., 1988a,b; Berman et al., 1989a; Douglas and Martin, 1990a; McCormick, 1990). These intrinsic adaptive currents act in concert with the synaptically-induced inhibitory currents to oppose the excitatory inward currents, which themselves arise both from intrinsic membrane currents and synaptic currents. In the adapted phase of the current-discharge curve, the response is dominated by the intrinsic adaptive potassium conductances (Schwindt et al., 1988a,b; McCormick, 1990). Under these conditions the effect of GABA is simply to shunt some excitatory current. This will displace the adapted current-discharge curve to the right on the current axis. The magnitude of this displacement will depend on the relative state of adaptation and the magnitude of GABA-mediated conductances. We have found in vitro that the iontopheretic GABA-mediated inhibition is still unable to prevent strong excitatory currents from reaching threshold and producing a discharge, despite the assistance of the adaptive conductances (Fig. 7). This is a remarkable finding that forces us to re-examine the whole basis of response selectivity meditated by GABA in the visual cortex.

Can GABAergic neurons inhibit visual responses?

Generations of physiologists have recorded the extracellular responses of single neurons in the visual cortex. These recordings have indicated that the firing frequency of cortical neurons in response to optimal stimuli can be in excess of

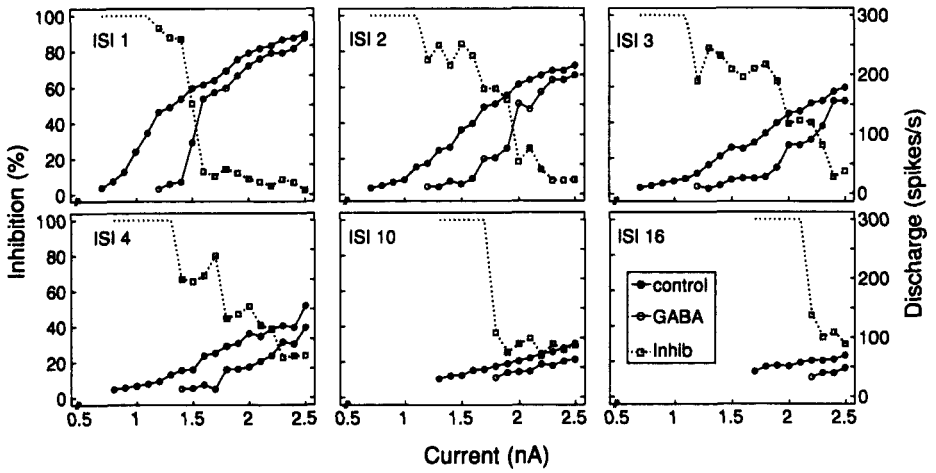


Fig. 7. Adaptation and inhibition of discharge in a layer 2/3 neuron in rat visual cortex in vitro. Current-discharge curves and percent inhibition plotted as for Fig. 5. Each graph shows the current-discharge curve before (closed circles) and during GABA application (open circles), and net inhibitory effect (open circles) for the interspike interval (ISI) indicated. GABA was iontophoresed in the vicinity of the soma. Full adaptation is reached by ISI 10. Control current threshold was 0.6 nA (note abscissa begins at 0.5 nA). Data was obtained after the GABA-induced conductance increase (38%, 9 nS) had stabilized (Scharfman and Sarvey, 1987). (Berman, N.J., Douglas, R.J. and Martin, K.A.C., unpublished.)

100 spikes/sec (Orban, 1984). Comparison of these responses with the current-discharge curves from similar neurons in vitro, gives some estimate of the magnitude of the excitatory current involved. Assuming the in vitro data is directly applicable to the in vivo situation, it seems unavoidable that at least 1–2 nA of excitatory current would be required to produce a reasonable discharge rate if the neuron was in the adapted state. Many of the proposed models for cortical selectivity require the inhibitory mechanisms to oppose this amount of excitatory current. For example, there are a number of models for selectivity of simple cells in layer 4 of visual cortex (Heggelund, 1981a; Wiesel and Gilbert, 1983) that use the same basic principles to achieve their effect: excitation is provided by thalamic afferents and inhibition via inhibitory interneurons, which themselves are excited by thalamic afferents or intracortical excitatory pathways.

The most transparent realization of these principles is Heggelund's circuit for the simple cell, illustrated in Fig. 8. We have pointed out previously that this circuit bears close resemblances to the circuit suggested by Barlow and Levick (1965) to account for directional selectivity in rabbit retinal ganglion cells, and later transplanted to the visual cortex by Barlow (1981). Here, as in most models of cortical selectivity, inhibition operates as negative excitation. That is, the purpose of the inhibition is to cancel out inappropriate excitatory responses. Using this inhibition, the concentric centre-surround fields of the thalamic afferents can thus be chiselled, filed, and smoothed into receptive fields of any desired size, shape, or texture (see Martin, 1988).

For the Barlow-Levick model, the spatial offset of the sets of excitatory afferents to the inhibitory neuron and the delay inserted by the additional synapses in the inhibitory circuit ensures that inhibition arrives after the direct excitation in the optimal direction of motion. In the non-preferred direction, excitation and inhibition arrive simultaneously and cancel. There is convincing evidence from a number of single unit

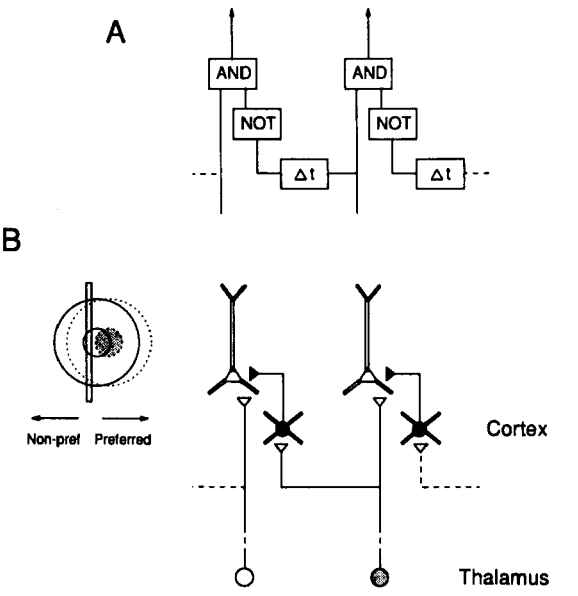


Fig. 8. Barlow and Levick (1965) model of direction selectivity. A. Two non-directional receptors (e.g., thalamic relay cells) are connected to a logical AND-NOT gate, one via a delay, Δt. B. Barlow and Levick (1965) model used to explain how the direction selectivity of cortical cells is derived from the non-directional thalamic input. The spatially displaced, circular symmetric receptive fields of thalamic neurons are shown on the left (shaded cell responds to shaded field). Pyramidal neurons receive direct excitation from thalamic neurons, and a synaptically delayed input from inhibitory cells (filled) in cortex.

studies that such a sequence of excitation and inhibition occurs (Bishop et al., 1971; Emerson and Gerstein, 1977; Palmer and Davis, 1981; Bullier et al., 1982; Ganz and Felder, 1984). Strong support for the circuit also arises from pharmacological studies in which directionality is lost when the action of inhibitory interneurons are impaired by blocking the $GABA_A$ receptors using bicuculline (Sillito, 1975). Further support arrives in the form of intracellular recordings, in which a sequence of depolarization followed by hyperpolarization was elicited when the stimulus moved in the optimal direction (Douglas et al., 1991).

Missing from these analyses of the directional response is a quantitative estimate of the relative magnitude of excitation and inhibition. Implicit in the Barlow-Levick model is that the inhibition is strong enough to cancel the excitation in the

non-preferred direction, but this calculation has not been made from experimental data. However, the separation of excitation and inhibition in simple receptive fields permits a relatively straightforward calculation to be made of the relative magnitudes of inhibition and excitation. This estimate can even be made from extracellular recordings in which the depth of the inhibition can be judged by the degree to which it can suppress the spontaneous discharge of the neuron. The estimate can also be made using intracellular recordings where the actual degree of hyperpolarization of the membrane can be assessed. Both these measures are illustrated for the same simple cell (Fig. 9). The neuron was located in layer 4 and was shown by electrical stimulation to be excited monosynaptically by thalamic afferents. In this example (Fig. 9A) it is clear that the inhibition following the excitation for the optimal direction of motion was not sufficiently strong to reduce the spontaneous activity to zero. The excitatory response was at least twice the baseline spontaneous activity, i.e., excitation in this neuron produced more action potentials than the inhibition suppressed.

This difference in the magnitude of excitation vs inhibition is also evident in the intracellular records (Fig. 9B). The magnitude of the hyperpolarization, which results in inhibition of the action potential discharge, is small. The amount of outward current required to produce a 5 mV hyperpolarization is about 0.2 nA for a neuron with an input resistance of 25 MΩ. The excitatory current required to drive the neuron to threshold is at least this value. Thus additional excitatory current is required to produce the frequency of action potential discharge seen here. It seems that for this simple cell at least, the directional response cannot be explained by the cancelling of inhibition and excitation: the apparent magnitude of the inhibition is insufficient. This simple observation and calculation, which could have been made at any time over the past 30 years, clearly raises a host of interrelated questions concerning the nature and mechanisms of action of GABA in generating response selectivity.

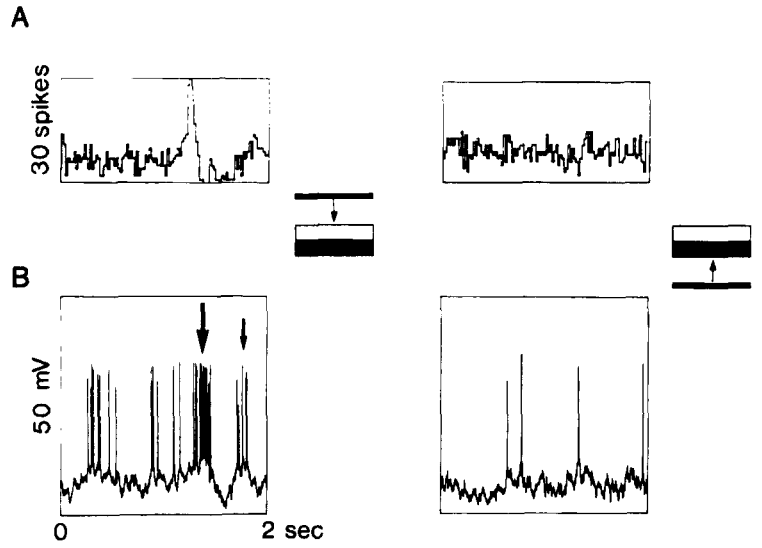


Fig. 9. Response of directional simple cell to a moving bar. Layer 4 neuron monosynaptically activated from LGN. A. Extracellular response to the moving bar in the preferred (left) and non-preferred direction (right). B. Intracellular record of membrane potential changes underlying the extracellular response in A. In the preferred direction the strong ON response (large arrow) is followed by a hyperpolarization and then a smaller excitatory response (small arrow). (From Douglas et al., 1991.)

