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AUGMENTING RESPONSES EVOKED IN AREA 17 OF THE CAT BY INTRACORTICAL AXON COLLATERALS OF CORTICO-GENICULATE CELLS

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SUMMARY

1. Evoked potentials were recorded in the visual cortex of the cat after electrical stimulation of the lateral geniculate nucleus (l.g.n.). The primary response, mediated by geniculo-cortical fibres, was depressed at stimulation frequencies above 7 Hz and replaced by a late potential, the incremental response, which gradually increased in amplitude with successive stimuli.

2. The incremental response was a negative–positive potential in the depth of the cortex with the negative component having maximal amplitude in layer 4. The response reversed polarity in layer 1 to become a positive–negative potential at the surface.

3. The latency of the negative component of the incremental response was about 3·5–4 ms in layer 4, compared to about 1·5 and 2·5 ms for the mono- and disynaptic components of the primary response.

4. The incremental response could only be evoked from the l.g.n. and the optic radiation, not from the optic tract, superior colliculus or other surrounding structures. Within the l.g.n., the effect was only evoked from stimulation sites in approximate retinotopic register with the recording site in the cortex. Low threshold points were found in the A laminae, completely overlapping with the low threshold points for the primary response. Thresholds increased steeply when the stimulation electrode was lowered into the C laminae.

5. The incremental response could still be evoked ten days after the destruction of all cells in the l.g.n. complex by kainic acid.

6. It is concluded that the described incremental response is identical to the augmenting response of Dempsey & Morison (1943) and is mediated by intracortical axon collaterals of antidromically activated cortico-geniculate neurones.

INTRODUCTION

In their classical studies of cortical evoked potentials, Morison & Dempsey (1942, 1943; Dempsey & Morison, 1943) recognized two types of responses to stimulation of

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specific thalamic nuclei – the primary response, easily shown to represent the synaptic effect of specific thalamocortical afferents, and the augmenting response. The augmenting response is a late incrementing potential evoked by repetitive stimulation at frequencies above 5–7 Hz. The potential is quite distinct from the primary response and may reach larger amplitudes. Although its mechanism and function remain obscure (Morin & Steriade, 1981), this is clearly an effect of great power, as well as one of unusual temporal properties.

Recently, in the course of our experiments on laminar patterns of connectivity in the primary visual cortex (Ferster & Lindström, 1983), we stumbled upon the augmenting response and the solution to the puzzle of its source. Part of our approach to cortical connectivity was to activate efferent neurones and their intracortical collaterals from their extracortical termination sites. Initially we had little hope of using this technique successfully on cortico-geniculate cells in layer 6; it seemed almost inevitable that any synaptic effect produced by antidromic activation of these cells from the lateral geniculate nucleus (l.g.n.) would be obscured by the large geniculo-cortical response produced simultaneously.

When stimulating in the l.g.n. at frequencies above 7 Hz, however, we noticed that large incremental potentials developed in the cortex at the expense of the primary response. Both the latency and threshold of the effect resembled those of similar incremental potentials evoked in the l.g.n. by repetitive orthodromic stimulation of cortico-geniculate neurones (Ahlsén, Grant & Lindström, 1982). It was reasonable to suppose that the cortical potentials were mediated by collaterals of the same cells, activated in the antidromic direction.

In this paper it is argued that the observed incremental response in the primary visual cortex is identical to the augmenting response of Dempsey & Morison (1943) and that the effect is mediated by intracortical axon collaterals of antidromically activated cortico-geniculate neurones. Our primary interest in this phenomenon arose from the possibility of using the response as a simple means of studying the synaptic effects of these collaterals. It is in fact possible to do so, and the companion paper contains a study of collateral effects on single neurones in different cortical layers (Ferster & Lindström, 1985).

METHODS

Adult cats were used for the experiments. They were anaesthetized with Pentothal sodium (initial dose 25–30 mg/kg, supplemented as needed), paralysed with gallamine triethiodide and artificially ventilated. End-expiratory CO_2, blood pressure and body temperature were continuously monitored and kept at normal physiological levels. The eyes were fitted with contact lenses of appropriate curvature to focus the eyes on a tangent screen on which visual stimuli were projected.

Unipolar stimulation electrodes were placed in the optic tract, l.g.n., and superior colliculus, using evoked potentials as a guide for proper placement (Ferster & Lindström, 1983). The electrodes in the optic tract and the superior colliculus were fixed to the bone of the skull, while the geniculate electrode was free to be moved with a micromanipulator. With a simple switch, this electrode could be changed to a recording electrode and visual responses were frequently used to determine the laminar position of the electrode and its position in the retinotopic map of the l.g.n. In tracking experiments, small electrolytic lesions were placed above and below the nucleus upon terminating a track. Nerve fibres were stimulated with constant current pulses (electrode negative), 0·2 ms in duration and with an intensity varying from 10 μA up to 2–3 mA. The stimuli were either delivered at a slow rate of 1–2 Hz or as 2–4 s long bursts of higher frequencies, separated by long resting periods.

Evoked potentials and unit activity were recorded intracortically with large glass micropipettes, filled with 3 M-NaCl, and having a resistance of 2–5 MΩ. In most experiments, depth readings together with the shape of the primary response were used to judge position of the recording electrode in different cortical layers (Mitzdorf & Singer, 1978). In some intracortical tracking experiments the electrodes were instead filled with 2% solution of Pontamine Sky Blue in 0·5 M-NaCl. Dye marks of about 50 μm in diameter were made at appropriate depths by passing 1–2 μA of negative current out of the electrode tip for 5 min. Tracks were reconstructed from serial frozen sections stained with Cresyl Violet. Recorded potentials were amplified, displayed with two different time bases on a double oscilloscope and photographed for subsequent analysis.

In two cats the geniculo-cortical neurones were selectively destroyed with kainic acid (Schwarcz & Coyle, 1977; Woodward & Coull, 1982). In each case four penetrations were made through the l.g.n. with a micropipette filled with 0·1% w/v kainic acid in artificial cerebrospinal fluid. The first penetration was made near the representation of the area centralis, 1 mm lateral to the medial border of the l.g.n. The 2nd, 3rd and 4th penetrations were made 2 mm rostral, caudal and lateral to the first. In each penetration four injections of 200 nl were made at an interval of 0·5 mm, starting at the top of lamina A. The animals were studied electrophysiologically ten days later in acute experiments. After the recording sessions, the animals received an additional large dose of the anaesthetic and were then perfused through the heart with saline, followed by buffered 3% w/v paraformaldehyde. The extent of cell destruction in the l.g.n. area was examined in serial frozen sections stained with Cresyl Violet, and the resulting fibre degeneration in the cortex was examined with a modification of the Fink–Heimer technique (Wiitanen, 1969).

