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THE IDENTIFICATION OF SINGLE UNITS IN CENTRAL VISUAL PATHWAYS

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There have been many single-unit studies on central visual pathways in recent years and several of these studies have been concerned with unit activity in or near the lateral geniculate nucleus (Tasaki, Polley & Orrego, 1954; Freygang, 1958; Lennox, 1958*a*, *b*; De Valois, Smith & Kitai, 1959; Erulkar & Fillenz, 1960; Grüsser-Cornehls & Grüsser, 1960; Hubel, 1960; Widén & Marsan, 1960; Hubel & Wiesel, 1961). In some cases the identification of the nature of the unit (i.e. presynaptic axon, cell-body, postsynaptic axon, etc.) has been made on a single criterion, e.g. latency. While this may suffice for many units, it is obviously desirable to improve the accuracy of identification by using as many criteria as possible. In connexion with our studies on the visual system we have found it essential to be able to distinguish between optic-tract axons, geniculate cells and radiation axons. The present paper is concerned with the criteria which we have found useful in this respect when recording extracellularly from central visual pathways. The criteria have been established for tract and radiation axons by recording directly from these pathways after having inserted the electrode under stereotaxic control. The information obtained in this way could then be applied to recordings obtained from the lateral geniculate nucleus (LGN) or its vicinity, situations in which a knowledge of the position of the electrode did not necessarily assist in the identification. In this way the three groups mentioned could be distinguished from one another. The possibility of other groups occurring will be discussed later.

The wave form of the extracellularly recorded response, its attenuation with distance from the unit and other features are critically dependent on the type of electrode used; hence our findings in this respect cannot be applied indiscriminately to situations where the electrodes are of different shape, material or resistance. These points are discussed below. Probably for this reason, our results are not in entire agreement with those of previous investigators in this field. Some of the material presented here has appeared in earlier shorter communications (Bishop, Burke & Davis, 1958, 1959; Bishop, Burke, Davis & Hayhow, 1958). The antidromic

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response of LGN neurones and the interpretation of the externally recorded cell response will be discussed in later papers (Bishop, Burke & Davis, 1962*a*, *b*).

METHODS

Adult cats were used in all experiments. They were anaesthetized with intraperitoneal allobarbitone (Dial, Ciba, 0·5 ml./kg) plus pentobarbitone (Sagatal, May and Baker, 0·1–0·3 ml./kg). The effect of the latter drug would probably have worn off by the time the first records were obtained several hours later. The cat's body temperature, read with a rectal thermometer, was controlled within normal limits (38–39° C) with the aid of an electric heating blanket. In nearly all experiments the cat was immobilized with gallamine triethiodide (Flaxedil, May and Baker) given intravenously at the rate of about 12–16 mg/hr after an initial dose of 8–12 mg. To avoid undue movement of the brain the respiratory tidal volume was kept at 20–30 ml. at a rate of 18/min but to prevent anoxia a stream of oxygen was passed down a thin plastic tube inserted to the level of the carina. This procedure maintains O_2 and CO_2 blood levels close to normal (cf. Darian-Smith, 1960).

Usually both optic nerves, but sometimes one only, were prepared for stimulation by resection of the eyeball and suspension clear of orbital tissue (for more complete details see Bishop & McLeod, 1954). The cat's head was fixed in the Horsley-Clarke planes. The skull was trephined directly over the LGN and the dura reflected. At the commencement of each experiment a steel micro-electrode was inserted vertically under stereotaxic control through the intact cerebral cortex on one side and the position of the LGN checked by inspection of the multineurone response resulting from electrical stimulation of one or other optic nerve. The steel electrode was then replaced by a glass capillary micro-electrode filled with 3 M-KCl or 4 M-NaCl and of d.c. resistance 1–10 MΩ. Such electrodes are too coarse for intracellular recording but are very suitable for extracellular recording because large unit potentials can be obtained well above the noise level or multineurone response and with a low risk of damage to the cell; in general, electrodes with a resistance of 2–5 MΩ were preferred. The choice of electrodes will be referred to in the Discussion.

Gross movements of the brain must be avoided when recording from units. These movements were considerably reduced by inserting the electrode through a sealed Perspex chamber (Fig. 1) mounted in a base made from dental impression compound (this material softens in hot water and can be moulded to the shape of the skull). To ensure a watertight fit dental impression paste was placed between the base of the chamber and the skull. The chamber, which was filled with either normal saline or liquid paraffin, was covered with a closely-fitting lid consisting of two plates of Perspex between which a sheet of thin rubber dam was glued. A hole large enough to take the electrode was drilled through the centre of the Perspex plates, but the rubber sheet had only a very much smaller hole through which the tip of the electrode was passed. On further advance of the electrode the rubber gripped its stem making a water-tight seal. The chamber could be filled and emptied through a side tube sealed into the base of the chamber. Air bubbles were extruded through the hole in the lid before sealing in the electrode. During the advance of the electrode pressure within the chamber remained atmospheric because the side tube was connected to an open-ended reservoir; when a unit was found this side tube was closed off.

The recording equipment was conventional and consisted of cathode follower probes close to the recording micro-electrode, pre-amplifier, amplifiers, cathode-ray oscilloscope, loud-speaker and camera. In a few experiments a neutralized-capacity input stage was used. In most experiments recording was via resistance-capacity coupling to the amplifier, the time constant being suitably adjusted, but in several experiments d.c. recording was used to check the relationship of membrane potential to type of response.

Stimuli to the optic nerves were 50 μsec rectangular pulses delivered through an isolating transformer, usually at 2–5 sec intervals. In all Figures oscilloscope records are negative upwards.

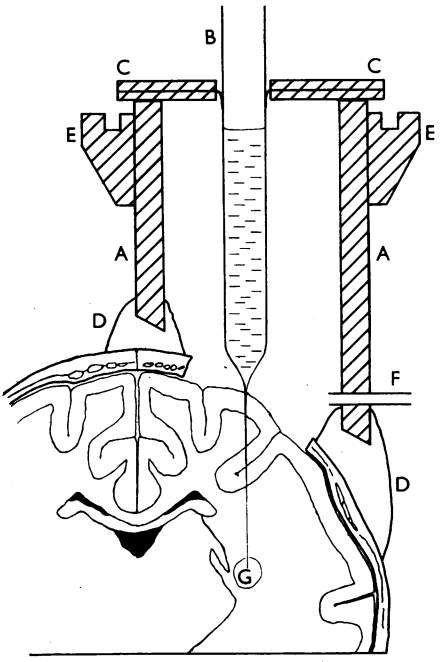

Fig. 1. Diagram, approximately to scale, to illustrate the chamber used to minimize brain movements. The brain is shown in coronal section at the level of the anterior part of the lateral geniculate nucleus (G). The chamber is shown in section at the same level, the walls (A) and lid (C) cross-hatched, the base mounted in dental impression compound (D). The chamber was held in position by attachments at E. The electrode (B) was inserted through the rubber sheet in the lid. Pressure within the chamber could be adjusted via the side tube (F). From median plane of brain to axis of electrode is approximately 9 mm. Further details in text.

