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THE INTERPRETATION OF THE EXTRACELLULAR RESPONSE OF SINGLE LATERAL GENICULATE CELLS

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In the two preceding papers (Bishop, Burke & Davis, 1962*a*, *b*) we have described the responses of single optic tract fibres, lateral geniculate cells and optic radiation axons to orthodromic and antidromic stimulation. In these papers also we discussed the part played by the type of electrode in determining the character of the response, the changes that occur in the responses with time and the wave form of unit responses from axons. In the present paper we provide an interpretation of the wave form of the extracellular responses of single cells in the dorsal nucleus of the lateral geniculate body (LGN) of the cat.

Although the wave form of intracellular responses has been discussed extensively, much less attention has been paid to the extracellular wave form. However, there have been valuable discussions by Svaetichin (1951, 1958), Tasaki, Polley & Orrego (1954), Fatt (1957*a*, *b*), Håkansson (1957), Freygang (1958), Freygang & Frank (1959) and Murakami, Watanabe & Tomita (1961). From the experimental point of view we have endeavoured to analyse the wave form by splitting it into its separate components. The results suggest that extracellular recording may reveal information not available from intracellular records. In view of the increasing use of implanted electrodes, further detailed study is very desirable (cf. Nelson, Frank & Rall, 1960).

METHODS

The preparation is essentially as described in the preceding papers (Bishop *et al.* 1962*a*, *b*). Lysergic acid diethylamide (LSD 25) was injected into the carotid artery through a cannula in the lingual artery (see Bishop, Field, Hennessy & Smith (1958) for further details).

RESULTS

Fractionation of the orthodromic wave form

As described previously (Bishop *et al.* 1962*a*), the extracellularly recorded response of the LGN cell to stimulation of the optic nerve is a

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positive/negative (sometimes positive/negative/positive) wave form in which, initially at least, the negative phase is larger than the positive phase. The positive phase is complex, consisting of two or three separate components. In the full response the only indication of these components is the appearance on the downstroke of the positive phase of one or two steps or inflexions (Figs. 1, 3–7).

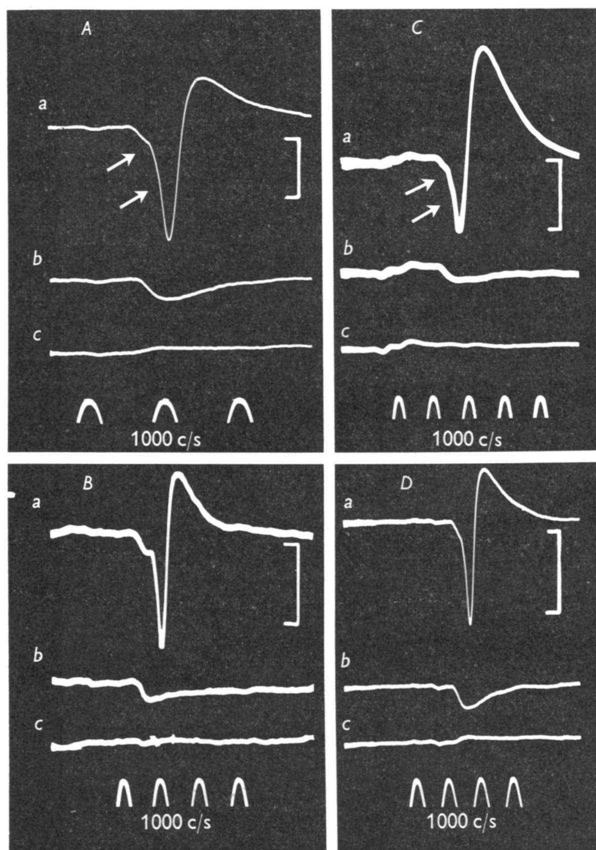

Fig. 1. Examples of S potentials in four LGN cells (*A*, *B*, *C*, *D*). Three records are shown for each unit: *a*, full orthodromic response; *b*, S potential; *c*, base line, including the multineuronal field response. In each full response there is a small step on the positive phase approximately at the level of the peak of the S potential. Note also the second inflexion on the positive phase, especially in *A*, *C* (arrows). Voltage calibrations: *A*, *B*, *D*, 2 mV; *C*, 5 mV.

For convenience in description we shall refer to the components of the response as the S potential, the A potential and the B potential, and the steps on the positive phase as the S–A and A–B steps respectively (Fig. 2). The justification for these terms will appear in the Discussion. The S–A

step is usually a distinct step or double inflexion whereas the A–B step may be a single inflexion as in the orthodromic response (Fig. 1*A*, *C*, arrows) or a double inflexion as in many antidromic responses (Figs. 7, 8; see also Bishop *et al.* 1962*b*).

When the positive phase of the response is small, that is, when the electrode is not yet very close to the cell, the steps may not be obvious (Figs. 12, 13 in Bishop *et al.* 1962*a*). With a larger positive phase (comparable in amplitude to the negative phase) the A–B step is only rarely undetectable. On the other hand, in several cases the S–A step could not

