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THE REFRACTORY PERIOD OF THE SENSORY SYNAPSES OF THE LATERAL GENICULATE NUCLEUS

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The character of the recovery of excitability in an axon following the discharge of an impulse has been the subject of intensive study and the essential features, both in the case of peripheral axons and some at least of the central axons, are now well known. Equally intensive studies have been made of the recovery of excitability of the structures associated with synaptic transmission, namely the presynaptic endings and the post-synaptic cell body, dendrites and initial portion of the axon, but there is still much that is obscure particularly in regard to the first few milliseconds of the recovery cycle as the structures concerned are passing out of the absolutely and relatively refractory periods. Although there are many indications in the literature that sensory neurones have an extremely brief absolutely refractory period, the only systematic studies that have been made on these problems concern the motoneurones of the spinal cord (cf. particularly Brooks, Downman & Eccles, 1950*a*, *b*; Eccles, 1955; Lloyd, 1951) and brain stem (Lorente de Nó, 1935*b*; Lorente de Nó & Graham, 1938). The present study, which is concerned with the sensory synapses of the dorsal nucleus of the lateral geniculate body, is limited to the recovery from the refractory state. The later stages of the recovery cycle will be dealt with elsewhere (P. O. Bishop & R. Davis, to be published).

In the case of axons, recovery of responsiveness can be used as a measure of recovery of excitability by reference to the unconditioned shock-response curve (Graham & Lorente de Nó, 1938). With the synapse, however, the amplitude of the presynaptic volley is more directly a measure of the stimulus applied to the post-synaptic cells than is the strength of the shock applied to the presynaptic fibres. Hence the degree of recovery of responsiveness, as indicated by the amplitude of the post-synaptic discharge, can be translated into excitability terms by using the curve relating the amplitudes of the unconditioned presynaptic and post-synaptic volleys. In this way allowance

may be made for fluctuations in the excitability of the presynaptic pathway during the recovery process. The main limitation of this method of analysis relates to the uncertainty associated with subliminal fringe excitation. The basic assumption is made that in the case of the lateral geniculate nucleus the subliminal fringe associated with a smaller presynaptic volley is completely discharged by a larger independent presynaptic volley. In other words, it is assumed that as the presynaptic volley grows in amplitude the geniculate cells are progressively brought into the discharge with no 'islands' of subliminal fringe excitation remaining; so that a second testing presynaptic volley, if sufficiently smaller than the conditioning volley, will find that all the geniculate cells with which it makes synaptic contact are recovering from discharge. This problem will be analysed in more detail later (see Discussion), but the evidence available here does not provide a conclusive answer.

METHODS

Adult cats were used in all experiments, and were anaesthetized with intraperitoneal allobarbitone (Dial, Ciba, 0·5 ml./kg). Very occasionally an additional small amount (totalling 0·3 ml.) of intravenous sodium pentobarbitone (Sagatal, May and Baker) was necessary during the course of the experiment. The cats' body temperature, read with a rectal thermometer, was controlled within normal limits (38–39° C) with the aid of an electric heating blanket. The general methods used have already been adequately described in previous communications from this laboratory (cf. particularly Bishop, Jeremy & Lance, 1953). The eyeball was resected and the optic nerve prepared for stimulation by being suspended clear of orbital tissue. The response of the opposite lateral geniculate body was recorded by means of a steriotaxically directed steel micro-electrode introduced down through the intact cerebral cortex. In this investigation a new stereotaxic instrument was used which made use of the Horsley-Clarke system of coordinates in a new way. Using this new instrument and a somewhat different technique the lateral geniculate body was generally found to occupy a position about 1·0–1·5 mm posterior to that indicated in an earlier study (Bishop & McLeod, 1954).

RESULTS

The notation used for referring to the principal structures contributing to the wave form of the geniculate response following optic nerve stimulation will be the same as that given in an earlier publication (Bishop & McLeod, 1954). Thus the term 'contralateral t_1' refers to the group of fibres of larger diameter in an optic tract that have come from the contralateral optic nerve, and 'contralateral r_1' the corresponding post-synaptic neurone with its cell body in the lateral geniculate and its axon proceeding in the optic radiation to the cerebral cortex. Since the investigation reported here has been restricted to the fibres that have crossed in the chiasma, it will be convenient for our present purposes to refer to the contralateral t_1 fibres simply as t_1 fibres and similarly with r_1. The form of the potentials associated with synaptic transmission in the lateral geniculate have already been analysed in detail (Bishop, 1953; Bishop & McLeod, 1954). The wave form of a typical response restricted to t_1 fibres is shown in the tracings of Fig. 1. Within the geniculate the arrival of the t_1

volley is recorded as a positive-negative diphasic spike, followed after a short delay by the post-synaptic discharge represented by the negative-positive diphasic spike (r_1). Usually the terminal positivity of the r_1 spike is of fairly low amplitude.

Selection of recording site and quantitative analysis of wave form

In each experiment a recording site in the geniculate body was chosen so that the wave form of the response to the t_1 impulses was convenient for measurement and relatively uncomplicated by t_2 impulses. In determining the recovery of the synapses from the refractory state, it is clear that the potentials following the r_1 spike of the conditioning response form the base line upon which the test response is written. Usually a recording site could be found such that, with a conditioning shock close to maximal for t_1 fibres, this base line was reasonably flat. In Fig. 3 A, the conditioning response (C.R.) was maximal for t_1. Occasionally the first response of the repetitive discharge (Bishop, Jeremy & McLeod, 1953) was troublesome and sometimes the r_1 spike had a fairly pronounced terminal positivity (C.R., Fig. 3 B).