RESULTS

The following results were obtained in 31 experiments in which recordings were made from 433 units. When a capillary micro-electrode is inserted into optic tract, LGN or optic radiation, the unit wave forms recorded are of several types. The two main types of record obtained are illustrated in Fig. 2. In agreement with other authors we consider that these recordings are from axon (*a*) and LGN cell (*b*), respectively. Before describing the various tests we have used to establish this conclusion and to distinguish tract axon from radiation axon it is desirable to describe the responses in more detail.

Response type 'a'

This response (Fig. 2*a*) appears suddenly with almost no warning even when the electrode is advanced in small steps. It is commonly, although not always, associated with a low resting potential of 5–30 mV (in one case up to 45 mV). It is initially of simple monophasic shape, positive-going, of short duration (1–3 msec) and rapid initial downstroke (0·15–0·30 msec). In amplitude the responses vary considerably, the majority

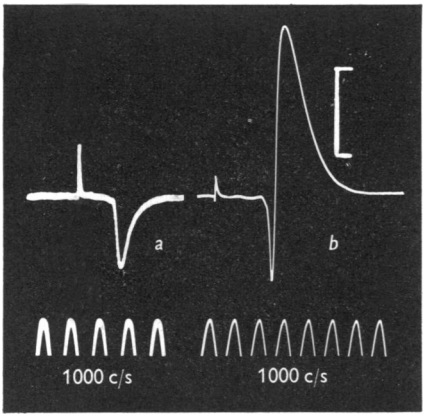

Fig. 2

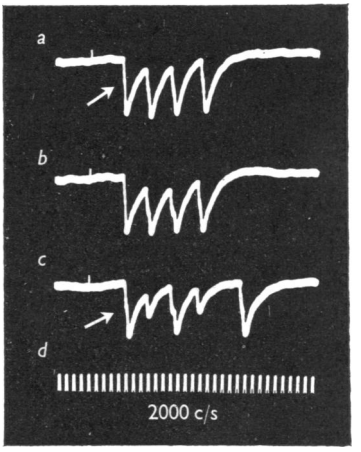

Fig. 3

Fig. 2. (*a*) Response type 'a'. (*b*) Response type 'b'. Voltage calibration 10 mV.

Fig. 3. Deterioration of response type 'a'. In each trace, *a–c*, the unit fires repetitively to a single stimulus. The first response in each burst shows a slight inflexion on the downstroke (arrows) which becomes more marked during the burst (e.g. *b*) and may lead to occasional failure of the second component (*c*).

lying between 1 and 10 mV. The response behaves in all-or-nothing fashion when the strength of stimulus is varied. It is rarely stable (with these electrodes) and may disappear suddenly or undergo a typical break-up of wave form. The sequence of events in this deterioration are: the appearance of a notch on the initial downstroke, increasing delay of peak and reduction of amplitude; eventually the response is clearly in two parts, the second of which fails intermittently; later the second component fails altogether; finally there is failure of the first component. This deterioration of wave form is illustrated in Fig. 3. This unit fired repetitively in response to a single stimulus. At first (Fig. 3*a*) there was only a slight inflexion on the downstroke. During the next few minutes the notch became more prominent particularly on the repetitive responses (Fig. 3*b*). Still later (Fig. 3*c*) the second component failed intermittently. Other examples of

responses of type 'a' in which a notch has appeared on the downstroke are shown in Figs. 14*B* and *C*. Figure 14*C* also shows the failure of the second component when the unit fires a second time.

Response type 'b'

This is a complex positive-negative wave form showing usually one or two steps on the downstroke of the positive phase (Fig. 2*b*). It is not associated with a resting potential. The exact wave form and the ratio of the amplitudes of the positive and negative phases as well as the over-all amplitude vary considerably and depend critically on the distance of the tip of the electrode from the unit. For example, Fig. 4 shows the change in wave form of two units as the electrode was advanced vertically down through the LGN. The sequence of changes is characteristic of many other

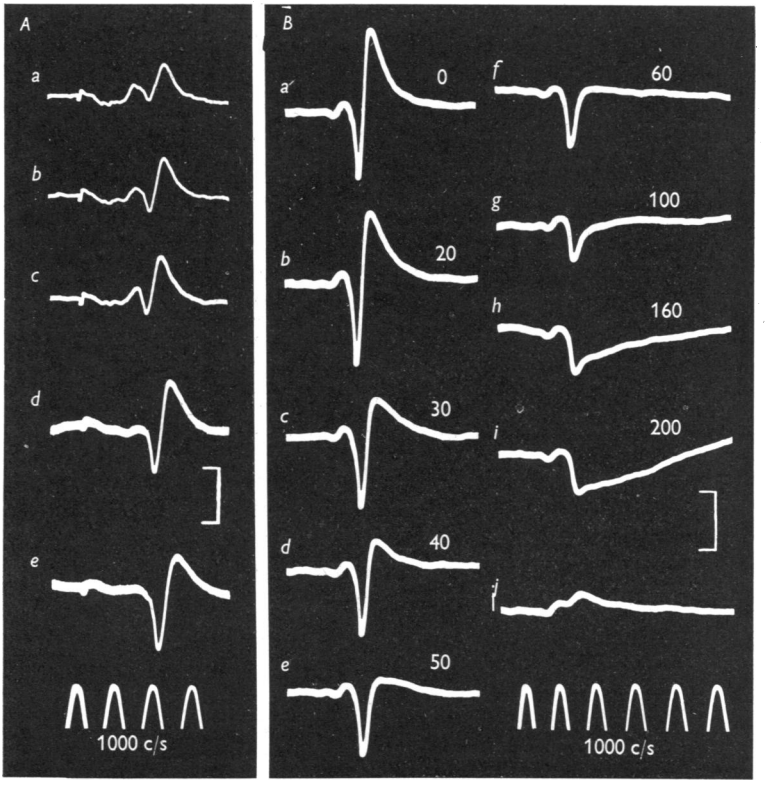

Fig. 4. Change of wave form of response 'b' as the electrode is advanced. *A*. Distance between steps not recorded. Note increase in amplitude of both positive and negative phases, but the relatively greater increase in positive phase, as the electrode advances $a \rightarrow e$. Voltage calibration 2 mV. *B*. Another unit. Figures indicate distances in micra from position in which record (*a*) was obtained. *j*, multineurone response only. Voltage calibration 2 mV.

experiments. When the unit is first detected the negative phase is large, usually larger than the positive phase (Figs. 4*A*, *a*; 4*B*, *a*). As the electrode is advanced the positive phase becomes progressively larger and inflexions appear on the downstroke. The negative phase also increases in amplitude at first but later becomes reduced whilst the positive phase is still increasing. The decrease in amplitude of the negative phase is accompanied by a slowing of its time course; eventually this phase disappears (Fig. 4*B*, *f*).