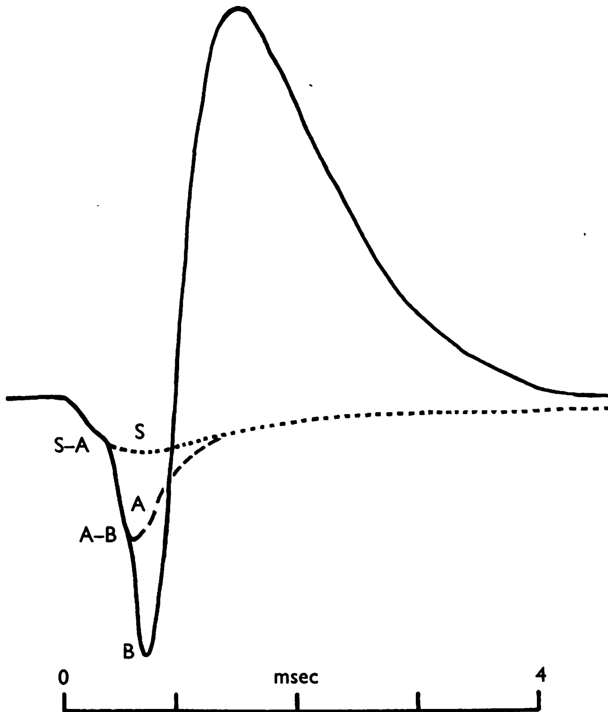

Fig. 2. Diagram to illustrate the nomenclature used in discussing the LGN cell responses. The solid line indicates the full response; it has two steps or inflexions on its positive phase; the S–A and A–B steps. The dotted line indicates the time course of the S potential (S), and the interrupted line the time course of the A potential (A) superimposed on the S potential. The A potential is shown as a monophasic wave but depending on the position of the electrode it may be diphasic (see text).

be noted with certainty. The reason for this may lie partly in the smaller amplitude of the S potential so that it may well have been present but submerged in the multineurone response. In general the S–A step becomes more obvious with closer approach to the cell, a procedure which usually leads to a reduction in amplitude of the negative phase (cf. Bishop *et al.*

1962*a*). However, there is not necessarily loss of negativity and several responses show an unmistakable S–A step as well as a large negative phase (e.g. Figs. 1C, 3A). In other instances despite a large amplitude of response the S–A step was apparently absent.

The S–A step or S potential is commonly about one fifth the amplitude of the positive phase, the largest ratio observed being 29 %; the A–B step or A potential is about 60 %, the range being from 44 to 66 %.

The S potential

The orthodromic wave form may be fractionated in several ways to reveal the S potential. The simplest method consists in reducing the strength of stimulus. At low strengths of stimulation the response may fail at the S–A step to leave a slow positive wave, the amplitude of which has varied considerably from one unit to another, the maximum value observed being 2·85 mV (Fig. 1). In the great majority of cases the S potential behaves in an all-or-nothing manner; it has a constant amplitude and cannot be graded. For example, all the superimposed records in Fig. 3*A* were obtained without varying the strength of stimulus applied to the optic nerve. Three types of response were obtained—the full positive/negative response, the S potential and a base line containing a small wave which was probably the multineuronal response (see also Fig. 1). Our interpretation of these and similar records is that the unit response results from the discharge of a single optic nerve fibre. The stimulus is at threshold strength for this fibre so that occasionally it fails to discharge. Furthermore, the resulting S potential is of critical amplitude for the generation of the full response. This finding is quite common and has been put forward as evidence that a single optic fibre can discharge a LGN cell (Bishop *et al.* 1958). Although the situation is usually as described above, in several cases the S potential could be graded by adjusting the strength of stimulus, e.g. the responses from two cells shown in Figs. 3*B*, *C*. In each case the S potential could be seen to consist of three components of similar time course, only the summed components being capable of discharging the cell. Even in these examples only a small number of optic tract fibres are necessary to discharge the LGN cell.

Measurements were made of some features of the S potential in six units. These units were chosen because the time course of the S potential could be clearly discerned and appeared to be uncomplicated by additional features (e.g. late responses); in each case the S potential was obtained by a single weak stimulus. The rise time ranged from 0·37 to 0·75 msec (mean 0·48 msec); time of half-decay from peak ranged from 0·36 to 1·40 msec (mean 0·83 msec). Measurements of total duration were unreliable but

varied from about 2 to 10 msec. Although these values show a considerable variation between units the mean values are very similar to those obtained for the multineuronal synaptic potential (Bishop, 1953; Bishop & McLeod, 1954).

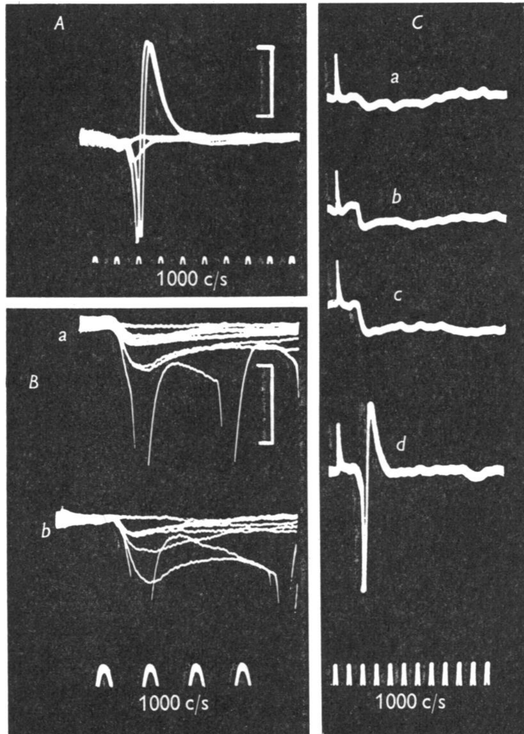

Fig. 3. Gradation of amplitude of the S potential. *A*. Most common result, no gradation. Four superimposed records at constant threshold stimulus strength (note absence of unit response on one sweep). No smaller or larger S potential was obtained in this unit. *B*, *C*. Less common result, gradation. *B*. Superimposed records, the stimulus strength being varied. The S potentials appear to fall into four or five amplitude steps. This recording was possibly intracellular. *C*. Another unit. *a*, *b*, *c*, graded S potentials obtained by varying the stimulus strength. Full response in *d*. Voltage calibrations 5 mV (none available for *C*).

