

## AN INTRACELLULAR ANALYSIS OF GENICULO-CORTICAL CONNECTIVITY IN AREA 17 OF THE CAT

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*(Received 29 April 1982)*

### SUMMARY

1. The latencies of excitatory and inhibitory post-synaptic potentials (e.p.s.p.s and i.p.s.p.s) evoked by electrical stimulation of afferents from the lateral geniculate nucleus were recorded in neurones of area 17 of the cat visual cortex. After application of an extrapolation procedure to compensate for the conduction time of the afferent axons, a histogram of latencies formed three distinct peaks. Potentials in each of these were interpreted as being mediated by mono-, di- and trisynaptic pathways.

2. Characteristic laminar differences in the extracellular field potentials evoked from the lateral geniculate nucleus (l.g.n.) and in the antidromic activation of neurones from the l.g.n. and superior colliculus were used to determine the laminar position of recorded neurones. It was found that within a given layer, all cells maintained similar connexions with relay cells in the l.g.n. Cells in layers 3, 4, upper 5 and 6 were monosynaptically excited by geniculate afferents, while cells in layers 2 and lower 5 received only indirect excitation via other cortical neurones. Layer 3 cells were unique in receiving a prominent disynaptic e.p.s.p. in addition to the direct excitation from the l.g.n. Late, trisynaptic e.p.s.p. components were seen in many layer 5 and 6 cells.

3. The orderly laminar arrangement of the connexions had the consequence that identified cortico-geniculate neurones were monosynaptically excited and cortico-collicular neurones di- and trisynaptically excited by geniculate afferents. Cortico-cortical neurones in layers 2 and 3 received di- or mono- plus disynaptic excitation, depending on laminar position.

4. Post-synaptic inhibitory potentials were evoked in all impaled cells, following stimulation of the geniculo-cortical pathway. Except for a few layer 2 cells, this inhibition was mediated through disynaptic pathways of the feed-forward type. There was a good positive correlation between conduction times for monosynaptic e.p.s.p.s and disynaptic i.p.s.p.s in the same cells, suggesting that cortical neurones receive excitation and inhibition from the same type of geniculate afferents.

5. The stimulating electrodes activated not only geniculo-cortical afferents, but antidromically activated cortical efferent neurones from their extracortical axons. These neurones possess intracortical collaterals, and care must be taken to distinguish

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the resulting potentials from those mediated by orthodromic activation of geniculate afferents. In doing so, evidence was obtained for excitatory connexions from layers 2 and 3 to layer 5, from layer 5 to layer 6, and from layer 6 to layer 4. Typical recurrent inhibition was not observed.

6. The receptive field properties of many neurones were examined before penetration. Simple cells were found in layers 4 and 6 and they all received prominent monosynaptic excitation from the l.g.n. Cells in layers 2, 3 and 5, as well as a few non-projecting cells in layer 6, had complex receptive fields. The connectivity of these cells varied with laminar position; complex cells in layers 6 and upper 5 received direct excitation, those in 3 direct plus indirect excitation and those in 2 and lower 5 only indirect excitation from the l.g.n. The significance of these results for the construction of cortical receptive fields is discussed.

#### INTRODUCTION

In an attempt to understand how the receptive fields of visual cortical cells are constructed by neuronal circuitry, many investigators have studied the projection to the cortex of the lateral geniculate nucleus (l.g.n.). There are several reasons for this approach. The l.g.n. is the major source of visual input to the cortex, and at the synapse between geniculate relay cells and cortical cells, receptive fields undergo the remarkable transformation from centre-surround organization to orientation selectivity. The geniculo-cortical projection is also more accessible than intracortical connexions for analysis with certain anatomical and physiological techniques. In addition, the organization of the connexion between the l.g.n. and the visual cortex has been the subject of several models that seek to explain cortical function, most notably the serial processing model of Hubel & Wiesel (1962) and the parallel processing model in its various forms (see Stone, Dreher & Leventhal, 1979). In these different models, the receptive field properties of cortical cells have been attributed either to the synaptic order of input from geniculate relay cells, to the receptive field type of the input (that is, either X or Y), or to the spatial organization of input from on and off relay cells.

Questions concerning the physiological organization of the cortex are inseparable from those of anatomical structure, and in particular, laminar structure. Cells with different receptive field properties and different intra- and extracortical projections are segregated into different layers. So also are the axon terminals of geniculate relay cells, and some classes of cells predominate in layers containing geniculate terminals, while others avoid these layers. Simple cells form an obvious example: they are found almost exclusively in layers 4 and 6 (Gilbert, 1977) where X and Y relay cells terminate (LeVay & Gilbert, 1976), suggesting that simple cells receive direct input from the l.g.n., in apparent agreement with the model proposed by Hubel & Wiesel. Yet, an intermingling of cell bodies and axon terminals does not necessarily imply that synapses are formed between them, nor does a separation between them imply the lack of connexion. Apart from cells in layer 2, almost all cortical neurones are in a position to make direct synaptic contact with geniculate terminals somewhere on their dendritic trees. It is only by the direct examination of single neurones that the connectivity of different cell classes can be understood.

In most previous physiological studies of geniculo-cortical connectivity (for references see Discussion), the latencies of potentials evoked by electrical stimulation of the visual pathway are used as an indication of connectivity. It is assumed that the more synapses interposed between the stimulated and recorded neurones, the longer will be the latency. No matter how simple in principle this experiment may be, it is a difficult one to perform and interpret. It may be for this reason that several previous studies (Singer, Trepper & Cynader, 1975; Bullier & Henry, 1979*a*) have found little correlation between geniculo-cortical connectivity and receptive field properties or laminar position despite the elegant anatomical organization of the cortex. We have now re-examined the synaptic connexions between geniculate relay cells and cortical neurones using intracellular recordings and an extrapolation procedure to determine connectivity. The method has allowed us to overcome some of the problems of previous studies and in addition to identify some intrinsic collateral connexions of cortical efferent neurones.

#### METHODS

*Animal preparation.* Experiments were performed on normal cats 4–12 months old, weighing between 2 and 3 kg. Anaesthesia was induced with ketamine HCl (25 mg/kg i.m.) followed by sodium pentothal (20–30 mg/kg i.v.). Additional small doses of pentothal were given as needed to maintain the animal in the state of slow wave sleep (1–2 mg/kg .h). A paralytic (gallamine triethiodide) was infused and the animal artificially respirated with positive end-expiratory pressure of 1–2 cmH<sub>2</sub>O. Tidal volume was adjusted to maintain end-expiratory CO<sub>2</sub> at 3.5–4.0%. To reduce movements of the brain due to respiration, the animal was suspended by a clamp placed on the vertebrae at the mid-thoracic level, and a pneumothorax was performed just before recording. Temperature was maintained at 38 °C. For visual stimulation, the eyes were fitted with contact lenses of a curvature appropriate to focus them on a tangent screen placed 57" in front of the cat. Accommodation was paralysed with atropine and the nictitating membranes retracted with Neo-synephrine.

*Recording.* Intracellular recordings were made with micropipettes whose tips had been broken back to a diameter of approximately 0.5  $\mu$ m. Filled with 3 M-potassium acetate, they had a d.c. resistance of 10–20 M $\Omega$ . In some experiments, extracellular recordings were made with electrodes filled with a 2% solution of Pontamine Sky Blue dye in 0.5 M-NaCl. Dye marks of about 50  $\mu$ m in diameter were made in an electrode track by passing 1–2  $\mu$ A of negative current out of the electrode tip for 5 min (Kaneko & Hashimoto, 1967). The electrodes were advanced through the brain with a stepping-motor driven manipulator. At the beginning of each track, the exposed brain was covered with a solution of warm agar (3% in 0.9% saline).

Most impaled cells were clearly damaged by the penetration: after an initial period of injury discharge, the spike mechanism was often blocked and synaptic potentials gradually declined in amplitude. If possible, the cell was hyperpolarized by current injection through the recording electrode to prevent injury discharge and to stabilize the recording. Current injection was also routinely used to reverse or enhance post-synaptic potentials (p.s.p.s). For this reason, d.c. membrane potential was not measured since polarization of fine electrodes by passage of current makes such measurements of little value. Despite the difficulties, it was still possible to measure reliably latencies of synaptic potentials. For each class of cell we also managed to obtain a few stable penetrations lasting up to 1 h with 70–90 mV action potentials. Measured latencies in these cells did not differ from the over-all population.

Receptive field properties were investigated with stimuli projected onto the tangent screen with a hand-held projector. Our scheme for identifying receptive field types was based on Hubel & Wiesel's (1962). This means that a cell was classified as simple only if it had at least two separate on and off discharge regions. Special attention was given to the off regions of simple cells in order to distinguish them from the inhibitory flanks of complex cells (Bishop, Henry & Smith, 1971). A true off response could be elicited from the former, while the latter produced only inhibition in

response to a flashing or moving slit (Ferster, 1981). Complex cells were further subdivided into special and standard types according to Gilbert (1977).

Although synaptic potentials were recorded intracellularly, receptive field properties were usually investigated extracellularly, before penetration of a cell. There was initially the question whether the electrode ever penetrated a different cell from the one whose properties had been identified, thus leading to the association of the receptive field properties with the wrong pattern of connectivity. This seemed to occur very rarely, if ever. In those cases where the cell was still capable of firing action potentials after penetration, the receptive field properties were identical before and after penetration. In addition, a large proportion of cells could be activated antidromically from one or more of the stimulating electrodes, and for each one the threshold and latency of the

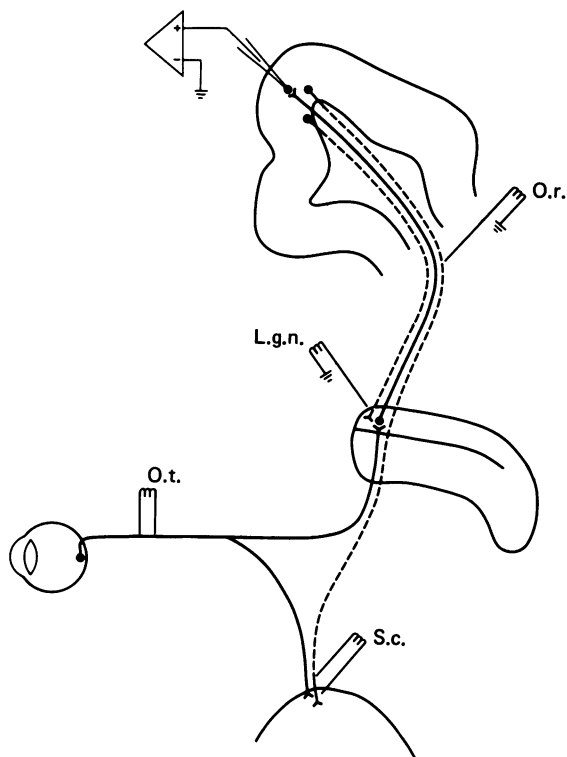


Fig. 1. Placement of the stimulating electrodes in the o.t., l.g.n., o.r. and s.c. Note that the s.c. electrode may activate both retinal ganglion cells and cortico-collicular cells antidromically. The l.g.n. electrode may stimulate the axons of cortico-collicular and cortico-geniculate neurones, as well as geniculo-cortical neurones, while the o.r. electrode may reach also cortico-cortical axons (not included in the diagram).

antidromic spike was the same before and after penetration. Neighbouring cells in extracellular recordings always differed at least in one of these parameters and usually in both. Therefore, errors of interpretation due to this aspect of the procedure seemed unlikely.

*Electrical stimulation.* The visual system was stimulated at four different points with cathodal current pulses (0.2 ms duration) delivered through tungsten electrodes placed as indicated in Fig. 1. A concentric electrode was positioned in the optic tract (o.t.) just posterior to the optic chiasm, in a point giving evoked potentials in the visual cortex and the l.g.n. at low threshold ( $< 30 \mu\text{A}$ ). A second unipolar electrode was placed in the upper part of lamina A of the l.g.n. This electrode was placed so that the receptive fields of surrounding l.g.n. cells were close in register with the receptive fields of recorded cortical cells. In practice, when misalignment of the electrodes separated

the receptive fields by more than the diameter of a geniculate receptive field centre, the threshold of evoked potentials in the cortex rose abruptly. Therefore, at the start of a new cortical penetration, it was usually necessary to move also the l.g.n. electrode.

A third electrode was placed in the optic radiations (o.r.) so that cells recorded through the l.g.n. electrode could be antidromically activated at low stimulus intensities. The final position of the tip was usually at lateral 5, about 5 mm below the surface of the cortex and 3–4 mm anterior to the recording electrode. The o.r. electrode was also moved at the start of new cortical penetrations. Finally, a concentric electrode was placed in the superior colliculus (s.c.), here again so that the receptive field positions of recorded collicular neurones matched those found for cortical cells. The depth of this electrode was adjusted to maximize the evoked potentials recorded at the cortical surface.

With the electrodes placed as described, the threshold of field potentials evoked in the cortex was less than  $50 \mu\text{A}$ , but to obtain maximal synaptic effects at the shortest latencies, stimulus currents frequently had to be increased to 500 or even  $1000 \mu\text{A}$ . We were therefore concerned over the possibility that the stimulation current might cause damage to the tissue surrounding the electrode tip. In fact, Nissl-stained sections often revealed small lesions at stimulation sites. These lesions were usually too small to cause a major problem, but at times it seemed that after long periods of stimulation at a single point it became difficult to evoke visual responses from cells in the corresponding region of the cortex. In these cases the penetrations were terminated and the recording electrode moved a few millimetres to a new region of cortex.