RESULTS

Properties of cortical incremental response

Comparison of primary and incremental responses in area 17. When the l.g.n. is stimulated at low frequency (1–2 Hz), a field potential consisting of two negative peaks followed by a positivity can be recorded in layer 4 of the cortex (Fig. 1*A–C*, upper traces). It has been known for a long time that this potential, usually referred to as the primary response, is due to stimulation of geniculo-cortical fibres. Not surprisingly then, a similar response, delayed by a millisecond, can also be evoked from the optic tract (Fig. 1*E*, upper trace). The two negative peaks (marked by an open triangle and circle in *D*) correspond in latency to monosynaptic and disynaptic excitatory post-synaptic potentials, seen in intracellular recordings from cortical neurones (Ferster & Lindström, 1983). A small inflexion representing the incoming afferent volley often precedes these negativities (Fig. 1*D* and *E*).

If the stimulation frequency is increased above 7–10 Hz, the primary response is greatly reduced in amplitude (Fig. 1*A* and *B*, lower traces). The disynaptic component, in particular, is very frequency-sensitive and is often virtually abolished; the early spike discharge of monosynaptically activated cortical neurones is suppressed at these rates of orthodromic stimulation (Ferster & Lindström, 1985). At higher stimulus amplitudes the disynaptic potential is instead replaced by a late negative–positive field potential that grows slowly in amplitude over several seconds (Fig. 1*C*, lower trace). No corresponding potential can be evoked by stimulation of the optic tract (Fig. 1*E*, lower trace). It is this late potential rising out of the remnants of the mono- and disynaptic components that we believe to be identical to the augmenting response of Dempsey & Morison (1943) and to be mediated by antidromically activated cortico-geniculate neurones. We will use the descriptive term 'incremental response' for the potential throughout the paper.

The differences in shape between the primary and incremental responses evoked

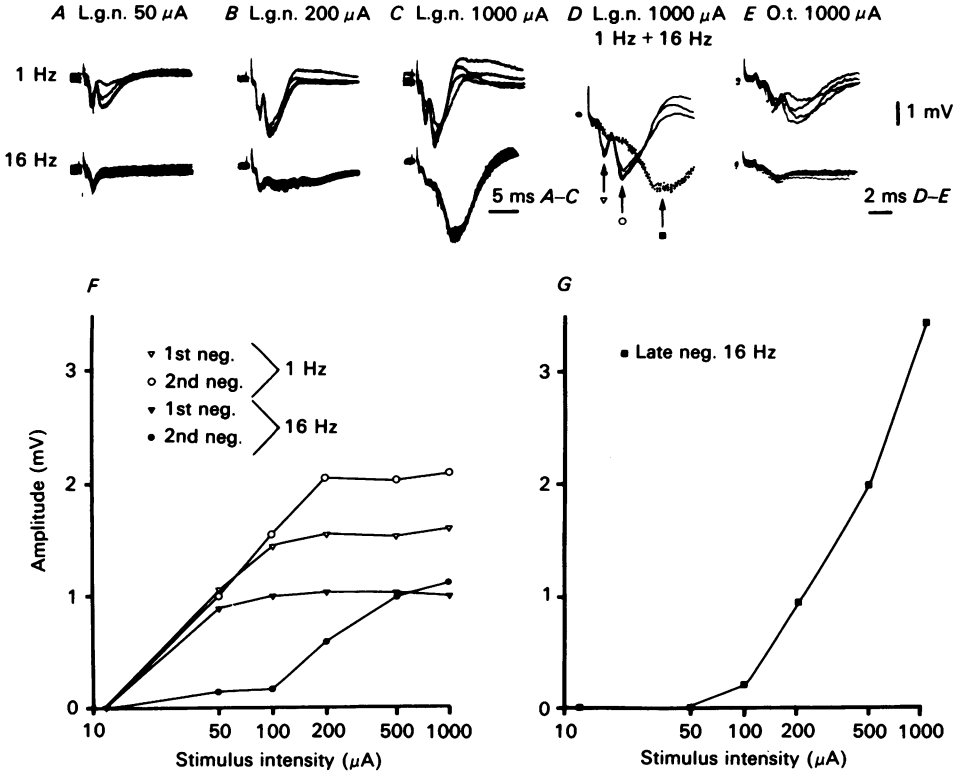

Fig. 1. Incremental response in the primary visual cortex. The records in *A–E* show extracellular field potentials recorded with a micro-electrode in layer 4 of area 17 upon stimulation of the lateral geniculate nucleus (l.g.n.) and the optic tract (o.t.). The l.g.n. stimulation intensity was increased from *A* to *C*. The records in the upper row were obtained with a stimulation frequency of 1 Hz and the two negative potentials represent the mono- and disynaptic components of the primary response, evoked by stimulation of geniculo-cortical fibres. The stimulation and recording electrodes were well aligned with overlapping receptive fields (as tested with visual stimulation) and the threshold for the primary response was quite low, 12 μA. Maximal response was obtained at about 200 μA (cf. *B* and *C*). A similar primary response, delayed about 1 ms, was obtained from the optic tract (*E*). The lower traces show corresponding steady-state potentials obtained with a stimulation frequency of 16 Hz. Note the suppression of the primary response in *A* and *B* and the development of a new delayed potential, the incremental response, in *C*. The two responses in *C* are shown superimposed with expanded time base in *D*. There was no corresponding late potential after o.t. stimulation at 16 Hz, only a decrease of the primary response. In this and all subsequent records, negativity of the micro-electrode tip with respect to an indifferent electrode in the temporal muscles is indicated downwards. Voltage calibration in *E* refers to all records. In the graph *F* the amplitude of the mono- and disynaptic components of the primary response (open symbols) is plotted against the l.g.n. stimulation intensity. The measurements were from the same experiment as the sample records and taken at the peak of the corresponding potentials, as indicated by arrows in *D*. Filled symbols indicate corresponding measurements at 16 Hz stimulation of the l.g.n. Note the profound suppression of the disynaptic field potential at this frequency; the apparent increase in the negativity (2nd neg.) above 100 μA is mainly an artifact: the negativity was measured at a time interval longer than the onset latency of the late incremental response. When scrutinizing the individual successive responses to the train of stimuli it was clear that the disynaptic component of the primary response was suppressed before the incremental response developed, suggesting that very little of the disynaptic component survived at 16 Hz. The graph *G* shows the steady-state amplitude of the late negativity at 16 Hz and different l.g.n. stimulation intensities.

by l.g.n. stimulation are best seen in Fig. 1*D*, where the traces of *C* are shown superimposed with an expanded time base. The leisurely rise and fall and broad peak of the late incremental potential compared to the components of the primary response were quite typical. The exact onset latency of the response was difficult to determine mainly because it developed on top of a partly surviving early component of the primary response. As in the illustrated case, however, there was often a clear inflexion at the beginning of the late potential. If this inflexion is taken to represent the onset of the incremental response then it had latencies in the order of 3·5–4 ms, i.e. more than twice the latency of the monosynaptic component of the primary response.