It was often not possible to fractionate the response by varying the stimulus strength, even when an S–A step was quite prominent. Evidently the S potential once evoked was capable of generating the full response. The S potential may also be revealed by other methods. Figure 4 illustrates block of synaptic transmission in a LGN cell brought about by (i) delivering the stimulus in the period of subnormality following a short train of stimuli at high frequency (Fig. 4*A*) and (ii) the use of lysergic acid diethylamide (LSD) (Fig. 4*B*). In Fig. 4*A*, (*a*) illustrates the normal

response and (*b*) and (*c*) the response 20 msec from the end of a burst of six stimuli at about 300/sec. The interval of 20 msec was chosen because at this time the subnormality is approximately at a maximum (Bishop & Davis, 1960*a*). Responses (*b*) and (*c*) show the S potential only without the A–B complex and in (*d*) the background multineurone response

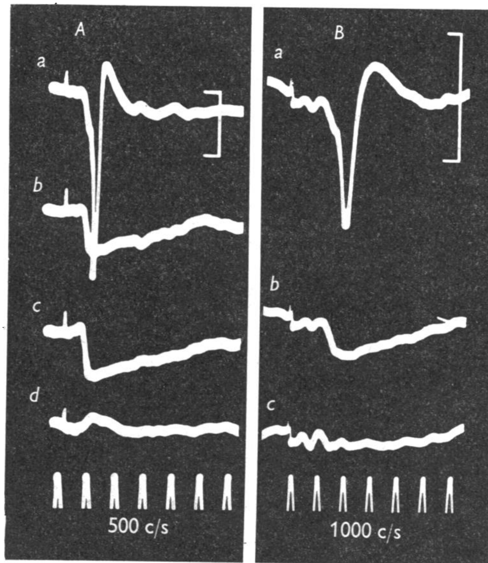

Fig. 4. S potentials obtained by synaptic block. *A*. Block during subnormal period following response of cell. *a*, full response. *b*, *c*, S potentials. *d*, multineurone field response only. *B*. Block due to action of LSD. *a*, full response. *b*, S potential. *c*, multineurone field response. See text for further details. Voltage calibrations 1 mV.

is shown for comparison. Fig. 4B shows the response (*a*) before, and (*b*) after, the injection of 100 μg LSD into the carotid artery. LSD has been shown to block transmission through the LGN without affecting the presynaptic response (Evarts, Landau, Freygang & Marshall, 1955; Bishop *et al.* 1958). It is thought to act by competitive block of the normal transmitter (Bishop, Burke & Hayhow, 1959). An S potential is revealed in response (*b*) and additional injections of LSD reduced the amplitude of this S potential still further. Figure 4*B*, *c* shows the multineurone response alone. The S potential obtained in either of these two ways does not appear to differ from that obtained by the use of a weak stimulus.

When the optic nerve is stimulated at a frequency of 250–500/sec, transmission through the LGN fails after 2–5 stimuli. The electrical events at the unit level are illustrated in Fig. 5*A*, *b*. In this unit the first response

to a series of stimuli at about 300/sec shows a prominent S–A step. This step is more prominent still in the second response, whilst the third and subsequent responses show only an S potential. Furthermore, the S–A step in the second response is smaller than that in the first and there is then a progressive decrease in the amplitude of the S potential with successive stimuli. These features are shown also in the records from another unit in Fig. 5*C*. In the case of the unit response illustrated in Fig. 5*A*, *b* it was possible to obtain an S potential by weakening the stimulus strength.

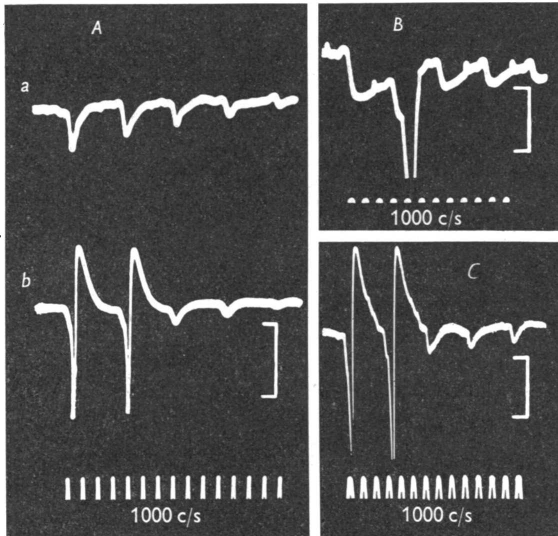

Fig. 5. S potentials during repetitive stimulation of the optic nerve at 250–500/sec. *A*. *a*, *b*, records from the same cell. *a*, weak stimulus strength, S potentials only. *b*, stronger stimulus but full response occurs only to first two stimuli, subsequent responses being S potentials. *B*. Another unit. Summation of S potentials to give spike response. *C*. Another unit. Response similar to *A*, *b*. Spikes off screen in *B*, *C*, Note progressive decline in amplitude of S–A step and/or S potential in all records. Voltage calibrations: *A*, *b*, 5 mV (higher gain in *a* but no calibration available); *B*, *C*, 1 mV.

When the optic nerve was stimulated repetitively using the weak stimulus the record shown in Fig. 5*A*, *a* was obtained, there being again a progressive decrease in the size of the S potential during the train of stimuli. The same phenomenon is illustrated in Fig. 5*B* from a third unit in which, however, there was summation of the first and second S potentials to give the full response (the negative phase had disappeared in this unit). The amplitude of the S potential declined further during the train of responses so that no full response was obtainable thereafter.

Although in the majority of cases there was a decrease in amplitude of the S potential during a short train of stimuli, in a few cases there was no

obvious decrease. The decrease in amplitude (where it occurred) was not due to failure to stimulate the optic nerve because the decrease occurred even when the stimuli were well above threshold. Optic nerve fibres also respond regularly to high-frequency stimulation (Bishop & McLeod, 1954; Bishop & Evans, 1956). Hence the failure must occur either in the fine nerve terminals or at the synapse.

