Generation of end-inhibition in the visual cortex via interlaminar connections

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To understand the mechanisms by which the receptive field properties of visual cortical cells are generated, one must consider both the thalamic input to the cortex and the intrinsic cortical connections. In the cat striate cortex, layer 4 is the main recipient of input from the lateral geniculate nucleus, yet the cells in that layer possess several receptive field properties that are distinct from the geniculate input, including orientation specificity, binocularity, directionality and end-inhibition, the last of which allows cells to respond to edges of a restricted length. These properties could be generated by connections within the layer, by its input from the claustrum or by the massive projection that layer 4 receives from layer 6 (refs 6–9). In the present study, we attempted to determine the functional role of the layer 6 to layer 4 projection by reversible inactivation of layer 6 using the inhibitory transmitter γ-aminobutyric acid (GABA). After inactivating layer 6, cells in layer 4 lost end-inhibition. Cells in layer 2 + 3, which receive their principal input from layer 4, were similarly affected. The elimination of end-inhibition was specific, other receptive field properties, such as direction selectivity or orientation specificity, remaining intact.

Recordings were made in cats maintained on sodium thiopental anaesthesia, paralysed with succinylcholine and artificially respirated with 100% oxygen. At the start of an experiment virtually all of the cells in layers 2, 3 and 4 were end-inhibited to some degree. The oxygen helped maintain the proportion of end-inhibited cells, which otherwise tended to decline during the experiment. The animals' electrocardiogram, electroencephalogram, temperature and expired CO₂ concentration were continuously monitored. A small hole was drilled in the skull above the visual cortex, and the dura and pia opened. To study the role of the layer 6 to 4 pathway, we inactivated layer 6 by injecting GABA and examined the effect of this treatment on the receptive field properties in layers 2 to 4. A similar approach has been used in other systems with inhibitory transmitters or analogues, local anaesthetics or cobalt. The advantage of GABA is that it affects cells and not afferents on the effects are reversible. In initial experiments to determine the area inactivated by GABA injections of various amounts in concentrations, the micropipette was placed about 300 μm below the layer 5/6 border and a second tungsten electrode, lateral displaced at various distances from the first electrode, was advanced to approximately the same laminar position as the micropipette. The entrance into layer 6 was recognized by a spread of cells possessing long receptive fields in this layer.

After the penetrations, electrolytic lesions were made to mark the placement of the electrodes histologically and to determine the horizontal displacement between the two electrodes. In one experiment, a 0.1-μl injection of 10 mM GABA inactivated an area of ~700 μm in diameter. The inactivation lasted for several minutes and was always reversible. Having established the conditions for blockade of the action of layer 6 cells, we recorded from cells in layers 2 + 3 and 4 while the GABA pipette was in a corresponding topographic position in layer 6. Figure 1 shows response histograms of a simple cell in layer 4. When tested with a stationary flashed bar, the cell's receptive field consisted of a separate 'on' region flanked by two antagonistic 'off' regions. The cell was end inhibited: its response to a long bar (8°) was reduced by 50% compared with its response to a bar of optimal length (1°). A injection of 0.1 μl of 10 mM GABA in layer 6 had no effect on the cell's response to the short bar, but greatly increased its response to the long bar, so that end-inhibition was completely eliminated. This blockade was reversible, for 3 min after GABA injection the cell's response was again reduced over 50% and end-inhibition.

Cells in the superficial layers showed similar effects to those seen in layer 4. Figure 2 shows response histograms of a cell in layers 2 + 3 with a complex receptive field, 0.5° × 1.5° in size, on-off responses to a stationary flashing bar. The cell responded briskly when a bar of optimal length (0.5°) was moved across its receptive field, but the response was reduced by 50% when...
Fig. 1 Effect of inactivation of layer 6 on a simple cell in layer 4. The cell's receptive field was 1° x 1° and had antagonistic on (+) and off (−) subfields when tested with a stationary flashing bar. Layer 6 was inactivated by pressure injection of 0.1 μl GABA (10 mM in 0.9% saline) using a glass micropipette with an insulated tungsten electrode glued to the side. Recordings were made either through the tungsten electrode or the micropipette, and were used to determine the duration of blockade. The injection pipette was positioned in layer 6 by advancing it until cells with receptive field properties characteristic of the layer were found and then advanced an additional 300 μm. A second tungsten electrode was placed in layer 4, directly over the pipette in layer 6. The positions of the injection pipette and tungsten electrode were later confirmed histologically. Response histograms from 10 stimulus presentations each were made before (top), immediately after (middle) and 3 min after (bottom) GABA injection in layer 6. The triangles under each histogram indicate the point at which the bar reversed direction. The cell was end-inhibited, such that its response to a long bar (8°) was 54% less than its response to a bar of optimum length (1°). This inhibition was eliminated by inactivation of layer 6 (right column), whereas the cell's response to a short bar was unchanged. Calibration: vertical mark, 10 spikes per bin; horizontal mark, 1 s.