The stimulation intensity required to evoke an incremental response was consistently 5–10 times higher than the threshold level for the primary response evoked from the same stimulation site (Fig. 1*A–C*). The difference is well illustrated by the graphs in Fig. 1*F* and *G*, where the amplitude of the different responses has been plotted against the l.g.n. stimulus intensity. In this particular case the stimulation electrode in the l.g.n. was well aligned with the recording site in the cortex (cells recorded with the two electrodes had completely overlapping receptive fields). Not surprisingly, the primary response had a threshold of only 12 μA and reached maximal amplitude at about 200 μA (Fig. 1*F*, open symbols). The same was true of the mono- and disynaptic components remaining at 16 Hz (filled symbols). Note that the apparent rise in the disynaptic component above 100 μA is an artifact caused by the development of the late potential (see Figure legend). The incremental response, in contrast, was barely detectable at 100 μA, and was still growing in amplitude at 1000 μA. Higher stimulation intensities were rarely investigated for fear that the high-frequency, high-intensity stimulus would cause a sizeable lesion at the stimulation site in the l.g.n. With 1000 μA, 0·2 ms pulses delivered at 16 Hz, the average current passed through the electrode is already 3 μA, enough to cause a small lesion in a few seconds if applied as constant current.

Frequency sensitivity of incremental response. The responses shown in the lower traces of Fig. 1 are steady-state potentials reached after several seconds of stimulation. The gradual development of the incremental response is illustrated in Fig. 2. At a stimulation rate of 16 Hz, the mono- and disynaptic components of the primary response are drastically reduced already by the second stimulus (Fig. 2*A*, upper trace). Almost immediately the late, high-threshold potential begins to grow and continues to do so for several seconds (Fig. 2*A*, lower trace). The rate of growth of this potential was dependent on both the stimulus intensity and frequency (Fig. 2*B* and *C*). The minimum frequency for the unequivocal development of the response was 7 Hz in the illustrated case, but traces of the response have been seen at 5 Hz in a few other experiments. Note that the ordinate of the graphs is stimulus number and not time; the potential develops much more slowly at 7 Hz than at 16 Hz.

Stimulation frequencies above 16 Hz were only rarely tried, but whenever they were it was clear that the incremental response developed even faster and to higher peak amplitudes than at 16 Hz. At rates of 25–50 Hz, however, there was also a very rapid 'fatigue' of the response, so that the amplitude was already down to a small fraction of maximal size after a few seconds of stimulation. Like the growth of the incremental response, the degree of fatigue depended on the intensity, amplitude and duration of the stimulation. Even prolonged stimulation (more than 5 s) at 16 Hz

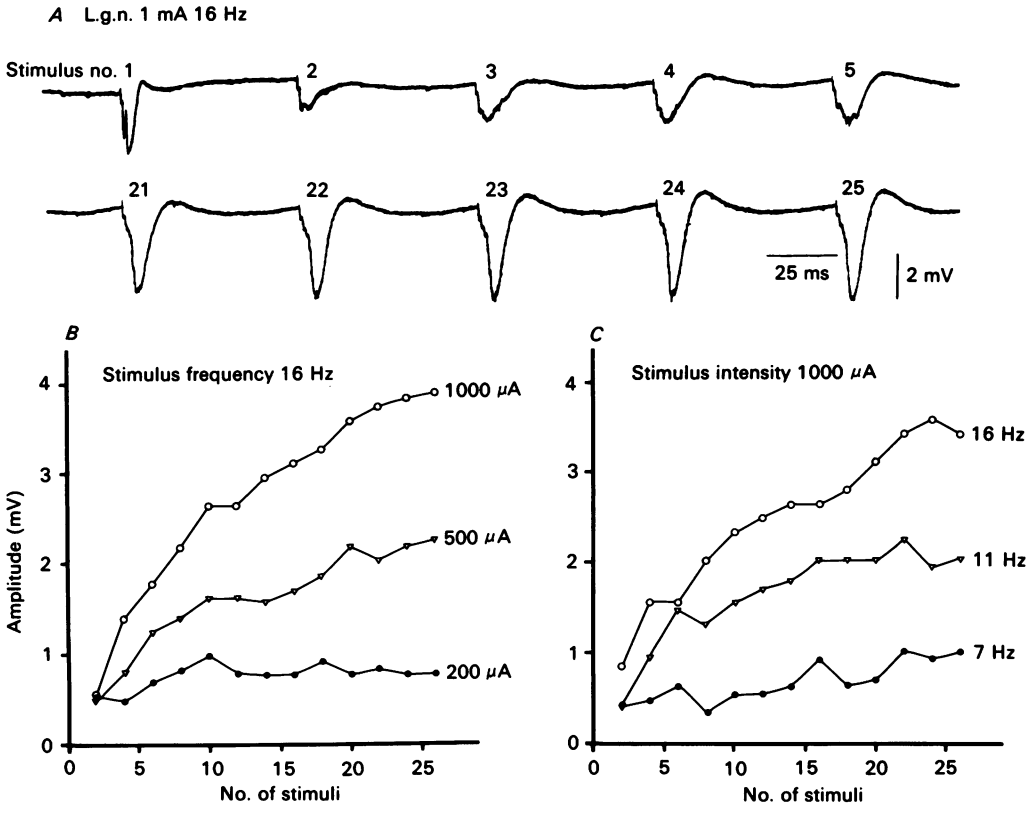

Fig. 2. Frequency sensitivity of incremental response. The traces show two sequences of responses in lamina 4, evoked by repetitive stimulation of the l.g.n. at 16 Hz, 1 mA. The upper trace is from the beginning and the lower from the end of the same run and the stimulus number is indicated above each response. The primary response was drastically reduced already after the second stimulus and from the third on, the late incremental response started to develop. The response was still growing in amplitude during the stimulation sequence 21–25. In the graphs the amplitude of the late negativity is plotted against the stimulus number, in *B* using a fixed frequency of 16 Hz and varying the l.g.n. stimulus intensity, in *C* varying the stimulus frequency but keeping the intensity constant at 1 mA. The incremental response developed faster and to higher amplitudes both when the stimulus intensity and frequency were increased. In this experiment the threshold intensity was about 200 μA and the lowest effective frequency 7 Hz. The small variability of the response is suggested by the two 1000 μA, 16 Hz curves which were obtained at different times during the same experiment.

and 1000 μA produced clear signs of fatigue. It was therefore necessary to use a test procedure where the l.g.n. was stimulated with 2–4 s long bursts separated by recovery periods of 30–60 s.