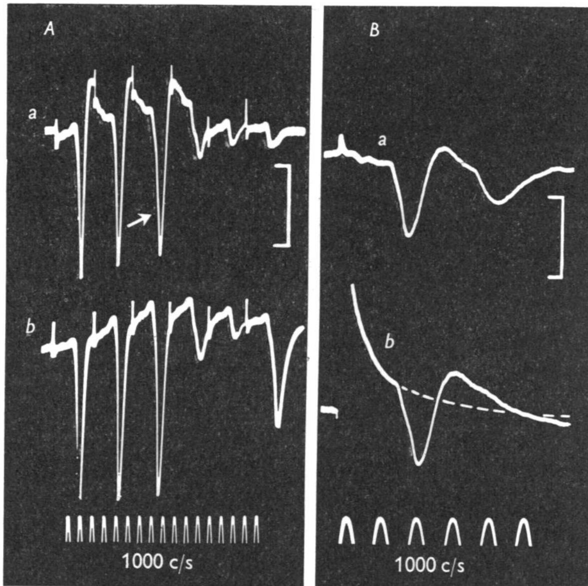

Fig. 6. Fractionation of the orthodromic response to reveal A potential. *A*. Repetitive stimulation causes failure of B potential after third stimulus in *a* and *b*. Attenuated A potential superimposed on S potential in both fourth responses, S potential only in fifth response in both records. A potential in sixth response of *b* probably full size; compare with height of A–B step in third response of *a* (arrow). *B*. *a*, repetitive discharge to single stimulus. The second response is A potential only. *b* shows antidromic response for comparison. Interrupted line, base line distorted by stimulus artifact. Voltage calibrations: *A*, 5 mV; *B*, 2 mV.

A and B potentials

The fractionation of the antidromic response to give an A potential has been described in the preceding paper (Bishop *et al.* 1962*b*). It is much more difficult to fractionate the orthodromic response. For example, reduction in strength of stimulus normally gives fractionation only at the S–A step. However, on rare occasions the response has been seen to fractionate at the A–B step. Figure 6*B* illustrates fractionation as a result of repetitive firing. LGN cells commonly fire repetitively to a single stimulus (Bishop, Jeremy & McLeod, 1953) but the second and subsequent

responses, although they may be reduced in amplitude with respect to the first response and show a more pronounced A–B step (see e.g. Fig. 10, Bishop *et al.* 1962*a*), seldom fractionate at this level. In Fig. 6*B* the second response in trace (*a*) is an A potential. The identity of the unit was confirmed by comparison with the antidromic response of the same unit (trace (*b*)) which has the characteristically more prominent A–B step.

It also happens occasionally that during repetitive stimulation the response fractionates to give an A potential. Thus in Fig. 6*A* the response is seen to fractionate in two stages. The first stage occurs in the fourth response of each record. In this response the S potential gives rise to an attenuated A potential whereas in the fifth response there is an S potential only (second stage). The full amplitude of the A potential may be gauged from the size of the A–B step in the third response in trace (*a*) (arrow). Probably the A potential in the sixth response in trace (*b*) is close to full size.

The examples just quoted are comparatively rare. They are usually obtained from cells whose responses have lost their negative phase, implying that the damage caused by the electrode has reduced the safety factor for propagation into the B area of the cell. On the other hand, the occurrence of an A potential to antidromic stimulation is extremely common. Evidently the structure responsible for the B potential is readily excited orthodromically but not so readily antidromically. However, if one evokes an orthodromic response soon after an antidromic response, at a time when the refractory period of the A potential is over but that of the B potential is not, then only an A potential results. Figure 7 demonstrates this effect, the two stimuli being applied at various intervals. At short intervals (*a* and *b*) only an A potential, graded in size, appears. In (*c*) and (*d*) there is very little increase in the A potential but a B potential is added to it, the positive phase seemingly small because it is delayed and on the upstroke of the positive phase of the A potential. In (*e*) and (*f*) the orthodromic response approaches the amplitude of the unconditioned response in (*g*).

In seven units an A potential has resulted from orthodromic stimulation, one or other of the above methods being used to suppress the B potential. All A potentials except one were monophasic. In three of these units, an A potential was also obtained by antidromic stimulation (Bishop *et al.* 1962*b*); in two cases the A potentials were monophasic to both routes of stimulation, in the other case the A potentials were both diphasic.

Altogether twenty units have yielded A potentials to either orthodromic or antidromic stimulation, fifteen of these giving A potentials which were positive monophasic responses, the other five giving diphasic positive/negative responses (Fig. 8, see also Fig. 3, Bishop *et al.* 1962*b*). However,

in nine of the former group, the responses were from cells in which the full cell response had lost all or most of its negative phase. Since it has been concluded (Bishop *et al.* 1962*a*) that loss of negativity indicates damage to a part of the cell it is reasonable to confine our consideration to those examples in which the full cell response showed a good negative phase.

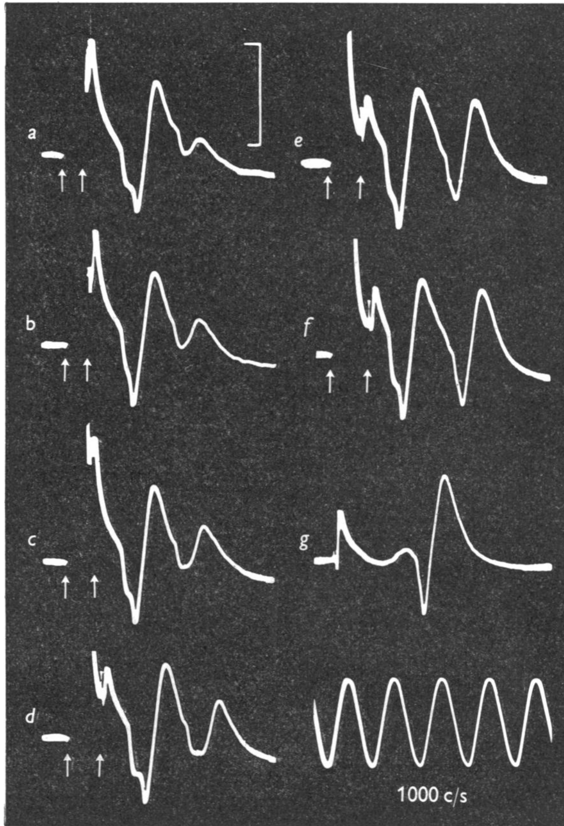

Fig. 7. Fractionation of orthodromic response when elicited shortly after antidromic response. A series of records at various short intervals between antidromic and orthodromic stimuli. The arrows indicate the commencement of the stimulus artifact in each case. *a* and *b*, A potential only to orthodromic stimulus. *c*, *d*, small B potential is added. *e*, *f*, response almost full size. *g*, unconditioned orthodromic response. Note the prominent A–B step in the conditioned responses. Voltage calibration 5 mV.