Further advance of the electrode causes an increase in duration of the positive phase with reduction in amplitude (Fig. 4*B*, *h*, *i*). At this point the response may suddenly become very large whilst remaining positive and a resting potential may appear. Resting potentials of up to 60 mV have been obtained. With the relatively low-resistance electrodes used this last wave form is usually not maintained for more than a minute or so. The indications are that this last stage represents the penetration of the membrane by the electrode followed by deterioration of the unit due to damage. It must be emphasized that prior to this last stage no resting potential is recorded; response type 'b' is certainly an extracellular recording in spite of the fact that its over-all amplitude may reach 30 mV. Normally responses with amplitudes 5–10 mV are readily obtainable. Penetration does not always occur and the unit may disappear or deteriorate with further advance of the electrode.

Very occasionally at the stage of penetration or slightly before, the unit may commence to discharge in the absence of stimulation of the optic nerve, evidently due to irritation by the electrode. Spontaneous discharge of physiological origin is believed to occur also and will be mentioned later. The loss of negativity undoubtedly indicates a loss of responsiveness by part of the membrane of the unit but it does not lead to the immediate death of the unit. Responses of the sort shown in Figs. 4*A*, *e* and 4*B*, *d* and *e* have been maintained for several hours in many units. However, it is unusual to obtain such responses without having had at any earlier stage a response in which the negative phase was larger than the positive phase.

The amplitudes of the positive and negative phases of the series partly illustrated in Fig. 4*B* have been plotted against the advance of the microelectrode in Fig. 5. The graph shows a complete loss of negativity about 110 μ before penetration of the membrane. It seems likely that loss of negativity due to damage by the electrode would occur only if the electrode made contact with the membrane. Failure to penetrate until an advance of 100–200 μ had been made implies either a tangential approach to the unit or, more probably, that the electrode pushed the unit before it for some distance and probably also invaginated the cell membrane. No systematic attempt has been made to study the distances over which a unit may be recorded; it is clear, however, that once the response starts

to lose negativity there must be a strong suspicion that the electrode is then touching the membrane; from then on the additional advance made by the electrode before penetration should probably not be regarded as an advance in the extracellular field of the unit.

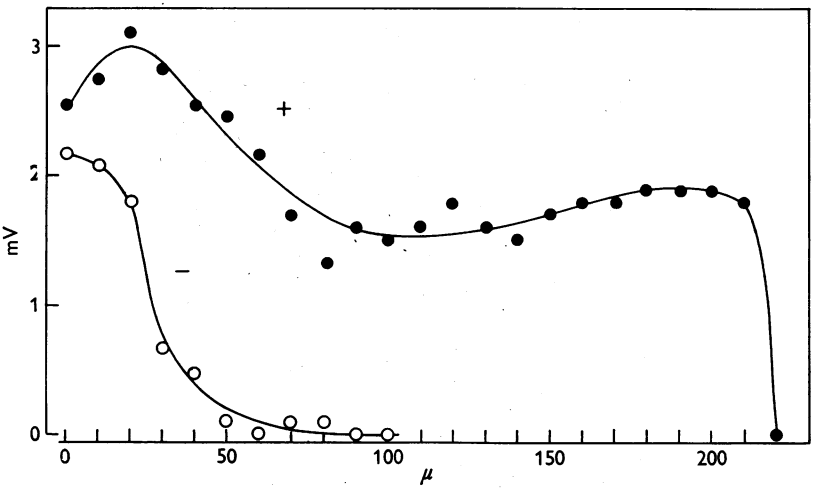

Fig. 5. Graph of amplitude of response (mV) against distance advanced by the electrode (μ) from a series of records partly illustrated in Fig. 4*B*. +, amplitude of positive phase, −, amplitude of negative phase. At a distance between 210 and 220 μ from the position at which the first record was obtained (Fig. 4*B*, *a*) the response abruptly changed to a much larger positive response which rapidly deteriorated. There is an interval of about 10 sec between successive readings.

The change of wave form which occurs on advance of the electrode may also occur in the course of time if the electrode is not moved, provided the response is not too small (Fig. 6). Sometimes the loss of negativity occurs with only minor changes in the amplitude of the positive phase. Thus in Fig. 6*A* the sequence of records *a* to *d* was obtained without movement of the electrode within a period of 2 min; in such cases we may suppose that there has been very little change in the relative positions of unit and electrode but their proximity has resulted in damage to part of the membrane. In other cases the positive phase has increased in size; here it seems probable that the unit has moved closer to the electrode owing to respiratory or arterial pulsations or for some other reason such as the 'yielding' of part of the brain. In Fig. 6*B*, for example, the records cover a period of 15 min, and the changes in wave form during this time are consistent with a slow movement of tissue.

The duration of responses of type 'b' is usually 2–5 msec but both this and the relative durations of the positive and negative phases vary with the wave form. Thus when the positivity is small its duration is less than

0·5 msec; at the time when the negativity has just disappeared it may be up to 3 msec and with further deterioration much longer. The negative phase in different units varies from about 1 to 4 msec but in any one unit this increases only slightly with reduction of amplitude.

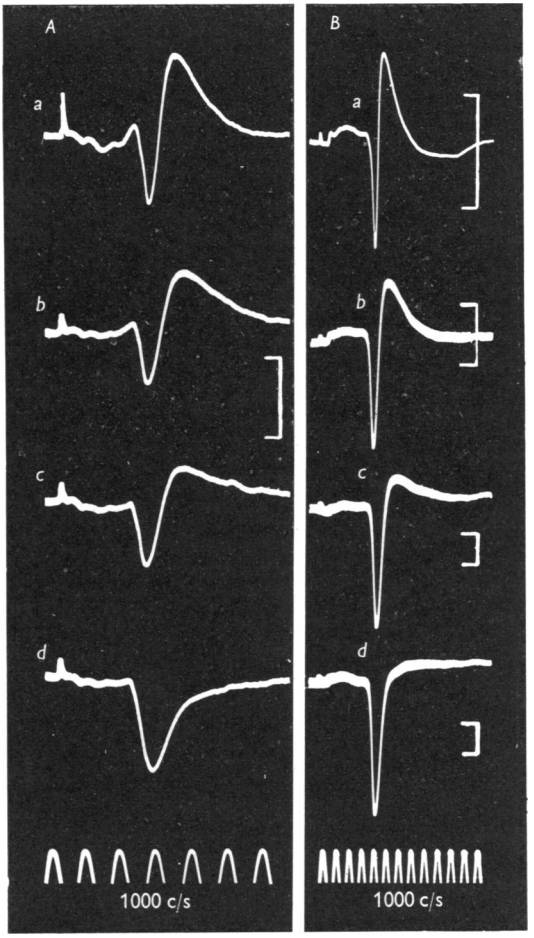

Fig. 6. Change of wave form of response 'b' with time, the position of the electrode being unchanged. *A*. Very slight changes in amplitude of the positive phase, loss of amplitude of the negative phase. *B*. Another unit. Increase in amplitude of positive phase. At first very little change, later loss of amplitude, of the negative phase. Voltage calibrations 2 mV.