The mechanism underlying the fatigue was not explored, although it is possible that a massive synchronous activation of cortical neurones by the stimulation was responsible for the effect (Ferster & Lindström, 1985). As a rule, the fatigue was not the result of damage in the l.g.n., since normal incremental responses could be evoked repeatedly given suitable recovery periods. At the same time it was clear that we were balancing close to the critical level for lesions at the stimulation site; long stimulation

sequences (more than 20–30 s) always resulted in elevated thresholds for later trials.

Pattern of incremental response in different cortical layers. The shape of the incremental response varied between different cortical layers as shown in Fig. 3 by records taken from the same experiment as that described in Ferster & Lindström

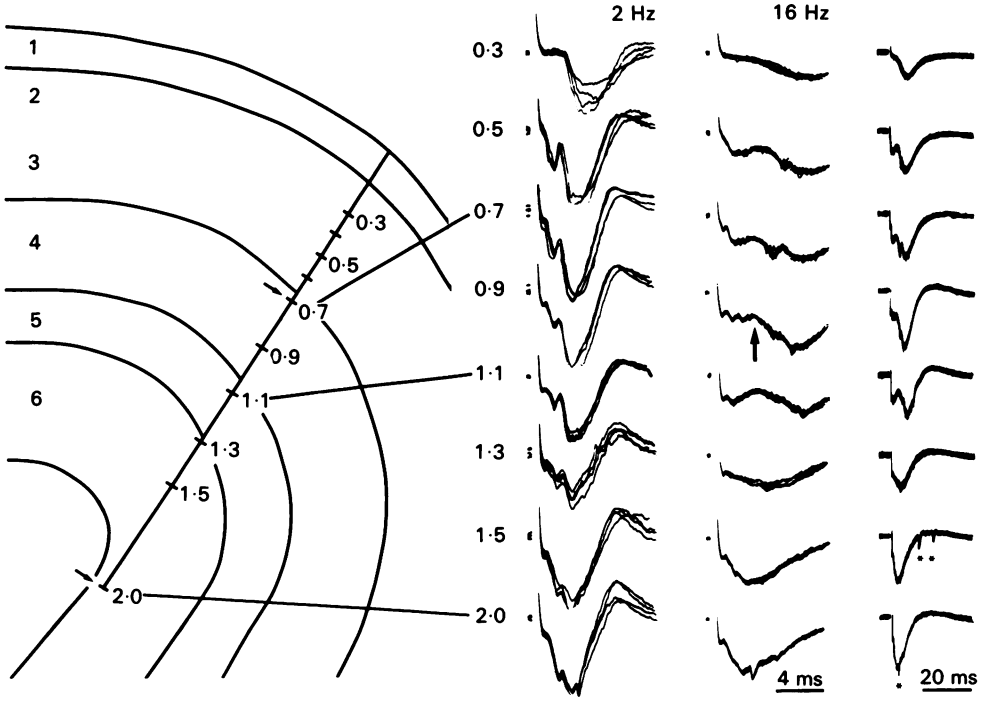

Fig. 3. Depth profile of incremental response in the cortex. The left diagram is a reconstruction of an electrode track made with a dye-filled electrode, the two arrows indicate the centres of two small dye spots. The records to the right show the extracellular field potentials evoked at the indicated depths by stimulation of the l.g.n. with an intensity of 1 mA. The left column shows primary responses at a stimulation frequency of 2 Hz, the middle and right columns the corresponding incremental responses at 16 Hz, recorded with two different time bases. The stimulation intensity was about 5 times the maximal for the primary response, but submaximal for the incremental response. The negative component of the incremental response had the shortest latency (arrow, middle column) and largest amplitude in layer 4. A clear, somewhat delayed potential was found in supragranular layers and in layer 5, while the potential was essentially missing in layer 6. The early negativity in this layer is presumably composed of a surviving monosynaptic geniculo-cortical field potential and spike fields from antidromically activated layer 6 cells. A few antidromic spikes are indicated by asterisks in the two lower traces of the right column. The time calibration below the middle column is valid also for the left.

(1983, Fig. 2). The recording electrode was filled with Pontamine Sky Blue and dye marks were made ionophoretically at the points marked by arrows in the reconstruction of the track. The l.g.n. was stimulated with 1 mA, which was supramaximal for the primary response but submaximal for the incremental response. As shown earlier, the laminar pattern of the primary response (first column) reflects the pattern of

connexions of geniculate afferents with cortical cells. The earlier monosynaptic component is largest in layers 3, 4 and 6 where monosynaptically activated neurones are found, and it is reduced or absent in layers 2 and 5, which contain cells with disynaptic input. (The small early negativity in layer 5 is mainly a spike field due to antidromic activation of corticocollicular neurones from the l.g.n. electrode at this high stimulation intensity.)

The incremental response was most prominent in the middle of the cortex (middle and right columns), in layer 4, where it also had the shortest latency (arrow in trace taken at the depth 0·9 mm). Here the negative potential often had a clear double-peak configuration. A large but somewhat delayed response was also found in layers 2 and 3. The potential reversed to a positive–negative response in layer 1 and at the surface (not illustrated). In the deeper cortical layers the potential was much attenuated, although it did not show a proper reversal. The layer 6 response was dominated by a large early negativity which seemed to be composed of a reduced monosynaptic geniculo-cortical field potential plus spike fields of antidromically activated cortico-geniculate neurones. A few antidromic spikes growing out of the field potential are marked by asterisks in the two lower traces of the right column. The lack of a late incremental negativity in this layer presumably reflects the poor synaptic driving of layer 6 cells during this kind of stimulation (Ferster & Lindström, 1985).