Of the eleven cells in this category, six gave monophasic A potentials whilst in five the A potentials were diphasic. As already explained, the B potential in the undamaged cell is also a diphasic potential.

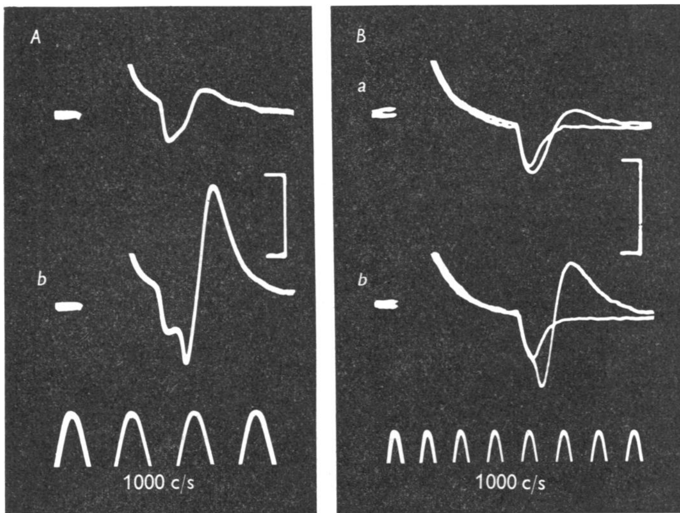

Fig. 8. Diphasic and monophasic A potentials. *A*. Response of LGN cell to antidromic stimulation. *b*, full response. *a*, A potential, but note inflexion on upstroke of positive phase suggestive of an abortive B potential (see Discussion). *B*. Another cell, antidromic stimulation. Two superimposed responses in each record. The cell usually gave either monophasic A potential or full response as in *b*, but occasionally gave diphasic A potential as in *a*. Voltage calibrations: *A*, 5 mV; *B*, 2 mV.

Refractory period of A and B potentials

In Fig. 9 the amplitude of the orthodromic response (the sum of the positive and negative components) in the series of records partly illustrated in Fig. 7 has been plotted against the interval between the commencements of the A potential in each response. The open circles indicate that the response was an A potential, the filled circles an A + B potential, whilst the crosses indicate a response in which there appeared to be the first trace of a B potential (e.g. Fig. 7*b*). At intervals less than about 1·1 msec, although there was a slight deflexion of the tracing as a result of orthodromic stimulation, it was not possible to say whether this was the response of the unit or the multineurone response. The amplitude of the A potential at short intervals varied by as much as 100 % but there was no definite tendency for it to grow in amplitude as the interval increased. With a comparatively small further increase in the interval (0·1 msec) the response changed from an A potential to an A + B potential which was almost full size.

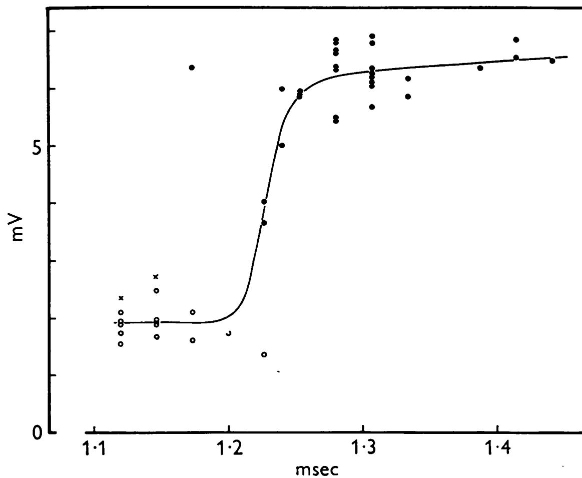

Fig. 9. Graph of amplitude of orthodromic response (mV) against interval between commencements of A potential of antidromic and orthodromic response respectively (msec), values taken from a series of records partly illustrated in Fig. 7. Open circles, A potential; filled circles, A+B potential; ×, responses in which there appeared to be the first trace of a B potential.

DISCUSSION

The S potential

Our interpretation of the S potential is that it is a synaptic potential, that is, the extracellular potential change resulting from the flow of current associated with an excitatory post-synaptic potential (EPSP). Our reasons for thinking this are as follows. The monophasic wave form of the S potential indicates that it is a non-propagating response. The time course of the S potential is relatively prolonged, about five times as long as the duration of the positive phase of the full response. Summation of S potentials occurs to initiate a spike response (Fig. 5*B*). Block of synaptic transmission by various means (LSD, repetitive stimulation, post-discharge subnormality) all reduce the response to an S potential (Figs. 4, 5). It is particularly relevant that the S potential can be graded. This gradation can occur in two ways: adjustment of the strength of stimulus occasionally shows the S potential to be made up of two or three elements of similar time course (Figs. 3*B*, *C*) and, secondly, the amplitude of the S potential normally declines progressively during a train of evoked responses (Fig. 5).