Identification tests on responses 'a' and 'b'

With the stereotaxic apparatus that has been developed in this department it is possible to place the electrode tip in the optic tract or optic radiation with a fairly high degree of accuracy. Hence it is possible to

record from optic tract or optic radiation axons with practically no doubt as to their identity. The responses of such units have been subjected to a variety of tests and these will be described below. The results of these tests enable us to distinguish between tract and radiation axons in situations where a knowledge of anatomical position is not an adequate guide, e.g. in the LGN in which tract and radiation axons as well as LGN cells may be encountered.

Responses of units in optic tract and radiation are exclusively of type 'a'. Responses of type 'b' are recorded only in the vicinity of the LGN and responses of type 'a' may also be recorded here. For this reason and for others which will appear later, response 'b' is considered to be from a LGN cell. However, as already mentioned, response 'b' may undergo a change of wave form, becoming a purely monophasic positive wave often resembling response 'a'. In the present work it has not been possible to distinguish a response as being from an interneurone; such a unit presumably responds either as a principal LGN cell or a radiation axon.

Latency

The absolute latency does not provide a certain test for many units because the optic nerve contains fibres with a wide range of conduction velocities (Chang & Kaada, 1950; Bishop, Jeremy & Lance, 1953; Bishop & Clare, 1955; Lennox, 1958*a*). Thus some LGN cells and radiation axons may have brief latencies while small tract axons may have longer latencies. More information is conveyed by the change of latency which occurs when the stimulus strength is varied. In post-synaptic units the change of latency is particularly marked with stimuli near threshold and if the stimulus is set at threshold the play of latency to successive stimuli may be quite large; on the other hand a presynaptic unit shows very little play of latency at threshold. Figure 7 illustrates these points in (*a*) a tract axon and (*b*) a LGN cell. Note that in (*b*) there is a small positive wave (corresponding to the first step on the 'b' response in Fig. 2) which shows only a slight change of latency; the large changes of latency are in the 'spike' portion. The greatest shortening in latency with increase of stimulus strength was 7·9 msec (from 13·6 to 5·7 msec; mean of six responses in each case) the stimulus being increased to 3·3 times threshold. Latencies both to threshold and supraliminal (2–5 times threshold) stimulation were measured in 45 post-synaptic units; in 19 of these the change of latency exceeded 1 msec, in 12 it was less than 0·2 msec, but in no case less than 0·1 msec. By contrast in tract axons the change in latency never exceeded 0·1 msec. The change in latency in the small positive wave of the cell response (see Fig. 7) is usually 0·1–0·2 msec, only rarely exceeding the latter value.

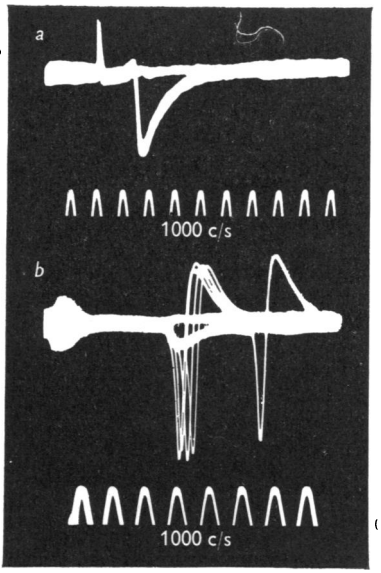

Fig. 7. Variation in latency of response of tract axon (*a*) and of LGN cell (*b*) when stimulus to the optic nerve is at threshold. About 10 sweeps superimposed in each case. Stimulus held constant at threshold strength. Note the occasional blank sweeps when the stimulus fails to excite. Negligible change of latency in response of tract axon, considerable change in spike portion of LGN cell response, slight change in small positive wave.

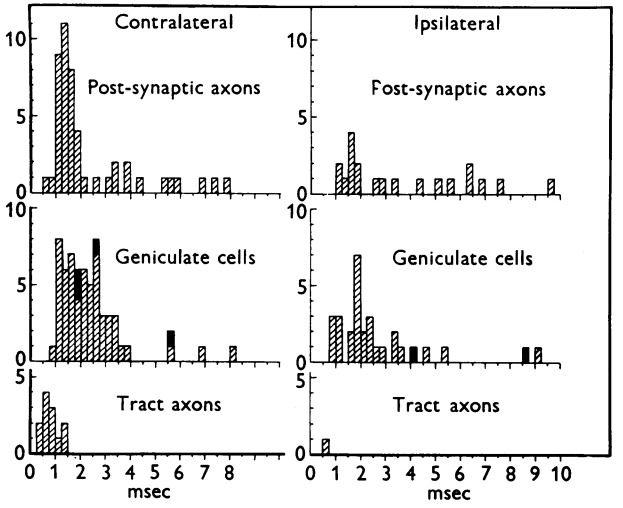

Fig. 8. Distribution of latencies of 175 units recorded in or near the LGN (not less than 11 mm below cortical surface). In all cases the stimulus was supraliminal (2–5 times threshold). The units are divided into groups of latency width 0·25 msec. The hatched columns of LGN cells represent cells with response type 'b', the solid columns, cells with response type 'c'. Tract and post-synaptic axons are distinguished by the tests described in the text. Ordinates, nos. of units.

Figure 8 shows the distribution of latencies in 175 units to supraliminal stimuli. The responses were recorded in or near the LGN. As might be expected, only tract axons have latencies less than 0·5 msec. No tract axon had a latency greater than 1·38 msec, corresponding to a conduction velocity of about 30 m/sec. However, the number of tract axons in the series is small (13). Since the optic nerve contains a large group of fibres with a mean conduction velocity of about 20 m/sec which certainly enter the LGN (Bishop, Jeremy & Lance, 1953), it is probable that our electrodes are not sufficiently fine to 'hold' small axons long enough for adequate study. This is probably true also for small LGN cells and radiation axons. Hence the histogram is almost certainly biased in favour of large units.