The story inside

The case for the simple cell described above is clear enough from the extracellular records. The intracellular recording serves only to confirm the conclusion that the inhibition does not seem strong enough to explain the direction selectivity. But, one swallow does not make a summer. In other neurons, for example, the spontaneous activity recorded by the extracellular electrode is reduced to zero by the inhibition. In such cases the magnitude of the inhibition can only be guessed at (Palmer and Davis, 1981). Only intracellular recording can resolve the issue. We have recorded intracellularly from neurons in the visual cortex, stimulating them with conventional visual stimuli. The general pattern of inhibition as it relates to the membrane potential can be summarized quite briefly. The magnitude of the hyperpolarizations was greatest when the neuron was stimulated with the optimal stimulus. Stimuli that are not optimally oriented, or which move in non-optimal directions, do not elicit strong hyperpolarizations. The intracellular records from the simple cell illustrated in Fig. 9 are then typical.

Hubel and Wiesel (1962) envisaged orientation selectivity as being set up once and for all in layer 4 and then relayed by excitatory connections to other layers. The spectre of their schema has haunted considerations of inhibition in the visual cortex. If the selectivity of the neurons has been generated at a prior stage of processing, then what appears to be inhibition would not be postsynaptic inhibition at all, but removal of excitation. Indeed, all the data presented above could potentially be explained in terms of removal of excitation. A variant of this has been provided by Nelson (1991), who has proposed, on the basis of extracellular recordings, that the inhibition present in the visual cortex acts by a presynaptic mechanism directed only at the thalamocortical synapse in layer 4. In other words, there is no postsynaptic inhibition, only a mechanism that removes the initial thalamocortical excitation. Against this, there are several lines of evidence that point to the existence and effectiveness of postsynaptic inhibitory mechanisms. One line is the accumulation of structural and physiological evidence, which shows that every neuron in the cortex receives a rich GABAergic input (see Somogyi, 1989). Add to this the extensive studies of the physiology of the GABAergic neurons, which have shown that they have quite normal receptive fields (Gilbert and Wiesel, 1979; Martin et al., 1983; Martin, 1988). In sum, there is no reason to suppose that the GABA neurons are not activated by the conventional stimuli that are used. On these grounds alone, it seems likely that postsynaptic inhibition would be evident under most stimulus conditions.

The presence of postsynaptic inhibition can be demonstrated using conventional biophysical techniques (see Ferster, 1986). The strategy is to current clamp the membrane potential at different values and show that the amplitude of the "inhibitory" potential changes in the direction expected for a postsynaptic mechanism. This is shown for a layer 5 pyramidal neuron (Fig. 10). A hyperpolarizing potential was evoked by flashing a dark bar on its "ON" subfield. If the "hyperpolarization" is due to a reduction in excitatory depolarization then the amplitude of the hyperpolarization should hardly be affected by a sustained polarizing current. When depolarizing current was injected into the neuron, we found that the amplitude of the inhibitory hyperpolarization increased in magnitude. This indicates that the inhibition is not presynaptic. The depolarization increases the difference between the membrane potential and the reversal potential of the inhibitory synapses, so providing a larger driving potential for the inhibitory current.

The central difficulty is that inhibition measured by these biophysical techniques does not appear to be powerful. Yet strong inhibition is required, it seems, if strong excitatory responses are to be quenched. However, there remain several escape routes for those wedded to the necessity of omnipotent inhibition. One is the possibility that the hyperpolarization does not accurately

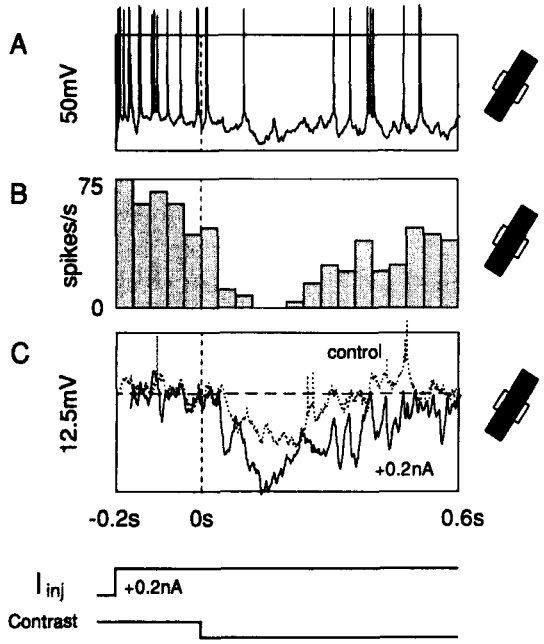


Fig. 10. Response of a layer 5 pyramidal cell with a simple receptive field (S1) to flashed bar. Excitatory current was injected to raise firing rate (I_{inj}, $t = -0.2$ sec, $+0.2$ nA), while a dark bar appeared (Contrast, $t = 0$ sec) which remained for the test duration. A. Intracellular response to the current step and flash onset (vertical dashed line). B. Histogram showing effect on spike rate averaged over 7 trials. Flash initially suppressed firing (latency 40 msec), but the effect diminished from 250 msec onwards. C. Average membrane polarizations, (7 trials) associated with flash-induced inhibition of firing, with (solid line) and without (dotted line) conditioning current step (I_{inj}). (From Berman et al., 1991.)

reflect the magnitude of the inhibition. If inhibition acts by shunting the excitatory current it would not induce much change in membrane potential. Shunting inhibition would at least account for directional and orientation selectivity in cases where the magnitude of the hyperpolarization appears too small to account for the elimination or reduction of response seen with non-optimal stimuli. Experimentally, the signature of shunting inhibition is a large increase in the input conductance of the neuron. For shunting inhibition to be effective against visually-driven excitation, it must be sustained for the duration of flow of the excitatory current. We could examine this in vivo using the standard technique of injecting a series of constant-current pulses to measure the conductance, while stimulating the neuron visually. If the inhibition was associated with large increases in conductance, then the amplitude of the voltage deflections produced by the constant current pulses should fall dramatically during inhibition.

We tested a range of neurons using this method (Douglas et al., 1988). The results were clear-cut. Large sustained conductance changes were not found, although a wide range of conditions were tested. Regardless of the contrast of the stimulus, the receptive field of the neuron (simple or complex), the receptive field property being examined (subfield antagonism of simple cells, orientation, or directionality), or whether the neuron was driven directly or indirectly by thalamic afferents, the input conductance of the neuron did not increase more than about 10–40% of the initial conductance. This was insufficiently large to account for the inhibition of the excitatory response.

To illustrate this point we give two examples: one using a stationary flashed stimulus, the other using a moving stimulus. We used a stationary stimulus to activate the inhibitory mechanisms that underlie the subfield antagonism of simple cells originally reported by Hubel and Wiesel (1959, 1962). A dark bar was placed over the "ON" field of a layer 5 neuron. With the onset of the stimulus, the membrane hyperpolarized and remained hyperpolarized for the entire duration of the stimulus (Fig. 11). Constant current pulses were then injected into the neuron through the recording pipette during a control and test period. The derived input conductance was plotted as a percent of the conductance of the neuron measured during the control period. The onset of the stimulus induced a clear increase in the conductance of the neuron, which endured for the duration of the stimulus. Thus the inhibition and hyperpolarization was associated with an increase in input conductance of about 20%.

We could further demonstrate that the degree of hyperpolarization seen with visual stimulation

does not reflect the limit of postsynaptic inhibition available to the neuron. Electrical stimulation is the most effective means we know of for inducing an IPSP, presumably because the pulse stimulus provides a volley of excitation to the cortex that is not matched even by the flashed stimulus. During electrical stimulation a large fraction of the cortical neurons are activated simultaneously and summate their effect. Comparison of the visual and the electrical response for the same neuron (Fig. 11) indicates that the magnitude of the hyperpolarization elicited by the electrical pulse is greater than that of the flash stimulus. The single pulse activation of the inhibitory synapses endures for several hundred msec. This is not due to repetitive excitation of the inhibitory population, since every neuron we have tested in this way shows the same pattern of a brief EPSP followed by an extended IPSP. Thus the whole visual cortex becomes silent during the period of an electrically-evoked IPSP.

The second example is that of a simple cell from layer 6, which we stimulated with a moving bar (Douglas et al., 1988). This neuron showed a sequence of excitation followed by inhibition in the preferred direction of motion (Fig. 12). In the reverse direction, inhibition was also evoked, but now superimposed on the excitatory response, as might be predicted from the Barlow and Levick (1965) model. Constant current pulses were injected to measure the conductance. The start of the regular pulse train was staggered from trial to trial. This reduced the sensitivity of the method, because we could not average several trials, but ensured that the entire period of recording was sampled. We estimated that conductance changes smaller than 15% of control would not be detected by our method.

These measurements showed that in neither the preferred direction, nor the reverse direction, was there any evidence of a marked increase in conductance, except during the period of maxi-

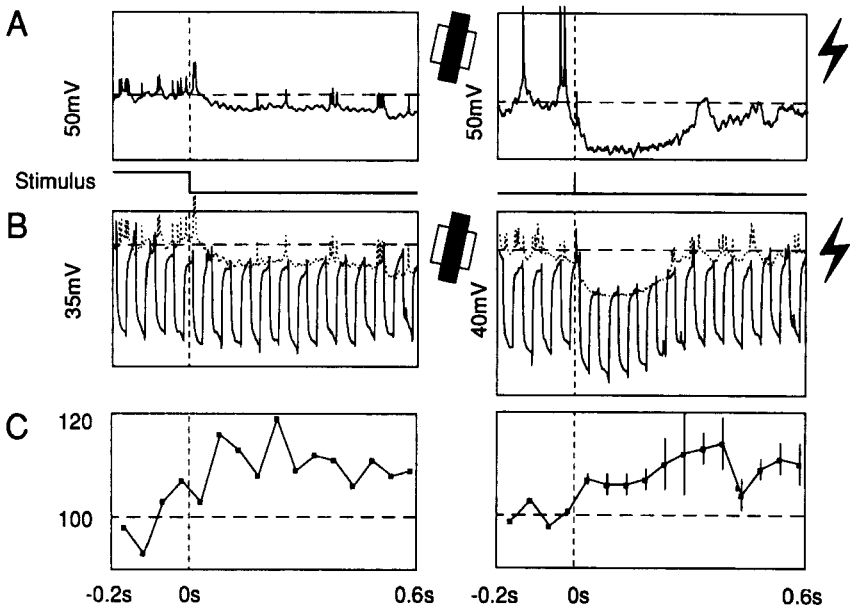


Fig. 11. Responses of layer 6 pyramidal neuron with simple (S1) receptive field to dark bar flash (left column) and electrical stimulation (bolt) of afferents from OR2 (right column). A. Intracellular membrane potential responses to flash (6 trials) and OR2 stimulation (single sweep). B. Averaged responses with hyperpolarizing constant current pulses (solid lines) to flash (6 trials) and OR2 (5 trials) stimulation. Dotted trace shows average responses without current pulses, for comparison. C. Mean percentage change in input conductance (normalized against control mean) derived from the average responses above for flash ($n = 7$) and OR2 response ($\pm$S.E.M., $n = 5$). (From Berman et al., 1991.)

mal excitation. The regions of distinct hyperpolarization were not associated with increase in the conductance of more than 20%. The results of the moving stimulus are compatible with those of the flashed stimulus. The flashed stimulus might be expected to produce a larger change in conductance because there is a more coherent volley of excitation arriving at the cortex, and because the response evoked is more "pure". The moving bar inevitably evokes both excitation and inhibition through its leading and trailing edges.