*Laminar position of recorded cells.* The usual method for determining the laminar position of recorded cells is to mark the position directly with horseradish peroxidase (HRP) or dye ejected from the micropipette. Unfortunately, electrodes filled with these substances proved impractical for the present investigation since the high resistance made it difficult to polarize cells in the course of examining synaptic potentials. We have instead relied on the fact that extracellular field potentials vary systematically from layer to layer in the cortex and that, in addition, cells in different layers project to different regions of the brain. Together these criteria provide a surprisingly accurate method for locating recorded cells. The method has the distinct advantage that the laminar position is known at the time of recording and does not require subsequent histology.

Penetrations were made in area 17, medial to the crest of the lateral gyrus, between Horsely-Clark coordinates P1 and P7. Electrodes were angled perpendicular to the cortical surface and subsequent reconstruction of electrode tracks verified that they had passed from layer 1 to layer 6 in sequence, as intended. Such a penetration through the cortex, made with a dye-filled electrode, is illustrated in Fig. 2 along with the extracellular field potentials evoked from the l.g.n. at several depths. The first change in the field potentials occurs in layer 2–3 exactly halfway between the lower border of layer 1 and the upper border of layer 4. Above this point, the field potentials consist of a single negative component at about 2.5 ms latency. Below it, an earlier negative component with a latency of just over 1 ms appears and grows to its full size within  $100 \mu\text{m}$  or less. This transition point has been marked with dye spots in nine penetrations and was always found to be at the same laminar position. The early potential probably reflects the monosynaptic activation of cells in the region where it is seen (see Results), but more importantly here, it precisely indicates when an electrode has passed from the upper to the lower half of layer 2–3.

All the cells encountered before the appearance of the early field component and up to  $250 \mu\text{m}$  below it had complex receptive fields. At greater depths, however, simple cells were encountered. We took the appearance of simple cells to be an indication of the electrode having entered layer 4, given the reports of Gilbert (1977) and Gilbert & Wiesel (1979) concerning the location of cells of different receptive field type. Complex cells were not encountered again until reaching layer 5.

Entrance into layer 5 was marked by a decrease in the early component of the field potentials (which was later found to correspond to a relative lack of monosynaptically activated cells in this layer). The change in the potentials is not as abrupt or as obvious as it is in the upper layers, however (Fig. 2). Layer 5 is more easily distinguished by the presence of cortico-collicular cells (Palmer & Rosenquist, 1974; Gilbert & Kelly, 1975). Immediately upon entering, several cells may be recorded extracellularly that can be antidromically activated from the superior colliculus. Even if an impaled cell could not itself be activated in this manner the antidromic activation of nearby cells could be used as an indication that the cell was in layer 5.

In layer 6, the early component of the extracellular field potentials reappears, no doubt reflecting

the activity of collaterals of geniculate afferents there (LeVay & Gilbert, 1976). Layer 6 cells project to the l.g.n. (Gilbert & Kelly, 1975) and upon entering this layer, dozens of neurones, antidromically activated from the l.g.n. but not the s.c., can be recorded extracellularly.

It should be noted that layers 5 and 6 could be distinguished even in the absence of an electrode in the s.c. Many cortico-collicular cells (31/39) could be antidromically activated from the l.g.n., presumably because their axons passed through the l.g.n. on their way to the s.c. Their threshold for activation from the l.g.n. was, however, much higher (0.5–2 mA) than for nearby cortico-geniculate neurones (10–200  $\mu$ A). There was also a striking difference in the latency of antidromic

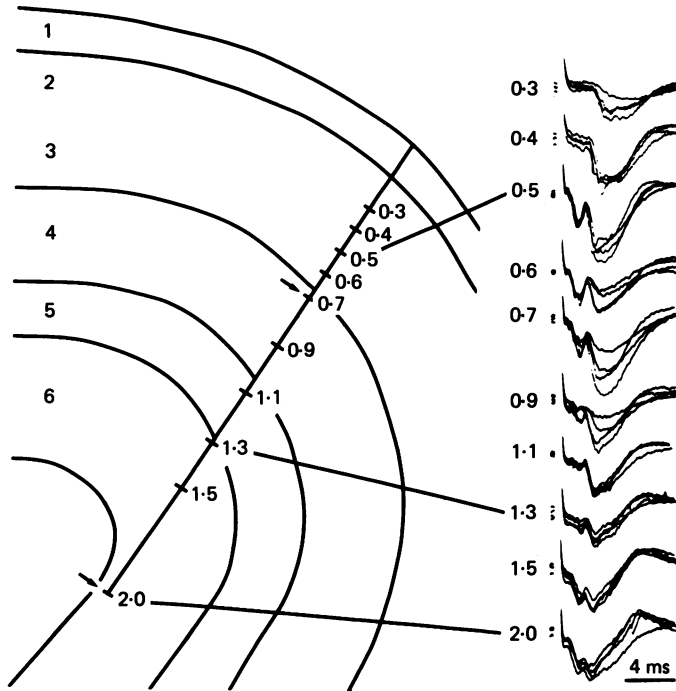


Fig. 2. Depth profile of extracellular field potentials evoked in the cortex by stimulation in the l.g.n. (0.2 mA, 0.2 ms). The electrode track was made with a dye-filled electrode and two spots were made by current ejection (arrows). Extracellular field potentials were recorded at the depths indicated in millimetres. The marked difference in the potentials between the upper and lower halves of layer 2–3 (compare records at 0.4 and 0.5 mm) was observed in nine similar experiments. Note that the early negative potential (latency 1.3 ms) that characterizes layers 3 and 4 is reduced in layer 5, but reappears in layer 6.

spikes evoked from the l.g.n. electrode. In dye-marking experiments such as the one illustrated in Fig. 2, it was found that many layer 6 cells had very long antidromic latencies, ranging between 2.5 and 45 ms (Fig. 3). For half the cells the latency shortened in one or more discrete steps of up to 2.5 ms as the stimulus intensity was increased. Such latency shifts are typical for terminally branching axons (Jankowska & Roberts, 1972). On the other hand, cortico-collicular cells that could be antidromically activated from the l.g.n. had antidromic latencies of 3.5 ms or less and the latency did not change significantly with increased stimulus intensity (although such behaviour was frequently observed when the same cells were activated from the s.c.). There is a slight overlap in the latencies of the two populations, but the appearance of many extracellularly recorded cells with antidromic latencies from the l.g.n. tens of milliseconds in length was an unequivocal indication that the electrode had reached layer 6.

Mislocalization of cells due to recordings from apical dendrites or axons seemed to pose a small problem since such structures were only rarely impaled with perpendicular electrode penetrations.

On one occasion only was an intracellular recording obtained from an identified cortico-collicular neurone above layer 5 (in upper layer 4), while cortico-geniculate neurones were never impaled outside layer 6. A few intra-axonal recordings were easily recognized by the shape of the action potential and by the slow rise and small amplitude of the synaptic responses.

*The source and synaptic order of recorded potentials.* Because of the elaborate interconnexion of the visual system, our electrodes stimulated not only axons of geniculate relay cells but also axons of cortical cells projecting to other parts of the brain. Many of these cells are known to have extensive collaterals within the cortex. For this reason, a potential was not accepted as being the result of

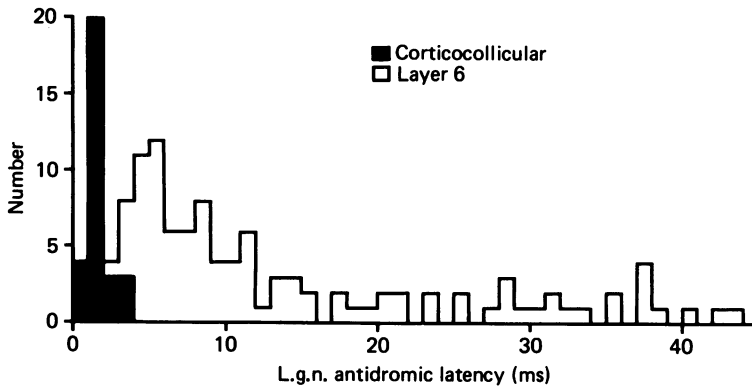


Fig. 3. Latencies of antidromic spikes evoked from the l.g.n. in 134 neurones. The thirty cells indicated by filled areas could also be antidromically activated from the s.c. electrode. Open areas indicate 104 cells recorded with dye-filled electrodes and subsequently found to be in layer 6. Half of the layer 6 cells had two or three different latencies depending on the strength of stimulation. For these, the earliest latency is shown.

input from the l.g.n. unless a similar potential could be evoked from the o.t. with a longer latency, corresponding to the extra conduction time plus the synaptic delay within the l.g.n.

Once a potential was identified as being due to an input from the l.g.n., its latency was used to determine its synaptic order, that is, the number of synapses the signal had to pass in the cortex before reaching the recorded cell. It is important to remember that the latency of interest here is the time between the arrival of the afferent volley at the terminals of the geniculate axons in the cortex, and the beginning of the synaptic potential. After stimulation in the l.g.n. the latency actually recorded includes at least two additional components: spike initiation time and conduction time in the afferent axons. All latencies were measured from the onset of the stimulus shock artifact and therefore include the spike initiation time, being 0.2 to 0.3 ms with the used stimulus intensities (1.5–2 times threshold for the critical fibres; Jankowska & Roberts, 1972).

To compensate for the conduction time in the geniculo-cortical pathway, an extrapolation procedure was used in which the latency of the evoked potentials was measured after stimulation at two different points of the pathway, in this case at its start in the l.g.n., and in the o.r. at about 2/3 of the way to the cortex. The difference in latency between the potentials evoked from the two sites should reflect the conduction time of the stretch of axon running between them, that is, of 2/3 of its length. The conduction time of the entire axon is therefore 3/2 the difference between the two latencies. Once determined, it can be subtracted from the total l.g.n. latency. This procedure is represented graphically by plotting the o.r. and l.g.n. latencies against the relative distance from the cortex of the two stimulation electrodes. After connecting these points with a straight line (see Figs. 5, 6, and 8), the intercept of the line with the ordinate represents the l.g.n. latency (including the spike initiation time) minus axonal conduction time in the optic radiations. This intercept was used to determine the synaptic order of a potential and it will be referred to as the *extrapolated latency*.

The extrapolation procedure assumes that the conduction velocity of a geniculate axon is constant along its length. To test this assumption, we recorded from a small number of relay cells in the l.g.n. and stimulated their axons at several points in the optic radiations. Over most of their

length, these axons indeed had a constant conduction velocity, but there was a clear decrease in velocity near their termination in the cortex, no doubt because the axons become thinner as they branch (Ferster & LeVay, 1978). The extrapolation therefore underestimates the real conduction time to the terminals by 0.1–0.2 ms, more for slowly conducting axons. This experiment also allowed us to trace out the course of the geniculate axons by finding low threshold points for antidromic activation of geniculate neurones. Their lengths, measured between the usual positions of the electrodes in the cortex and l.g.n., was about 21 mm. The distance to the o.r. electrode from the cortex was about 7 mm. Hence, the value of 2/3 was used in the extrapolation for the distance between the stimulating electrodes relative to the total length of the axon.

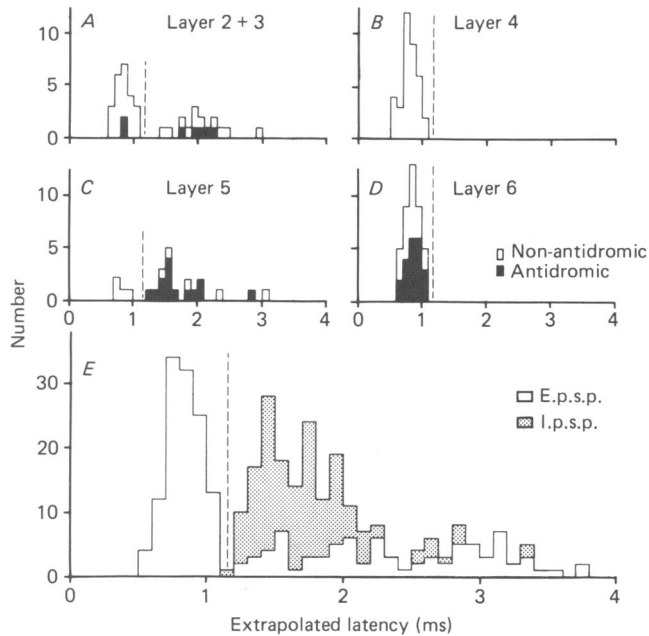


Fig. 4. Extrapolated latencies for p.s.p.s mediated by afferent fibres of relay cells in the l.g.n. *A–D*, the earliest e.p.s.p. recorded in 139 neurones for which laminar position was determined (see Methods). Shown by filled areas are cells activated antidromically from any of the stimulation electrodes. *E*, extrapolated latencies for 201 e.p.s.p.s and 124 i.p.s.p.s recorded in 180 cells impaled in these experiments. Besides the cells in *A–D*, a number of functionally unidentified neurones were included in this material. The distribution was used to determine the latency ranges for potentials of different synaptic order. The division between mono- and disynaptic potentials was taken to be 1.05 ms and is indicated by a dotted line on each histogram. The di-/trisynaptic division is less clear but was taken to be 2.4 ms.

The extrapolated latencies for a large number of synaptic potentials of geniculate origin in an unselected sample of cortical neurones were used to determine the latency ranges for potentials of different synaptic order (Fig. 4*E*). In this histogram there are three more or less distinct peaks, which were interpreted as representing potentials mediated by mono-, di- and trisynaptic pathways (division points 1.05 ms and 2.4 ms). The interpretation is strengthened by the observation that all inhibitory p.s.p.s (i.p.s.p.s) fall into the second and third peaks, implying that geniculate afferents have only excitatory effects on cortical cells. The average extrapolated latency of potentials in the first peak is 0.7 ms, which as mentioned, includes the spike initiation time and an extra delay due to the lower conduction velocity in the terminal branches of the axon. The actual synaptic delay is therefore more likely to be about 0.3–0.4 ms.