There is a tendency for the contralateral cells to form groups with peak latencies at 1·1 and 2·6 msec (Fig. 8). When recording from populations of LGN cells Bishop & Davis (1960*a*) found that, with maximal stimulation of the contralateral optic nerve, the mean latencies at the LGN of the two main groups of fibres were 0·75 and 1·7 msec, respectively. The corresponding latencies for the onset of post-synaptic activity were 1·05 and 2·22 msec, respectively, with latencies to peak of about 1·3 and 2·7 msec. These findings are consistent with our unit studies considering the small numbers sampled. Too few units have been recorded to give a reliable indication of whether ipsilateral fibres are faster, group for group, than the contralateral fibres (Bishop, Jeremy & Lance, 1953). As regards long-latency cells, usually we cannot decide whether they are innervated directly or indirectly from the optic nerve. If directly, this would imply the existence of optic fibres with conduction velocities as low as 4 m/sec (see Discussion).

It should be mentioned that units are frequently obtained with extremely long latencies of the order of 200 msec (Bishop *et al.* 1958; cf. Erulkar & Fillenz, 1960). Such units have not been included in the histogram because they appear to form a distinct group, there being no units with intermediate latencies. However, a unit may discharge both at short latency (not more than 10 msec) and long latency (about 200 msec).

Spontaneous discharge

The method of preparation of the optic nerve for stimulation probably destroys most of the retina. Under these conditions none of the 39 tract axons studied was found to be spontaneously active. On the other hand, LGN cells and radiation axons often discharge spontaneously. This provides some indication that the spontaneous activity is not due to the presence of the electrode, unless we suppose that tract and radiation axons are radically different in their properties. It might be supposed that cells would be more easily irritated to discharge by the presence of the electrode

than are axons. Our evidence does not support this supposition. Of 137 LGN cells in experiments in which both optic nerves were prepared for stimulation, 51 cells (37 %) were spontaneously active. In the same experiments 78 post-synaptic axons were studied and of these 28 (36 %) were also spontaneously active. Hence it seems that the electrode does not bring about discharge in any significant proportion of units (cf. Bornschein, 1958). No attempt was made to determine the source of the 'spontaneous' activity.

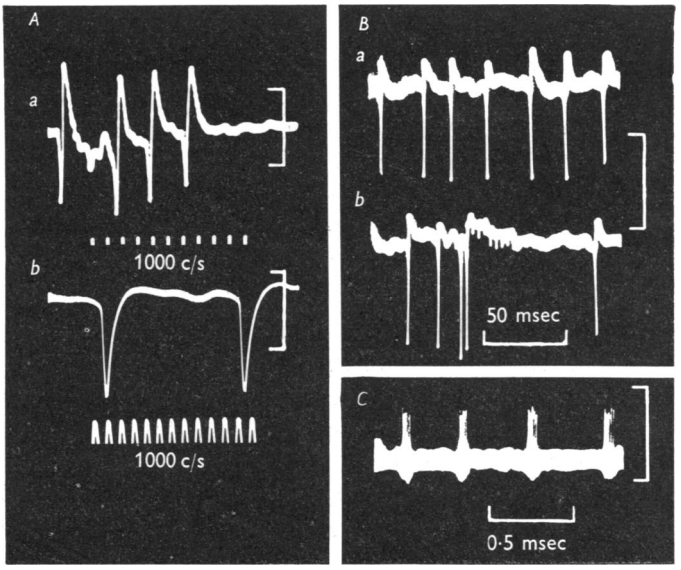

Fig. 9. Examples of spontaneous activity in visual units. *A*. Most usual type, mean frequency up to about 20/sec. *a*, LGN cell. The first response is spontaneous, the subsequent repetitive discharge is evoked. *b*, post-synaptic axon. *B*. High rate of discharge in LGN cell probably due to injury. *a*, portion of record. *b*, effect of stimulating the optic nerve (10 stimuli at about 350/sec). This stimulus evoked only two responses; the small deflexions are the stimulus artifacts. A pause of 78 msec occurs in the discharge. *C*, rhythmical 'bursts' in LGN cell (response type 'c'). Each burst contains about six responses. Voltage calibrations: *A*, *a*, 1 mV; *A*, *b*, 5 mV; *B*, *C*, 2 mV.

Examples of three types of spontaneous discharge are shown in Fig. 9. Figure 9*A*, *a* shows a spontaneous discharge in a LGN cell just preceding an evoked response; Fig. 9*A*, *b* is from a radiation axon. In both cases the discharge was regarded as 'physiological', being relatively infrequent and not associated with any deterioration of the response or death of the unit. The rate of such discharge varies considerably from 1/several sec to about 20/sec. Occasionally the firing occurred in short high-frequency bursts containing 1 to 5 or more spikes in the burst. This type of discharge is by

far the commonest. The second type is shown in Fig. 9*B*, *a*. Here the rate is high (about 40/sec), the discharge is sensitive to movement of the electrode and the unit soon deteriorates.

Spontaneous activity may be inhibited by stimulation of the optic nerve. This is most easily demonstrated when the rate is high, of course. Figure 9*B*, *b* shows the effect of applying a train of 10 stimuli at about 350/sec to the optic nerve. Actually only two responses were evoked and thereafter the unit did not fire for 78 msec. The small positive spikes were due to stimulus escape. This effect has also been noted by Tasaki *et al.* (1954). In the Renshaw cell spontaneous discharge is suppressed for some time after synaptic activation (Curtis & Eccles, 1958). A difficulty in the way of an explanation of this result as due to the inhibitory effect of the positive after-potential that follows a discharge (Bishop & Davis, 1960*a*) is that the spontaneous firing when it occasionally reaches such a high rate is not associated with comparable periods of inhibition. Moreover, the effect can be demonstrated using stimuli which do not evoke the unit at all. It is possible that the inhibition is due to after-currents from other cells or to the activation of inhibitory fibres. Neither possibility has been tested.

A third type of spontaneous discharge appears as short bursts repeated at regular intervals. The bursts consist of about six impulses firing at a mean rate of several hundred per second while the burst intervals vary from 0·1 to 1·0 sec. An example is shown in Fig. 9*C*. This type of discharge is not due to injury nor does it follow the rhythm of the pulse. A similar rhythmical discharge may be initiated by a stimulus to the optic nerve. This type of activity has been described previously (Bishop & Davis, 1960*b*; Hubel, 1960).