The evidence that visually-evoked inhibition is not associated with large changes in membrane potential or input conductance, added to the case against selectivity being produced by strong inhibition. We remained concerned that we were underestimating the magnitude of the inhibition. Perhaps the intracellular recording pipette lo-

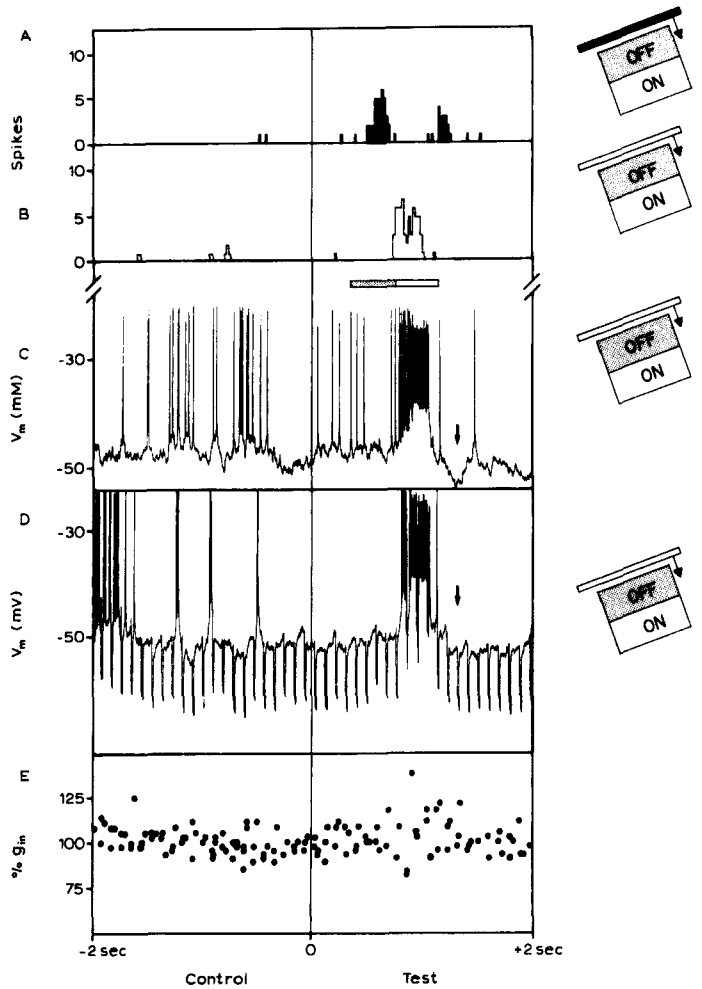


Fig. 12. Responses of layer 6 pyramidal neuron with S2 receptive field to moving bars. Neuron received monosynaptic activation from LGN. Same protocol as Fig. 1. A and B. Extracellularly recorded action potential discharge evoked by light and dark bars in most preferred direction and orientation. C. Intracellular recording of membrane potential response underlying extracellular response in B. Rapid discharge is followed by a hyperpolarization (arrow). D. Membrane potential response to visual stimulus with injection of current pulses (−0.1 nA, 30 ms, 10 Hz). E. Input conductances (normalised against control mean) derived from 3 trials similar to D. There is no large and sustained change in the conductance of the neuron anywhere in the response. (Bar width, 0.3°; length, 13.1°; velocity, 3.3°/sec). (See Douglas et al., 1988; Berman et al., 1991.)

cated in the soma was unable to detect conductance changes occurring out on the dendrites. We needed to secure quantitative estimates of two central issues: firstly, how visible are inhibitory conductances and, secondly, how large do they need to be to sink the excitatory currents? The quickest way of answering these questions seemed to be to do the calculations.

Visibility of synaptic conductances

We derived theoretical expressions for the change in input conductance produced by synapses, which could be positioned at any location on a passive dendritic tree (Koch et al., 1990). For the case of an idealized neuron with an infinite dendritic cylinder, the analysis demonstrated that the change in conductance produced by a single synapse decays exponentially with the distance of the synapse from the recording site. As we might expect, the visibility of a conductance change was highly dependent on the relative location of the active synapses and the recording pipette. The visibility of the conductance change decayed more rapidly with distance in neurons with long thin dendrites (e.g., beta-type retinal ganglion cells) than in those with shorter thicker dendrites (e.g., pyramidal neurons). Fortunately, the visibility did not depend on the synaptic reversal potential, so that conductance changes that were hyperpolarizing, depolarizing or shunting, were all equally visible. The changes in the somatic input conductance produced by multiple synaptic activation were always less than the sum of the conductance changes produced by individual synapses acting on their own. This was a case of more giving less due to the non-linear interactions between the multiple conductance inputs. Finally, we found that the absolute change in the somatic input conductance was independent of the leak produced by damage to the somatic membrane by the recording pipette.

We then used our anatomical data to simulate the effects of activating basket cell synapses made at known locations on the dendritic trees of actual pyramidal cells (Fig. 13). We selected examples of the largest (from layer 5) and smallest (from layer 2) pyramidal cells from our catalogue of physiologically-characterized and morphologically-identified neurons. For these simulations we did not reduce the detailed 3-dimensional recon-

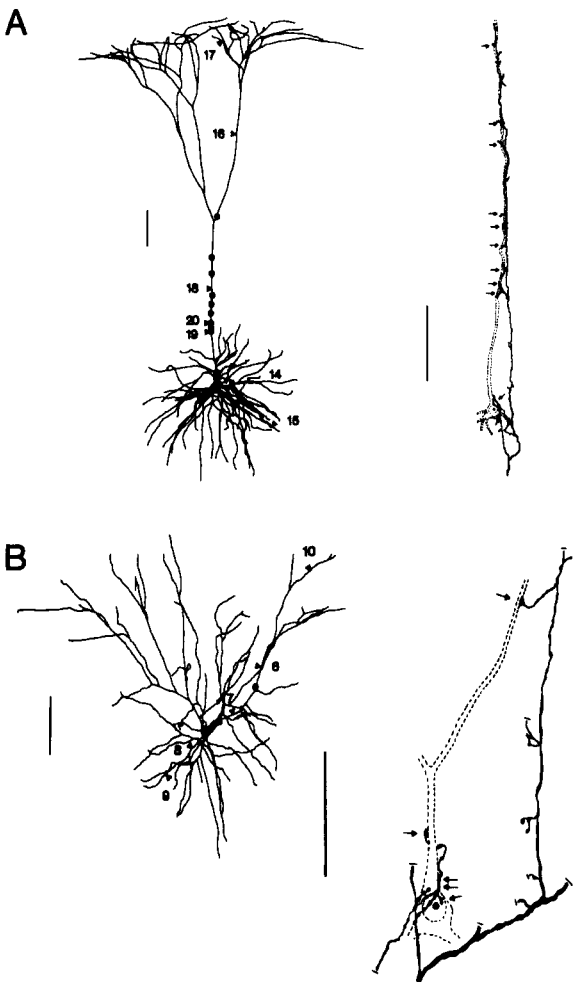


Fig. 13. Location of inhibitory inputs on pyramidal cells used in simulations of conductance visibility. A. Computer plot of a reconstructed layer 5 pyramidal cell (left) showing siting of inhibitory inputs (filled circles) and excitatory inputs on spines (numbers 15–20). Locations of inhibitory sites were based on the synaptic contacts (arrows, right) formed by an HRP-filled GABAergic basket cell axon on a layer 5 pyramidal cell. B. Reconstruction of an HRP-filled layer 2 pyramidal cell (left) and siting of inhibitory (circles) and excitatory (numbers 6–10) inputs. Inhibitory site location based on identified basket cell input to a similar cell (right, arrows) in layer 2/3. Scale bars: 100 μm in A; 50 μm in B. (See Koch et al., 1990.)

struction of the dendritic tree to the simplified compartmental model (see Fig. 2). The specific locations of basket cell synapses made on such pyramidal cells were obtained from our catalogue of neurons (Somogyi et al., 1983; Kisvárday et al., 1987). The basket cell synapses were assumed to act by either $GABA_A$ (chloride-mediated, shunting) or both $GABA_A$ and $GABA_B$ (potassium-mediated, hyperpolarizing) synapses.

Various combinations of excitatory and inhibitory interactions were simulated. In all the various conditions, the inhibitory synapses had to increase the input conductance of the neuron by at least 100% to reduce the excitatory current by 50%. To achieve complete inhibition of moderate excitation required very much larger increases in conductance: up to 1600% in one example. In most cases, over 80% of the total inhibitory conductance would be visible to a recording pipette located in the soma. This is perhaps not surprising, given that the basket cell synapses are located on the proximal dendrites and soma. Of the reduction in visibility that occurs, most is due to proximal $GABA_A$ conductances shunting the inhibitory synapses that are located more distally on the dendritic tree.