The scatter in the extrapolated latencies of presumed monosynaptic potentials may be due to



factors including errors in determining onset of the intracellular potentials. An uncertainty of 0.1 ms for latencies from the l.g.n. and o.r. could make a maximum error of 0.3 ms in the extrapolated latency. The value used for the length of geniculate axons is also an average; the actual length is certain to vary a few millimetres from cell to cell. Finally, the slowing of conduction velocity in the afferent terminals is likely to vary slightly from axon to axon. For di- and trisynaptic potentials a fourth factor is introduced, namely the spike initiation time and axon conduction time in the intervening cortical cells. The variability in this extra delay no doubt widens the second peak compared to the first and may account for the greater degree of overlap between the second and third peaks in Fig. 4A. Despite all these sources of error the separation between the three peaks is relatively clean and it simply remains to determine into which peak a given potential falls in order to determine its synaptic order.

## RESULTS

The effect of electrical stimulation of the visual pathway was tested in over 500 cortical neurones. For analysis of connexions with geniculate afferents, however, we have restricted our consideration to 139 intracellularly recorded cells, since the latencies of extracellularly recorded spikes proved to be too unreliable in determining synaptic order. Before penetrating some cells, no spikes at all could be evoked by electrical stimulation. Yet after penetration, prominent excitatory p.s.p.s (e.p.s.p.s) were observed. Even sizeable monosynaptic e.p.s.p.s were sometimes incapable of bringing a cell to threshold. Thus with extracellular recording it was not always possible to detect a connexion. Another serious problem was the variability in the delay between the onset of e.p.s.p.s and spikes. The average delay was 0.4 ms, but it varied by over 0.8 ms, not only from cell to cell, but between responses from different stimulating sites in the same cell. As a result, no clear separation in latency between extracellular spikes of different synaptic order was apparent, while e.p.s.p. latency gave an unambiguous indication of the number of synapses in the path between the l.g.n. and any given cell (Fig. 4E). One potential problem with limiting the analysis to intracellularly recorded cells was the possibility that the sample would be biased towards larger cells. Such a bias cannot be excluded, but there was no significant difference in the receptive field properties or laminar positions of the extra- and intracellularly recorded samples.

### *Laminar organization of geniculo-cortical connectivity*

*Layer 4.* The bulk of the terminals of geniculate relay cells is located in layer 4, and from electron microscopic studies, it appears that most (Davis & Sterling, 1979), if not all (Hornung & Garey, 1981) of the neurones in the layer do make synapses with these terminals. Not surprisingly, therefore, it was found that layer 4 cells receive monosynaptic excitation from the l.g.n. (Fig. 4B). Typical examples of synaptic potentials in a layer 4 cell are shown in Fig. 5. A large e.p.s.p. was evoked from the l.g.n. electrode (Fig. 5B) with a latency of 1.3 ms (compare the intracellular record with the extracellular record lowermost in Fig. 5H). An e.p.s.p. of similar time course and amplitude was also evoked from the o.r. electrode with a latency of 0.9 ms (Fig. 5C). The geniculate origin of these potentials is demonstrated by the fact that a similar potential could be evoked from the o.t. electrode. The latency of this e.p.s.p. was 1.5 ms longer than that of the e.p.s.p. from the l.g.n., just long enough to account for the conduction time of the retinogeniculate axons and the synaptic transmission

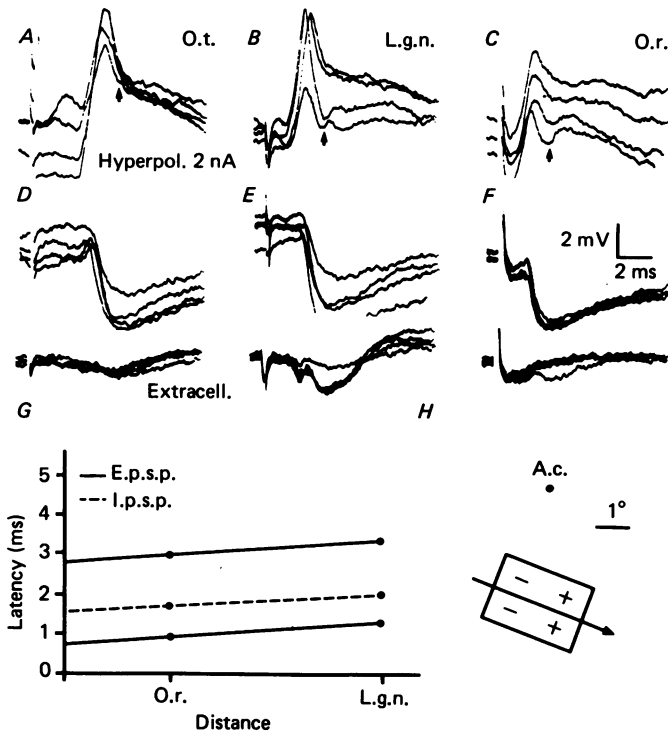


Fig. 5. P.s.p.s recorded in a layer 4 simple cell. Records of potentials evoked from each stimulation electrode are arranged in columns as marked above. In each row, records were taken under the same conditions (gain, sweep speed, injected current, etc.). *A-C*, e.p.s.p.s evoked from the o.t., l.g.n. and o.r. with latencies of 2.8, 1.3 and 0.9 ms. Small trisynaptic e.p.s.p.s are also visible. The latencies indicated by the arrows were estimated by comparing several records. These records were taken while a small hyperpolarizing current was injected to reduce i.p.s.p. amplitudes. *D-F*, i.p.s.p.s evoked in the same cell from corresponding stimulation sites. The lower traces are extracellular field potentials taken just outside the cell. *G*, extrapolation of the latencies of the intracellular potentials (see Methods). The latencies of the p.s.p.s evoked from the l.g.n. and o.r. are plotted against the distance of each electrode from the recording site in the cortex (7 and 21 mm). The point at which the connecting line intercepts the ordinate is the extrapolated latency from which connectivity is judged. The values for the e.p.s.p.s, 0.7 and 2.8 ms are in the mono- and trisynaptic ranges. The i.p.s.p. is disynaptic (1.6 ms). *H*, the cell's receptive field. Plus and minus signs indicate on and off subfields. The arrow indicates preferred stimulus direction. A.c. area centralis.

within the l.g.n. The extrapolation procedure used to determine the intracortical latency for the e.p.s.p. (see Methods) is shown graphically in Fig. 5*G*. Since the 0.4 ms difference in latency between the l.g.n. and o.r. potentials represents the conduction time of 2/3 of the afferent axons, the entire conduction time must be 0.6 ms. The remaining portion of the l.g.n. latency, 0.7 ms, is the extrapolated latency from which the connectivity is judged. This value clearly falls in the range for monosynaptic potentials in the histogram of Fig. 4*E*.

Apart from the prominent direct excitation from geniculate relay cells, several other synaptic potentials were evident in layer 4 cells.

(1) Stimulation in the o.t., l.g.n. and o.r. evoked a large i.p.s.p., which became visible

when the cell was more depolarized (Fig. 5*D-E*). This i.p.s.p. had an extrapolated latency of 1.6 ms, which is in the middle of the disynaptic range (Fig. 5*G*, dotted line). The pattern of a monosynaptic e.p.s.p. and disynaptic i.p.s.p. was common to all thirty-six cells impaled in layer 4.

(2) Ten layer 4 cells responded to stimulation of the superior colliculus with an e.p.s.p.-i.p.s.p. sequence similar in shape and latency to that seen in response to o.t. stimulation. These neurones were recorded in the upper part of layer 4 where axons of Y-type geniculate relay cells terminate (Ferster & LeVay, 1978). The effect was presumably the results of antidromic activation of Y-type retinal ganglion cells from their collaterals in the s.c. (Fukuda & Stone, 1974).

(3) In addition to monosynaptic excitation from relay cells in the l.g.n., di- or trisynaptic e.p.s.p.s were occasionally observed (four cells). An indication of an e.p.s.p. with trisynaptic latency is visible in Fig. 5*A-C* (arrows). These late e.p.s.p. components were much smaller in amplitude than the monosynaptic response in the same cells.

(4) All the potentials described above were due to activation of geniculate relay cells as demonstrated by the response to o.t. stimulation. With high intensity stimulation in the l.g.n., an additional e.p.s.p. appeared in every cell tested that was not matched by appropriate responses from the o.t. This potential was mediated by the intracortical axon collaterals of antidromically activated cortico-geniculate cells and is examined in detail in a subsequent paper (Ferster & Lindström, 1983 and in preparation).

Receptive field properties were studied in twenty-four of the thirty-six cells impaled in layer 4. The remaining twelve cells were impaled suddenly, before their receptive field was properly analysed, and the damage caused by the penetration prevented a reliable intracellular investigation. All studied layer 4 cells (including many that were not impaled) were typical simple cells, giving on and off discharges in separate regions of the receptive field, and mutual antagonism between these regions. The cells had fields with two or three subregions and could be end-stopped. As pointed out by Gilbert (1977), simple receptive fields in layer 4 differ from those in layer 6 in being relatively short along the axis of orientation. In fact, the subfields of most layer 4 cells in the present study were approximately square and of the same size as the receptive field centres of geniculate relay cells, recorded simultaneously at the same eccentricity with the stimulating electrode in the l.g.n. (see Methods).

*Layers 2 and 3.* In area 17, the border between layers 2 and 3 cannot be easily distinguished anatomically. Nor is there any great difference in the receptive field properties of cells in the upper and lower parts of the supragranular layers. With the possible exception of a few special complex cells located at the lower border of layer 3, cells in layers 2 and 3 have standard complex receptive fields and the position of a cell within the layers is not correlated with ocular dominance, orientation tuning, direction selectivity or end-stopping (Gilbert, 1977). It was therefore surprising to find a striking dependence of the connectivity of the cells upon position within layers 2 and 3. The first hint of such a dependence came from the extracellular fields recorded in these layers. As an electrode reaches the point halfway between the lower border of layer 1 and the upper border of layer 4, an early extracellular field potential with a latency of 1.0–1.3 ms suddenly appears in the response to stimulation in the l.g.n. (Fig. 2, Methods).

The region below this transition point will be called layer 3. All the cells recorded in layer 3 whose visual responses were studied had standard complex receptive fields of relatively small size (average width  $2^\circ$ ). Of the eighteen cells impaled in this region, all received monosynaptic input from the l.g.n. An example is shown in Fig. 6. That this cell was located in layer 3 can be seen in the extracellular records in the lower part of *F*, which show a negative inflexion at just over 1 ms. (The field potentials

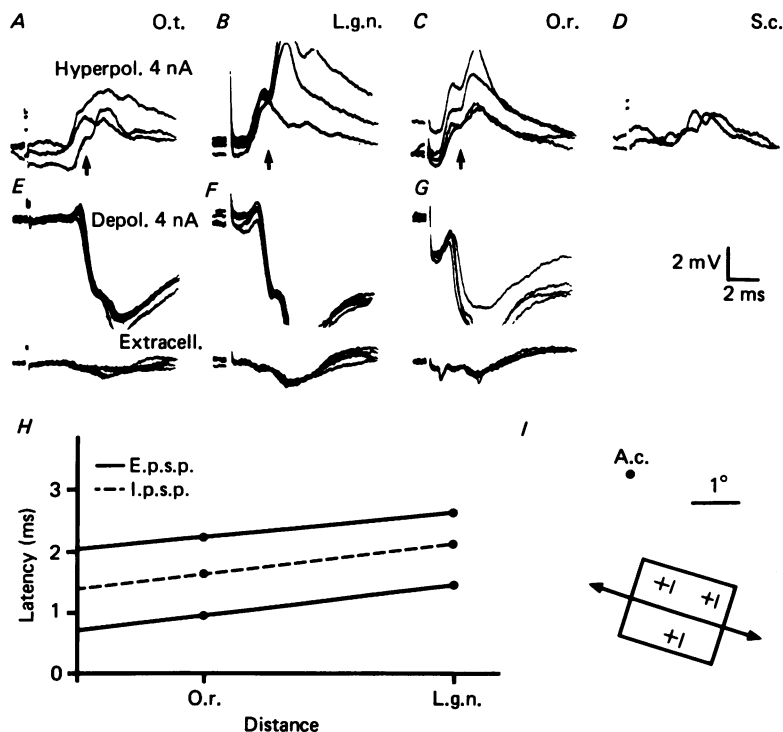


Fig. 6. P.s.p.s recorded in a layer 3 complex cell. *A–D*, e.p.s.p.s evoked from the four stimulating electrodes. In addition to a large monosynaptic e.p.s.p., the cell received prominent disynaptic e.p.s.p.s from the o.t., l.g.n., and o.r. (arrows). *E–G*, i.p.s.p.s revealed by injection of depolarizing current. Note the small inflexions near the bottom of the i.p.s.p.s, representing the remnant of the disynaptic e.p.s.p.s. Below are the extracellular traces; in *G*, an antidromic spike from a distant cortico-cortical cell is visible. *H*, extrapolation. *I*, the cell had a standard complex receptive field and gave on and off discharges throughout its receptive field.

in Fig. 6 are much smaller than those in Fig. 2, primarily because the electrode used for intracellular recording was much finer than that used for dye-marking.) The early e.p.s.p.s evoked from the o.t., l.g.n. and o.r. had short latencies, little jitter and extrapolated to 0.7 ms (Fig. 6*H*). In addition, a large disynaptic e.p.s.p. was evoked from all three electrodes (arrows). This combination of equally large mono- and disynaptic e.p.s.p.s was unique to layer 3 complex cells. Typically, both components were strong enough to bring the cell to threshold and in extracellular records, double firing was often observed. Note that the cell received also a double component e.p.s.p. after s.c. stimulation (Fig. 6*D*). This effect was presumably due to antidromic

activation of retinal ganglion cells in the s.c. (cf. above). As for layer 4 cells, a disynaptic i.p.s.p. was revealed at more depolarized membrane potentials (Fig. 6E-G). The small humps near the bottom of the i.p.s.p.s are the remnants of the disynaptic e.p.s.p. component.