A possibility which must be considered is that the S potential is a presynaptic potential, the response of the optic tract fibres ending on the cell

in question. Grüsser-Cornehls & Grüsser (1960) claim that the response from a LGN cell contains a presynaptic component (α-component) but from their records this component resembles more the A potential than the S potential; they do not appear to have recorded an S potential. However, some of their responses have very brief latencies, as short as 0·55 msec, and it is difficult to see that these could be other than presynaptic; our earliest post-synaptic response was 0·76 msec. Although the S potential is rather variable in time course, in general it is much slower than the response of a myelinated axon, e.g. in optic tract or optic radiation (Bishop *et al.* 1962*a*). However, it is possible that a slower time course and a graded amplitude might be characteristics of fine nerve endings. As recorded with an electrode inside or outside the motoneurone, the presynaptic potential is a small brief positive/negative wave (Brock, Coombs & Eccles, 1952). It is not clear what parts of the presynaptic fibres generate this potential. It may well be that the fine non-myelinated nerve endings, because of their random arrangement, contribute very little. In any event it is unlikely that our electrodes will record any larger presynaptic potential than that found in motoneurones, particularly one that is about one fifth of the spike response; nor that its time course would be any different. Furthermore, if one supposes that the S potential is presynaptic then presumably the decrease in amplitude during a brief tetanus (Fig. 5) represents either a decrease in trans-membrane action potential or a progressive failure of some of the fibres contributing to the potential (cf. Krnjević & Miledi, 1958, 1959). The reduction in amplitude often appears to be too great to be due to the former cause (e.g. Fig. 5*A*) and Krnjević & Miledi (1958, 1959) have found that presynaptic failure of transmission does not commence for some time after the start of the tetanic stimulation.

It therefore seems unlikely that the S potential is presynaptic. We take the gradation of the S potential with strength of stimulus to mean that in this case the cell is innervated by a few optic fibres each separately capable of depolarizing the cell but probably not of initiating an action potential except by summation of depolarizations. The decrease in amplitude of the S potential during repetitive stimulation resembles similar changes in junctional potentials elsewhere, e.g. end-plate potentials in mammalian muscle (Liley & North, 1953) and suggests either a progressive decrease in output of transmitter or desensitization of the post-synaptic membrane.

The most unusual feature of the S potential is the fact that it is a positive wave form. The synaptic potential recorded from a population of LGN cells by means of a rather coarser steel electrode is a negative monophasic wave of similar time course to the unit S potential (Bishop, 1953; Bishop & McLeod, 1954). The explanation must be sought in the relative sizes of the tips of the two types of electrodes (cf. Bishop *et al.* 1962*a*). The coarser

type of electrode, suitable for recording multineurone potentials, probably integrates the potential over quite a large distance, e.g. 100 μ or more. This is equivalent to recording at a relatively large distance from the cell by means of a fine capillary micro-electrode. Thus the unit responses obtained by the latter type of electrode at a distance from the cell (type 'c'— Bishop *et al.* 1962*a*) resemble the multineurone potentials obtained by coarser electrodes. The propagated spike of the multineurone potentials may be regarded as being compounded of unit responses of type 'c'.

During synaptic transmission the dendrites and soma will be depolarized by the transmitter substance or agent. The dendritic shafts arborize extensively in all directions away from the cell body (Taboada, 1927) so that they occupy a very much larger volume in the nucleus than does the initial portion of the axon. The radial arrangement of the dendrites means that the initial portion of the axon will be electrically hidden from a distant electrode during the EPSP, and net outward flow of membrane current will only be found as the axon approaches and leaves the limits of the field of distribution of the cell's dendrites. It is to be expected therefore that a coarse electrode will record a negative synaptic potential from among the LGN cells and a positive synaptic potential among optic radiation axons just above the nucleus (Bishop, 1953; Bishop & McLeod, 1954; Bishop & Davis, 1960*b*). On the other hand a fine capillary micro-electrode can 'approach' the cell much more closely; it integrates the potential over a very much smaller distance and hence can detect the small potential gradients which occur over the surface of the cell body during activity (cf. Efron, 1959). Many type 'c' unit responses appear to rise out of a negative synaptic potential. The association of the type 'c' unit response with what appear to be negative synaptic potentials has been repeatedly observed in subsequent studies in this laboratory using photic stimulation and tungsten micro-electrode recording. This would explain why Fatt's (1957*a*, *b*) extracellular recordings from motoneurones have a similar time course to our 'b' type unit responses but were of opposite polarity. They were recorded at a distance from the motoneurone.

If one regards the A and B potentials as arising in the general region of the initial segment of the axon and the adjacent part of the soma then the fact that the S potential is found only in association with them means that the S potential must also arise in the same region. The fact that the S potential is always positive means that the part of the cell from which the electrode records is always a source of current during the EPSP. Two possible explanations suggest themselves. Either the electrode has damaged the membrane in this area or the soma has a less dense innervation than the dendrites. The former alternative would imply that the electrode always damaged the cell because in no case did we record a

negative S potential. However, on many occasions S potentials were recorded simultaneously with cell responses showing large negative phases (e.g. Figs. 1, 3). This implies that the cell membrane beneath the electrode was undamaged. It is possible, however, that the electrode destroys some of the afferent nerve branches overlying part of the cell so that the part is artificially denervated. Such a partial denervation would reduce the density of innervation of the soma. In this case the cell body would be depolarized to a lesser degree than the dendrites and hence would act as a source of current to them during the EPSP.

It is also true that damage to the cell membrane such as nearly always occurs at a later stage in the recording would enhance the effect. Indeed the largest S potentials were obtained at a stage when the full response had lost much of its negative phase (see e.g. Fig. 4). For example, in almost all cases in which the S potential was more than one fifth the amplitude of the positive phase of the full response, the negative phase had been considerably reduced or had disappeared. Also on several occasions despite the appearance of a large response it was impossible to see any S potential. If we assumed uniform innervation over the dendrites and soma then during the EPSP current would be drawn from the initial segment by both dendrites and soma and the S potential would be negative. Damage to soma membrane or afferent nerves would reduce the size of the S potential and then reverse it. Because the first stage, a negative S potential, has not been detected the possibility should be considered that the soma has a density of innervation genuinely less than that of the dendrites (cf. Freygang, 1958). Glees (1941) had the impression that axo-dendritic contacts predominated over axo-somatic contacts.