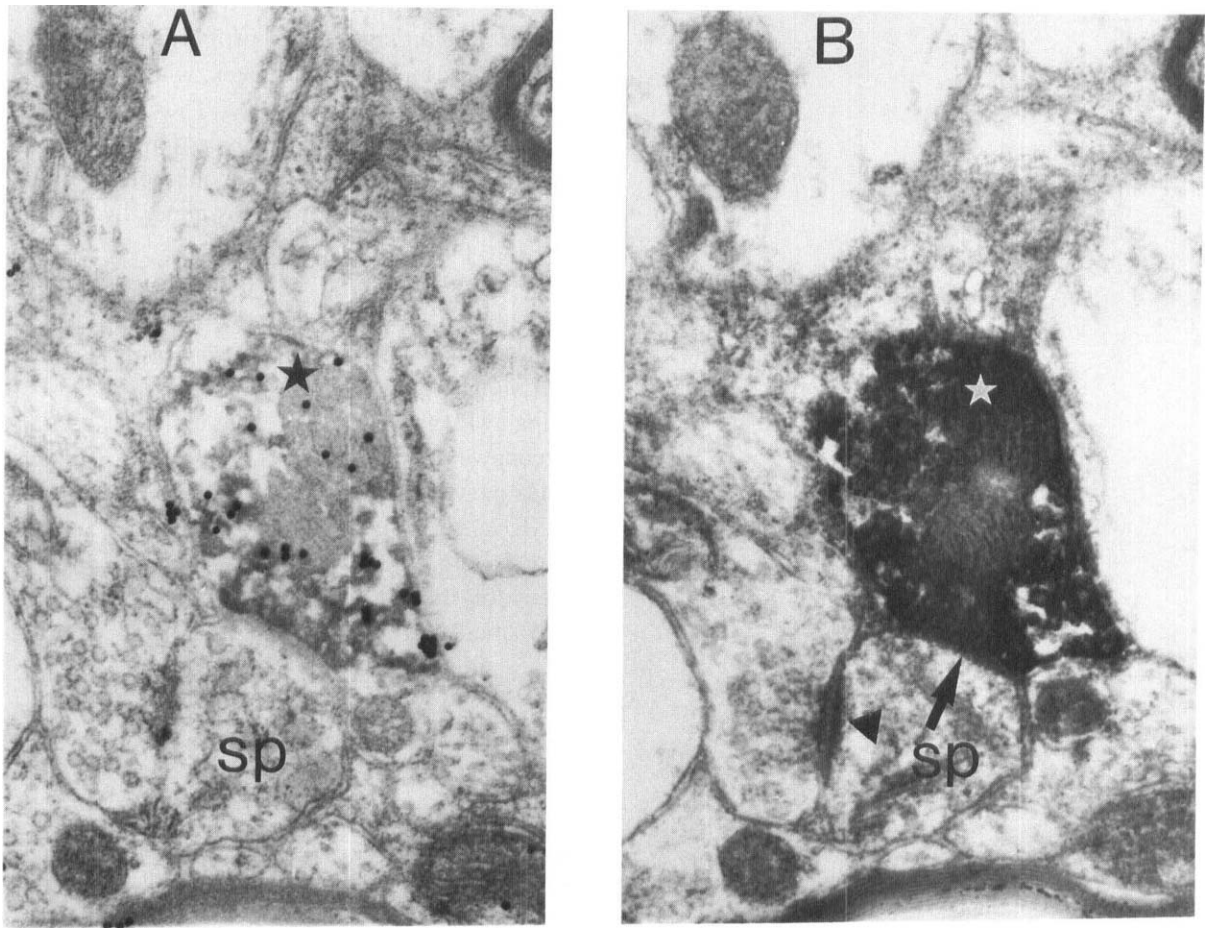
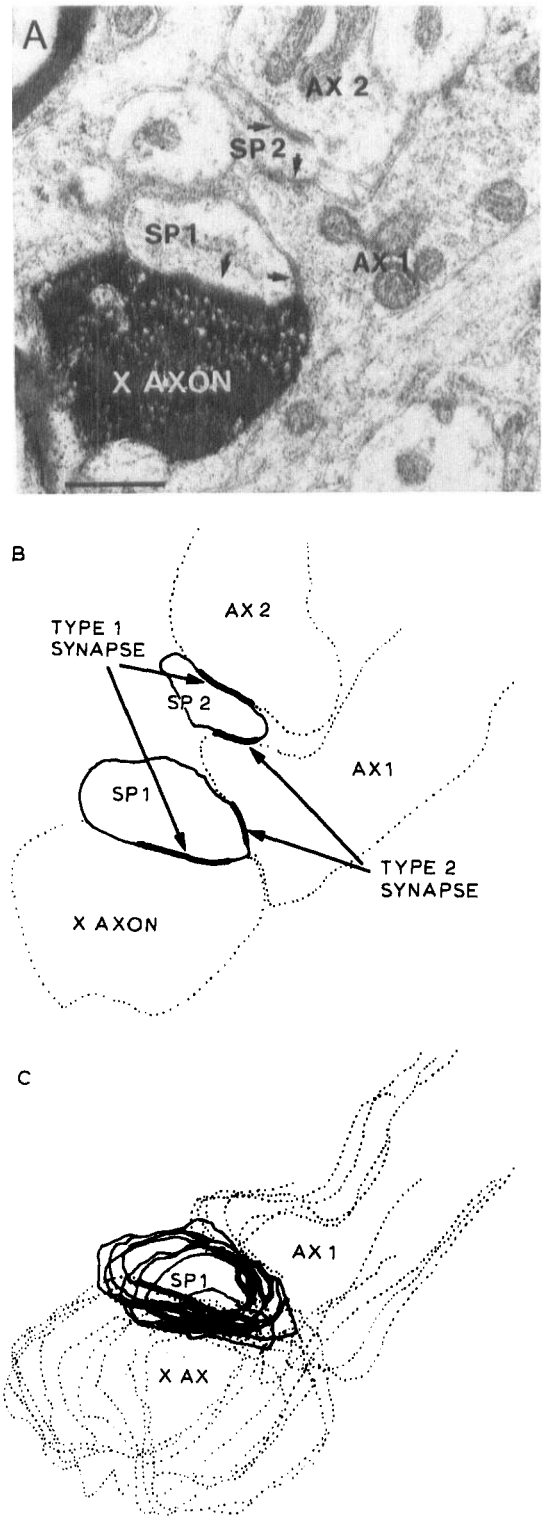


Fig. 14. GABA-immunoreactive input to a spine. A and B. Serial EM micrographs of an HRP-filled bouton (*) from a clutch cell that formed a type 2 synapse (inhibitory) on a spine (sp). The same spine also receives a type 1 synapse (presumably excitatory) from another bouton. The HRP-filled bouton (*) was shown to be GABA-positive in the adjacent section in A, by GABA-immunoreactive labelling. Scale bar: 0.5 μm. (modified from Somogyi and Soltész, 1986.)



Synaptic "veto" on dendritic spines

One further issue needs consideration, and that is whether significant inhibition occurs at the level of the spine. The notion of an inhibitory veto operating on the dendritic spines has been mooted repeatedly (Diamond et al., 1970; Jack et al., 1975; Torre and Poggio, 1978; Koch and Poggio, 1985). Spines are the site of most of the excitatory input to cortical spiny neurons (Garey and Powell, 1971; Szentágothai, 1973), and thus a shunting inhibitory synapse located on a particular spine would provide a means of selectively inhibiting the excitatory input to that spine. Spines are distanced electrotonically from the soma by the intervening impedance properties of the dendritic shaft, and the spine neck, which is thought to have a high axial resistance. Thus, the conductance changes wrought by the action of synapses on the spine head would be masked from our recording pipette, whose tip is generally impaled in the soma or proximal dendrites.

It is impossible to test the hypothesis that a selective inhibitory veto acts at the level of a spine using contemporary electrophysiological techniques. However, we could do the next-best thing, which was to see whether the necessary circuitry exists (Dehay et al., 1991). There is clear evidence that spines do receive synapses from GABAergic boutons (see Fig. 14) (e.g., Somogyi and Soltész, 1986). We also know the source of many of these GABAergic synapses: the large and small (clutch) basket cells both provide be-

Fig. 15. Dual input to a thalamorecipient dendritic spine. A. EM micrograph showing a spine head (sp1) receiving asymmetric (type 1) input from an identified HRP-filled thalamic X-axon, and symmetric (type 2) from an unidentified axon (AX 1). B. Computer plot of profiles show in A. A nearby spine head (SP 2) also receives both type 1 and type 2 input. C. Wire frame montage of several sections through the structures contacting spine 1 in A, showing the extent of the dual inputs. Scale bar 0.5 mm. (See Dehay et al., 1991.)

tween 20–40% of their output to spines (Somogyi et al, 1983; Kisvárday et al., 1986; Somogyi and Soltész, 1986). The double bouquet cell also provides input to spines (Somogyi and Cowey, 1981). Only about 7% of spines receive a synapse from a GABAergic bouton in addition to the excitatory synapse (Beaulieu and Colonnier, 1985; Dehay et al., 1991). In layer 4 it seems most likely that the excitatory input needs to inhibited at the earliest stage possible. This means that the excitation provided by the thalamic afferents themselves has to be inhibited, because it is out of their non-oriented, non-directional receptive fields that the selective cortical fields are built. It seems possible that the inhibitory synapses were targeting the spines that received an excitatory synapse from a thalamocortical bouton. We examined this possibility using a combined physiological and anatomical method (Dehay et al., 1991).

We recorded from single axons as they coursed through the optic radiations, and identified them physiologically as arising from either X- or Y-type relay cells in the lateral geniculate nucleus of the thalamus. The axons were then injected intra-axonally with horseradish peroxidase, which fills the entire axonal arbour. Using the electron microscope we were then able to identify spines that received excitatory synapses from the horseradish peroxidase labelled boutons (Fig. 15A). Most spines received only a single synaptic input. Occasionally the spine received a second synapse, whose morphology identified it as originating from a smooth GABAergic interneuron (Fig. 15). When the results were totted-up from a total of 76 boutons examined from 4 axons, we found that less than 7% of the spines that made synapses with thalamic afferent boutons received a second synapse (Dehay et al., 1991). For the vast majority of thalamocortical synapses on spines, the excitatory current they provide flows unscathed by shunting inhibition to the parent dendritic shaft. Even for spines that receive a GABAergic synaptic input, there is some doubt as to the selectivity of the inhibition. In some instances we were able to trace the axon making the synapse on the spine and show that it made an additional synapse on the parent dendritic shaft. These observations provide a clear and quantitative answer to the issue of whether a synaptic veto mechanism was located at the level of the dendritic spine and could be used to gate effectively the primary source of excitation to visual cortex. We cannot explain away our experimental failure to find large inhibitory conductances by supposing they are located on electrotonically distant spine heads. Nelson (1991) has suggested that the thalamocortical synapse may be subject to presynaptic inhibition. This convenient explanation of our physiological result is ruled out by our morphological observation that none of the thalamic afferent boutons received a synaptic input, as would be required for presynaptic inhibition.