Using histological controls, studies in this laboratory have shown that, in general, recording from within the cell layers of the geniculate body, the t_1 component of the wave form increases in amplitude as the recording site moves laterally and posteriorly. This increase in amplitude is largely confined to the t_1 negativity and the positivity may show a relative decrease. A similar change may be noted as the electrode is inserted from above downwards through the cell layers along a single electrode track. In general, also, the reverse is true with respect to the response by the cells in the geniculate, the r_1 spike being more prominent anteriorly and medially. The t_1 and t_2 fibres are not evenly distributed within the geniculate body. In the case of the contralateral fibres the response to t_1 impulses predominates anteriorly, the t_2 component being relatively minor. The t_2 fibres are distributed to more posteriorly placed situations, although the t_1 contribution is always prominent. In the case of the ipsilateral fibres, however, the t_2 contribution at all recording sites is always more prominent than that of t_1, although again t_2 increases posteriorly.

In order to minimize the complication due to t_2 potentials the micro-electrode was usually inserted at approximately the junction of the anterior two-fifths and the posterior three-fifths of the antero-posterior extent of the lateral geniculate body and at approximately the mid point of its medio-lateral extent. Small adjustments of the electrode position in this general location enabled the typical wave forms used to illustrate this paper to be obtained. However, the results obtained at a variety of positions were similar so that there is no reason to suppose that the actual recording sites chosen had any significance beyond convenience of measurement.

The tracings of Fig. 1 show the way in which the various measurements were made. In Fig. 1 A, two tracings of a typical response have been superimposed, one showing the response to a single sub-maximal shock applied to the optic nerve, and the other the same response in which the synapses have been blocked by preceding repetitive stimulation (Bishop & McLeod, 1954). In the latter instance the r_1 spike is replaced by the slow synaptic potential s_1. The peak-to-peak amplitude of the tract spike was used rather than the amplitude of the negative component because it was easier to measure and generally more satisfactory for the purposes of the present study. In regard to the r_1 spike, however, the measurement of the negative component was found to be more satisfactory than the peak-to-peak measurement. It has already been observed (Bishop & McLeod, 1954) that the peak of the positive-going dip between the t_1 and r_1 spike negativities bears a fairly constant relationship to

the pre-stimulus base line, changing little with wide variations in stimulus strength and physiological state of the preparation. The r_1 spike was measured from this point (labelled a in Fig. 1 A) because not infrequently the pre-stimulus base line was unsatisfactory as a reference level.

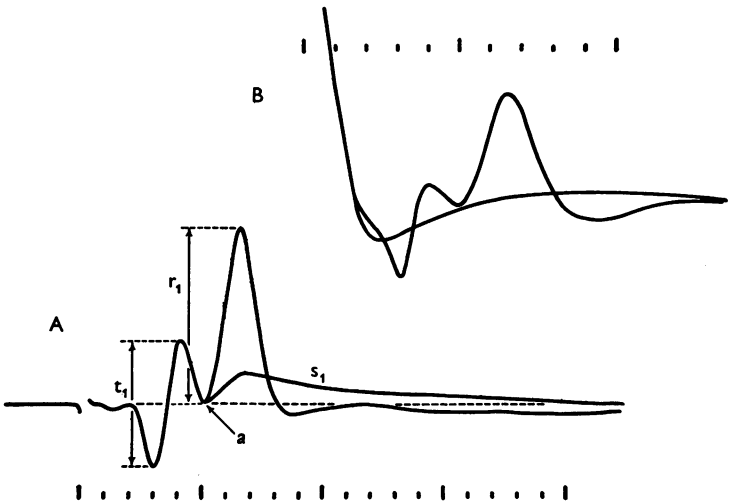

Fig. 1 A: tracing of a typical lateral geniculate response to a t_1 afferent volley showing how the measurements were made. t_1, presynaptic spike; r_1 post-synaptic spike; s_1 superimposed synaptic potential; B: tracings from another preparation with the conditioned test response superimposed upon the terminal part of the wave form of the conditioning response to show how corrections were made for the fluctuating base line. Time marks, 0·2 and 1·0 msec.

Fluctuations in excitability of geniculate neurones

The geniculate responses were always obtained against a background of 'spontaneous' slow fluctuations of potential and the responses themselves showed 'random' fluctuations in amplitude. As might be expected the amplitude of the t_1 spike in response to a constant stimulus was usually fairly stable, but occasionally the r_1 spike showed quite marked fluctuations. This is well shown in the top record of Fig. 3 B, in which ten successive responses have been superimposed. Usually, however, for a given level of anaesthesia, the fluctuations of the r_1 spike, expressed as the standard deviation of its amplitude, remain fairly constant despite a progressive increase in the height of the r_1 spike with increasing t_1 volleys, so that the ratio of the standard deviation to the amplitude of the r_1 spike progressively diminishes as the stimulus is increased.

With the levels of anaesthesia used in this series of experiments the mean standard deviation of the r_1 fluctuations expressed as a percentage of the maximal r_1 spike was 3·4, 3·5 and 3·9 % respectively in three different animals. A testing shock which was about 50 % maximal for t_1 usually gave a response

whose amplitude, though small in relation to the conditioning response, was nevertheless large enough for measurement and sufficiently constant.