The A potential

In the preceding paper (Bishop *et al.* 1962*b*) it was shown that the antidromic response of the cell occasionally fractionated to yield a smaller unit response, the A potential. Reasons were given for thinking that this A potential corresponded to the intracellular A potential recorded in the spinal motoneurone (Fuortes, Frank & Becker, 1957; IS spike of Coombs, Curtis & Eccles, 1957*a*, *b*). In this paper it is shown that the orthodromic cell response can also be made to fractionate and yield an A component (Figs. 6, 7). Although a single orthodromic stimulus never produces an A potential only, except in a damaged cell, the fact that the A–B step is always at the same level in both orthodromic and antidromic responses (Bishop *et al.* 1962*b*) strongly suggests that the A potential arises in the same part of the cell whatever the route of excitation. Because of the many similarities between the behaviour of the A component of the extracellular response and that of the intracellular record (Phillips, 1959; Bishop

et al. 1962*b*) there is little doubt that the former is the extracellular counterpart of the latter.

It is, however, difficult to fractionate the orthodromic response to produce an A potential. In the case of the responses graphed in Fig. 9 the range of response intervals over which the A potential was obtained in this unit was very small (0·1 msec) and even within this range the response occasionally reached full size. Both Fig. 9, and Fig. 7 in the preceding paper (Bishop *et al.* 1962*b*), illustrate the very short refractory period of the A component (less than 1·1 msec) and the only slightly longer refractory period of the B component (1·3–1·6 msec) of the LGN cell measured as a response interval in each case. Similar brief refractory periods were found in the earlier multineurone-potential studies of Bishop & Evans (1956). These findings are of importance when considering the phenomenon of repetitive firing in these cells (Bishop, Burke & Davis, unpublished).

Using similar techniques Eccles and his colleagues (Eccles, 1957, pp. 53–55) have shown that the orthodromic response of the motoneurone usually fractionates to an EPSP, not to an A potential. They regard fractionation at the IS (A) potential level as occurring particularly in a deteriorating cell. Our experience is in agreement with this, the responses illustrated in Fig. 6, for example, having lost a considerable portion of their negative phase (Bishop *et al.* 1962*a*).

Generation of impulses

The suggestion has been made by several authors (Forbes, 1934, 1939; Gesell, 1940; Bishop, 1953) that the local currents due to the synaptic potential excite primarily the initial segment of the axon, which then discharges the soma and dendrites. This suggestion has received considerable support from detailed investigations on the spinal motoneurone (Araki & Otani, 1955, 1959; Coombs *et al.* 1957*a*, *b*; Fuortes *et al.* 1957). In the LGN cell we have shown that the A potential precedes the B potential whether the excitation is orthodromic or antidromic. This indicates that the part of the cell in which the A potential arises has a lower threshold than the rest of the cell. A recording from this part of the cell should show a negative A potential. Failure to find such a response when good negativities can be recorded elsewhere suggests that the area of membrane in which the response arises is very small. This conclusion would be consistent with the impulse arising in the unmyelinated part of the axon as in the crustacean abdominal stretch-receptor (Edwards & Ottoson, 1958).

The relatively common occurrence of the A potential as a result of antidromic excitation is explained by the difficulty of propagation past the junction between initial segment and soma, the greatly expanded surface

of the soma at this region reducing the safety factor considerably. On the other hand with orthodromic excitation the action potential can propagate from the initial segment into a soma already partially depolarized by the EPSP. The longer A–B interval and slightly reduced amplitude of the antidromic response (cf. Bishop *et al.* 1962*b*) are readily explained by the same considerations (Brock, Coombs & Eccles, 1953). The records, both intracellular and extracellular, from other cells indicate that this method of initiating the cell response is of widespread occurrence (e.g. Betz cells (Phillips, 1956, 1959); frog spinal and sympathetic ganglion cells (Svaetichin, 1958; Nishi & Koketsu, 1960); hippocampal neurones (Kandel, Spencer & Brinley, 1961)).

Wave forms of A and B potentials

Our interpretation of these wave forms is that there is a propagation of the impulse, first over the initial segment and then over the soma and possibly the larger proximal parts of the dendrites. We have not been able to assign any portion of the wave form of our unit responses to propagated activity in the dendrites and our interpretation does not involve invasion of the dendrites by a propagated impulse to any extent.

It has been suggested (e.g. Freygang, 1958) that the extracellular response has the same time course as the membrane current at the site of recording and that it is therefore related to the derivative of the voltage transient across the membrane. The similarity between the extracellular record and a wave form calculated essentially by differentiation of the transmembrane action potential on the assumption that the electrical constants of the membrane are invariant has led to the view that the membrane in proximity to the electrode does not become active. The further conclusion is drawn that in the case of the LGN cell (Freygang, 1958), the spinal motoneurone (Freygang & Frank, 1959) and the pyramidal cell (Efron, 1959), the soma-dendritic membrane is electrically inexcitable, depolarization occurring either by synaptic (chemical) activity or electrotonically from the electrically excited initial segment. This hypothesis is supported by a comparison of simultaneously recorded intracellular and extracellular responses with concentric electrodes (Freygang & Frank, 1959). However, there is evidence that an intracellular electrode may damage the membrane in the vicinity of the point of impalement (Murakami *et al.* 1961). This damage is not evident from the intracellular record, presumably because the intracellular electrode, 'sees' a larger area of membrane than the extracellular electrode, but the outer of two concentric electrodes is particularly well placed to see the damage caused by the inner electrode. However, Freygang & Frank (1959) considered that the damage was not a significant factor.

Our own experience is that a 'normal' extracellularly recorded B potential has a large negative phase which is lost as a result of proximity of the electrode to the cell (Bishop *et al.* 1962*a*). If the B potential were regarded as the derivative of the transmembrane action potential then for many responses this would mean that the latter had a repolarization phase faster than its depolarization phase. In our experience therefore there is no reason to think that the soma is not normally invaded by the impulse. It is true that when the response has deteriorated and lost negativity, presumably because the electrode has damaged the cell membrane, it resembles a differentiated action potential (cf. Fatt, 1957*b*). Similarly, since a diphasic A response may be recorded at the same site and at the same time as a B response that has a large negative phase it is unlikely that the negativity of the A response is due solely to differentiation across an inactive membrane.