We seem to have returned to the place where we first began: faced with the problem of understanding what GABAergic inhibition is doing in the cortex. However, we are wiser for the exercise we have summarised above. Through experiment and simulation we have been able to define some of the limits of the behaviour when excitatory and inhibitory synapses interact. Our failure to find significant increases in conductance during non-optimal stimulation cannot be explained by an inherent inability of our technique to detect such conductance changes if they are occurring: it would be impossible, even for physiologists of limited competence, to miss conductance changes of the magnitude required (Koch et al., 1990). The anatomical investigation confirmed that there is no special synaptic organization that might prevent inhibition being visible to normal recording methods. Taken together, the theory and experiment imply that synaptically-mediated inhibition cannot play the dominant role that has traditionally been assigned to it. Instead, the excitatory gain of the cortex appears to be controlled by a relatively weak inhibition. To make further progress on this problem we scrambled out of the thickets of synaptic interactions and headed for higher ground. What we needed was a wider view of the operation of local circuits in the cortex.

Solutions through cortical microcircuits

From time to time, neurophysiologists and neuroanatomists have suggested that the circuitry of the neocortex is highly conserved, with the same basic "bauplan" being used in all cortical areas (Szentágothai, 1975; Creutzfeldt, 1977; Powell, 1981). Amongst physiologists, Hubel and Wiesel (1977) found evidence for repeated functional units in the primary visual cortex, which they named "hypercolumns". They introduced the notion of the functional uniformity of striate cortex and suggested that the reason for this might be that cortical area 17 consists of a series of stereotyped machines, repeated over and over (Hubel and Wiesel, 1974). Their idea that area 17 has a crystal-like structure has not been explored in depth by physiologists or neuroanatomists, and certainly no-one has gone much beyond speculating that the concept might be extended to include the entire neocortex.

Neuroanatomists, to be sure, have provided a great deal of information about the types of neurons that are found in neocortex, and their relative numbers (Lorente de Nó, 1949; Scholl, 1956; Rockel et al., 1980; Jones, 1984; Gabbott and Somogyi, 1986; Peters, 1987). It has largely been left to physiologists to assemble these components into functional circuits. But, their circuits have been configured for particular functions. For example, the layer 6 pyramidal neurons send a local collateral projection to the middle layers of the cortex. Wiesel and Gilbert (1983) and Bolz and Gilbert (1986) used this anatomical information to show how the specific property of end-inhibition might be achieved by this circuit. However effective their explanation is for the visual cortex, and it is controversial (Murphy and Sillito, 1987; see Martin, 1988, Somogyi, 1989), it sheds no light on what the equivalent projection might be doing in all the other cortical areas.

The natural question arises as to why we should not be content with explaining the role of a stereotyped connection in terms of a specific function. It is self-evidently difficult enough to tease out a single function for any of the intracortical circuits even in well-worked areas like the visual cortex. Our saga with GABA-mediated inhibition showed that we might be in danger of becoming too focused on GABA-mediated inhibition alone. Unless we deliberately took a wider look, with GABA-mediated inhibition forming a part rather than the whole of the picture, we might miss out on understanding basic principles of cortical operation. Hence our present endeavour to find a more general solution to the problem of cortical function, rather than simply to explain the orientation or direction selectivity of neurons in the visual cortex. The task we set ourselves was to develop a "canonical" microcircuit for neocortex (Douglas et al., 1989; Douglas and Martin, 1991a,b). "Canonical" is meant in the mathematical sense of simplest, or clearest form of microcircuit. The general properties that emerge from the abstract circuit we have developed, have provided novel insights into the possible mechanisms underlying the response selectivity of neurons in the visual cortex and the role of GABA-mediated inhibition in particular.

Ten years ago it was not possible to envisage such a project. There were still too many unknowns. Now the situation is very different. A compendium of new techniques has produced a veritable flood of information about the nuts and bolts of neocortex. In particular, methods that enabled the anatomical identification of neurons that had been studied physiologically meant that, for the first time ever, a direct bridge could be built between the microstructural studies and the physiological studies of single neurons. Another major tool is the computer, whose value we have demonstrated here for the analysis of interneuronal interactions. In the development of the canonical microcircuit the computer simulations were especially invaluable for exploring facets of the function of the circuitry that could not be studied experimentally.

The starting point for the microcircuit was the biology. The first step was to decide on the components to be included in our preliminary sketch

of the cortical circuits. For this, we could draw on an extensive database of neurons whose receptive fields were mapped, whose afferent connections were studied using electrical stimulation, and whose morphology was identified by intracellular filling with horseradish peroxidase (Martin and Whitteridge, 1984). The major additional advantage of filling the neuron with horseradish peroxidase is that the intracortical collaterals of the axon can be completely labelled. This is not true with any other method, including the invaluable Golgi-staining technique. Thus the three-dimensional reconstructions of the dendritic arbours allowed us to derive the "simplified" neurons that were used here and in the theoretical analysis of inhibition described above. The detailed reconstructions of the axonal arbours of these neurons allowed us to produce an accurate picture of the stereotyped interlaminar connections of the various types of neurons in the different

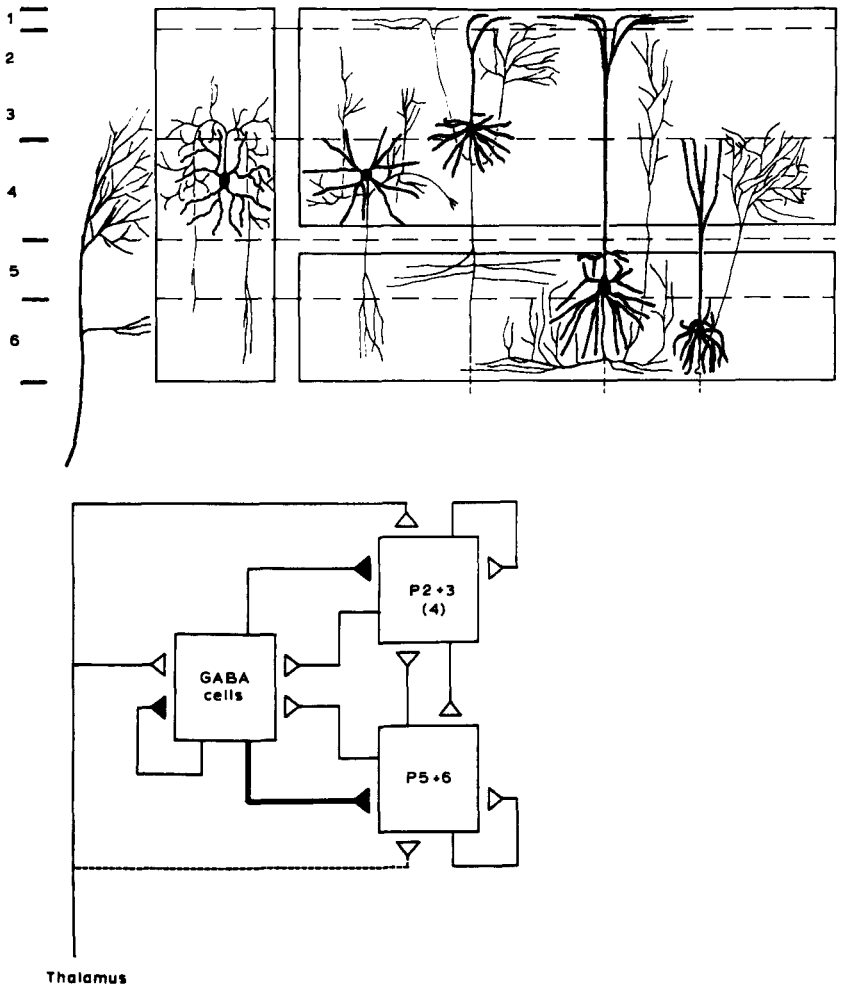


Fig. 16. Cortical circuitry and the canonical microcircuit. Top diagram shows the basic neuronal types in cortex, their typical axonal arborizations and the laminar boundaries. The cell types grouped in the 3 boxes are represented below in their respective positions in the canonical circuit. Superficial (P2 + 3(4)) and deep (P5 + 6) pyramidal cells are interconnected with a single pool of GABA cells. Heavy solid line indicates stronger inhibitory input, and dashed line indicates weaker thalamic input, to deep cells. (See Douglas et al., 1989; Douglas and Martin, 1991a,b.)

cortical layers (Martin and Whitteridge, 1984). These connections, and their reduction to the canonical form, are shown in Fig. 16. We are obviously aware that there are many varieties of smooth, GABAergic neurons, but in the light of our theoretical analysis of axoaxonic and basket cell inhibition, we are as yet unable to offer any compelling argument for differentiating them into functional subgroups. To avoid unnecessary overcomplication we have used a basket cell that makes connections to superficial and deep cortical layers as the generic functional representative of the population of GABAergic neurons.

Ultrastructural studies of the synaptic connections made by identified neurons provide the statistics of the basic connections between the different components of the neocortex (see Martin, 1984, 1988). These studies show that spiny cells receive about 85% of the synapses made by any cell type in the cortex. Since 70% of the cortical neurons are pyramidal, this means that pyramidal neurons are the focus of connectivity. Even in layer 4, the main thalamorecipient zone, we found a surprising number of "star" pyramidal neurons (Martin and Whitteridge, 1984). The spiny stellate neurons, which are found only in layer 4, probably form less than 10% of neurons even in the granular cortical areas, such as visual cortex. Their percentage falls even lower in the agranular areas such as somatomotor cortex, where layer 4 is virtually absent and layer 3 becomes the main thalamorecipient zone (see Jones, 1984). The traditional view of layer 4, and the spiny stellate cells in particular, as unique recipients of thalamic input is quite incorrect. Both physiological studies (Hoffman and Stone, 1971; Bullier and Henry, 1979; Ferster and Lindström, 1983; Martin and Whitteridge, 1984) as well as detailed anatomical studies (Freund et al., 1985a,b; see White, 1989) show that neurons in every lamina receive direct thalamocortical input.

Our electrophysiological studies (Martin and Whitteridge, 1984; Douglas et al., 1989) provided estimates of the conduction times and synaptic delays, which were incorporated into the simulations. What we have not yet been able to establish is how the weighting of the thalamic input varies for the different target neurons. Layer 5 pyramidal cells, whose apical dendrite pass unbranched through layer 4, probably receive fewer thalamocortical synapses than neurons that have a large fraction of their dendritic arbour in layer 4 (see White, 1989). It should be noted that even in layer 4 of visual cortex, the thalamic afferents contribute only 5–30% of the synapses (Garey and Powell, 1971; LeVay and Gilbert, 1976). The majority of excitatory synapses, even in layer 4, come mainly from the intracortical collaterals of spiny cells.