Random fluctuations in the amplitude of the response are reduced by increasing the depth of anaesthesia. Thus in one preparation using an 85% maximal r_1 spike the standard deviation of the fluctuations (expressed as a percentage of the maximal r_1 spike) was 5·3 and 2·6% respectively immediately before and after intravenous administration of 0·5 ml. of Sagatal. However, it was decided to work with the animal as lightly anaesthetized as possible and to rely on a sufficiently large number of records to offset the fluctuations. The depth of anaesthesia was usually not more than was necessary to prevent reflex movements when the optic nerve was stimulated.

Spontaneous background activity usually shifted the response bodily up or down the cathode-ray tube screen without significantly affecting the wave form. For this reason the superimposition of successive traces, as shown in Fig. 3 B, in order to obtain a mean response, had a rather limited usefulness owing to the great increase in the width of the combined trace that frequently occurred. The experiment illustrated in Fig. 3 B was, however, reasonably successful from this point of view, each trace being obtained by superimposing ten consecutive geniculate responses. Usually, however, mean amplitudes were obtained from a number of individual responses at each interval between the conditioning and testing shocks. The latter were increased by small steps to give a large number of records. Unconditioned testing responses were always taken before and after each group of conditioned responses to make allowance for any change that may occur during the course of the experiment. Using the photographic enlarger, a large number of conditioning responses, similarly taken at intervals during the experiment, were superimposed to give a mean conditioning response. Significant points on this mean curve were checked by measurement of the individual records. As shown in Fig. 1 B, the conditioned testing responses were each projected upon this mean curve so that, in measuring the amplitudes of the t_1 and r_1 spikes, corrections could be made for irregularities in the base line provided by the conditioning response. Usually, however, only small corrections were necessary as a base line similar to that shown in Fig. 3 A was obtained.

Recovery from the refractory state

Because of the variability of the response under constant experimental conditions, and the long interval (5 sec) which had to be allowed for full recovery between trials, it was not practicable to use methods in which the strength of the test shock has to be adjusted to give a constant response (either pre- or post-synaptic) at each chosen value of the interval between conditioning and testing shocks. We therefore measured both the pre- and post-synaptic responses to a test shock of constant strength applied to the optic nerve. This means that the effective 'stimulus' applied at the synapse, which, as pointed out earlier, is better measured by the amplitude of the presynaptic spike than by the strength of the test shock, was not constant but varied with the degree of recovery of the presynaptic fibres. The curve of recovery of responsiveness obtained in this way may be spoken of as the 'functional recovery cycle' because it is not directly comparable to the curve obtained for axons by the methods commonly used. This arises from the fact

that the time taken for the recovery of the t_1 spike of the test response is also available for the recovery of the post-synaptic cells, so that it is possible that the r_1 spike of the test response might be relatively smaller at a given shock interval if the t_1 test spike could have been made constant throughout the recovery cycle. The degree of recovery of a test response at a given shock interval may be estimated by reference to the unconditioned t_1/r_1 curve, but corrections for the variations in the size of the t_1 volley in this way do not provide information about the degree of recovery that would have occurred had the afferent volley been kept constant. The 'functional recovery cycle' is thus concerned with the presynaptic fibres as well as the post-synaptic cells. The curves of Fig. 5 have been presented without correction for the degree of recovery of the presynaptic fibres.

In the case of the lateral geniculate synapses the graph relating the amplitudes of the presynaptic and post-synaptic spikes is approximately linear at levels of afferent stimulation from threshold upwards till the shock is close to maximal for t_1 fibres (Bishop & McLeod, 1954). It is clear that if the graphs are to be used to correct for variations in the size of the t_1 spike they must remain unchanged throughout the experiment and, furthermore, the parameters of the unconditioned test response must be constant and remain consistent with the t_1/r_1 curve for that position. If these criteria are not fulfilled a spurious supernormality or subnormality may be introduced into the recovery phase of the geniculate synapses. Occasionally the curve of the t_1/r_1 relationship deviates from a linear relationship: but whatever its form it was found to remain constant for a particular recording site, provided the preparation remained in good condition.

Curves b of Fig. 2, which were constructed from records taken at the same recording site, show the usual nearly linear t_1/r_1 relationship. The oscillograph traces for each curve took about one hour to record, starting about three hours later for the second curve than for the first. Curves a, obtained from another preparation and again separated by an interval of three hours, illustrate the constancy of the t_1/r_1 relationship even when this deviates more markedly from linearity. An experiment yielding such a curve would, however, not be regarded as satisfactory for the purpose of this study unless the amplitude of the unconditioned test response was small enough not to correspond to the portion of the curve where the r_1 spike changes rapidly for a small change in t_1. Usually the wave form of the geniculate response was chosen to give the curve a slope of approximately 45°. Each point in Fig. 2 represents the mean of (usually) six records.