Judging by their distances from the point at which the early field potential develops, the impaled cells with monosynaptic input from the l.g.n. were distributed throughout layer 3. The cell in Fig. 6, for example, was recorded less than 50  $\mu\text{m}$  below the transition point, while others were found as far as 250  $\mu\text{m}$  below it. Near the crest of the lateral gyrus, where most penetrations were made, layer 3, as defined here, is between 200 and 250  $\mu\text{m}$  thick.

The region above the point at which the early extracellular field component appears will be called layer 2. The extrapolated latencies of the thirteen cells impaled in this region all indicated a di- or in one case a possible trisynaptic connexion with the l.g.n. The receptive fields of eight cells were examined extra- or intracellularly, and all were standard complex in type. An example of a layer 2 cell is shown in Fig. 7. Not a trace of an early field component is visible in the extracellular records, the earliest potential having a latency of 2.7 ms. In the intracellular records, the earliest e.p.s.p. and i.p.s.p. both extrapolate to disynaptic latencies. Six of the layer 2 cells with disynaptic e.p.s.p.s from l.g.n. relay cells had i.p.s.p.s which seemed to be mediated by a trisynaptic pathway.

The extrapolated latencies of the earliest e.p.s.p. in layers 2 and 3 cells are plotted in the histogram of Fig. 4A. As stated above, the cells in layer 3 all fall into the first peak representing a monosynaptic connexion, while the cells of layer 2 come later. Some cells of both categories were antidromically activated from the o.r. with latencies of 0.6–1.3 ms (filled areas Fig. 4A). The found proportion of such cells presumably does not reflect the true proportion of projecting cells in these layers, since the o.r. electrode was not optimally placed to activate their axons. Thus, the antidromic spikes usually had very high thresholds (0.2–1 mA), often 5–10 times the threshold for e.p.s.p.s in the same cells and many projecting axons might have been beyond reach for the electrode.

Eight cells, included in Fig. 4A, were located at the point of transition in the extracellular fields in the middle of layer 2–3, or at least close enough so that it was impossible to assign them to a particular layer on the basis of position alone. Five of these cells received monosynaptic and three disynaptic excitation from the l.g.n. It is difficult to know how sharp the dividing line is between the region of mono- and disynaptically activated cells in the supragranular layers. The apparent mixture of the two cell types near the border between layers 2 and 3 may be due to the limitations of the method for locating cells, but if real, the zone containing both cell types occupies only a small part of these layers.

*Layer 5.* Though it is surrounded by terminals of relay cells of the l.g.n., layer 5 itself contains few such terminals. Accordingly, intracellular records from the majority of cells in layer 5, including all cortico-collicular cells, revealed that they were without monosynaptic input from the l.g.n. The synaptic responses of layer 5 cells were among the most complex encountered in the cortex and care must be taken to avoid errors in interpretation. Some of the problems are illustrated in Fig. 8 with records from a cortico-collicular cell.

This neurone was activated antidromically from the s.c. with a latency of 4.0 ms (Fig. 8*G*), and with relatively high stimulus strengths and shorter latencies from the l.g.n. (Fig. 8*E*), and o.r. (Fig. 8*F*) as well. The synaptic potentials evoked from the different sources are quite unlike those of the monosynaptically activated cells in layer 4. First, the e.p.s.p. from the l.g.n. (Fig. 8*B*) has a rather long latency, 3.1 ms at its minimum, which is longer by a millisecond than the geniculate latency of mono-

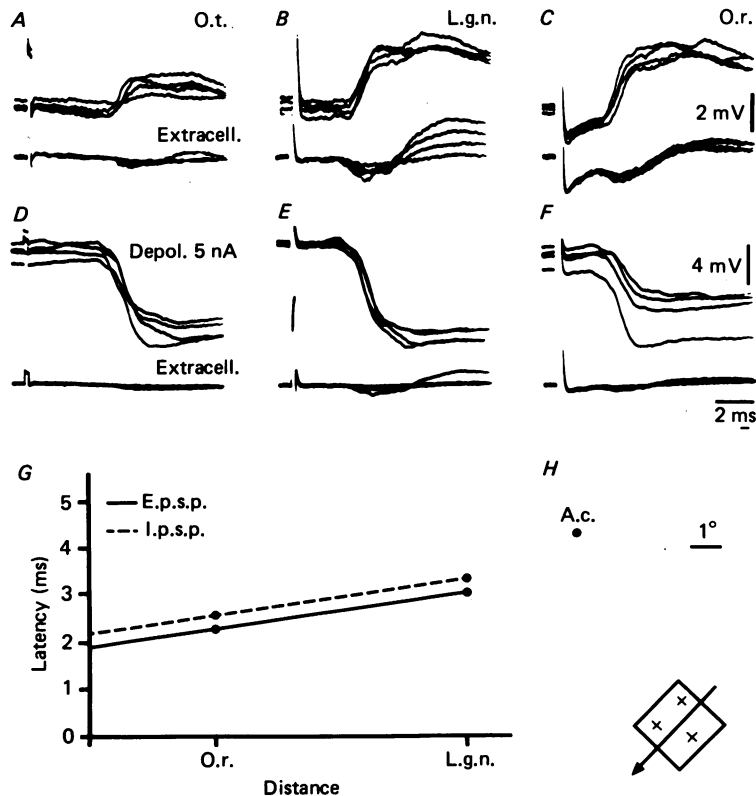


Fig. 7. P.s.p.s in a layer 2 complex cell. *A-C* and *D-E* show e.p.s.p.s and i.p.s.p.s evoked from the o.t., l.g.n. and o.r. with corresponding extracellular traces. The cell was encountered in layer 2, before the appearance of the early component of the extracellular fields (*B*, lower trace). *G*, extrapolation of the earliest e.p.s.p. and i.p.s.p. components. *H*, receptive field; the cell was 100% end-stopped and responded best to a leading edge moved down and to the left. Only on responses were evoked by flashing stimuli.

synaptic potentials. Secondly, there is a large scatter in the time of onset of the e.p.s.p. from trace to trace and a pronounced fluctuation in amplitude. These characteristics immediately suggest a disynaptic input, with the jitter probably due to the variability in the latency of spikes in the neurones interposed between the geniculate afferents and the recorded cell. From the o.t. electrode, a corresponding e.p.s.p. component can be evoked at the appropriate latency (5.1 ms). Thus, it seems likely that most, if not all of the potential evoked from the l.g.n. is mediated by the retinocortical pathway.

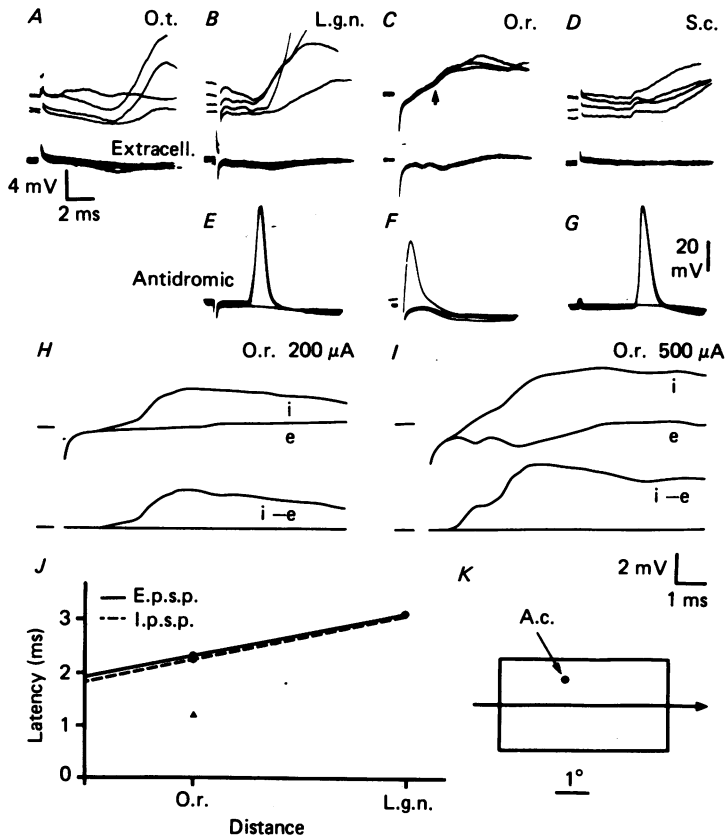


Fig. 8. E.p.s.p.s recorded in a layer 5 cortico-collicular neurone. *A-D*, e.p.s.p.s and extracellular field potentials evoked from the four stimulating electrodes. *E-G*, antidromic spikes evoked from the same stimulation sites. *H* and *I*, superimposed tracings of intracellular (*i*) and extracellular (*e*) potentials evoked from the o.r. at two different stimulus intensities. The lower traces give the transmembrane potential obtained by subtracting (*i*) from (*e*). The tracings in *I* are taken from the records in *C*. *J*, extrapolation of the l.g.n. latency with the latency of the second o.r. component (arrow in *C*). The latency of the early o.r. component due to antidromic activation of cortico-cortical neurones is indicated by the triangle. A disynaptic i.p.s.p. was also present in this cell (see Fig. 10). *K*, the cell had a large standard complex receptive field and preferred leading and trailing edges moved towards the right.

Given this, the extremely early e.p.s.p. seen in response to stimulation in the o.r. (Fig. 8*C*) is somewhat puzzling. The exact latency of this e.p.s.p. is difficult to judge by examining the intracellular potential alone, but when tracings of the intra- and extracellular responses are superimposed (upper part of Fig. 8*I*; note the expanded time base) it can be seen that the two potentials begin to diverge at 1.2 ms. This means that there is an e.p.s.p. in the o.r. response with a latency short enough for a monosynaptic connexion. Yet an attempt to extrapolate this e.p.s.p. with the e.p.s.p. evoked from the l.g.n. would result in a much longer conduction time and shorter extrapolated latency than for any monosynaptic e.p.s.p. seen in other cells (see

triangle in Fig. 8J). It follows that the early o.r. potential probably has a different origin than the e.p.s.p. evoked from the l.g.n.

A more likely candidate for an o.r. potential corresponding to the e.p.s.p. from the l.g.n. is indicated by the slight inflexion on the rising phase of the o.r. response (Fig. 8C, arrow). This second e.p.s.p. is difficult to resolve in the original record because it rides on top of the remains of the earlier potential, and on the later components of the extracellular field potential. That this small inflexion represents a separate e.p.s.p. component is more obvious in the lower tracings of Fig. 8I, where the extracellular field potential has been subtracted from the intracellular response in order to obtain the transmembrane potential. This procedure reveals more clearly the two components of the o.r. potential. The second of the two e.p.s.p.s, when connected with the geniculate potential, gives an extrapolated latency of 1.9 ms, which is well within the disynaptic range (Fig. 8J).

What then, is the source of the early potential evoked from the o.r. electrode? Most likely this e.p.s.p. originates from upper layer neurones projecting to other cortical visual areas. From anatomical experiments, it is known that projecting cells in layers 2 and 3 send axon collaterals to layer 5 (Gilbert & Wiesel, 1981) and several cells in these layers were antidromically activated from the o.r. electrode at short latency, but not from the l.g.n. or s.c. electrodes (cf. above). The threshold for antidromic activation of cortico-cortical cells from the o.r. electrode was usually much higher than the threshold of the geniculo-cortical responses. The early and late potentials evoked from the o.r. showed the same relationship, as can be seen by comparing Fig. 8H and I. At 200  $\mu$ A stimulation of the o.r., the presumed disynaptic e.p.s.p. was almost maximal, while the monosynaptic component was barely visible; only the latter increased when the stimulus intensity was increased to 500  $\mu$ A. The opposite effect would be expected if the two components represented mono- and disynaptic inputs from geniculo-cortical fibres.

Though the predominant excitatory input from the l.g.n. to the cell in Fig. 8 was disynaptic, most layer 5 cells had in addition a trisynaptic e.p.s.p. component, either in combination with a disynaptic e.p.s.p. or occasionally as the only visible component. An example of a cell with only trisynaptic excitation is illustrated in Fig. 9. In this case the larger delay in the o.r. record between the geniculo-cortically mediated potential (Fig. 9F, arrow) and the early potential makes it particularly clear that the early component does not correspond to the response seen from the l.g.n. (and o.t.); connecting these two responses in the extrapolation diagram in Fig. 9J would in fact result in a negative intercept. The extrapolated latencies of the earliest visible e.p.s.p. in each recorded layer 5 cell is shown in Fig. 4C. The majority of cells in layer 5 with indirect excitation from the l.g.n. could be antidromically activated from the s.c., or from the l.g.n. with the short latency characteristic of cortico-collicular cells (filled areas).