In the absence of sufficient information concerning the distribution and conductances of the NMDA receptor in the visual cortex, we assumed only an AMPA-like receptor for the excitatory synapses. In our simulations the thalamocortical afferents provided 10–20% of the total excitatory conductance of spiny cells of the superficial layers (including layer 4), and 1–10% for the deep layer pyramids. As for the superficial layer spiny cells, the thalamocortical afferents supplied 10–20% of their total excitatory conductance. The remainder of the excitatory conductance comes from the intracortical collaterals of the spiny neurons. We arranged that spiny cells in layer 2 + 3 + 4 received half their intracortical excitation from spiny neurons in layer 2 + 3 and half from neurons in layer 5 + 6. A similar arrangement applied to the pyramids of layers 5 + 6. We did not assume that all neurons receive the same pattern of inputs. If we open up the boxes (Fig. 17) we find various patterns of connectivity of the components, e.g., some receive direct thalamocortical input, some none. For the purposes of the simulation it was convenient to consider the activity of the "average" neuron, which had the "average" connectivity. In the comparisons given below we compare the response of individual neurons recorded in vivo, and the average simulated response of the superficial or deep layer pyramidal cell populations.

All the pyramidal neurons appear to have both

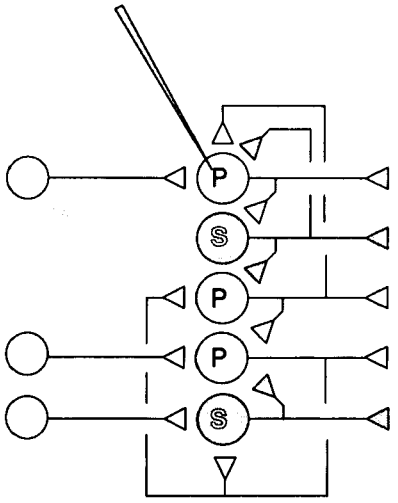


Fig. 17. Principles of connectivity between the different neurons in the microcircuit. Excitatory (open triangles) input from thalamus innervates some of the pyramidal cells (P) and smooth cells (S) in cortex. They in turn are interconnected with excitatory (open triangles) and inhibitory (shaded triangles) synapses. The performance of the circuit is monitored by "recording" the averaged response of one group of neurons.

$GABA_A$ and $GABA_B$ receptors (Connors et al., 1988; Douglas et al., 1989), and these were incorporated into the canonical microcircuit. In terms of the function of the GABA conductances, the microcircuit we constructed required one important assumption for which we have only a physiological justification. When we recorded from identified pyramidal neurons in the superficial and deep layers we found what so many before us had found, the standard receptive field types described originally by Hubel and Wiesel (1962). However, there was one clear and robust difference between superficial and deep layer pyramidal neurons that was independent of receptive field type (Douglas et al., 1989; Douglas and Martin, 1991a,b). When the cortex is activated by a brief electrical pulse stimulus to the white matter, an action potential is generated simultaneously in a large number of thalamocortical afferents. The arrival of this synchronous volley of action potentials in the cortical grey matter evokes in every neuron the well-described sequence of an EPSP followed by an IPSP (Fig. 18A). The difference we found was that in the deep layer pyramidal cells, the latency from stimulus onset to maximum hyperpolarization was consistently much shorter than for the superficial layer cells (Fig. 18B). It appeared functionally as if the $GABA_A$-mediated inhibition was stronger for pyramidal cells in the deep layers. This aspect was incorporated in our simulation by making the $GABA_A$ inhibition twice as strong for the deep layer compared to the superficial layer pyramidal neurons. A structural explanation for this difference has yet to be found.

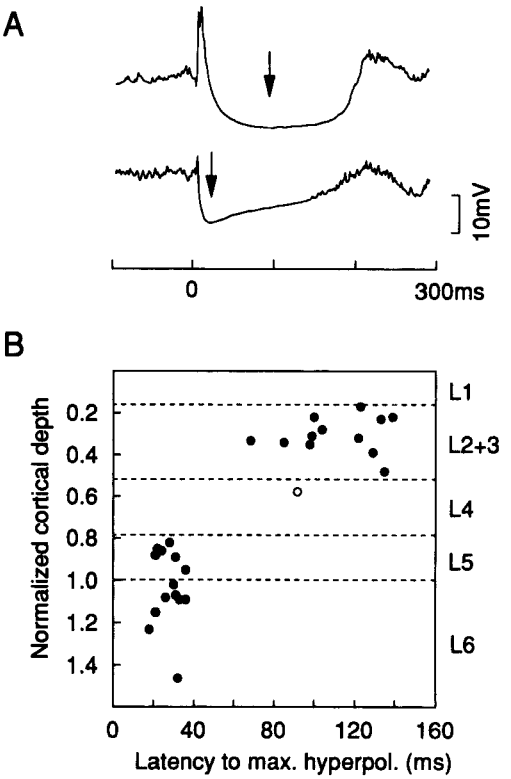


Fig. 18. Response of cortical neurons to stimulation of the optic radiation. A. Averaged intracellular response of typical superficial (top trace, layer 2/3) and deep (bottom trace, layer 5/6) neurons. Arrows indicate the position of maximum hyperpolarization. B. Relationship between cortical depth (layer on right) and latency to maximum hyperpolarization for 26 identified pyramidal (filled circles) and one spiny stellate cell (open circle). (See Douglas and Martin, 1989, 1991a,b.)

Validation of the canonical microcircuit

The simplest way of comparing the performance of the microcircuit to that of the visual cortex was to simulate the electrical pulse stimulus applied to the thalamic afferents. This avoided all the complications and computational overload of simulating a visual stimulus. The pulse stimulus could also be used in combination with the pharmacological tools that now exist for manipulating the GABA receptors. By blocking or activating components of the GABA receptors in actual cortical neurons in vivo, and stimulating the afferents with an electrical pulse, we could see whether the canonical microcircuit would predict similar behaviour. In the event the microcircuit predicted the in vivo results with remarkable accuracy. For example, iontophoresing the $GABA_A$ antagonist *N*-m-bicuculline onto the neuron appeared to eliminate the early portion of the IPSP, allowing more excitation to predominate (Fig. 19A). In the deep layer pyramidal cell population, this had the effect of producing a response that resembled that of the superficial layer pyramidal cells. The latter part of the IPSP appeared to be insensitive to bicuculline application, even after 5–10 min of iontophoresis. The rapidly developing and short-duration component of the IPSP is probably due to the bicuculline-sensitive $GABA_A$ receptors, while the slower-developing and longer-lasting component is probably due to the bicuculline-insensitive $GABA_B$ receptors, as has been found in vitro (Connors et al., 1988). Clearly, in our in vivo recording, both the $GABA_A$ and the $GABA_B$ responses are hyperpolarizing. In in vitro recording the reversal potential of the $GABA_A$ synapse is nearer the resting membrane potential.

In our experiments, the tip of the multibarrel pipette containing the drugs was only 20–30 μm from the tip of the intracellular pipette. The localized effect of *N*-m-bicuculline iontophoresis was simulated by modifying the effect of the $GABA_A$ conductances in a subset of pyramidal and smooth neurons in the appropriate layers. In this subset the $GABA_A$ component of the inhibitory conductance was reduced to 20% of its original value. The simulations show that the modelled responses reflect quite accurately the

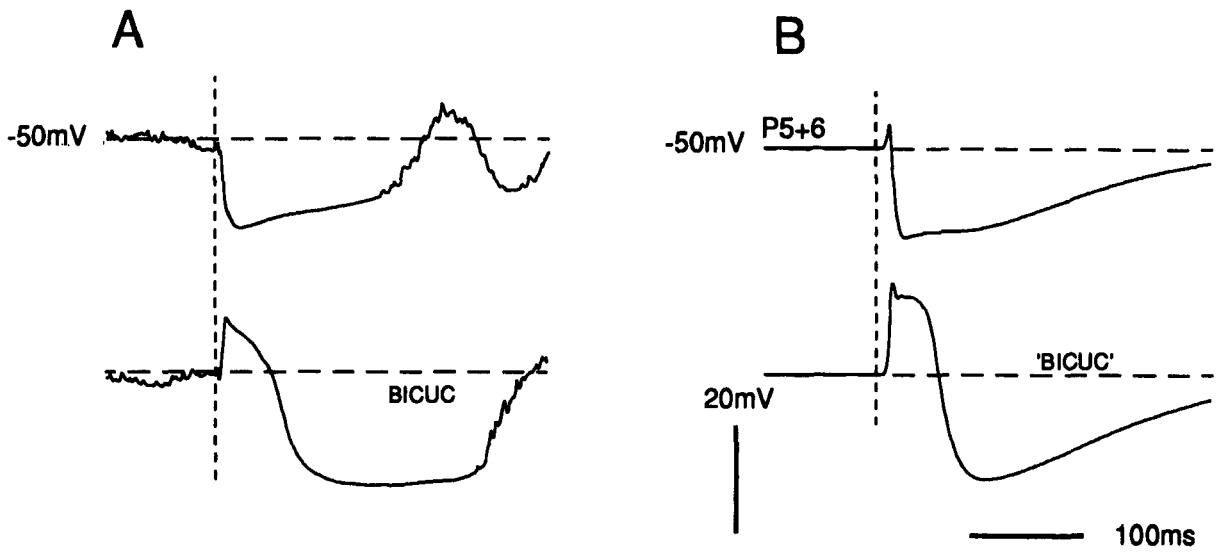


Fig. 19. Comparison of neuron and canonical model response to afferent stimulation and the effect of bicuculline. A. Normal response of a deep layer neuron (top trace) to afferent stimulation (vertical dashed line in all figures) and during iontophoresis of bicuculline (BICUC, bottom trace). B. Response of a deep neuron in the model representing the average response to afferent stimulation (top). Simulation of blockade of $GABA_A$ "receptors" ("BICUC") (bottom). (See Douglas and Martin, 1991a)

response obtained in the actual neurons (Fig. 19B). The effect of GABA, or GABA in combination with *N*-m-bicuculline was also examined (Fig. 20). In the in vivo recordings, application of GABA produced a hyperpolarization of the membrane. The absolute size of the IPSPs decreased, presumably because the resting potential had moved closer to the GABA reversal potential (Fig. 20A). When *N*-m-bicuculline was added to the GABA iontophoresis, the membrane hyperpolarized further and the amplitude and duration of the EPSP increased. We interpret the latter as the release from $GABA_A$ inhibition, and the former as evidence for different reversal potentials of the $GABA_A$ and the $GABA_B$ receptor (Connors et al., 1988; Douglas and Martin, 1990a). The $GABA_B$ response is thought to be mediated by potassium, which has a reversal potential of about -90 mV, whereas the $GABA_A$ response is thought to be mediated by chloride, which has a membrane reversal potential of about -70 mV (for review, see Douglas and Martin, 1990a). Thus, when the chloride-mediated response is blocked by bicuculline, the membrane moves towards the still-active $GABA_B$ reversal potential. The responses to GABA in the microcircuit were simulated by activating 50% of the $GABA_A$ and the $GABA_B$ conductances (Fig 20B). *N*-m-Bicuculline effects were modelled as described above. The simulated responses provided as remarkable agreement with the experimental data.