Source of incremental response

Low threshold points in the l.g.n. region. As already described, the incremental response could be evoked from the l.g.n. but not from the optic tract. From geniculate stimulation sites the threshold for the effect was considerably higher than for the primary response. One could seek to explain these results by invoking complex mechanisms in which the effects of a single pathway could be altered by changing stimulus parameters. But a simpler explanation by far would be that the two effects were mediated by different sets of fibres. Corticocollicular neurones were one alternative to consider, since such cells can be antidromically activated from the l.g.n. at high thresholds (Ferster & Lindström, 1983) and have extensive intracortical collaterals (Gilbert & Wiesel, 1979). This possibility was ruled out by the fact that no incremental response could be elicited by stimulation of appropriate sites within the superior colliculus at 16 Hz, not even at stimulation intensities of several milliamperes.

Systematic series of penetrations were made through the region of the l.g.n. in four experiments in order to narrow down the alternative sources for the incremental response. The result of one such experiment is illustrated in Fig. 4. In this case, five tracks were made through the region of the l.g.n. with the stimulating electrode. Three made in the same frontal plane (T1–3) are indicated in the diagram to the left. The other two were made 1 mm anterior and 1 mm posterior to the centre track. The thresholds for the primary and incremental responses were determined at closely spaced intervals (200 μm) along each track, while the recording electrode was kept in the same place in layer 4 of the cortex. A good indication of the relative position of the different l.g.n. penetrations with respect to the cortical recording site was obtained by mapping the receptive fields. The centre position of the receptive fields recorded in each l.g.n. track is indicated in the inserted diagram in the upper left

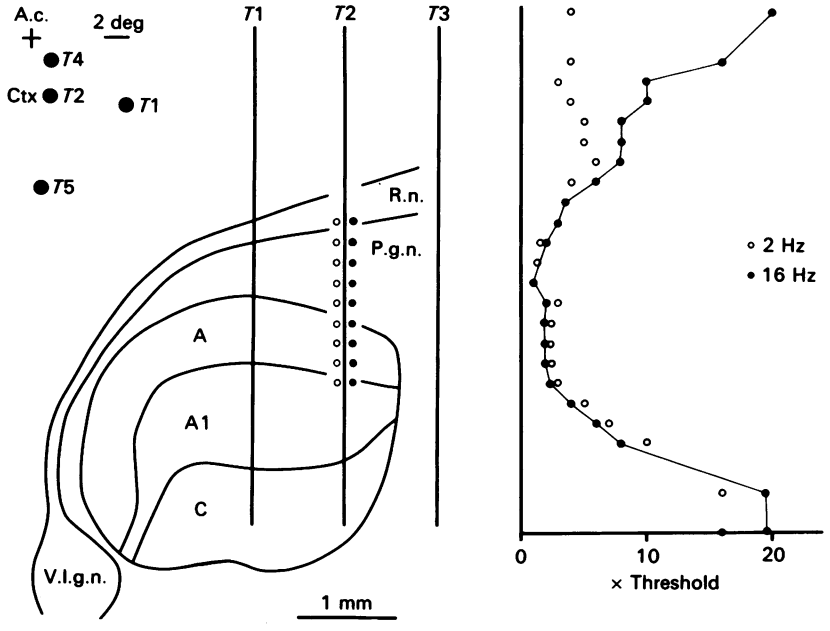

Fig. 4. Low threshold points in the l.g.n. region for incremental responses. The left diagram is a reconstruction in the frontal plane of three penetrations through the anterior part of the l.g.n. Two more penetrations were made 1 mm posterior (T4) and anterior (T5) of the middle track (T2). In each penetration the thresholds for primary and incremental responses were determined at intervals of 0·2 mm, while the recording electrode was kept in a fixed position in layer 4 of the cortex. Small electrolytic lesions were placed above and below the l.g.n. so that stimulation points could be related to the histology. During the actual experiment the depth of energy into lamina A and the transition points between the different layers could easily be identified by recording cell activity. By determining the receptive field position of cells in each penetration it was also possible to judge the relative position of the penetrations with respect to the recording point in the cortex. The centre point of the fields in each case in relation to the area centralis (a.c.) is indicated in the inserted diagram in the upper left corner of the Figure. As seen here, the receptive fields of geniculate cells in the T2 track were completely in register with the fields at the cortical recording site (ctx). The lowest thresholds for both the primary (10 μA) and incremental (50 μA) responses were found in this track just above lamina A. Open and filled circles indicate points with thresholds no more than three times the minimal for the primary and incremental responses, respectively. The threshold for the incremental response was more than 5 times higher than the minimal in the other penetrations. The right diagram shows threshold profiles for the two responses in the same penetration, plotted as multiples of the minimal value for each response; same symbols as before. R.n., reticular nucleus of the thalamus; p.g.n., perigeniculate nucleus; V.l.g.n., ventral lateral geniculate nucleus.

corner. As seen here, the receptive fields of the middle penetration (T2) were completely in register with the receptive fields at the cortical recording site (ctx). Not surprisingly then, the primary response had lowest thresholds (10 μA) in this penetration. The open circles to the left of the T2 track represent stimulation sites with thresholds below 30 μA, i.e. below three times the lowest value. A more detailed 'threshold profile' for the primary response in this penetration is shown in the

diagram to the right, where the required stimulation intensity, expressed in multiples of the absolute minimum, is plotted for each tested stimulation site. The thresholds increased dramatically below lamina A, but remained at a moderately low level for several millimetres in the optic radiation indicating that the responsible fibres did not deviate too far from the electrode. Threshold levels were at least twice the minimum of the centre track in all other penetrations.

The incremental response also had its lowest thresholds in the centre (T2) penetration, although the absolute level (50 μA) was 5 times higher than for the primary response. The threshold profile was almost identical to that of the primary response, however, as seen by the filled circles in the right diagram. The only notable difference was seen in the optic radiation above the l.g.n., where the relative threshold level for the incremental response increased rather steeply. In the other penetrations, the lowest threshold varied between 300 and 1000 μA – more than 5 times as high as in the centre track. Similar results were obtained in the three other tracking experiments. Thus, the fibres giving rise to the incremental response seem to be organized in a retinotopic fashion, just as the geniculo-cortical pathway.