In all, eleven of the nineteen layer 5 cells with disynaptic, trisynaptic or mixed input exhibited the early e.p.s.p. component evoked from the o.r. This combination points to the identity of the cortical neurones that mediate the di- and trisynaptic e.p.s.p.s evoked from the l.g.n. in the layer 5 cells. There is little doubt that at least a part of the latter potentials are also mediated by the cortico-cortical neurones in the supragranular layers, which themselves receive mono- or disynaptic excitation



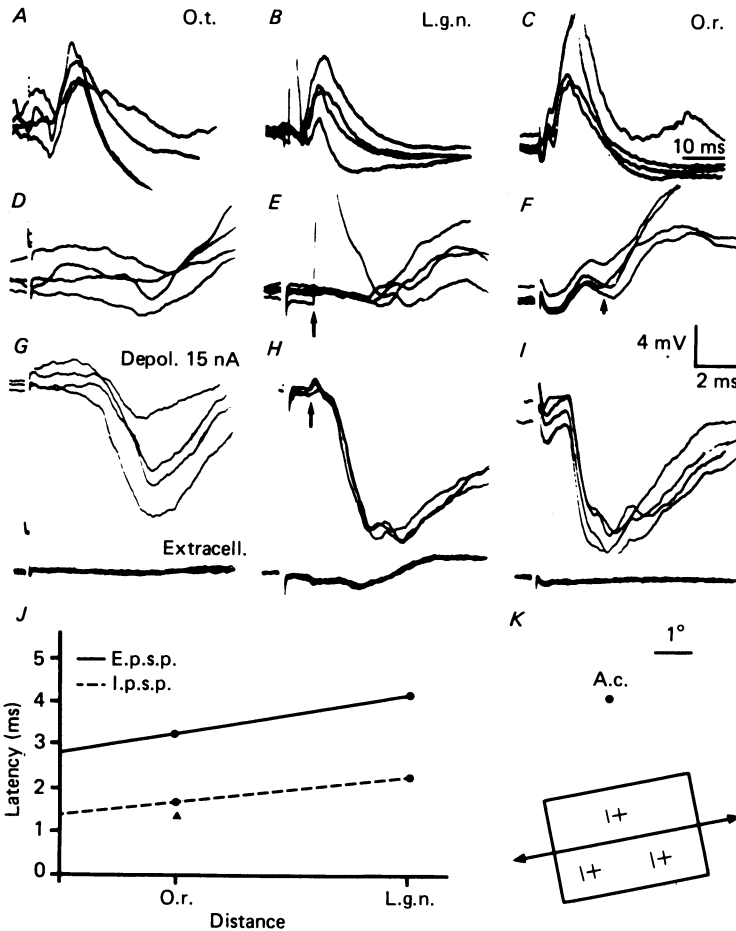


Fig. 9. A layer 5 cell with trisynaptic e.p.s.p.s evoked from the l.g.n. *A-F*, e.p.s.p.s recorded at slow and fast sweep speeds. In response to o.r. stimulation two e.p.s.p. components are seen. The second (arrow) corresponds in latency to the l.g.n. and o.t. potentials, while the first is probably mediated via collaterals of antidromically activated cortico-cortical neurones. *G-I*, i.p.s.p.s revealed by injection of depolarizing current into the cell. This cell could be antidromically activated from the l.g.n. (*E, H*, arrows), but not from the s.c. The short latency (1.4 ms) and the fact that the cell was located in layer 5 indicate that it was a cortico-collicular neurone. That it could not be activated antidromically from the s.c. was probably a result of damage to its axon by the l.g.n. electrode. *J*, extrapolation of the p.s.p.s. The triangle indicates the latency of the early component in the o.r. trace. *K*, the cell had a standard complex receptive field.

from the l.g.n. (see above). Therefore, the same supragranular cells will give rise to an early potential in layer 5 cells when antidromically activated, but di- and trisynaptic potentials when activated synaptically.

Layer 5 cells also received sizeable i.p.s.p.s from the o.t., l.g.n. and o.r. stimulation sites, as shown in Fig. 9*G-I*. The i.p.s.p. in this cell had a disynaptic latency (dotted line Fig. 9*J*), much shorter than the trisynaptic e.p.s.p. evoked from the same sites. Disynaptic i.p.s.p.s were also present in layer 5 cells with disynaptic e.p.s.p.s, although

they were more difficult to demonstrate since they were superimposed exactly on the e.p.s.p. The i.p.s.p.s appeared, however, as large negative-going potentials whenever the cells were depolarized by current injection or injury. Since the d.c. membrane potential could not be measured reliably, there was initially a possibility that the observed response was actually the reversed e.p.s.p. Fortunately, the fluctuation in the amplitude of the e.p.s.p.s made it possible to observe e.p.s.p.s and i.p.s.p.s at the same level of polarization. This is illustrated in Fig. 10 with records from the same

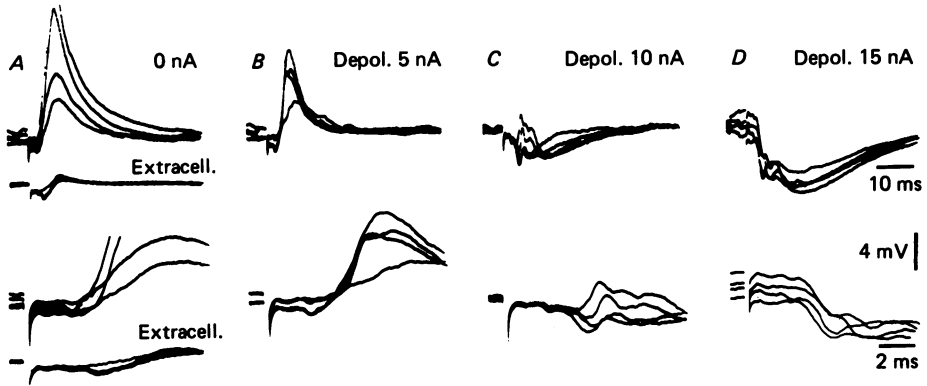


Fig. 10. *A-D*, synaptic potentials recorded at different levels of polarization in a layer 5 cell after stimulation in the l.g.n. (same cell as in Fig. 8). Upper and lower traces show the same responses taken at different sweep speeds. Note that the cell received an e.p.s.p. and i.p.s.p. with the same latency.

cell as in Fig. 8. A sequence of responses to stimulation of the l.g.n. was recorded at four different levels of depolarization. The critical traces are shown in *C*, taken at a level where the e.p.s.p. and i.p.s.p. currents were nearly balanced. Owing to the large fluctuation of the e.p.s.p. (also visible in *A* and *B*), the i.p.s.p. and e.p.s.p. dominated in alternate traces, each having the same latency. This alternation was not caused by slow drift in the membrane potential since a long sequence of similar records was obtained. In *D* the i.p.s.p. completely dominates although the peak of the e.p.s.p. caused a small positive hump near its bottom. Records like those in Fig. 10 convinced us that most layer 5 cells received both excitation and inhibition from relay cells via disynaptic pathways. The i.p.s.p.s had a more complicated time course and longer duration than the disynaptic i.p.s.p.s in layer 4 cells, suggesting that the inhibition in these two layers is at least partly mediated by different pathways. We found no evidence for recurrent inhibition of layer 5 cells following antidromic activation of cortico-collicular cells from the s.c. electrode.

A small number of cells with monosynaptic excitation from the l.g.n. were also encountered in layer 5. So far, four of them have been recorded intracellularly. Their extrapolated latencies are indicated in the histogram in Fig. 4*C*, along with the rest of the cells recorded in layer 5. An example of a monosynaptically excited layer 5 cell is illustrated in Fig. 11, which shows e.p.s.p.s of similar shape and size evoked from the o.t., l.g.n. and o.r. The cell also received a disynaptic i.p.s.p. (not illustrated). The pattern of p.s.p.s in these cells was less complicated than in disynaptically excited

layer 5 cells and more like that of the cells in layer 4. During extracellular recording, these cells were activated very effectively by a stimulus in the l.g.n. with very little delay between the spike and the e.p.s.p. None of them could be activated antidromically from the s.c. or l.g.n. Three of these cells were clearly encountered in the upper part of layer 5 just above the first cortico-collicular cells and below the simple cells of layer 4, leading us to believe that this cell type is concentrated near the border between layers 4 and 5. The presence of pyramidal cells without corticofugal axons

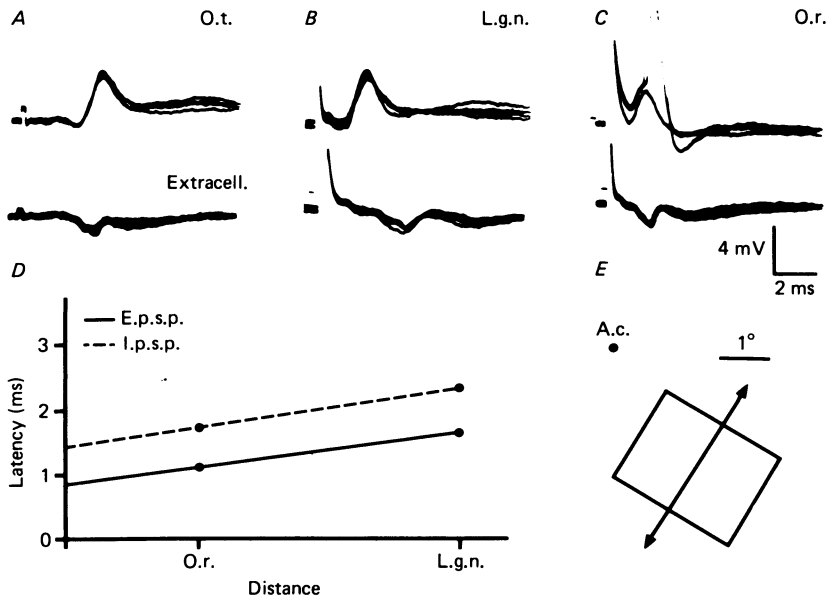


Fig. 11. A layer 5 complex cell with monosynaptic excitation from the l.g.n. *A-C*, e.p.s.p.s and extracellular potentials evoked from the o.t., l.g.n. and o.r. electrodes. The cell was not antidromically activated from any of the stimulation electrodes. *D*, extrapolation of the l.g.n. and o.r. latencies for e.p.s.p.s and i.p.s.p.s (not shown). *E*, receptive field.

in the upper part of layer 5 has been reported earlier (O'Leary, 1941; Martin & Whitterage, 1982). Lund, Henry, MacQueen & Harvey (1979) have further observed that no cells are labelled in upper layer 5 after injections of HRP in the s.c.

The cells of layer 5 can thus be divided naturally into two classes on the basis of their connectivity with the l.g.n.: those that receive di- and/or trisynaptic excitation, and those that receive monosynaptic excitation. The di-/trisynaptic group included cells with and without projections to the s.c. and cells with special and standard complex receptive fields. In our limited material there was no obvious correlation between the relative strength of the di- and trisynaptic components and receptive field type. Receptive fields of cells with monosynaptic input from the l.g.n. were standard complex in type, giving vigorous on and off responses to a flashing slit. They measured about 2° in each direction, and though few such cells were recorded, it would seem that their receptive fields are among the smallest in layer 5, which boasts cells with receptive fields as large as 6° across near the area centralis.

**Layer 6.** Layer 6 is distinguished by the presence of cells projecting to the l.g.n. As does layer 4, it contains terminals of geniculate relay cells (LeVay & Gilbert, 1976). These terminals are restricted to the upper part of the layer, however, and it was somewhat surprising to find that every cell encountered in layer 6 received monosynaptic excitation from the l.g.n. An example is illustrated in Fig. 12. This particular cell projected to the l.g.n. as indicated by the antidromic spike elicited from

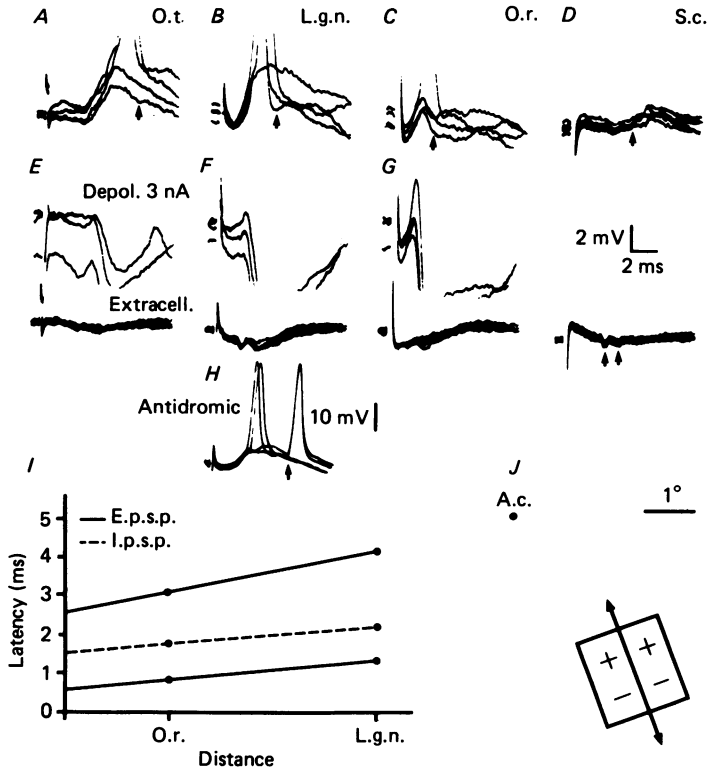


Fig. 12. P.s.p.s recorded in a layer 6 simple cell. *A-C*, e.p.s.p.s evoked from the o.t., l.g.n. and o.r. electrodes. In addition to the large monosynaptic component, a small trisynaptic component is visible. Arrows indicate latencies estimated from several records. *D*, a small e.p.s.p. evoked from the s.c. The latency (arrow) is best judged by superimposing the record on the extracellular trace below. The cell was recorded in the upper part of layer 6 and in the extracellular records the antidromic spikes of two distant cortico-collicular cells are seen (arrows). *E-G*, i.p.s.p.s visible when the cell was depolarized by current injection. Below are the extracellular field potentials. *H*, antidromic spikes evoked from the l.g.n. with a latency of 5.7 ms (arrow). In two of the four traces the antidromic spike is collided out by a preceding orthodromic spike rising from the e.p.s.p. *I*, extrapolation of the p.s.p.s. *J*, the cell's receptive field.

the l.g.n. with a latency of 5.7 ms. Despite the very long latency it was clear from the distinct threshold, lack of jitter in latency and the ability to follow high frequency stimulation that the spike was antidromic. It could also be collided out by preceding synaptic spikes, as seen in Fig. 12*H*: there are four traces in the record, and in two of them the antidromic spike is present (arrow), while in two others the antidromic spike is blocked by a preceding orthodromic spike rising off the e.p.s.p.