A final test was to use baclofen to activate the $GABA_B$ receptors (Fig. 21). When baclofen was iontophoresed onto neurons, the membrane hy-

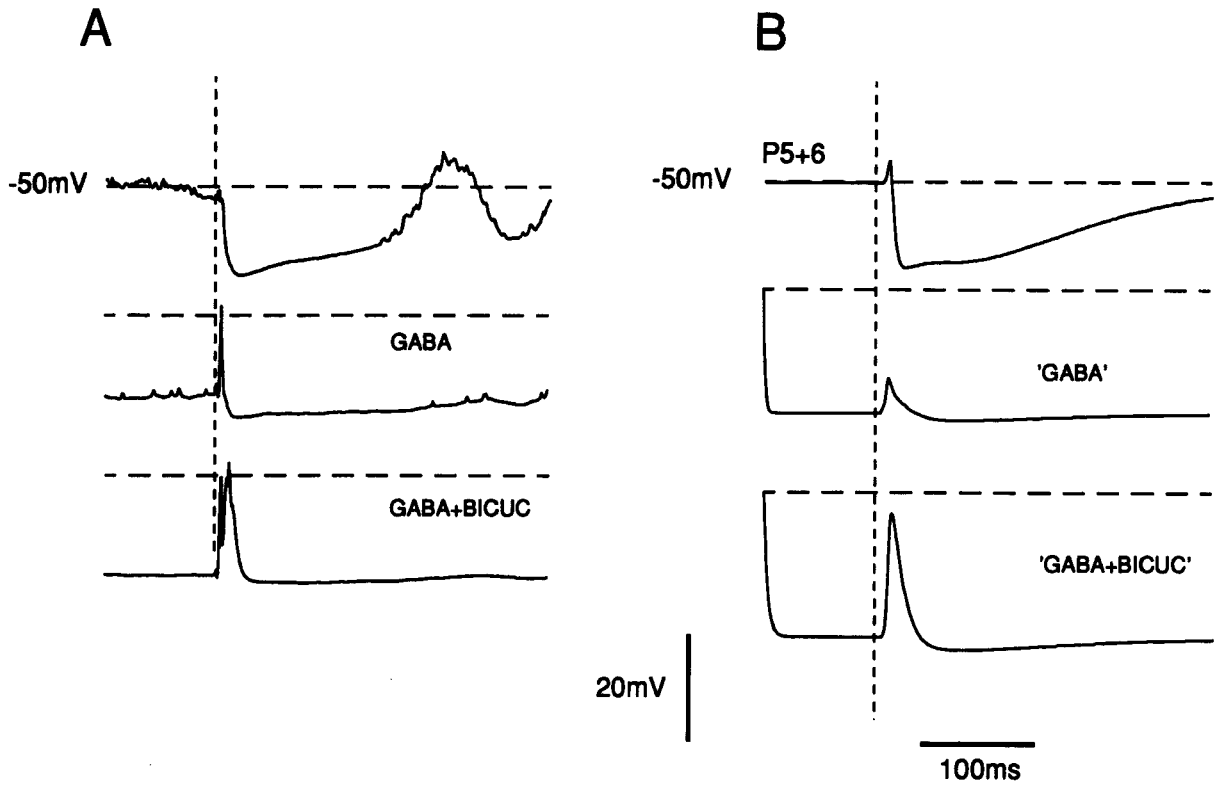


Fig. 20. Comparison of the effects of GABA and bicuculline application on a deep cortical neuron and the canonical model. A. Normal response of deep layer neuron (top), response during GABA iontophoresis (middle) and with additional iontophoresis of bicuculline (bottom). B. Response of a deep layer neuron in the canonical model (top). Simulation of GABA application (middle) and additional bicuculline application (bottom). (See Douglas and Martin, 1991a.)

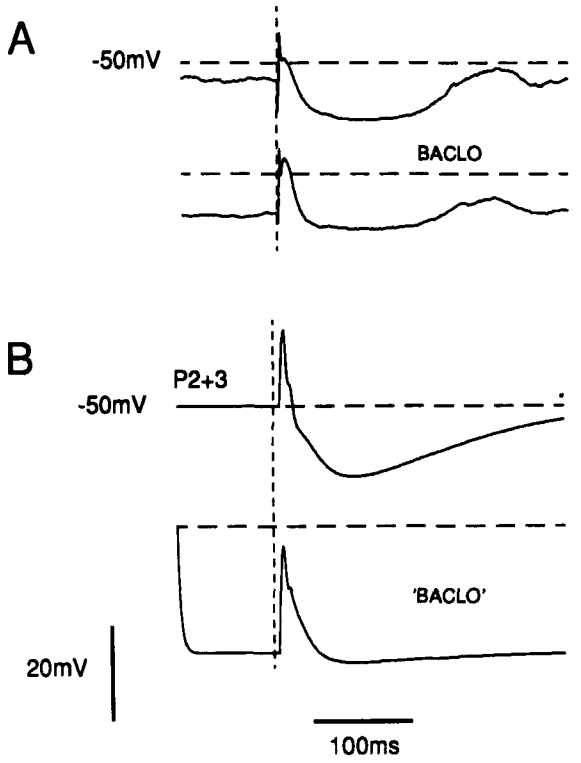


Fig. 21. Comparison of the effects of baclofen application on a cortical neuron and the canonical model. A. Typical response of layer 2 neuron to afferent stimulation (top) and during iontophoresis of baclofen (bottom). B. Response of a superficial neuron in the canonical model (top) and response during simulation of baclofen application (bottom). (see Douglas and Martin, 1991a.)

perpolarized and the late component of the IPSP flattened. This effect was simulated by activating 50% of the $GABA_B$ conductances in the test neurons of the microcircuit. The response of the model neuron differed from the actual neurons only in that activation of the $GABA_B$ receptors appeared to be more effective in the simulation. In other respects the response of the microcircuit to the simulated action of baclofen agreed well with that of the experimental data.

These results show, inter alia, that the dynamics of the $GABA_B$ inhibition are quite different from that of $GABA_A$. The $GABA_B$, acting as it does through a second messenger system, has a response that is governed by time-constants that are an order of magnitude greater than those of the $GABA_A$ response. Consequently, the $GABA_B$ mechanism behaves like a leaky integrator. With continued inhibitory stimulation, the inhibitory effect accumulates, gradually hyperpolarizing the membrane over hundreds of milliseconds, so increasing the current threshold for action potential generation. In this context, $GABA_B$ inhibition can be viewed as an adaptive process that acts via an inhibitory interneuron. The adaptive modification of the threshold depends both on the activity of the neuron being inhibited, and on the averaged activity within the population of neurons in which it is embedded. This latter aspect means that the response of a particular neuron can be referenced against the average activity of the population. The $GABA_B$ system might therefore be involved in adaptive processes, such as contrast gain control (Ohzawa et al., 1986). Indeed, the work of DeBruyn and Bonds (1986) suggests that the $GABA_A$ receptors are not involved in contrast gain control. The selective responses of neurons are generally thought to be exclusively mediated by the $GABA_A$ system (Baumfalk and Albus, 1987, 1988), but in the canonical microcircuit the $GABA_B$ system is inextricably involved in the structuring of receptive fields. This may explain why blocking $GABA_A$ does not always eliminate the selectivity of the neuron for the orientation or direction of the stimulus (e.g., Sillito, 1977; Nelson, 1991).

Canonical criticisms

The similarity of the model responses to the experimental data gave further confidence in the values of the parameters we had used for the model. Sceptics might put forward the proposition that with so many parameters to tweak it would be inept indeed not to have achieved such a close correspondence between experiment and theory. It is the nature of complex systems that they are under-determined by data. We have attempted to constrain as many parameters as possible by reference to biological data. Another line of criticism would say that the microcircuit is

obviously too simple, it lacks NMDA receptors, it does not differentiate between the different GABAergic neurons, it takes no account of the corticothalamic projection, or any of the many other projections to cortex that are known to exist. Of these deficiencies we are well aware, since these aspects were deliberately not included. Nevertheless, it is difficult for us to see the microcircuit as an oversimplification. Instead we view it as an example of our "minimalist" approach, a necessary use of Occam's razor to shave the number of variables to a minimum.

Our "minimalist" approach is an alternative to the "rococo" approach used by others to explain the selectivity of cortical neurons. Adherents of the rococo approach include everything (kitchen sink excepted) in their models (e.g., Wehmeier et al., 1989; Wörgötter and Koch, 1991). In contrast, we are not offering a comprehensive description of the cortical microcircuits. Rather, the canonical microcircuit is the minimum set of necessary connections from which more complex patterns of interconnections can be elaborated. In deriving the microcircuit we have made the fewest possible assumptions about the circuitry and the synaptic functions, while keeping our hypothetical circuit closely matched to the quality of the actual anatomical and physiological data from which it was derived.

In support of our approach there are several further points worth considering. One is that over 100 years of study of the cortical machinery has not produced a consensus as to what the cortical microcircuits are, or even how many of them there are. We offer a microcircuit that seems to represent the average connectivity and physiology of a patch of cortex. We do not claim that the connectivity of cortex is homogeneous. Indeed our own results from reconstructed neurons (see Martin and Whitteridge, 1984; Martin, 1988) show that it is not. The claim is simply that over the temporal scale of the electrical pulse response, the cortex behaves as if it had a rather simple connectivity. We anticipate that such canonical microcircuits are elaborated to perform many different functions in the different cortical areas. Our view contrasts with the view of neocortex as a collection of ad hoc specialist circuits, with different circuit designs for the different cortical areas, each surviving by evolutionary opportunism and designed to perform a special function like form or motion analysis. By suggesting the form of a canonical microcircuit we have at least provided a clear target by which alternatives can be judged, so that through this process we may arrive at some consensus as to what the form of the cortical microcircuits actually are.