In the case of myelinated axons the period of latent addition of stimuli is much shorter than the duration of the absolutely refractory period. Thus, in studying the recovery from the refractory state, as long as the conditioning shock is greater than the testing shock, the latter will not activate fibres which

had not previously been discharged. In the case of synapses, however, the duration of the synaptic potential will ensure that cells subliminally excited by the conditioning volley will retain an enhanced excitability for some time after the presynaptic fibres have largely recovered to normal. By intracellular recording Brock, Coombs & Eccles (1952*a*) found that the synaptic potential of spinal motoneurones had a duration of about 14 msec. Even with a shock supramaximal for both the t_1 and t_2 groups applied to the optic nerve, the

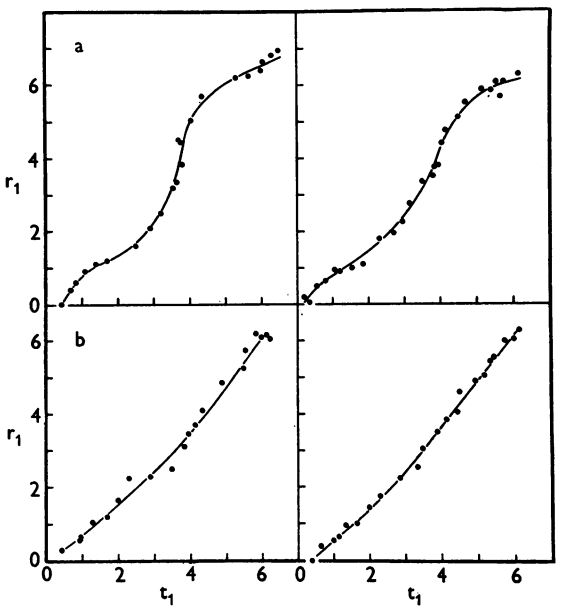

Fig. 2. Data from two preparations (a, top pair, and b, bottom pair) showing the relationship between the amplitudes of the t_1 and r_1 spikes in response to increasing afferent stimulation. An interval of 3 hr elapsed between the curves in each preparation. Abscissae and ordinates in arbitrary units.

possibility of a small subliminally excited fringe is difficult to exclude. Such a large stimulus cannot be employed, however, because the testing response would be obscured by the t_2 conditioning response. In order to minimize the possibility of subliminal fringe excitation, the conditioning shock was always made as large as possible and the testing shock as small as possible.

Absolutely refractory period

The absolutely refractory period of the mechanisms concerned in synaptic transmission through the geniculate was determined by progressively increasing the interval between conditioning and testing shocks in the usual way and ascertaining the least interval at which the latter produced an r_1 spike. In ten preparations the absolutely refractory period measured as the least

shock interval varied from 0·70 to 0·92 msec (mean 0·81 msec). Typical experiments are illustrated in Fig. 3 A, B. Whenever the t_1 spike could be discerned with reasonable assurance it was always followed by a clear r_1 spike.

The optic nerve at the site of stimulation was, however, suspended in air and so must have been at a temperature less than that of the brain. Lowering the temperature increases the absolutely refractory period of nerve fibres (Amberson, 1930). By stimulating the optic tract fibres within the brain,

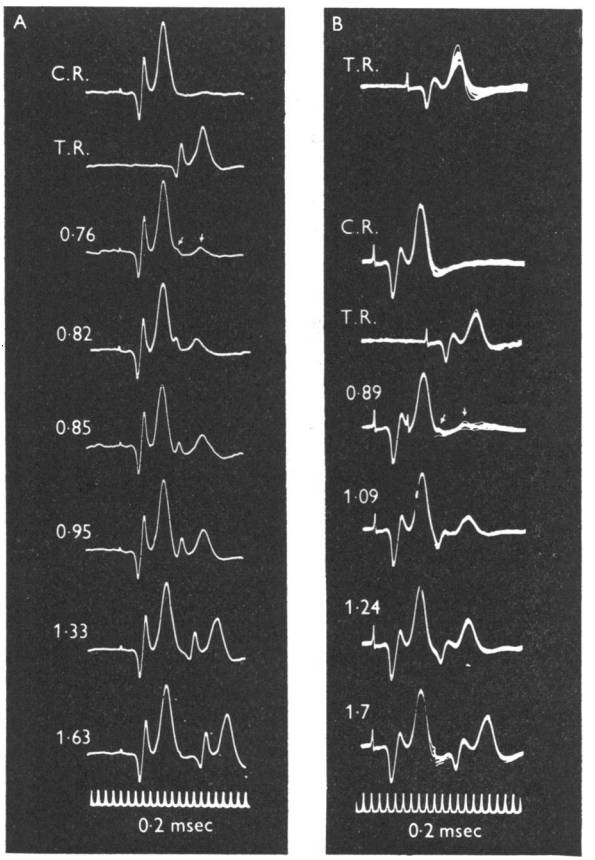

Fig. 3. Recovery of responsiveness in two preparations A and B. C.R., conditioning response; T.R., unconditioned test response. Shock intervals in msec are indicated in each case.

Bishop, Jeremy & Lance (1953) showed that the absolutely refractory period of the t_1 fibres was about 0·47 msec. It remained a possibility therefore, that the absolutely refractory period of the t_1 spike may be briefer than that of the r_1 spike at normal body temperature (38–39° C). In order to test this possibility, stimulating electrodes were placed in the optic tract about 10 mm from the nucleus and the geniculate response was recorded as before. In the

experiment illustrated in Fig. 4, both a t_1 and an r_1 response (indicated by arrows) were clearly present at a shock interval as brief as 0·65 msec. It is obvious that it would be difficult to be sure of an earlier appearance on the part of the t_1 spike because it would fall near the peak of the conditioning r_1 spike which itself may show small 'spontaneous' fluctuations. Although the two spikes could not be consistently observed at shock intervals less than 0·65 msec it was nevertheless possible to discern an r_1 spike as early as 0·6 msec.