Repetitive discharge

Recording directly from tract fibres reveals that, although they will discharge repetitively to a long pulse of current, they will not do so to a short pulse (e.g. 50 μsec). In confirmation of earlier work (Bishop, Jeremy & McLeod, 1953) it was found that LGN cells (and radiation axons) frequently fire repetitively in response to a single stimulus to the optic nerve (Table 1). We regard the presence of repetitive firing in a unit as a strong indication that it is post-synaptic. Examples of repetitive firing are shown in Fig. 10 (see also Figs. 3, 9, 12–14). Figure 10*a* and *b* show records from two LGN cells, Fig. 10*c* from a radiation axon. It is interesting that relatively more radiation axons fire repetitively than do LGN cells. This suggests that in some cases repetitive discharge arises in a part of the cell remote from the soma, possibly the initial segment or first node. This question of repetitive firing will be dealt with more fully in another paper.

Response to repetitive stimulation

It is known from previous work (Bishop & Evans, 1956) that optic nerve fibres will respond to tetanic stimulation at rates of up to 1000 or more/sec at least for short periods of time. LGN cells, on the other hand, fail to respond after only three or four stimuli at rates of a few hundred per second. Unit recording from tract or radiation axons (Fig. 11*a* and *c* respectively) confirms this finding. Units of type 'b' likewise cannot follow these high frequencies of stimulation (Fig. 11*b*); in the case of these units there is frequently a fractionation of the wave form, the response being reduced to a small slow positive wave (see also Fig. 7*b* and the later paper, Bishop *et al.* 1962*b*).

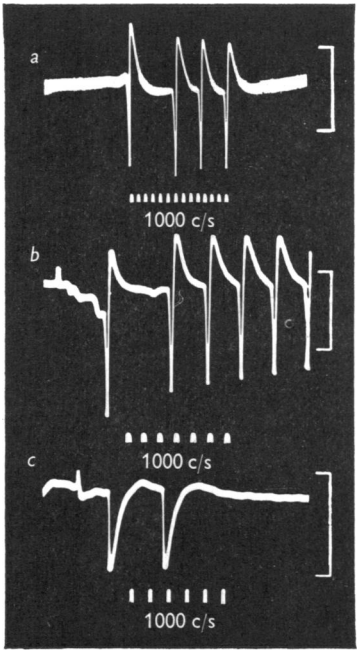

Fig. 10

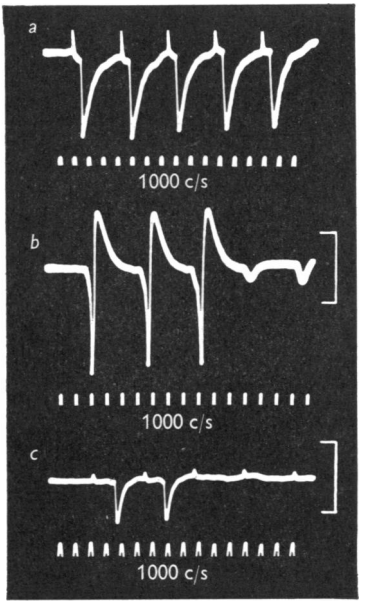

Fig. 11

Fig. 10. Examples of repetitive firing to single stimulus. *a*, *b*, LGN cells. The pause between first and second response is a common but not constant feature. *c*, post-synaptic axon. Voltage calibrations, 2 mV.

Fig. 11. Response to repetitive stimulation at about 300/sec. *a*, tract axon. *b*, LGN cell. *c*, post-synaptic axon. The post-synaptic units fail after two or three stimuli. In *b*, a step on the positive phase of the LGN cell response becomes more prominent in the second and third responses and evidently corresponds to the small responses that result from the fourth and fifth stimuli. Voltage calibrations, 5 mV (none available for *a*).

Other tests

Several other tests may be used to confirm the identification. Activation of the unit by stimulation of the cortex is strong, although not conclusive, proof that the unit is post-synaptic (see Bishop *et al.* 1962*a*). Activation from both eyes is even stronger proof (Bishop *et al.* 1958, 1959). Finally, the fractionation of response 'b' supports the idea that this is a cellular response (see Bishop *et al.* 1962*b*).

Other types of response

Whereas responses of types 'a' and 'b' are by far the commonest recorded with the present electrodes, other responses are encountered and must be briefly described.

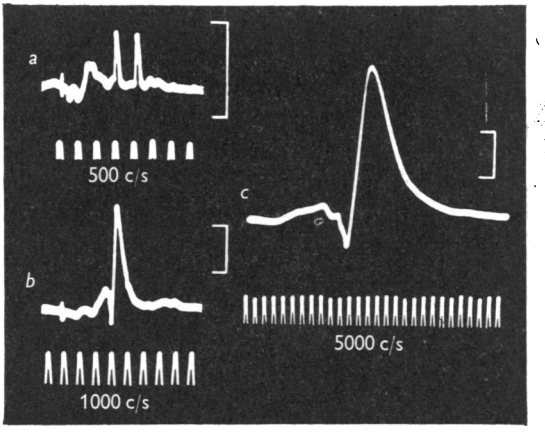

Fig. 12. Conversion of response type 'c' to type 'b'. *a*, multineurona response in LGN followed by two type 'c' responses from single unit. *b*, slight advance of electrode converts the response to type 'b'. Note change of gain and time base. *c*, response of same unit about 2 min later, the electrode remaining in the same position. Note further change in the time base. The response is similar to that in *b* but has increased in amplitude. Voltage calibrations, 2 mV.

Response type 'c'

This type of response is almost entirely negative in polarity being sometimes preceded by a small positive wave and usually followed by a low positivity of somewhat longer duration than the negativity (Fig. 12). Occasionally the negativity has an inflexion or notch on the rising limb. This type of response is the one commonly recorded with electrodes of large size, e.g. the steel micro-electrodes used for the recording of multineurone potentials in earlier studies from this laboratory (Fig. 13). It is in fact the only type of unit activity that may be recorded with these

larger-tip electrodes. Figure 13*c* and *d* show the usual LGN multineurone responses due to submaximal optic nerve volleys, followed in each case by a small single-unit response of type 'c' (arrows). The example in Fig. 12 shows that this type of response arises from a cell rather than an axon. A very slight advance of the electrode transforms the response from type 'c' (Fig. 12*a*) into type 'b' (Fig. 12*b* and *c*; note the change in gain). Further, responses of this type have never been obtained from optic tract or radiation.