In this particular cell, the e.p.s.p. was evoked from the l.g.n. electrode with a latency of 1.2 ms (Fig. 12*B*), and from the o.r. electrode with a latency of 0.8 ms (Fig. 12*C*). Combining the two gives an extrapolated latency of 0.6 ms, which falls in the early part of the monosynaptic range (Fig. 4*E*). The required criterion for identification of the e.p.s.p. as excitation from relay cells in the l.g.n. is seen in Fig. 12*A*; a similar e.p.s.p. was evoked from the o.t. at 3.0 ms. An i.p.s.p. became apparent in the illustrated cell upon injecting depolarizing current (Fig. 12*E-G*). All layer 6 cells received similar disynaptic i.p.s.p.s after activation of the geniculo-

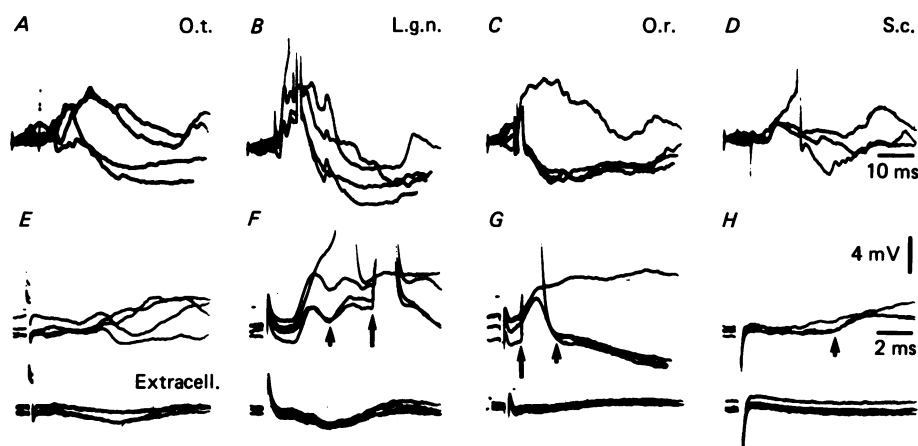


Fig. 13. E.p.s.p.s recorded in a second cortico-geniculate layer 6 cell. The responses in *A-D* are the same as those in *E-H* but were taken at 5 times slower sweep speed. Both mono- and trisynaptic e.p.s.p. components are present and the latency of the latter is marked in *F* and *G* (short arrows). Though also present in *E*, the latency of the late e.p.s.p. could not be determined accurately. Long arrows in *F* and *G* indicate antidromic spikes. The slower records clearly show the extreme variability and long duration of the trisynaptic e.p.s.p. component suggesting that the presynaptic cells are firing irregularly. An e.p.s.p. evoked from the s.c. (*D*, *H*) is probably caused by intracortical collaterals of cortico-colicular neurones. The cell had a simple receptive field (not shown).

cortical pathway; cf. dotted line in Fig. 12*I*. The histogram of the extrapolated latencies of e.p.s.p.s in the forty-one cells recorded in layer 6 is shown in Fig. 4*D*. All fell within the monosynaptic range, no matter what their properties were. About half (21) could be activated antidromically from the l.g.n. (filled area) with latencies within the range illustrated in Fig. 3 (3.1–30 ms).

Cells in layer 6 also received late e.p.s.p.s that could be evoked from the o.t., l.g.n. and o.r. electrodes. The earliest of these had extrapolated latencies indicating a trisynaptic pathway from geniculate relay cells. As a rule, these e.p.s.p.s were more prominent than any di- or trisynaptic e.p.s.p. components observed in layer 4 cells. This was especially apparent in records taken with slow sweep speed, as illustrated for a second layer 6 cell in Fig. 13. Typically these late potentials had long durations (30 ms or more), fluctuated markedly from trace to trace and had several ripples on their rising and falling phases, as if the presynaptic neurones were firing repetitively at irregular intervals. The fluctuation often made the latency difficult to determine in single traces, but it could be judged with reasonable accuracy by comparing several

records (short arrows in Fig. 12*A–C* and 13*F,G*). Extrapolated latencies for the trisynaptic components in the two illustrated cells were 2.6 ms (Fig. 12*I*) and 2.9 ms (not shown).

There is substantial evidence pointing to cortico-collicular cells in layer 5 as the source of these trisynaptic e.p.s.p.s. Cells in layer 5 have extensive axon collaterals in layer 6 (Gilbert & Wiesel, 1979) and as shown above their earliest excitatory input from the l.g.n. is disynaptic. Furthermore they often give an irregular response to stimulation of the retino-cortical pathway, with several spikes at long variable intervals, a behaviour which matches the fluctuation of the trisynaptic e.p.s.p.s in layer 6 cells.

If cortico-collicular cells are excitatory to layer 6 cells, one would expect to find an e.p.s.p. in layer 6 cells after antidromic activation of cortico-collicular cells from the s.c. Such potentials were in fact observed in twelve layer 6 cells with trisynaptic e.p.s.p.s. Before concluding that these potentials represent the input from layer 5 cells, the possibility that they were caused by antidromic activation of retinal ganglion cells projecting to both the s.c. and l.g.n. must be considered. This latter source is the likely explanation for potentials evoked from the s.c. in layer 3 and upper layer 4 cells (see above). In these cells the collicular potentials were identical in shape and latency to those evoked from the o.t. (compare, for example, Fig. 6*A* and *D*). In layer 6 this was not the case. Instead, collicular stimulation gave rise to small e.p.s.p.s, which were never followed by an i.p.s.p. and which usually differed in latency from the o.t. response by 0.5–2.0 ms. In addition, they were largely unaffected by increases of the stimulus frequency to 10–15 Hz, while the o.t. responses were drastically decreased (as well as the s.c. responses in layer 4 cells).

Examples of s.c. responses in the two illustrated layer 6 cells are shown in Fig. 12*D*, 13*D* and 13*H* (arrows). These small e.p.s.p.s had latencies of 4.7 and 5.6 ms, compared to 3.0 and 3.6 ms for the corresponding o.t. e.p.s.p.s. (The small size of the potential in Fig. 12*D* makes it necessary to superimpose the intra- and extracellular traces in order to determine the latency.) The cell in Fig. 6 was recorded near the border of layers 5 and 6. Less than 100  $\mu\text{m}$  above this cell, two cortico-collicular neurones were recorded extracellularly, and their distantly recorded antidromic spikes can be seen as small humps in the extracellular record of Fig. 12*D* (arrows). This record illustrates that the stimulus which evoked the described e.p.s.p. from the s.c. was sufficient to activate layer 5 neurones antidromically. In general, the latencies of e.p.s.p.s in layer 6 cells from the s.c. (2.3–6.0 ms) corresponded well with the antidromic latencies of cortico-collicular neurones from the s.c. (1.6–6.5 ms; not illustrated).

Although the cells in both layer 4 and 6 received direct input from the l.g.n., the effectiveness of the input in driving the cells was not equal under the conditions of the experiment. In layer 4, spikes could be recorded extracellularly with latencies only a few tenths of a millisecond greater than the e.p.s.p. latencies, while in layer 6, it was often so that no extracellular synaptic spikes could be recorded despite the large mono- and trisynaptic e.p.s.p.s. This was especially true for identified cortico-geniculate cells. The cells in Figs. 6 and 7, for example, exhibited only antidromic spikes in response to stimulation during extracellular recording. When they were depolarized by the penetration, action potentials did rise out of the e.p.s.p.s, but delayed by over a millisecond with respect to the onset of the e.p.s.p. The e.p.s.p.s in layer 6 cells appeared to be somewhat smaller than those in layer 4, but it seems unlikely from our recordings that the poor responsiveness of layer 6 cells can be explained by this difference alone. More likely their strange behaviour was caused by other factors, such as a tonic inhibitory input.

Reliable receptive field maps were obtained from sixteen of the impaled layer 6 cells. Fourteen of these were simple, with either two or three subfields and often the long narrow shape unique to cells in layer 6 (Gilbert, 1977). Length summation was visible in some cells with stimuli as long as  $10^\circ$ , while others, such as the cell in Fig. 6, gave a maximal response to a slit  $2^\circ$  in length. Thirteen of the impaled simple cells could be activated antidromically from the l.g.n. Though complex cells were rare in both our extra- and intracellularly recorded samples of layer 6 cells, it was clear that they differed from simple cells in more than just receptive field type. In extracellular recordings, they responded reliably to electrical stimulation with orthodromic spikes of short latency, while they never responded with antidromic spikes from any of the stimulating electrodes. Care was taken to make sure that this failure was not due to collision with preceding orthodromic spikes.

It has been suggested earlier that layer 6 contains cortico-geniculate cells with complex receptive fields and short antidromic latencies (0.5–3.0 ms) from the l.g.n. (Gilbert, 1977; Harvey, 1980b; Tsumoto & Suda, 1980). This suggestion was not confirmed by the present experiments. The described short latencies are instead typical for our cortico-collicular neurones (Fig. 3). In the entire material of extra- and intracellularly recorded cells, only six complex cells with short antidromic latencies from the l.g.n. were found that on testing did not respond with antidromic spikes also from the s.c. We believe that these cells belonged to the cortico-collicular system of layer 5 but that antidromic spikes did not reach them from the s.c. because of damage to the axons near the l.g.n. electrode. Several observations support this interpretation. (1) These six cells were all recorded in layer 5 or at the border between layers 5 and 6 as judged by their distances from other identified cortico-collicular and cortico-geniculate cells. (2) The threshold for antidromic activation from the l.g.n. was much higher than that for nearby layer 6 simple cells (see Methods). (3) The stepwise decrease in latency with increasing intensity typically observed when stimulating terminal axon branches was not seen. (4) The majority of identified cortico-collicular cells (31/39) could be activated both from the l.g.n. as well as the s.c. In one case, it was observed directly that conduction block of the efferent axon could occur between the l.g.n. and the s.c.: at the beginning of the penetration of the cortico-collicular cell illustrated in Fig. 8., antidromic spikes could be evoked from the o.r., l.g.n. and s.c. electrodes, but 30 min later, only the o.r. and l.g.n. spikes remained. In all other respects, the cell properties remained unchanged. The earlier observed short latency responses in layer 6 might represent axonal recordings from cortico-collicular cells, since such recordings are not unusual with tangential electrode penetrations.

#### *Synaptic effects of X- and Y-type geniculate afferents*

It is well known that retinal ganglion cells and geniculate relay cells projecting to area 17 are segregated in three main types, usually referred to as X, Y and W cells. The original, and no doubt most selective, criterion for distinguishing between X and Y cells is the linearity of spatial summation within the receptive field (Enroth-Cugell & Robson, 1966), but the cells also differ in conduction velocity of their axons, spatial frequency sensitivity, and size of receptive field centre (see Lennie, 1980, for review). The finding that the X, Y and W pathways remain segregated in the l.g.n. has prompted the suggestion that these functional systems are processed separately at the cortical level as well (Stone, 1972). This idea is given support by the observation that afferent terminals of the different types are largely segregated into different layers of the cortex (Ferster & LeVay, 1978; Gilbert & Wiesel, 1979).

It was hoped that the known difference in conduction time of the X, Y and W cells could be used to investigate the type of afferents providing input to a cortical cell. In the past, o.t.-l.g.n. latency difference, which reflects the conduction time of retino-geniculate axons and relay time in the l.g.n., has been used in this connexion

(Bullier & Henry, 1979*b*). Unfortunately, there is some overlap in the conduction times of the axons themselves, added to by the errors in measuring the o.t. and l.g.n. latencies of p.s.p.s. In our data, a histogram of o.t.-l.g.n. latency differences for monosynaptic e.p.s.p.s in the impaled cortical neurones formed a single peak in which the different afferent types are not immediately visible (Fig. 14*B*). Assuming that the range of overlap between the two types of afferents extends from 1.4 to 1.7 ms

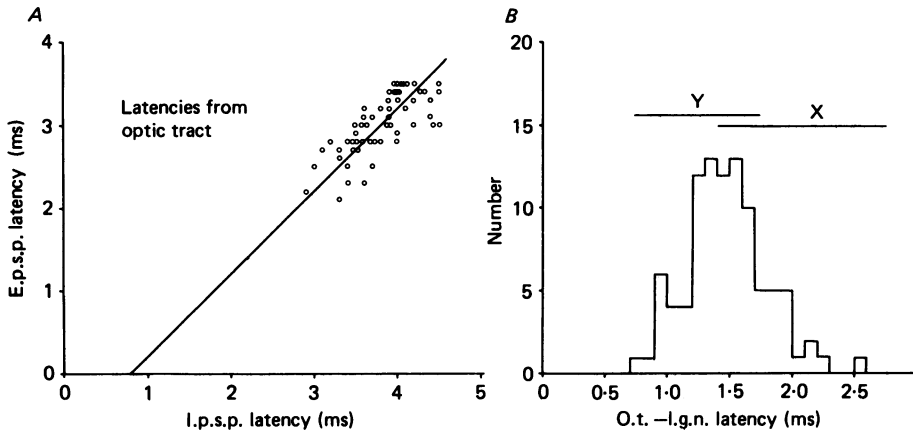


Fig. 14. *A*, latencies of e.p.s.p.s and i.p.s.p.s evoked from the o.t. electrode plotted against each other for individual cells. Only cells with monosynaptic excitation from the l.g.n. are included. A line with a slope of 1 was fitted by eye; the resulting *y*-intercept is 0.8 ms which corresponds to the mean time delay between mono- and disynaptic potentials obtained with the extrapolation of l.g.n. and o.r. latencies (see Fig. 4*E*). *B*, the distribution of the difference between the o.t. and l.g.n. latencies for e.p.s.p.s with monosynaptic extrapolated latencies. The horizontal lines indicate estimated ranges for X and Y relay cell axons.