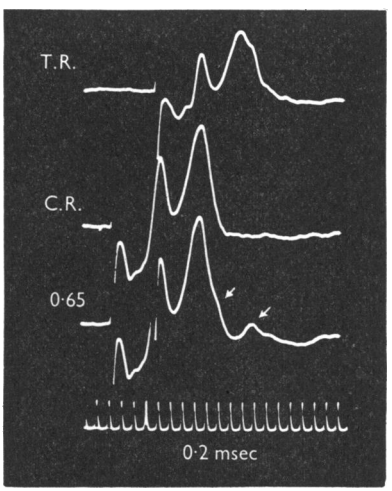

Fig. 4. Absolutely refractory period of geniculate synapses. Responses to t_1 volleys initiated by stimulating the fibres close to the geniculate. T.R., unconditioned test response; C.R., conditioning response; 0·65, shock interval in msec.

The slowing in the conduction time of the t_1 spike at the least shock interval at which a measurement could be made (0·65 msec) was about 0·25 msec. This measurement is necessarily rather uncertain, but it is unlikely to be far astray because, as can be seen from Fig. 4, the increased latency of the peak of the r_1 spike was no more than about 0·3 msec, a value which includes the increased delay associated with the generation of the post-synaptic response as well as that due to slowed conduction in the presynaptic pathway. Thus with a presynaptic pathway of about 10 mm the absolutely refractory period of the synapse was slightly less than 0·85 msec. It is probable, however, that the geniculate synapses have an absolutely refractory period less than this, approximating to that of the presynaptic fibres (0·47 msec, Bishop, Jeremy & Lance, 1953), but it would be necessary to reduce the length of the presynaptic path in order to demonstrate it. This would introduce the possibility of direct stimulation of the sensory neurones.

Relatively refractory period

The way in which the response recovers to normal during the relatively refractory period of the tract fibres is illustrated in the two series of records, A and B of Fig. 3, taken from different preparations. As the interval between the conditioning and testing shocks is increased the t_1 and r_1 spikes grow in amplitude together, their percentage recovery being closely similar until supernormality on the part of the r_1 response supervenes. Thus in series A at

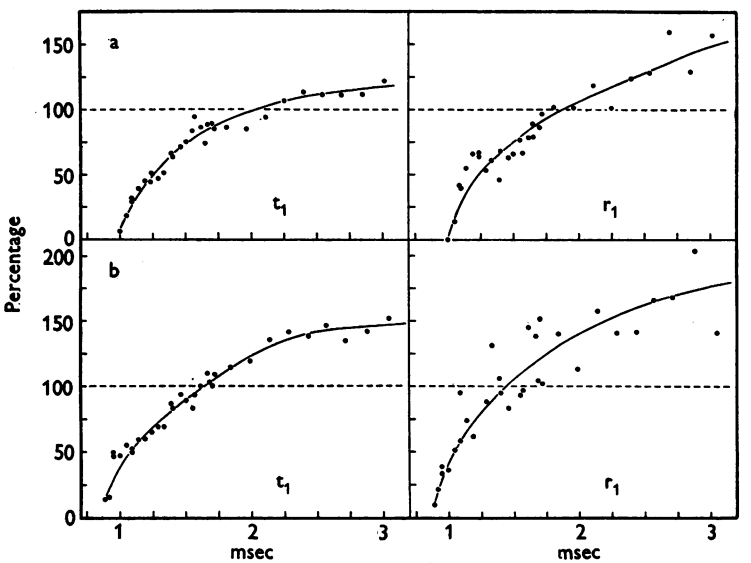

Fig. 5. Percentage recovery of responsiveness following conditioning on the part of the t_1 (pre-synaptic) and r_1 (post-synaptic) components of the geniculate response in two preparations (a, top pair and b, bottom pair). Abscissae, shock interval in msec.

0·82 msec t_1 is 24% recovered and r_1 is 28% recovered. The corresponding figures for the traces at the other intervals used in the illustration are: 0·85 msec, 40 and 45%; 0·95 msec, 60 and 58%; 1·33 msec, 77 and 102%, and 1·63 msec, 100 and 125%. In this series the t_1 spike of the unconditioned test response is 55% maximal. The recovery shown in series B is similar.

In Fig. 5 the percentage recovery of the t_1 and r_1 components in two preparations, a and b, is plotted against time. The traces shown in Fig. 3 B were taken from the same series used to provide the data for the curves b of Fig. 5. It can be seen from Fig. 5 that in each case the t_1 and r_1 spikes recover back to normal at roughly the same rate. The time taken for the t_1 spike to recover varied from 1·4 to 2·0 msec (mean 1·7 msec) and the corresponding figures for the r_1 spike were from 1·35 to 1·85 msec (mean 1·6 msec). Thus the t_1 and r_1 spikes had always returned to normal by 2 msec and with one

exception (Fig. 6b) the r_1 spike recovered earlier than the t_1 spike. The conduction velocity of the afferent volley returned to normal with the recovery of spike amplitude so that no correction for delayed conduction was necessary (cf. Graham & Lorente de Nó, 1938). These recovery times are likely to be slower than normal, however, by reason of the fact that the optic nerve was cooler than the normal body temperature.