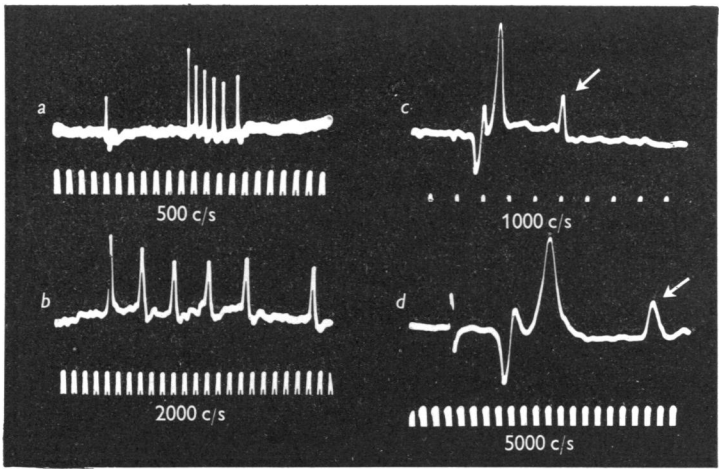

Fig. 13. Examples of response type 'c' recorded with steel micro-electrode (see text). *a*, unit firing repetitively at long latency. *b*, same unit, discharge photographed on faster sweep to show negative/positive wave form. *c*, another unit. The initial discharge is the multineuronal response (the positive/negative wave is the response of the tract fibres, the negative wave the response of the LGN cells). The unit fires relatively late (arrow) but possibly may have fired initially with the other LGN cells. *d*, a third unit. The remarks made concerning *c* apply to this unit also.

Responses of type 'c' are rarely of large amplitude being usually only a few times the noise level when high-resistance electrodes are used. They are obtained when the electrode is some distance from the cell. This explains why they are readily recorded by low-resistance metal electrodes and why such responses can usually be held for long periods without deterioration. It is this type of unit response which presumably forms the basis of the multineurone potentials recorded in the LGN with steel micro-electrodes of relatively large tip-size.

Other responses

As already mentioned, advance of the electrode towards a cell may result in the sudden development of a resting potential the response becoming a large positive monophasic wave, similar to the intracellular records

obtained from other cells (cf. Eccles, 1953, 1957). With these electrodes this type of response is not well maintained, rapidly decreasing in amplitude and increasing in duration before disappearing. It is conceivable that some of our slow positive wave forms may be from dendrites but their unstable character has prevented us from examining them satisfactorily.

DISCUSSION

The tests which we have found useful in distinguishing optic tract axons, LGN cells and post-synaptic axons from one another are summarized in Table 1. In general our conclusions agree with those of previous workers (Tasaki *et al.* 1954; Freygang, 1958; Grüsser-Cornehls & Grüsser, 1960; Hubel, 1960). However, it does not seem to have been fully appreciated that the response from axon or cell body can alter because of a change in

TABLE 1

Test	Tract axon	LGN cell	Post-synaptic axon
Wave form	Positive, monophasic, short-lasting (1–3 msec), rapid downstroke (about 0·25 msec). Often shows 'notching'. May lose second component	Negative, positive/negative or positive/negative/positive. Usually 1 or 2 steps on downstroke of first positive phase	As for tract axon
Latency	May be less than 0·50 msec	Never less than 0·50 msec	As for LGN cell
Shortening of latency when stimulation increased from threshold to supramaximal	Not more than 0·1 msec	Spike component not less than 0·1 msec, often exceeds 1·0 msec	As for LGN cell
Spontaneous discharge	Not if retina destroyed	37 % spontaneously active (both retinas destroyed)	36 % spontaneously active (both retinas destroyed)
Repetitive discharge to 50 μsec pulse	Never	33 % fire repetitively	79 % fire repetitively
Response to repetitive stimulation of optic nerve at high frequency (250–500/sec)	Follows the rate of stimulation	Fails after 3 or 4 stimuli but small component may be present and follow the rate	Fails after 3 or 4 stimuli
Stimulation of visual cortex (see Bishop *et al.* 1962*a*)	Not examined. One might expect some efferent fibres activated in this way present in tract	May respond if appropriate area of visual cortex is stimulated. Characteristic wave form	May respond if appropriate area of visual cortex is stimulated
Binocular stimulation	Unilateral activation	A small percentage of units activated separately from either eye (see Bishop *et al.* 1958, 1959)	As for LGN cell

the position of the cell relative to the electrode or because of damage to the cell membrane. In spite of the examination of a large number of units we have no satisfactory criteria for dendrites. Although responses of very slow time course were occasionally recorded these were not stable and we were never sure that their slow time course was not due to damage.

Although it was our endeavour to differentiate the responses of optic-radiation axons from those of LGN cells, the tests do not distinguish between short-axon neurones on the one hand and neurones discharging to the cerebral cortex on the other. The method of antidromic stimulation via electrodes in the visual cortex may identify optic-radiation neurones. We were unable to establish any criteria for the identification of interneurones. However, interneurones may be involved in transmission in the LGN. This is indicated by the fact that of 18 units in which the latency of the response to supramaximal stimulation exceeded 5·0 msec, five were evoked by weak stimulation of the optic nerve, i.e. such as to stimulate only the fast-conducting group of fibres (t_1 fibres, mean conduction velocity 34 m/sec, cf. Bishop, Jeremy & Lance, 1953). Of these five units, three were cells. In some other units in the long-latency group the threshold for response was low but we were not able to ascertain its relation to the main groups of fibres. Thus there is evidence that some LGN cells are activated polysynaptically from the optic nerve. One such cell was also activated by cortical stimulation but with a long and variable latency (range from 2·5 to 7·7 msec) and a wave form not typical of genuine antidromic activation (see Bishop *et al.* 1962*a*) so that the activation was probably via one or more synapses.

In general, responses of type 'a' are easily distinguished from responses of type 'b'. As shown in Fig. 14, however, there is a possibility of confusion if the response has deteriorated. Figure 14*A* shows the response of a LGN cell (*a*) soon after recording commenced, (*b*) some time later when the negative phase had disappeared. Figure 14*B* shows a response from a tract axon, Fig. 14*C* from a radiation axon. There is a marked similarity between Figs. 14*A*, *b* and 14*B*, *C*. In each case the response is monophasic and there is a slight inflexion on the downstroke of the response. In Fig. 14*C* the axon has fired a second time, fractionating at about the level of the inflexion in the first response in a manner similar to that described for Fig. 3. A fractionation of the cell response in this way may occur to orthodromic stimulation if the response has deteriorated (see Bishop *et al.* 1962*b* for a discussion of this feature).

In some cases it may be possible to distinguish between the two types of response. For example, the time course of the cell response is usually slower than that of the axon. In eleven cells in which the response had lost its negativity the duration of the downstroke of the positive wave

form ranged from 0·27 to 0·95 msec (mean 0·51 msec). In axons, the downstroke was usually less than 0·5 msec even when the response had commenced to fail. Obviously there is considerable overlap; if the downstroke was less than 0·25 msec one could be reasonably certain that the unit concerned was an axon, if more than 0·50 msec then the unit was probably a cell. The most satisfactory criterion for a cell wave form is the presence at some stage of a good negative phase.