The fibres are organized according to ocular dominance as well (Hubel & Wiesel, 1962). In the experiment discussed above the cortical electrode was placed in a column dominated by the contralateral eye. This explains why the primary response had low thresholds in lamina A, which contains geniculate relay cells activated from the contralateral eye. The incremental response in this and in one other similar experiment also had low thresholds in lamina A. In the remaining two tracking experiments the cortical recording electrodes were in a column dominated by the ipsilateral eye; then, the low threshold points for the incremental response were found in lamina A1, the ipsilateral layer.

The first conclusion to be made from this experiment is that the incremental responses are unlikely to be caused by stimulation of a fibre system unrelated to the retinocortical pathways. Such systems passing through or close to the l.g.n., but unrelated in function, would not be expected to have the remarkably specific topographic organization observed. The experiment also made the possibility that the primary and incremental responses were mediated by the *same* fibres (e.g. geniculo-cortical fibres) highly unlikely. Theoretically, the difference in absolute threshold of the two effects could be due to an intracortical frequency-modulated process, but such a mechanism cannot easily explain the deviation of the two relative threshold curves in the optic radiation.

Though the incremental response is unlikely to be mediated by the same set of fibres as the primary response, we still have to consider an input from W cells in the C laminae of the l.g.n. These geniculo-cortical neurones do not contribute overtly to the primary response (Mitzdorf & Singer, 1978) and thus may be responsible for the incremental response. The long latency of this potential is certainly consonant with this idea since the conduction velocity of the W cells is much lower than that of X and Y cells in laminae A and A1. This possibility was all but ruled out, however, by the very high thresholds for the incremental response evoked from the C laminae.

Incremental response after destruction of l.g.n. neurones with kainic acid. The most reasonable remaining candidate for the projection responsible for the incremental response is the cortico-geniculate pathway and the intracortical collaterals of layer 6 cells activated antidromically from the l.g.n. This is the only known system besides

the geniculo-cortical projection to have the required topographic (Gilbert & Kelly, 1975) and ocular dominance organization (S. LeVay & H. Sherk, personal communication). However, since many of the arguments leading to this conclusion are somewhat indirect, a more definitive test of the hypothesis is desirable.

Ideally, one would like to stimulate antidromically the layer 6 cells from the l.g.n. independently of the geniculo-cortical fibres. As illustrated above, this is not possible because of the much lower electrical threshold of geniculo-cortical fibres. We chose kainic acid, therefore, as a means of destroying the latter while leaving the former intact; from its reported properties, kainic acid injected into the l.g.n. should do just that (Schwarcz & Coyle, 1977; Woodward & Coull, 1982). Plate 1 *A* shows a low-power photomicrograph of the anterior portion of the l.g.n., seen in sagittal section, of an animal that had massive amounts of kainic acid injected into the nucleus ten days prior to the experiment (see Methods). Large amounts were used since it was important to ensure that no trace of the forward projecting pathway from the l.g.n. remained. Though at low power the familiar shape of the l.g.n. is evident, at higher power (Pl. 1 *C*) it can be seen that the entire structure is made up of large numbers of densely packed glial cells. Upon careful inspection of each section through the l.g.n., not a single neurone was found either in the l.g.n. itself or in adjacent portions of surrounding structures, including the medial interlaminar nucleus, the lateral part of the pulvinar and the posterior complex of thalamus. For comparison, a high-power photomicrograph from an uninjected l.g.n. is shown in Pl. 1 *B*.

The complete destruction of geniculate neurones was accompanied by the requisite degeneration of their terminals in layers 4 and 6 of the straite cortex, as seen with a silver degeneration stain (Pl. 1 *D*). One would expect, therefore, that stimulation in the l.g.n. in this animal at 1–2 Hz would not elicit the normal primary response, and in fact, no detectable primary response was seen at stimulation intensities as high as 1 mA (Fig. 5, upper traces). By comparison, in normal animals with properly aligned electrodes, the primary response had thresholds below 20 μA (Figs. 1 and 4). Of course, in this experiment appropriate alignment could not be found directly since it is normally determined by comparing the receptive field positions of the cells found at the recording and stimulating electrodes; while one could determine the topographic position of the geniculate electrode by recording multi-unit activity from undamaged retinal terminals, the cortex was visually silent. One could nevertheless find the approximate corresponding position in the cortex using published topographic maps (Tusa, Palmer & Rosenquist, 1978). The records in Fig. 5 are taken with the electrodes in such an estimated correspondence yet no primary response is evident. Nor could any primary response be elicited from numerous sites along several tracks surrounding the illustrated one, some of which are shown in the diagram of Fig. 5.

In contrast to low-frequency stimulation, higher frequencies elicited potentials resembling the normal incremental response. At 16 Hz the response had the usual configuration with two late negativities in layer 4, the latency of the first component being about 4 ms (Fig. 5, arrow in first column). The potential had the lowest thresholds from the dorsal layers of the l.g.n. in presumed register with the recording electrode (Fig. 5, filled circles of the diagram) and it grew slowly over several seconds of repetitive stimulation (not shown). Similar results were obtained in one other experiment with kainic acid destruction of the l.g.n. In both cases the evoked response differed from the normal incremental response in two ways: its threshold was even

higher than normal (about 400 μA) and its maximum amplitude was smaller. There are three possible explanations for this behaviour. First, the stimulating and recording electrodes could have been poorly aligned. Secondly, geniculate terminals of cortical cells may have been damaged, particularly in the area of the injections, either by the kainic acid itself or simply by the large volume of fluid injected. Thirdly, the excitability of cortical cells may be depressed by the loss of input from geniculo-cortical neurones. Perhaps the activation of cortico-geniculate axons from a wider area of the l.g.n. by the larger stimulus overcomes the problem; hence the higher thresholds.