Demonstration of the competence of the microcircuit

In describing the testing of the canonical microcircuit we concentrated on the effects the GABA agonists and antagonists had on the IPSP. Indeed, the pulse response is so dominated by the substantial and long-lasting hyperpolarizing IPSP, that it is not difficult to miss the brief and relatively small EPSP that precedes the IPSP. However, in many ways, it is the excitatory response that is most critical. The most notable facet of the circuit is that the thalamic neurons only provide a small (10–20%) component of the cortical excitation (Douglas et al., 1989; Douglas and Martin, 1991a,b). The major drive to cortical neurons is from other cortical neurons. What we need most to understand is how this excitation is controlled by the GABA-mediated inhibition.

We have approached this problem in several ways, using both experimental and theoretical approaches. The clearest understanding is perhaps provided by returning to the example of direction selectivity. In our consideration of the conventional models for directionality, we provided evidence from both experiments and theory that these models could not account for the response seen. In particular, the magnitude of the inhibition was not large enough to quench the strong excitatory response evoked by stimuli moving in the optimal direction. This problem is pointed up by a glance at the schematic circuit

(Fig. 16), which shows that positive, excitatory feedback is everywhere in evidence. But, it is the rich interconnections between the excitatory populations that are at the heart of our solution to the problems of deriving the selective responses of cortical neurons.

The paradoxical absence of strong inhibition in our in vivo data may be explained if we reconsider the traditional role of the thalamic afferent excitation. In all other models, the thalamic afferents provide the major drive to simple cells, and perhaps some complex cells as well (Hubel and Wiesel, 1962; Heggelund, 1981b). In the canonical microcircuit, the thalamic afferents serve only to provide a primary source signal that is strongly amplified by the recurrent collaterals of the pyramidal cells. This provides the key to reconciling the apparently weak inhibition with the control of strong excitation. The simple explanation that emerges is that if the relatively weak excitatory input from the thalamic afferents is inhibited, then the pathways available for cortical re-excitation will not be engaged, or only weakly so. Rapid activation of inhibitory neurons by the thalamic afferents can shape the cortical response by controlling access to the cortical amplifier. If no inhibition is present, the weak geniculate excitation will be amplified by the local excitatory circuitry, which will eventually produce the strong excitation associated with optimal stimulation. The prime advantage of this process is that the inhibition need only control a small component of the excitation, not the full-blown response.

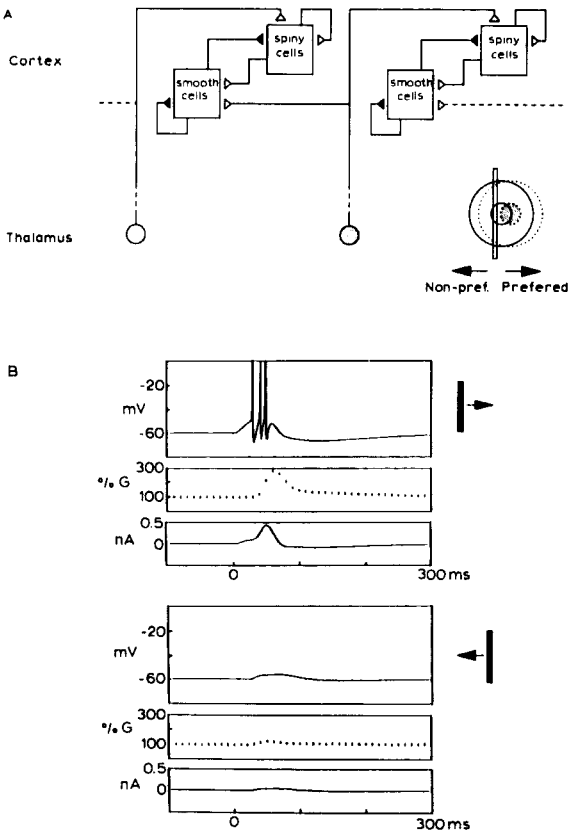


Fig. 22. Simulation of directionality with the canonical microcircuit. A. Two identical modules of the canonical microcircuit are linked to simulate directionality. For simplicity, only neurons in layer 4 were simulated. The two modules received thalamic input from "cells" with displaced receptive fields (shaded and unshaded field). B. Directional responses of the model. Top three windows: Response of an average "neuron" to preferred direction; membrane potential (top), input conductance changes (middle) and total current arriving at the soma (bottom). Bottom three windows: Response of an average "neuron" to non-preferred direction. Membrane potential (top), associated conductance increase (middle), and total current (bottom) arriving at the soma. (See Douglas and Martin, 1991a.)

Selectivity explained

To demonstrate how stimulus-selectivity might be generated by this mechanism, we have taken the example of a direction-selective neuron that is monosynaptically excited by the non-directional thalamic neurons (Douglas and Martin, 1991a,b). This is the most difficult example to account for in terms of conventional models, since the thalamic excitation is identical for both directions of motion, only the timing of the inputs is different. To simulate this we have linked together two modules, each containing the identical canonical microcircuit (Fig. 22A). To simplify the calculations we considered only the neurons within layer 4, but the same principles apply to the whole microcircuit. To achieve the bias necessary for direction-selectivity we arranged that the smooth inhibitory neurons were excited by thalamic afferents whose receptive fields were slightly displaced

from those of the afferents supplying the spiny neurons in the same module. Obviously, this is not the only manner in which a bias could be built into the circuit, but it is simple and consistent with the non-uniform distribution of the synaptic boutons of single thalamic afferent arbours (Gilbert and Wiesel, 1983; Freund et al., 1985a; Humphrey et al., 1985).

It is evident from the circuit that when the stimulus is moved in the preferred direction, the spiny neurons will be excited slightly ahead of the smooth neurons in the same module. Inhibition is then too late to prevent intracortical re-excitation, resulting in a strong response. In the non-preferred direction the smooth neurons will be excited slightly in advance of the spiny neurons they inhibit. Now the smooth neurons are able to inhibit the primary excitation provided by the thalamus, and so prevent the cortical amplification circuits from being engaged. The simplified neurons used for the simulations had biophysical properties that were, in the required respects, indistinguishable from the actual reconstructed neurons from which they were derived. We were therefore able to make precise measurements of the magnitude of the various currents and conductances during the different stimulus conditions. The net synaptic currents that arrive at the axon hillock, and the normalized conductance changes associated with these currents are shown in Fig. 22B. In the preferred direction of motion the net synaptic current is initially inward and leads to a depolarization and action potential discharge. After the discharge, the net current is outward and leads to a post-discharge hyperpolarization similar to that seen in actual neurons (Berman et al., 1991; Douglas and Martin, 1991a). This outward current is due to the extended time-course of the $GABA_B$ response. The high conductance changes associated with the response are mainly due to the excitatory currents. In actual neurons some of the conductance change at the spine head would be masked from the soma recording site by the impedance of the spine neck and dendritic shaft. In the non-preferred direction the net synaptic current is small and produces a depolarization of 4 mV. The conductance change is mainly due to the activity of the inhibitory synapses. In this case it is an increase of about 20% above control, which is near the lower limit of what we were able to measure experimentally in vivo. Thus the model provides a resolution to the paradox of the absence of strong inhibition.

The concept we have introduced here, of inhibition acting against a small primary excitation, not the full-blown secondary excitation, provides elucidation of a number of morphological observations we have made during our studies of the cortical microcircuitry. From the design of the canonical microcircuit it follows that the spiny cells operate in tandem with the smooth cells in the same module. Thus the smooth cells will produce the strongest recurrent inhibition when the spiny cells they inhibit are driven optimally. Conversely, non-optimal stimulation, which provides weak re-excitation, will drive the smooth cells weakly. This means that the smooth cells and the spiny cells they inhibit can co-exist in the same cortical column and have the same stimulus selectivity. This explains the physiological observation of simple cell responses, where the strongest inhibition is seen with the optimal stimuli (Bishop et al., 1973; Ferster, 1986; Douglas et al., 1991). Most models predict that a lack of response is due to strong inhibition (e.g., Sillito, 1984), while a strong response is taken to mean that inhibition is absent (e.g., Ferster, 1987). Because the canonical microcircuit functions in the opposite manner, it alone can explain the otherwise puzzling observation that smooth neurons provide their strongest innervation to their own cortical columns (Freund et al., 1983; Kisvárday et al., 1985a,b).

The necessity for the primary excitation to be inhibited effectively during non-optimal stimulation, also provides an explanation for the innervation of smooth neurons by thalamic afferents. In our study of X and Y-type afferents, we found that the only neurons that received direct synap-

tic input onto their somata were the GABAergic neurons (Freund et al., 1985b). We also noted that the collateral branches of the afferents that formed these somatic synapses were myelinated up to the bouton. The morphological picture suggested that thalamic excitation of the smooth neurons has to be very secure and rapid, hence excitation is delivered by myelinated axons as close to the axon hillock as possible, by-passing the cable properties of the dendrites. From the example of directionality given here, it is clear that inhibition cannot be delayed or else the intracortical circuits will engage and amplify the small thalamic signal.

Canonical benefits

The canonical microcircuit provides a secure and definable place for the GABA-mediated inhibitory system. Indeed, from the perspective of the canonical microcircuit, inhibition and excitation are as inseparable as white on rice. Structurally, inhibitory and excitatory neurons are richly interconnected. Functionally they operate in tandem. Changes in the bias or strength of one has immediate and profound consequences for the other. Thus, when viewed from the perspective of the canonical microcircuit, debates about whether the inhibitory system is involved in a particular response property of neurons in visual cortex, fade into history.

The insights we have gained into the GABAergic inhibitory system have come by looking at the system from both a structural and a functional viewpoint. In clarifying our intuitions, the importance of formal modelling cannot be over-emphasized. The theoretical work has provided a landscape in which the experimental results appear in sharp relief. In this landscape many of the current conceptual confusions about cortical processing appear to be due to a fixation on the single neuron. It is now clear to us that to think only in terms of single neurons is doomed to frustration. It is its context in the local circuits that determine the behaviour of the single neuron. Herein lies perhaps the canonical microcircuit's greatest strength, for if nothing else it draws attention to the properties of circuits, not single units.

The microcircuit provides the means of linking many different pieces of experimental data and offers novel explanations of operations at subcellular, cellular and microcircuit levels. The principles of operation of the canonical microcircuit, demonstrated here for direction selectivity, can be directly applied to other cases of selectivity, such as orientation, end-inhibition, and depth tuning. In the context of the microcircuit many of the apparently contradictory experimental results concerning GABA inhibition have been reconciled. The canonical microcircuit also offers a route through to important, but little explored areas, like adaptive gain control, recurrent pathways, and analogue parallel processing. It may also serve as a basis for understanding the microcircuits in areas where pathways other than the thalamic afferents provide the "seed" excitation. It thus provides strong direction for the coherent development of unifying theories and experimental work across a broad front.

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