The stimulating bar was lengthened to 8°. The cell showed preferential preference, responding 2.3 times more to movement at the optimal distance than to movement in the opposite direction. When layer 6 was inactivated by injecting 0.2 μl of 10 mM GABA, the cell's response to the short bar was unchanged, but its response to the long bar was strongly increased, so that end-inhibition was eliminated. There was no effect on direction selectivity. Thus, end-inhibition was selectively eliminated, decreasing from 58% before GABA injection to 3% during layer 6 inactivation. Seven minutes after GABA injection the cell had almost completely recovered (45% end-inhibition).

For most cells, the reduction of end-inhibition after a GABA injection was readily recognized within the first few responses to a long bar swept across the receptive field. The maximal effect lasted 1-5 min and the cell's response then gradually recovered, being complete within 3-40 min. The effects were reversible after several injections. In one case, 11 injections were made without causing permanent changes in the receptive field properties of the recorded cell and without causing damage visible in Nissl-stained sections.

Fig. 2 Effect of 0.2 μl GABA (10 mM) injection in layer 6 on a complex cell in layer 2+3. Same procedure as in Fig. 1. The cell's receptive field was 0.5° x 1.5° and gave mixed on (+) and off (−) responses when tested with a stationary flashing bar. When the stimulating bar was 8° long (top right), the cell's response was reduced by 58% of its response to a bar of optimal length (0.5°, top left). During layer 6 inactivation, the cell was only 3% end-inhibited (middle right). Seven minutes after GABA injection the cell was again 45% end-inhibited (bottom right). Inactivation of layer 6 had no effect on the response to the bar with an optimal length of 0.5° (histograms in left column). Note that inactivation of layer 6 had no effect on the cell's direction selectivity. Each histogram was obtained from 10 stimulus presentations. Calibration: vertical mark, 50 spikes per bin; horizontal mark, 2 s.

To determine the spatial distribution of the effect, we compared length response curves before and during inactivation by producing and reversing the effect many times and obtaining histograms before and during inactivation for each of a number of bar lengths. An example is shown in Fig. 3. The cell showed summation until the stimulating bar was increased up to the full length of the excitatory part of the cell's receptive field; further lengthening of the bar inhibited the cell's response until the response was completely suppressed by a bar 4° in length. Inactivation of layer 6 uncovered a vigorous response to long bars, but had no effect on the response to bars equal in length or shorter than the excitatory portion of the receptive field.

We also investigated the effect of layer 6 inactivation on orientation tuning and directionality. The full range of orientations over which we could elicit a response was determined using a hand-held projector, and we determined this range before and during layer 6 inactivation. While layer 6 was inactivated, the orientation tuning to a long bar was sharper than to a short bar, as it was before the blockade. A few cells showed slight increases in width of orientation tuning, although even when end-inhibition was completely eliminated they were still clearly orientation-selective. For the entire population studied directionality was unaffected during layer 6 inactivation (Fig. 2).

The effect of layer 6 inactivation was examined on a sample of 49 end-inhibited cells from 12 cats. We selected only those cells with moderate to strong end-inhibition, and limited the size of the injection to that required for a clear reduction of end-inhibition, but never applied more than 0.3 μl in a single injection. The reduction of end-inhibition and the time course of the effect depended on the alignment of the two electrodes and the size of the GABA injection. For example, end-inhibition...
of the cell in Fig. 2 was reduced from 58% to 40% by 0.1 μl GABA and abolished by 0.2 μl. However, the amount of GABA necessary to reduce end-inhibition was independent of the laminar position of the cell, with complex cells in layer 2+3 requiring the same average dosage as simple cells in layer 4. Out of our sample of 49 cells, end-inhibition was significantly decreased for all but 3 of the cells. For the cells where no effect was seen, later histological reconstruction of the positions of the recording and injection electrodes showed that their horizontal displacement was greater than 500 μm. Figure 4 shows the extent of the change in end-inhibition for our sample population of cells in layers 2+3 and 4.