(Bullier & Henry, 1979*b*), it is impossible to determine the afferent type for fully 40% of the monosynaptic e.p.s.p.s. An equally bad segregation was obtained when the geniculo-cortical conduction times, taken from the extrapolation diagram, were used. The reason for the poor result may be that the latency was dominated by the fastest conducting fibres converging onto a particular cortical neurone. Thus, the distribution of conduction times was far from representative for the entire population of afferents.

Apparently, latency measurements alone are ineffective in separating cells into different classes on the basis of afferent input type. It is still informative, however, to examine the distribution of o.t.-l.g.n. latency differences in cell classes previously discriminated by other means, such as laminar location or receptive field properties. Complex cells in layer 3, for example, might receive their monosynaptic geniculate input exclusively from Y cells since their dendrites probably extend only as far down as the terminals of Y cells in layer 4ab. The o.t.-l.g.n. differences of this population (0.8–1.6 ms, mean 1.2, cf. Fig. 6*A, B*) were in fact limited to the early part of the range for monosynaptic cells (Fig. 14*B*), consistent with an exclusive Y input. Simple cells in upper layer 4 had similar o.t.-l.g.n. latency differences (below 1.5 ms), while cells in lower layer 4 had latency differences of 1.3 ms or more, consistent with an X input. The presence in some cells in layers 3 and upper 4 of e.p.s.p.s evoked from



the s.c. with latencies similar to o.t. potentials (Fig. 13*A* and *D*) is in keeping with this interpretation. These e.p.s.p.s are presumably due to the antidromic activation of Y-type retinal ganglion cells projecting to the s.c.

Despite the apparent segregation of the two afferent types the possibility for convergence still exists for cells in the overlap region of Fig. 14*B*. The cells with the longest o.t.-l.g.n. latency differences did certainly not receive additional Y-type input, however, and those with shortest o.t.-l.g.n. delays had no trace of later unitary e.p.s.p. components that would suggest an additional monosynaptic X-type input. Furthermore, there were clear differences in the receptive field properties of simple cells in the upper and lower halves of layer 4 which also suggest that single cells do not receive monosynaptic excitation from both X- and Y-type afferents. The subfields of eleven cells in the upper part of layer 4 were about twice as large as those of ten cells in the lower part, paralleling the difference in receptive field centre size of Y and X cells in the l.g.n. (Bullier & Henry, 1979*b*, *c*).

The o.t.-l.g.n. delays of disynaptic e.p.s.p.s and i.p.s.p.s covered the same range as those of monosynaptic e.p.s.p.s, but the determination of afferent type is rendered more unreliable by the scatter that the extra synaptic delay adds to the individual latencies. It is interesting, however, that latency measurements may provide some information about the organization of inhibitory systems. For cells with monosynaptic excitation and disynaptic inhibition, the o.t. latencies of e.p.s.p.s and i.p.s.p.s are plotted against each other in Fig. 16*A*. While here again, the afferent type of individual p.s.p.s cannot be determined because of the scatter, the close correlation between the two latencies indicates that in a given cell, e.p.s.p.s and i.p.s.p.s are mediated by afferents with the same conduction velocity and therefore of the same functional type. Thus, the X and Y pathways seem to remain segregated in the cortex to an extent previously unsuspected.

Finally, we have found no trace of e.p.s.p.s or i.p.s.p.s that with reasonable certainty can be attributed to input from W-type relay cells in the C laminae of the l.g.n. Most likely, W-cell axons were not effectively stimulated by the l.g.n. electrode placed in the A laminae. Though W-cell axons pass through the A laminae on their way to the cortex, the effective intensity range between threshold and cathodal block for fine axons is extremely small. Therefore it is possible that only a few fibres were activated at any one stimulus intensity and the resulting e.p.s.p.s too small to be detected.

#### DISCUSSION

It is a comfort to find in the results of this study that the synaptic input to the visual cortex from the l.g.n. is as systematically organized as the internal and efferent projections of the cortex itself. Within a given layer or sublayer, or within a given functional class, all neurones maintain virtually identical connexions with geniculate relay cells. Briefly stated, all impaled cells in layers 3, 4 and 6 received direct excitatory connexions, while those in layers 2 and 5 (with the exception of some cells in the upper part of the latter) received their visual input only via other cortical neurones. This arrangement is indicated in Fig. 15 along with some of the intracortical pathways investigated in this study. From the laminar organization of the efferent projections of the cortex, it follows that cortico-geniculate neurones receive mono-

synaptic excitation, cortico-collicular neurones receive indirect excitation, while cortico-cortical neurones receive either, depending on their position in layer 2 or 3.

These conclusions, and the results on which they are based, differ considerably from those of more recent examinations of geniculo-cortical connectivity. Among previous studies, that of Toyama, Matsunami, Ohno & Tokashiki (1974) comes closest to our own in methods. They found a close correlation between laminar position and connectivity with the l.g.n., and described a pattern of input to cells in layers 2 to

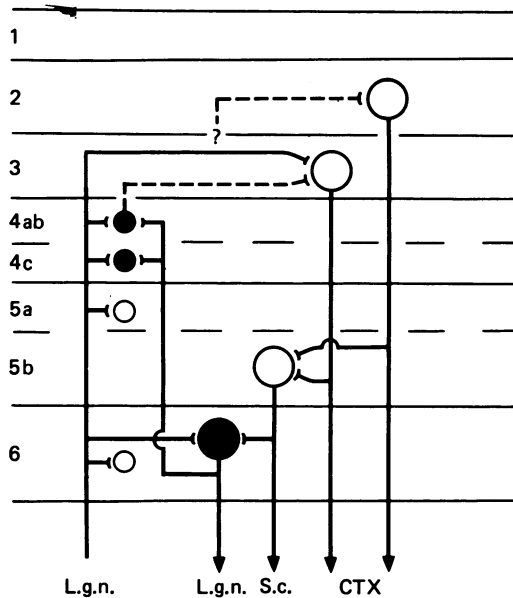


Fig. 15. A schematic illustration of excitatory synaptic connexions in area 17. The filled lines represent direct connexions from l.g.n. relay cells and intracortical collateral connexions identified by antidromic activation of the presynaptic neurones. The input to layer 3 cells from layer 4ab is suggested on the basis of a disynaptic e.p.s.p. evoked from the l.g.n. and from anatomical evidence (see text). The disynaptic excitatory input to layer 2 cells presumably originates from layer 4c cells. Open and filled circles indicate complex and simple cells; large and small circles represent cells with and without extracortical projection.

4 in partial agreement with the one described here. However, their suggestion that cortico-collicular neurones in layer 5 are monosynaptically excited from the l.g.n. may stem from a misinterpretation of the short latency e.p.s.p.s evoked in these cells by stimulation in the optic radiations. As argued in Results, these e.p.s.p.s are caused by antidromic activation of upper layer cells rather than by the orthodromic activation of geniculate afferents.

The remaining studies concerned with geniculo-cortical connectivity have relied either partly (Singer, Tretter & Cynader, 1975) or completely (Stone & Dreher, 1973; Henry, Harvey & Lund, 1979; Bullier & Henry, 1979*a, b, c*) on extracellular recordings. The problems associated with the use of extracellular recording in the study of synaptic connexions have been outlined in the Results section. Not only do many cortical cells fail to respond to a particular set of electrically evoked e.p.s.p.s, but

when they do respond, there is an almost unpredictable delay between the onset of the e.p.s.p. and the initiation of a spike. Accordingly, extracellular studies are characterized by a large scatter in measurements, which poorly reflect the true synaptic connectivity. When a histogram of the latencies of extracellularly recorded spikes was compiled from our data, whether measured from the o.r. or extrapolated from the l.g.n. and o.r., a single peak resulted with no clear segregation between mono- and disynaptical potentials. It is therefore largely to the use of extracellular recordings that we attribute the conclusions reached in the previous studies that connectivity bears little relation to cell type or to laminar position.

One additional extracellular study, in this case of area 18, does report a relatively close correlation of laminar position with connectivity (Harvey, 1980*a*). Area 18 has the advantage that no X-type afferents project there, reducing the scatter in the arrival time of the incoming spikes. Synchronous arrival of afferent spikes may also reduce the scatter in the e.p.s.p.-spike interval if it results in e.p.s.p.s with faster rise times. Excluding those cells not driven by electrical stimulation in Harvey's report, the majority of the cells in each layer of area 18 form similar connexions with the l.g.n. as do their counterparts in area 17. The two areas also resemble each other in the laminar distribution of efferent projections (Gilbert & Kelly, 1975) and receptive field properties (Dreher & Cottee, 1975; Ferster, 1981), the major difference being that cells in area 18 have larger receptive fields (Hubel & Wiesel, 1965) and are sensitive to lower spatial frequencies (Movshon, Thompson & Tolhurst, 1978). Area 18 may therefore perform a similar analysis of the visual field as area 17, but on a different scale.

#### *Laminar position and geniculo-cortical connectivity*

One of the more striking results of this study is the large proportion of cortical neurones monosynaptically excited from the l.g.n. Layers 3, 4 and 6 are among the thicker and more densely packed layers of area 17, and together may contain more than 70% of all cortical neurones. In layer 4, it is known from electron microscopic reconstructions that the large majority of cells make synapses with geniculate terminals (Davis & Sterling, 1979; Hornung & Garey, 1981). It now appears that those few cells for which such synapses were not found, made them on unreconstructed portions of the cells, either farther out on the dendritic tree or on dendritic spines.

Besides neurones within the termination field of geniculate relay cells, many cells outside these layers made direct synaptic contact with geniculate afferents. Some of the cells in the lower part of layer 6 may have been close enough to geniculate terminals in the upper half of the layer to make synaptic contact on their basal dendrites. All of them, though, send their apical dendrites through the upper half of layer 6, and into layer 4 where they may branch repeatedly (O'Leary, 1941; Lund *et al.* 1979; Gilbert & Wiesel, 1979). Thus, layer 6 cells may make synapses with geniculate terminals in layer 4 as well as in layer 6. Certainly, these apical dendrites do not seem to receive a significant excitatory input from two other sets of terminals in layer 4, those of layer 4 cells themselves (given the lack of disynaptic e.p.s.p.s in layer 6 cells) or axon collaterals of other layer 6 cells (Ferster & Lindström, 1983).

The dendrites of cortico-collicular neurones in layer 5 seem to pass through the terminal fields of X and Y geniculate afferents completely untouched, that is

'untouched' to within the limits of our technique. If monosynaptic e.p.s.p.s were present, they must have been smaller than the noise in the recordings, or about 25–50 times smaller than monosynaptic e.p.s.p.s in layers 3, 4 and 6. Despite our small sample we are rather confident of this negative finding with respect to cortico-collicular neurones. The quality of our intracellular recordings was better for these cells than for any other group of cortical neurones, and we believe that even synapses far out on distal dendrites should have been detected if present. There is good evidence from other types of pyramidal neurones that synaptic input to distal apical dendrites is easily detectable in soma recordings (Andersen, Silfvenius, Sundberg & Sveen, 1980). While anatomical experiments do indicate that geniculate afferents make synapses with apical dendrites of layer 5 cells (Hornung & Garey, 1981; Peters, Proskauer, Feldman & Kimerer, 1979) our findings suggest that at least for X and Y afferents these occurred exclusively on pyramidal cells without extracortical axonal projection (see Results). It follows from these results that the terminals of geniculate relay cells do not make contacts indiscriminately with all dendrites in their vicinity, but rather select among them according to functional specificity.

The terminals of geniculate neurones in the A laminae of the l.g.n. do not reach higher than approximately 100  $\mu\text{m}$  above the border between layers 3 and 4 (LeVay & Gilbert, 1976; Ferster & LeVay, 1978). Yet impaled cells as far as 250  $\mu\text{m}$  above this border received monosynaptic excitation possibly because they sent their basal dendrites down into the terminal region. Alternatively, afferents from Y cells in the C laminae contribute to this monosynaptic input. After injections of radioactive amino acids into the C laminae of the l.g.n., patches of label can be seen extending much higher up into layer 3 than after injections of the A-laminae (LeVay & Gilbert, 1976).

Geniculate afferent terminals are not only restricted to certain layers of the cortex, but within layer 4, terminals of Y and X afferents are segregated from each other, Y in 4ab and X in 4c. This anatomical arrangement seems to be reflected in the synaptic connectivity; the cells in these two sublayers may receive exclusive connexions from one type of afferents (Bullier & Henry, 1979*b, c*), as indicated by their receptive field properties and e.p.s.p. latencies. A selective input from X and Y afferents to separate cortical neurones may result in a parallel processing of these systems (Stone, 1972), but does not exclude a simultaneous sequential treatment of the visual input.