Following the return to normal the t_1 fibres exhibit a prolonged phase of supernormality, only the initial portion of this phase being shown in Fig. 5 (cf. Bishop, Jeremy & Lance, 1953). Similarly, the geniculate cells exhibit a phase of supernormality, but it is clear that the greater part of this supernormality is to be attributed to the increase in the number of optic nerve fibres stimulated by the test shock during the supernormal period of the nerve. There is, however, a definite though short-lived phase during which the r_1 response is increased by an amount which cannot be accounted for by an increased afferent discharge. As has already been observed this supernormality on the part of the geniculate cells actually begins while the optic nerve fibres are still relatively refractory, generally commencing when the interval between conditioning and testing shocks is little more than 1 msec. The r_1 response is regarded as supernormal if it has an amplitude greater than that appropriate to the amplitude of its t_1 spike as determined from the unconditioned t_1/r_1 curve. The detailed description of the supernormal phase in the recovery of the geniculate synapses is reserved for a later paper (P. O. Bishop & R. Davis, to be published).

The relationship between the recovery of the tract fibres and that of the geniculate cells is also illustrated in the graphs of Fig. 6. Graphs (a) and (b) relate to one preparation and (c), (d) and (e) to another, the abscissae and ordinates in each case being plotted in common but arbitrary units. The points determining the graphs (a) and (b) represent the mean of 4 or 5 traces, while those for (c), (d) and (e) were obtained from the superimposed traces. Selections from the latter form the series of Fig. 3 B. Graphs (a) and (c) indicate the relationship between the amplitudes of the t_1 and r_1 spikes of the unconditioned response at different levels of afferent stimulation. For easy comparison these graphs (i.e. a and c) have been drawn in on (b) and (d) respectively as broken lines. The open circles in graph (b), obtained from records of the unconditioned response to increasing afferent stimulation taken at the end of the particular experiment, again illustrate the constancy of this t_1/r_1 relationship. Graphs (b), (d) and (e) show the relationship of the amplitudes of the t_1 and r_1 spikes of the conditioned response as it recovers through the relatively refractory period of the tract fibres. The points shown as dots surrounded by a circle, in each case indicated by an arrow, represent the mean unconditioned test response. In the curves shown the response has been followed until the t_1 spike has recovered approximately to normal, the longest interval between the

conditioning and testing shocks, however, being still less than 2 msec in each case. In the early phases of the relatively refractory period the tract fibres and the geniculate cells recover in such a way that the slope of the t_1/r_1 graph is the same as that obtained by increasing afferent stimulation without conditioning. In other words, the synapses behave as though they had not been conditioned but were responding normally to increasing afferent stimulation.

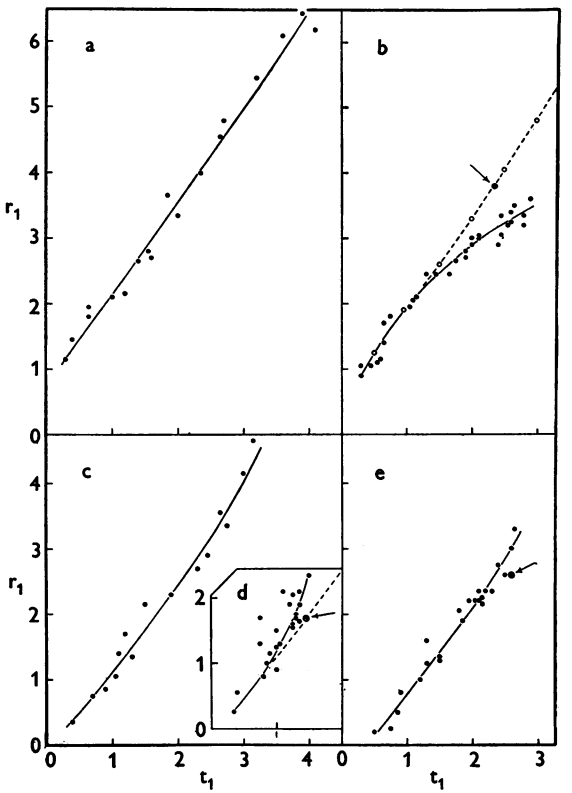

Fig. 6. Relationship between amplitude of presynaptic spike (t_1) and post-synaptic spike (r_1) in two preparations, (a) and (b) on the one hand, (c), (d) and (e) on the other. (a) and (c), relationship with increasing stimulus strength applied to the optic nerve. (b), (d) and (e), relationship during recovery of responsiveness following conditioning. For details see text. Abscissae and ordinates in arbitrary units.

In the later stages of the t_1 spike's recovery to its unconditioned amplitude the conditioned t_1/r_1 graph almost invariably diverged upwards from the unconditioned t_1/r_1 graph (Fig. 6 d) indicating a relative supernormality on the part of the r_1 spike. Moreover, this supernormality on the part of the geniculate cells was still present even after the tract fibres had themselves become supernormal and due allowance had been made for the latter circumstance. The

degree of absolute r_1 supernormality was, however, very variable and never very large. In only one instance (Fig. 6 b) did the curve diverge downwards before the t_1 spike had recovered back to its unconditioned amplitude. Even in this case the two curves coincided until the t_1 spike was approximately 50% recovered.

Fig. 6 e requires a brief comment. It will be seen that a larger test response was used for (e) than for (d). Graph (e) was, however, constructed from records obtained at the end of the experiment and presumably the electrode had moved slightly before they were taken because the mean unconditioned response no longer fell on the line of graph (c). However, in this case also there was apparently some degree of r_1 supernormality because the curve passes upwards to the left of the mean unconditioned response.