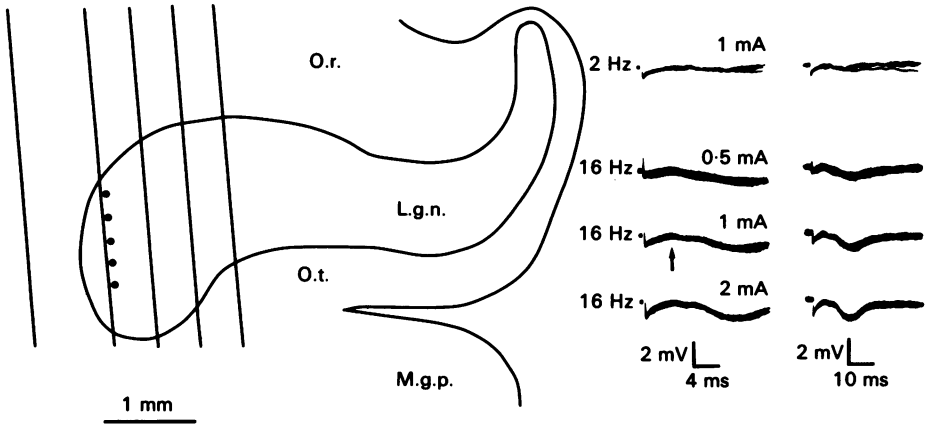

Fig. 5. Remaining incremental response after destruction of the l.g.n. with kainic acid. The diagram to the left shows the outline of the nucleus as seen in a parasagittal section together with the reconstruction of five electrode tracks through the region. All neurones in the l.g.n. complex had been destroyed by massive kainic acid injections ten days prior to the experiment (see Pl. 1). The responses to the right, shown with two different time bases, were recorded in layer 4 of the visual cortex after stimulation in the l.g.n. at indicated intensities and frequencies. The stimulation point was in the second track from the left, presumed to be approximately in retinotopic register with the cortical recording site. Note the lack of primary response at 2 Hz, despite the very high stimulus intensity and the development of late incrementing potentials at 16 Hz. This response resembles in latency (about 4 ms, arrow) and shape the incremental response seen in normal animals, the only difference being its higher threshold (about 400 μA) and smaller amplitude. Similar responses could only be evoked from the points indicated by filled circles in the diagram, in all other penetrations the thresholds exceeded 2 mA, the highest intensity tried. O.r., optic radiation; m.g.p., medial geniculate nucleus.

It should be noted here that while the incremental response is greatly diminished at low frequencies of stimulation (1–2 Hz), it is not completely absent. Careful inspection of the upper right-hand trace of Fig. 5 reveals a small but detectable response with the approximate shape and latency of the incremental response. A similar response is probably present in normal animals but obscured by the larger primary response. The appearance of the incremental response then requires two changes, a suppression of the primary response and a potentiation of the late high-threshold potential. From the observation that the incremental response remains and exhibits its usual properties in the absence of geniculate principal cells, we conclude that it is, in fact, the cortico-geniculate neurones and their collaterals that mediate the incremental response.

DISCUSSION

There seems little doubt that the phenomenon we have studied is identical to the augmenting responses described by Dempsey & Morison (1943). There are several points of similarity. (1) Augmenting responses and our incrementing potentials are both evoked from the specific thalamic nuclei of the cortical area studied, in our case the l.g.n. (2) The frequency sensitivity of the different components of the primary and augmenting responses are similar. The mono- and disynaptic components of the primary response are diminished at frequencies above 7–10 Hz, while the delayed incremental response develops only at these or higher frequencies. In addition, the higher the frequency above this lower limit, the greater the final amplitude of the response and the more quickly it develops. (3) The depth profile of the effect examined here is identical to that of the augmenting response as recorded in motor cortex after stimulation of the ventrolateral thalamus (Spencer & Brookhart, 1961; Sasaki, Staunton & Dieckmann, 1970), the delayed component at 16 Hz being a negative–positive wave within the middle cortical layers but reversing to a positive–negative wave at the surface. (4) The thresholds of the augmenting response and the present incremental response are both greater than that of the primary response (Bishop, Clare & Landau, 1961). (5) Both responses can only be elicited from the appropriate relay nuclei and their projection pathways to the corresponding region of cortex, not from the primary afferent pathways (Bishop *et al.* 1961).

From earlier reports on the augmenting response, it is clear that there has been some confusion as to how to delineate the augmenting response from other potentials evoked by thalamic stimulation. Such difficulties may even explain the original claim by Dempsey & Morison (1943) that the augmenting response could sometimes be evoked in the somatosensory cortex by stimulation of the medial lemniscus or peripheral nerves. Other investigators (Matsuda, Sasaki & Mitzuno, 1972) maintain that augmenting responses cannot be evoked in the primary sensory areas of the cortex, only in the surrounding areas. They report occasional incremental responses, however, and it is not clear to us why these responses were disqualified as augmenting responses. The lack of good effects in their experiments may simply be related to the difficulty of aligning stimulating and recording electrodes properly. Certainly, their finding is discordant with the original description of the augmenting response (Dempsey & Morison, 1943) as well as with several other studies (Bishop *et al.* 1961; Morin & Steriade, 1981) including our own. Thus, we do not think that possible disagreements in the literature as to the proper identification of augmenting responses invalidate our conclusion that the observed incremental responses are in fact augmenting responses in the primary visual cortex.

Despite a number of experiments on the augmenting response over the past forty years, the mechanism responsible for it has remained obscure. Morison and Dempsey originally proposed a thalamic system in parallel to that underlying the primary response but which receives substantial indirect excitation from cortical efferents as well as from lemniscal afferents. The state of increased excitation of the augmented response was suggested to involve 'mutual reinforcement' between thalamus and cortex, but the 'principal mechanism underlying augmentation . . . lies in the thalamus' (Morison & Dempsey, 1943). Other authors have also interpreted the effect in terms of separate thalamic projection systems (Bishop *et al.* 1961; Spencer & Brookhart,

1961; Grossman, Clark & Whiteside, 1967; Sasaki *et al.* 1970; Matsuda *et al.* 1972), others still have sought to explain the phenomenon in terms of a cortical mechanism (Purpura, Shofer & Musgrave, 1964; Morin & Steriade, 1981). While the pathway from the site of stimulation is said to be the same as that underlying the primary response, the intracortical effects of the input change dramatically with stimulus frequency. Thus, the cortical circuits are assumed to undergo functional reorganization depending on stimulus frequency.

While there remains a possibility of change in the functional state of the cortex during repetitive stimulation (Ferster & Lindström, 1985), it is now clear that the thalamocortical pathway is not directly involved. Instead, the response is due to the unexpected mechanism of antidromic activation of cortico-geniculate fibres and their intracortical collaterals, at least as far as the visual system is concerned.