#### *Connexions of efferent neurones*

The orderly laminar arrangement of synaptic connexions with the l.g.n. has important consequences for cortical cells with efferent projections. All layer 6 cells, for example, received direct excitation from geniculate afferents, and therefore so did all cortico-geniculate neurones. Layer 6 also contains cells projecting to the visual claustrum (LeVay & Sherk, 1981). Although these cells were not studied specifically in the present investigation, the uniform monosynaptic excitation of layer 6 cells indicates that they also receive direct excitation from the l.g.n.

Cortico-geniculate neurones form direct excitatory connexions at the geniculate level with relay cells (Ahlsén, Grant & Lindström, 1982) and two types of inhibitory interneurones: intrageniculate interneurones, which mediate feed-forward inhibition

to relay cells (Dubin & Cleland, 1977; Ahlsén *et al.* 1982) and perigeniculate neurones which are interposed in a recurrent inhibitory pathway (Ahlsén & Lindström, 1982, 1983). Given the retinotopic and ocular dominance specificity of the cortico-geniculate projection, each projecting layer 6 cell most likely directly excites the relay cells that provide much of its own input. This means that the cortico-geniculate system forms an excitatory recurrent feed-back pathway with respect to relay cells, very similar to the recurrent inhibitory system of the l.g.n. (Ahlsén, Lindström & Lo, 1982).

The efferent projection from layer 5 reaches not only the superior colliculus but also the pulvinar (Lund *et al.* 1979) and the pontine nuclei (Albus & Donate-Oliver, 1977), possibly via axon collaterals of the same neurones. Only the collicular projection was studied in this investigation and identified cortico-collicular neurones differed in almost every possible respect from cortico-geniculate neurones; their earliest input from the l.g.n. was disynaptic, they fired repetitively in response to electrical stimulation in the l.g.n., and they had large complex receptive fields approximately equal in length and width (Gilbert, 1977). It was originally suggested that the cortical input to the superior colliculus supplies collicular neurones with receptive field properties lacking in the direct retinotectal pathway (Wickelgren & Sterling, 1969) but, alternatively, the cortical input may gate the flow of impulses from the upper to the lower layers of the colliculus (Finlay, Schiller & Volman, 1976). Whatever their role, a major visual input to cortico-collicular neurones comes from cortico-cortical neurones of the supragranular layers. The activity reaching the colliculus thus reflects the activity relayed from area 17 to other cortical areas.

The synaptic drive to efferent neurones in the upper layers was more heterogeneous than that to neurones with subcortical projections; the shortest pathway from the l.g.n. could be either mono-, di- or may be even trisynaptic. It is possible that the difference in connectivity of cortico-cortical neurones in layers 2 and 3 is associated with a difference in extracortical projection; from HRP studies it seems that cells projecting to areas 18 and 19 are largely segregated into layers 3 and 2, although there is some overlap (Gilbert & Wiesel, 1981). Therefore, area 18 projecting neurones may receive convergence of mono- and disynaptic excitation from the l.g.n. while cells projecting to area 19 may be disynaptically activated.

#### *Intracortical connexions*

Different layers of the cortex are distinguished by specific intracortical, as well as extracortical projections, and it seems that in many cases, it is the same neurones that participate in both functions. In fact, the intracortical collaterals of efferent neurones may be responsible for a substantial part of the excitatory connexions within the cortex. Extracortical projecting axons, therefore, provide access to intracortical pathways independent of the normal geniculate projection. We have studied the intracortical effects of three major efferent systems, those projecting to the l.g.n., to the s.c. and to other cortical areas (Fig. 15).

Cortico-cortical cells lie predominantly in layers 2 and 3 and are known to project heavily to layer 5 (Gilbert & Wiesel, 1981). Antidromic activation of their efferent axons from the optic radiations produced a short latency e.p.s.p. in over half of the neurones in layer 5, including many cortico-collicular neurones. It is likely that this connexion was present in even more layer 5 cells since the placement of the optic

radiation electrode was not optimal for stimulation of cortico-cortical axons. The upper layer neurones, which themselves respond to stimulation of the l.g.n. with mono- or disynaptic e.p.s.p.s, may thus be responsible for a large portion of the di- and trisynaptic e.p.s.p.s observed in layer 5 cells. Although the e.p.s.p.s produced by antidromic stimulation of supragranular neurones were somewhat smaller than those produced by the orthodromic activation of geniculate afferents (Figs. 8 and 9), the difference may be explained by repetitive firing of neurones in layers 2 and 3 in response to stimulation in the l.g.n.

Cortico-collicular neurones in layer 5, in turn, project to layer 6, and stimulation of their axons in the s.c. produce characteristic e.p.s.p.s in layer 6 cells. Orthodromic stimulation of geniculate afferents results in disynaptic activation of layer 5 cells and subsequent trisynaptic e.p.s.p.s in layer 6 cells. Collaterals of layer 5 cells that could mediate this potential have been observed to extend for long distances in layer 6 by Gilbert & Wiesel (1979), who suggest that this projection could account for the unusual length summation in receptive fields of layer 6 cells, something that the spatially limited direct input from the l.g.n. cannot do. It is interesting here that the layer 6 cell, illustrated in Fig. 12, which had an unusually short receptive field also had a trisynaptic component much smaller than other layer 6 cells with good length summation.

Finally, layer 6 is the source of a massive collateral projection to layer 4 cells. The axons of layer 6 cells in the l.g.n. usually have higher threshold than relay cells themselves, and it is difficult to observe this collateral effects independently of the effects of the geniculo-cortical system. The demonstration of this projection therefore required additional techniques, and the findings will be described in a succeeding article (Ferster & Lindström, 1983 and in preparation).

The observed effects of collaterals of cortical efferent cells account for only part of the intracortical circuits. Di- and trisynaptic e.p.s.p.s in layer 2 and 3 cells, for example, seem to be supplied primarily by intrinsic cortical neurones. The identity of these neurones has yet to be determined, though anatomy provides several candidates. A proportion of layer 4 spiny stellate cells have collaterals projecting upwards (O'Leary, 1941; Gilbert & Wiesel, 1979; Lund *et al.* 1979), and these cells should produce disynaptic e.p.s.p.s in their target neurones, since they themselves receive monosynaptic excitation from the l.g.n. (cf. dotted lines in Fig. 17). Both the anatomy and physiology suggest that the majority of these layer 4 cells do not project out of area 17. A second possible source for disynaptic e.p.s.p.s is the monosynaptic complex cells in upper layer 5 without extracortical projection. Finally, the source of the disynaptic potentials may be other neurones in layer 3 itself, since they do have locally ramifying collaterals (Gilbert & Wiesel, 1981; Martin & Whitteridge, 1982) and receive monosynaptic input.

Inhibitory pathways are unamenable to investigation by electrical stimulation of efferent axons since few if any inhibitory neurones send axons out of the cortex. This is unfortunate since inhibitory interneurones and prominent i.p.s.p.s are a characteristic of every layer of the cortex. It is the spine-free neurones that are thought to have inhibitory functions since they contain the synthetic enzyme for GABA (Ribak, 1978) and make symmetrical type 2 synapses with their post-synaptic neurones (LeVay, 1973). The axons of many of these cells ramify locally and therefore may provide inhibition to neurones in the same layer. In layers where all cells have

the same connectivity with the l.g.n., one would then expect the inhibitory pathway to have one more synapse than the excitatory one. This is in fact the case in layer 2, where many cells have disynaptic e.p.s.p.s and trisynaptic i.p.s.p.s, and in layers 3, 4 and 6 where monosynaptic e.p.s.p.s and disynaptic i.p.s.p.s are the rule.

Not all cells followed the pattern of having i.p.s.p.s that reached them through longer pathways than the e.p.s.p.s. Cells in layer 5 with disynaptic excitation from the l.g.n. and also some layer 2 cells received i.p.s.p.s through disynaptic pathways. The neurones producing these i.p.s.p.s are probably located outside layers 2 and 5. Many cortical neurones also received later i.p.s.p. components although we were unable to analyse these effects in any detail. Whatever the source, cortical inhibitory systems seem to be mainly of the feed-forward type. Thus, we found no evidence for recurrent inhibition after antidromic activation of efferent neurones in layers 5 and 6. Whether the locally ramifying collaterals of upper layer neurones are part of a recurrent circuit still remains to be determined. In any case, it should be stressed that, although the inhibitory potentials described in this study may appear rather stereotyped throughout the cortex, this belies a diversity as rich as that observed for the excitatory connexions. This is clear both from the anatomical diversity of spine-free stellate cells and from physiological observations indicating that inhibitory connexions are responsible for many important receptive field properties, including end-stopping, direction selectivity and disparity sensitivity (Sillito, 1975; Ferster, 1981).

#### *Receptive field type and geniculate connectivity*

There is no doubt, from our results and from previous work (Hoffman & Stone, 1971; Toyama, Maekawa & Takeda, 1977), that substantial numbers of both simple and complex neurones in area 17 of the cat receive monosynaptic excitation from relay cells in the l.g.n. There is, however, an important difference between the two types in that *all* recorded simple cells received monosynaptic excitation, while complex cells occurred in two forms: those with and those without monosynaptic input. The result concerning simple cells was predicted by Hubel & Wiesel (1962), primarily because the responses of the subfields to stationary stimuli resemble the responses of geniculate relay cells to the same stimuli.

The experiments performed in this study say nothing new about spatial organization or even receptive field type (on or off) of geniculate afferents projecting to simple cells. Several observations, however, suggest that the on and off subfields are constructed by separate excitatory input from on- and off-centre relay cells with spatially displaced receptive fields (Toyama *et al.* 1977) and not only from the centre and surround mechanism of a single relay cell type. The widths of the subfields correspond closely to the receptive field centres of relay cells at the same eccentricity, and many simple cells have only two subfields, whereas input from a line of relay cells of one type would produce three. In addition, the responses from subfields of opposite type are often equal in strength, while the response evoked from the surround of a relay cell is always much weaker than that from the centre (Palmer & Davis, 1981). In fact, small stimuli that were effective in evoking a good response from each subfield of simple cells in the present study were completely ineffective in activating relay cells from their surrounds (see also Schiller, 1982).

Inhibitory mechanisms may also play a role in the receptive field organization of

simple cells, for example, in strengthening the mutual antagonism between subfields (Bishop, Coombs & Henry, 1973; Palmer & Davis, 1981; Heggelund, 1981). Such inhibition could be supplied by a simple inhibitory neurone whose receptive field was exactly superimposed on that of its target neurone, but with the sign of its subfields reversed. Simple cells with mirror image receptive fields are often found together and there is ample evidence for local inhibition within layers 4 and 6 (see above). Thus, inhibition from on cells would be superimposed on excitation from off cells and vice versa. Such an arrangement could explain why cells in layer 6 retain their 'simple' properties despite an excitatory input from complex layer 5 cells. Certainly, the inhibition would also sharpen the orientation selectivity that was initially set up (albeit crudely) by the spatial separation of on and off excitatory input from the l.g.n. (Sillito, 1975; Sillito, Kemp, Milson & Berardi, 1980).

For complex cells with monosynaptic excitation from the l.g.n., the situation is different. The uniform response to flashing stimuli obtained throughout the receptive fields implies that the excitatory input from each type of geniculate relay cell is distributed rather uniformly throughout the receptive field. Complex cells with both on and off responses over the entire receptive field may receive convergent excitation from both on and off centre relay cells with overlapping receptive fields (Toyama *et al.* 1977). This has been found to be the case for the on-off discharges of perigeniculate neurones (Ahlsén, Lindström & Lo, 1980). These neurones, however, respond equally well to stimuli of all orientations. Accordingly, one would expect a direct input to be entirely inadequate to account for the typical 'cortical' properties of complex cells, including orientation and direction selectivity. These properties would require an additional input from other orientation selective neurones, either excitatory or inhibitory. An inhibitory input may be the critical one for the few non-projecting monosynaptic complex cells in layer 6 and upper layer 5. But for layer 3 cells, the only efferent complex neurones with monosynaptic excitation, the additional input could be in the form of excitation; layer 3 cells are unique in receiving a cortically mediated excitatory input comparable in size to their monosynaptic excitation from the l.g.n. (see Fig. 6). If this excitation comes from layer 4, as suggested above, simple cells may be shaping the receptive field properties of these complex cells in the manner originally suggested by Hubel & Wiesel (1962; see, however, Malpeli, 1981).

From the present results, it is clear that the simple and complex receptive field properties are not diagnostic for the synaptic order of connexions from the l.g.n. These properties rather reflect the spatial distribution of excitation from on and off centre relay cells, whether mediated by direct connexions, or indirectly via other cortical neurones. At the same time it seems clear that even though the cortex cannot be described as a simple chain of neurones linked in sequence, the important features of complex cells are shaped by intracortical processes to a larger extent than for simple cells. This is true not only for complex cells in layer 3 as described above, but also for those in layers 2 and lower 5 which receive no direct excitation from the l.g.n. This is important since all efferent neurones in area 17, with the exception of cortico-geniculate cells, have complex receptive fields. The layer 6 cells may be considered as a special case, since the function of their efferent projection is to control the input to the cortex. Thus, in agreement with one of the important concepts of



the hierarchy model, visual information emerges from area 17 only after significant reorganization by cortical circuits.

We would like to thank Ms Nan Wallace for excellent technical assistance at many stages of this project. Support was provided by Magnus Bergvalls Stiftelse and the Swedish Medical Research Council (Project No. 4767). D.F. was supported by a Swedish Medical Research Council Postdoctoral Research Fellowship (No. 5864).

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