Our experiments suggest that layer 6 is the source of end-inhibition for cells in layers 4 and 2+3. Many layer 6 pyramidal cells have axon collaterals that project to and ramify within layer 4 (refs 6-8). As mentioned above, these cells have long receptive fields, often requiring relatively long bars for activation and showing increased response bar is lengthened up to 8° or more. Cells in layer 4 and above have small receptive fields, and show progressive inhibition as the stimulating bar is lengthened, up to sizes equivalent to the excitatory portion of layer 6 cell receptive fields. A model for generating end-inhibition from cells with long receptive fields was originally suggested by Hubel and Wiesel\(^{17}\). The projection pattern and receptive field properties of layer 6 cells led to the hypothesis that these cells are the main pool of the excitatory pool of the excitatory, either by direct inhibitory action or by making contact with inhibitory interneurons within layer 4 (refs 6, 14).

Layer 6 cells are likely to be excitatory, because they take up and transport aspartate, a putative excitatory transmitter\(^{18}\), and form asymmetrical synapses with round vesicles, a morphology usually associated with excitatory synapses\(^{18}\). However, using serial electron microscope reconstructions, McGuire et al.\(^{18}\) demonstrated that many of the processes that are postsynaptic to layer 6 collaterals belong to either smooth or sparsely spiny cells, which are thought to be inhibitory, as they form symmetrical synapses\(^{17,19}\) and are GABAergic\(^{16,20}\). A role for GABAergic interneurons in end-inhibition is supported by the iontophoresis of the GABA antagonist bicuculline while recording from end-inhibited cells in layer 2+3, though usually the effects were weak\(^{21}\). The fact that the response to short bars was unaffected by layer 6 inactivation is consistent with our model in that layer 6 cells are frequently not activated by short bars and therefore are unlikely to influence the response of layer cells to short bars. Finally, this hypothesis predicts that some inhibitory interneurons in layer 4 will have long receptive fields. These neurons may have been missed because interneurons are smaller and less common than principal neurons, and it is difficult to determine the receptive field length without appropriate quantitative methods. However, using intracellular recording and dye injection, A. Humphrey (personal communication) recently found a smooth stellate cell in layer 4 possessing a long receptive field similar to those of layer 6 neurons.

Our findings are comparable to those of Sherker and LeVay\(^{12}\) who studied the role of the projection from claustrum to cortex. The claustrum projects to all layers of visual cortex, but most heavily to layer 4 (ref. 5). Cells in the claustrum have similar receptive fields to layer 6 (ref. 6), and stimulating the claustrum lesioned, cortical cells lose some of their end-inhibition. The effects of inactivating layer 6 seen in the present study were stronger than those caused by lesioning the claustrum. Since layer 6 is the source of input to the claustrum and since this pathway is retinotopically organized\(^{12}\), inactivating layer 6 could effectively block the claustral input to layer 4 as well as the direct layer 6 to 4 projection, thus removing both sources of end-inhibition with a single injection.

Inactivating layer 6 abolished end-inhibition in layer 2+3 readily as in layer 4, although layer 6 projects predominantly to layer 4. However, spiny stellate cells in layer 4 have a strong presumably excitatory projection to layer 2+3 (refs 6, 9). Our results therefore suggest that cells in layer 4 endow layer 2+3 neurons with end-inhibition, an idea which is further supported by the finding that layers 2+3 and 4 have the same proportions of end-inhibited cells showing a comparable degree of end-inhibition. This may explain why bicuculline ionsphoretically could not eliminate end-inhibition in the superficial layers, since cells to which the bicuculline was applied may not have generated end-inhibition themselves, but instead may have inherited it from cells distant from the ionsphoretic pipette. Our findings provide indirect evidence for an excitatory link between layer 4 and 2+3, and suggest that at least one property is transferred.
though that pathway. This conflicts with the finding that layer 1 cells appear normal when layer 4 is silenced by inactivation of a lamina of the geniculate, requiring all receptive field properties to be generated anew in each cortical layer. Instead, results are consistent with the hypothesis of Hubel and Wiesel of a sequence of processing from simple cells to complex cells representing a functional interaction between cortical layers.

In conclusion, we have shown an association between one component of the intrinsic cortical circuit and a specific receptive field property. Our results demonstrate how a functionally defined class of cortical cells participates in the transformation of geniculate input occurring within the striate cortex.

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