There is ample evidence to support this conclusion. (1) the augmenting response can be evoked only from the l.g.n. and not from the optic tract, superior colliculus or surrounding structures. (2) The projection of the pathway mediating the augmenting response, like the cortico-geniculate pathway is specific for retinotopy and ocular dominance. (3) Within the l.g.n., however, the threshold for the augmenting effect is much higher than that of the primary response, comparable in fact to the threshold for antidromic activation of cortico-geniculate neurones (Ferster & Lindström, 1983). (4) The relatively long latency of the augmenting response in comparison to the primary response is easily accounted for by the long latency of antidromic activation of layer 6 cells from the l.g.n., about 3 ms for the latter and slightly longer for the former. Similarly, the slow rise time of the late response is likely to result from the large spread in antidromic latencies (Ferster & Lindström, 1983). While the C laminae projection might seem at first also to account for these properties, the lowest threshold for the augmenting response is found not in the C laminae, but in the A laminae. (5) The maximal early negativity of the augmenting response, at a given stimulus amplitude, is always seen in layer 4, as is the minimum latency for single cell responses at 16 Hz (Ferster & Lindström, 1985). Layer 4 is just where the terminals of layer 6 cells are found (O'Leary, 1941; Gilbert & Wiesel, 1979). The location of the field potential to the terminal region of layer 6 cells also demonstrates that the response is primarily a synaptic field potential, not a spike field from antidromically activated layer 6 cells. (6) Similar incremental responses can be evoked in the lateral geniculate nucleus on repetitive stimulation of the cortico-geniculate pathway in the orthodromic direction. (7) Finally, the augmenting response remains after the complete elimination of the visual thalamic projection to the cortex.

Its underlying mechanism having been identified, the augmenting response itself becomes less interesting from a functional point of view; it is unphysiological, not only because it is evoked electrically but because it involves antidromic activation of the neurones responsible for the effect. The intracortical pathway it represents is not unphysiological, however, and the augmenting response raises several questions about the function of this collateral system. Why, for example does it possess such unusual temporal properties. The augmenting response provides an experimental probe by which one may explore some of these questions. In the following paper (Ferster & Lindström, 1985), we begin to do so by examining the synaptic effects exerted on individual cortical cells by the collaterals of layer 6 cortico-geniculate cells.

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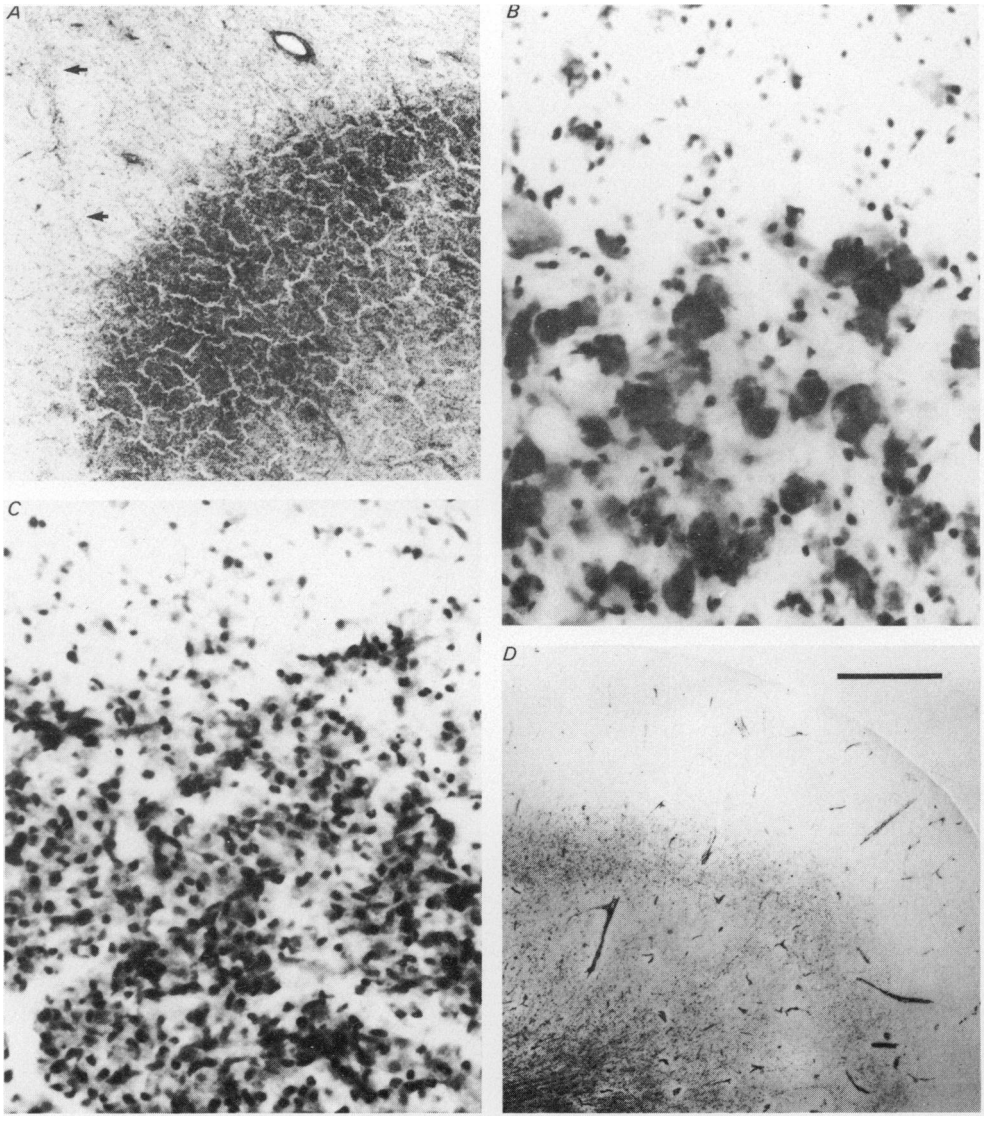
EXPLANATION OF PLATE

Destruction of neurones in the l.g.n. by kainic acid. The photomicrograph in *A* is a low-power view of the anterior pole of the l.g.n., injected with kainic acid ten days earlier. The section was obtained from the experiment illustrated in Fig. 5, and the two arrows point to the second track from which incremental responses could be evoked. The high-power micrograph in *C* straddles the upper border of the l.g.n. in the same section. Note the lack of neurones, the l.g.n. being filled with densely packed glial cells. In *B* a similar region of a normal l.g.n. is shown for comparison. All micrographs are from 50 μm frozen sections stained with Cresyl Violet. The section in *D* is from the primary visual cortex near the recording site in the kainic acid experiment. The section was stained to show degenerating fibres and terminals with a modification of the Fink–Heimer procedure. The calibration bar in *D* is 500 μm for *A* and *D* and 50 μm for *B* and *C*.


A
B
C
D