The following is a detailed interpretation of the potentials we have recorded. To be able to record distinguishable A and B potentials the electrode must be very close to the membrane of the cell body. The initial positivity of the A potential is due to activity occurring towards the distal end of the initial segment. The negative phase of the A potential has never been more than about 20% of the amplitude of the negative phase of the B potential (Bishop *et al.* 1962*b*). In such circumstances the electrode is recording from a region which does not become fully active during the A potential. It is supposed that in such cases the electrode lies close to the boundary between initial segment and soma, whereas when the A potential is purely positive the recording site is further away.

If the A potential is diphasic, the B potential is also diphasic. The observations which are difficult to explain are: (i) the negative phase of the A potential in isolation commences at a time when the positive phase of the B potential is largely over in the full response (Fig. 8*A*; cf. also Fig. 3*B* in Bishop *et al.* 1962*b*); (ii) the positive phase of the B potential increases in amplitude more or less *pari passu* with that of the negative phase (e.g. Fig. 6; cf. also Fig. 7 in Bishop *et al.* 1962*b*). The positive phase of the B potential is evidently due to the excitation of some more distant region of the cell and thus can occur before the partial response at the recording site. For example, the dendrites might discharge before the soma. We think this is unlikely because it would imply that the dendritic membrane was more excitable than the soma membrane and one would then expect to see an additional component in the response; besides, this explanation would not fit with observation (ii) above.

Nor is it possible to explain these records by postulating a transitional zone between initial segment and soma of gradually changing properties (Fuortes *et al.* 1957); this also would not give the result in (ii) above. We

suggest that the boundary between initial segment and soma is highly irregular, strands of the more excitable A-type membrane extending for considerable distances into the soma. Such a possibility has been considered by Coombs *et al.* (1957*a*). In conditions where invasion of the cell is difficult, e.g. when an impulse reaches the cell shortly after a preceding one, a portion only of the intervening B-type membrane may respond to give a small positive/negative wave. As recovery proceeds more of the B-type membrane responds and the positive/negative wave increases in amplitude. Such responses can be regarded as abortive responses of the B-area. It is not infrequently the case that an inflexion occurs on the upstroke of the positive phase of the diphasic A potential (Fig. 8*A*, *a*; cf. also Fig. 3*B* in Bishop *et al.* 1962*b*). In the response of one unit the A potential varied slightly, sometimes being diphasic, sometimes monophasic (Fig. 8*B*, *a*). These prolongations of A-type membrane would have the effect of grading transmission from the A to the B area and so of increasing the safety factor for invasion of the cell body. The existence in the chromatolysed motoneurone of patches of membrane of greater or less excitability (Eccles, Libet & Young, 1958) makes the above suggestion not improbable.

Certain objections to this scheme must be considered. There is a general belief that the inside of the cell body has such a low impedance relative to the membrane that it can be regarded as virtually isopotential (Rall, 1953, 1959*a*, *b*; Fatt, 1957*b*; Eccles, 1957; Freygang & Frank, 1959). If, as is generally assumed, the extracellular medium also has a low impedance relative to that of the cell membrane, then the membrane will change potential almost simultaneously over its entire surface. The experimental evidence on this point is conflicting. Svaetichin (1958) was able to position an electrode accurately on the dorsal root ganglion cell. Here the I-component (which seems to be equivalent to the positive part of the B potential) is very brief and Svaetichin considered that the cell-body membrane is depolarized almost instantaneously. Similarly the very brief positive phase of the B component in the response of the supramedullary neurone of the puffer (Bennett, Crain & Grundfest, 1959) suggests that, in this cell also, invasion of the soma is virtually instantaneous. However, in at least one cell, namely, the lobster stretch-receptor cell (Edwards & Ottoson, 1958), it has been shown that the impulse does propagate over the surface of the soma. In this cell, Edwards & Ottoson were able to place the external electrode on different parts of the cell under direct visual control.

Whatever be the correct explanation for the discrepancy it appears that the impulse may propagate relatively slowly over the soma of some cells (e.g. LGN cell, lobster stretch-receptor), and rapidly over others (e.g. puffer supramedullary cell, frog dorsal-root ganglion cell). The differences

may well be related to the shape of the cell, since the latter group are approximately spherical with either minute dendrites or none at all, whereas the cells of the former group are of more complicated shape with larger dendrites (Cajal, 1911; Alexandrowicz, 1951).

SUMMARY

1. Extracellularly-recorded orthodromic responses of single cells in the lateral geniculate nucleus (LGN) can be fractionated to reveal three components, the S potential, the A potential and the B potential.

2. The S potential, considered to be a synaptic potential, is a small monophasic positive wave of relatively slow time course. It may be obtained in isolation by various procedures which reduce the possibility of synaptic transmission.

3. The A potential may be a monophasic positive wave or a diphasic positive/negative wave. It is considered to be the response of the initial segment of the axon and is seen in isolation from the B potential only when the response of the cell is deteriorating or when special procedures are adopted, e.g. discharging the cell antidromically immediately before the arrival of an orthodromic volley.

4. The B potential is regarded as the response of the soma-dendritic membrane. It is a diphasic positive/negative wave but deteriorates to a positive wave probably because of damage to part of the membrane by the electrode.

5. To explain the positive wave form of the S potential it is suggested that the density of presynaptic endings is greater on the dendrites than on the soma. A diphasic A potential could arise if the boundary between initial segment of axon and soma was not straight but irregular, the two types of membrane interdigitating with one another.

6. It is suggested that in the LGN cell and other cells there is propagation of the impulse over the surface of the soma. We have been unable to assign any portion of the unit wave forms as indicating propagated activity in dendrites. The evidence is in favour of the soma membrane being electrically excitable.

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