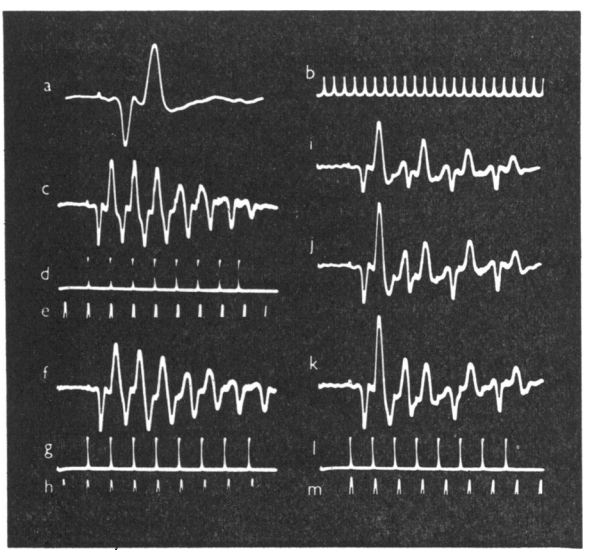

Fig. 7. Repetitive stimulation. (a), individual geniculate response to show the wave form. (b), time scale for (a), 0·2 msec. (c), (f) and (i), (j) and (k), repetitive geniculate responses to eight afferent volleys: (i), (j) and (k), responses to increasing stimulus strength with shock intervals kept constant. (d), (g) and (l), verticals indicating the actual intervals between the stimuli in each tetanus. Corresponding time scales; (e), 900 c/s; (h), 1000 c/s; (m), 1100 c/s.

Repetitive stimulation

The properties displayed by the geniculate synapses during their recovery from stimulation are well illustrated by using high rates of repetitive stimulation. In Fig. 7 the lateral geniculate has been activated at successively increasing rates using groups of eight afferent volleys in each case. Record (a) shows the form of an individual geniculate response on an expanded time base corresponding to scale (b) (each division 0·2 msec). In (c) the geniculate has been activated by afferent volleys at 920/sec, the verticals of (d) indicating the

actual times at which the stimuli were applied to the optic nerve and (e) being a corresponding 900/sec time scale. The two remaining groups of traces, (f), (g) and (h) on the one hand and (i), (j), (k), (l) and (m) on the other, are to be interpreted in the same way, the rate of stimulation in the former being 1024/sec (time scale h) and in the latter 1170/sec (time scale m). In (c) the r_1 spike of the geniculate response is well maintained for the first three tract volleys, but thereafter rapidly declines to fail with the sixth volley. The t_1 spike also declines somewhat over the series, but the marked reduction of the eighth t_1 spike in comparison with its immediate predecessor is to be attributed to the fact that, owing to an instrumental error, the interval between the seventh and eighth stimuli is rather less than those in the remainder of the tetanus. In (f) the t_1 spike is again fairly well maintained during the tetanus even although the rate of stimulation is now greater than 1000/sec and a small r_1 spike still survives with the sixth stimulus. It must always be borne in mind that the actual decline of the r_1 spike is less than is at first apparent in proportion to the failure of the t_1 spike. At 1170/sec the responses in (i) show alternation, the first, third, fifth and seventh t_1 spikes being well maintained with the corresponding r_1 spikes declining more markedly over the series. The alternate stimuli, second, fourth, etc. give rise only to very small and barely recognizable responses, again declining over the series. In (j) and (k) the stimulus strength has been successively increased. It will be seen that, although alternation is still present, the stimulus strength now becomes sufficient to produce a very much larger second response, the remainder of the tetanus being much the same as before. In the case of the increasing second response both the t_1 and the r_1 components grow in proportion.

Thus with the very rapid rates of repetitive stimulation used above, each of the first three responses behave more or less as a unit, the t_1 and r_1 components increasing or decreasing in proportion. It will be shown in a later paper that the much more rapid failure of the r_1 spike in the later responses is due to the fact that in the case of the geniculate cells the negative after-potential is short-lived and of low amplitude, rapidly reversing to a deep and prolonged positive after-potential. By contrast the tract fibres have a relatively long-lasting large-amplitude negative after-potential and a very shallow positive after-potential (Bishop, Jeremy & Lance, 1953).

Synaptic and peak delays

In order to make a more detailed comparison between the recovery of the geniculate response during the refractory period with the response following increasing afferent stimulation without conditioning, the synaptic and peak delays were measured in both circumstances. The synaptic delay, which here is regarded as the interval between the point at which the t_1 spike crosses the base line from positive to negative on the one hand to the beginning of the

r_1 spike (a in Fig. 1 A) on the other, has a normal value of about 0·3 msec (Bishop & McLeod, 1954; cf. also Brock, Coombs & Eccles, 1952*b*). It should be noted that both Lorente de Nó (1938) and Renshaw (1940) have used the term 'synaptic delay' to denote the time interval between the arrival of the presynaptic impulses and the commencement of the post-synaptic propagated discharge. The latter definition includes the utilization time of the synaptic potential as well as the interval regarded as the synaptic delay in the present study. The term 'peak delay' is here used to denote the interval between the peaks of the t_1 and r_1 negativities respectively. The value of 0·7 msec given by G. H. Bishop & Clare (1953) for the synaptic delay in the lateral geniculate presumably represents a peak delay time, though this is rather longer than that found in the present study.