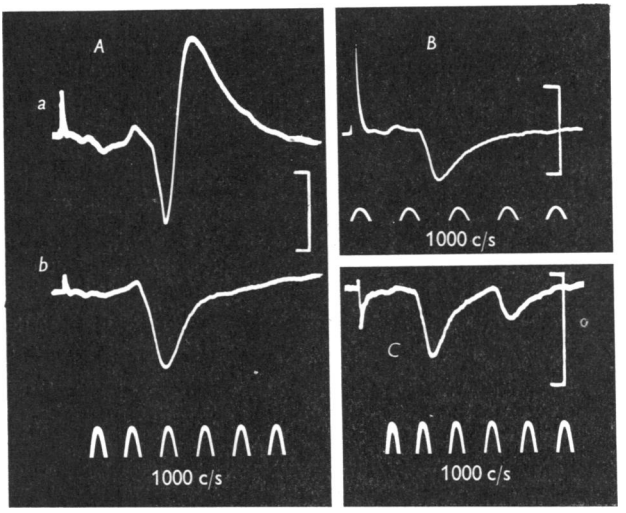

Fig. 14. Similarity of wave forms of 'a' response and deteriorated 'b' response. *A*, response of LGN cell. *a*, initially type 'b' response. *b*, 10–15 min later. *B*, response of tract axon. *C*, response of post-synaptic axon, firing repetitively. Note similar time course and inflexion on downstroke of positive phase in *A*, *b*, *B* and *C*. The second component fails in the repetitive discharge of *C*. Voltage calibrations, 2 mV.

Our interpretation of the fractionation of the axon response is identical with that of Tasaki *et al.* (1954) who suggest that there occurs a lowered safety factor for propagation along the axon at the recording point, presumably due to damage by the electrode. As the damage extends, there is first delay then failure of propagation to the distal node; finally the proximal node may fail. This interpretation is supported by the appearance of similar 'notchings' in the responses from peripheral nerve when the electrode was applied to the axon at various positions (Tasaki, 1952).

Axon responses are almost certainly recorded with the electrode tip in contact with either axon or myelin sheath. This conclusion is supported by the fact that this type of response appears suddenly as the electrode is advanced, that it is commonly associated with an apparent resting potential (probably a change in tip potential in many cases) and that the

response deteriorates more or less rapidly (out of 125 units classified as axons, only 18 were held for longer than 15 min). Since the node presents a very small target area to the electrode most of the recordings must be from the internode. The electrode probably pierces the myelin and may or may not pierce the cell membrane. Because of the presence of myelin around the tip of the electrode, the response is probably considerably distorted and attenuated. The positive wave form suggests that the response is either intracellular or that the recording area is solely a source, not a sink, of external current. In the latter event this could be due to damage to the membrane or perhaps because the internodal membrane is normally electrically inexcitable.

As discussed earlier, the loss of the negative phase of the 'b' response does not necessarily cause death of the cell. The most likely explanation of this change of wave form is that the electrode has damaged a small part of the cell membrane. Murakami, Watanabe & Tomita (1961) have produced evidence that penetration of the membrane of squid giant axon or frog muscle by a fine micro-electrode may cause a progressive loss of responsiveness in this part of the cell although the intracellular response does not change. It seems probable that our electrodes, which would be coarser than those of Murakami *et al.*, would be capable of causing similar damage by simple pressure on the membrane of LGN cells without penetration.

It is at present a debatable point whether or not the properties of the cell are changed to a significant degree by local damage due to impalement or pressure. In our experiments we were not able to correlate any change of properties with loss of negativity. On the other hand there can be little doubt that if the information required from a unit recording is that of frequency of discharge then a response of type 'c' is the most satisfactory since in this case it is fairly certain, on grounds both of amplitude and wave form, that the electrode is not actually in contact with the cell.

There is little doubt that the actual wave form of the response and its amplitude and duration depend on the type of electrode used. This makes it difficult to compare records from different laboratories. The following generalizations, however, seem to us to be true. An electrode with a large tip (d.c. resistance less than 1 MΩ and tip diameter more than 0·5 μ) tends to record only wave forms with a large negative component and very small positive components (cf. Efron, 1959). These are almost always cell responses and are associated with the large current flow into a cell during activity. The extracellular current flow around myelinated axons is less than this probably by a factor of 100 or more (Tasaki *et al.* 1954) and remains undetected by a large electrode, although if many axons are orientated in the same direction and activated synchronously a multi-

neurone response can be recorded; such conditions occur in optic tract and radiation and elsewhere.

The potential at the tip of an electrode is integrated over a progressively smaller volume of tissue as the tip size is reduced. This makes it possible for a fine electrode to detect gradients of potential over distances smaller than the dimensions of the cell soma. That these gradients may be quite large is attested by the large potentials (up to 30 mV) obtained by ourselves and others. However, the fine electrode must be very close to the cell to record these gradients, with the consequent danger that slight movements of the tissue may cause either large fluctuations in potential or impalement of the cell. The electrode selected for extracellular recording must represent a compromise between amplitude and detail of wave form on the one hand and stability of response on the other. The interpretation of the extracellular response of the cell will be considered in a later paper (Bishop *et al.* 1962 *b*).

SUMMARY

1. Extracellular records from single units in or near the lateral geniculate nucleus (LGN) of the cat have been obtained with capillary microelectrodes. Responses were evoked by electrical stimulation of the optic nerve.

2. The wave forms of the responses obtained from single optic tract and optic radiation axons (type 'a' responses) are identical but the behaviour of the units differs.

3. Responses have also been distinguished as arising from the region of the cell body of LGN cells (type 'b' responses).

4. A series of tests is described which enables tract axons, LGN cells and post-synaptic axons (mainly radiation axons) to be distinguished one from another in the LGN where all occur together.

5. Response type 'a' is a monophasic positive wave. It is usually unstable, the wave form breaking up in a characteristic way. Response type 'b' is a positive/negative wave form with occasionally a small terminal positive phase. Its compound nature is indicated by the presence of inflexions on the initial positive phase and by the occasional fractionation of the response at these steps. Response type 'b' deteriorates by loss of the negative phase, the deteriorated response coming to resemble a type 'a' response.

6. A third type of response, type 'c', is a small negative wave, sometimes preceded or followed by a small positive phase. This is the type of response commonly recorded with electrodes of larger tip-size and is obtainable only in the vicinity of the cell body.

7. As yet no criteria have been obtained for distinguishing between short-axon and long-axon neurones, nor for dendritic recording.

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