In two preparations (*A* and *B*) the synaptic and peak delays were measured both in the unconditioned response with increasing afferent volleys and also in the conditioned response as it recovered from refractoriness. In both preparations the unconditioned synaptic delay became more constant with increasing stimuli, though in *A* remaining about 0·27 msec while in *B* decreasing from 0·27 msec with small stimuli to 0·24 msec with maximal t_1 volleys. During recovery from refractoriness the corresponding delays were 0·27 msec in each case without any significant decrease as recovery progressed. The values for the peak delays showed a fairly wide scatter both at lower levels of stimulation without prior conditioning and also during the early part of the relatively refractory period. With increasing stimulation on the one hand and recovery from refractoriness on the other the peak delay times became very much more constant. The mean values of the peak delay times with maximum unconditioned t_1 volleys (*A*, 0·57 msec and *B*, 0·51 msec) were approximately at the lower limit of the scatter of the delay times at threshold, the mean decrease being of the order of 0·1 msec. Throughout the refractory period the peak delay times showed a fairly wide scatter but the values were approximately the same as for the unconditioned response, again decreasing slightly as recovery proceeded. As the synaptic delay is fairly constant and unaffected by refractoriness, the relative lability of the peak delay time is probably due to variations in the utilization time of the synaptic potential.

DISCUSSION

In his study of the refractory period of ocular motoneurones Lorente de Nó (1935*b*) assumed that the testing volley reached the synapse 'without appreciable delay' and he therefore concluded that the absolutely refractory period was 0·6 msec. The presynaptic pathway in his case was also about 10 mm in length (Lorente de Nó, 1935*a*). From the present study it is, however, clear that the major part of the increased latency of the post-synaptic spike of the test response is to be attributed to slowed conduction in

relatively refractory presynaptic fibres. This fact allows agreement to be achieved between Lorente de Nó's findings and those reported here. Together they place an upper limit of about 0·85 msec for the absolutely refractory period of oculomotor and geniculate synapses. The absolutely refractory period of the synapses on spinal motoneurones has been shown by Lloyd (1943) and Brooks, Downman & Eccles (1950*a*) to be rather longer than this, probably about 1·5 msec. These investigators used an antidromic conditioning volley followed by an orthodromic testing volley, but allowance had to be made for the failure of some of the antidromic impulses to invade the cell body.

It has been assumed above that all the geniculate neurones contributing to the test response had previously been discharged by the conditioning volley. With his preparation Lorente de Nó (1935*b*) was able to ensure that this was the case, but his method is not applicable to the lateral geniculate because of the complication due to t_2 impulses. Indirect evidence is, however, available which indicates that the conditioning volley probably does not leave any subliminally excited cells available to the testing volley. The fluctuations in the excitability of the neurones lying in a subliminal fringe make them generators of 'random' potentials. If islands of subliminally excited cells remain as an unconditioned r_1 response grows in amplitude, the standard deviation of the fluctuations of the r_1 spike will, assuming the fluctuations to be random, be given by the expression

$$\text{S.D.} = \sqrt{[\Sigma(\sigma_1^2 + \sigma_2^2 + \ldots + \sigma_n^2)]},$$

where σ = standard deviation of the fluctuations due to the individual island generators. Even if the r_1 fluctuations are not truly random the standard deviation is still likely to show a progressive increase as the r_1 spike grows in amplitude because each increment of r_1 would add further neurones to a growing subliminal fringe.

In five experiments on three cats the relationship of the mean r_1 responses to their respective standard deviations were examined. In no case did the regression coefficient of the standard deviation on the mean r_1 response approach the 5% significance level. In one experiment the regression coefficient was negative ($P = 0{\cdot}07$), indicating that there was a tendency for the standard deviation to diminish as the mean r_1 response increased. In two experiments on the second cat the probability of the regression coefficient differing significantly from zero was 0·50 and 0·23 respectively, indicating that the fluctuations in the standard deviation were entirely random over the range of mean r_1 values. In two experiments on a third cat there was some suggestion of a positive association between the standard deviation and the mean r_1 values ($P = 0{\cdot}06$ and 0·10) with the possibility of an increase of about 10% in the value of the standard deviation for unit increase in the mean r_1 value. Thus if one assumes that, at any level of afferent stimulation, cells which may

form islands of subliminal excitation are randomly distributed (from the point of view of excitability) among the other cells in the subliminal fringe which would eventually be added to the response by increasing the afferent stimulation, then the above analysis indicates that subliminal fringe excitation plays, at most, a negligible part in the responses during recovery from refractoriness. Such an analysis, however, must face the criticism that it is difficult to gauge the extent of a subliminal fringe in which there may be cells whose excitability is sufficiently below the discharge level for them to play no part in the random variations of the response, but which may still be brought to discharge by a summation of synaptic potentials. Furthermore, the cells which may form islands of subliminal excitation could well belong largely to the latter category. However, studies on the supernormal period in the recovery cycle of the geniculate synapses carried out in this laboratory indicate that the total subliminal fringe is probably very little larger than that suggested by the analysis of random variations above. In addition it has been shown that the increase in the r_1 spike of the test response due to supernormal excitability on the part of the geniculate neurones is again independent of the amplitude of the r_1 response over a fairly wide range of afferent bombardment. The increase in the r_1 spike due to supernormal excitability is again a measure of the size of the available subliminal fringe. Hunt (1955) has recently shown that the standard deviation of the variations of the response amplitude of unconditioned spinal motoneurones due to excitability fluctuations is also constant over a considerable range of afferent stimulation.

The curves of Fig. 6 indicate that relatively little spatial summation is required to discharge geniculate neurones. A post-synaptic discharge can usually be seen to follow a threshold or near-threshold t_1 response and thereafter the r_1 response increases in amplitude along with the tract spike. Variations in the geniculate discharge however indicate the presence of a subliminal fringe. It is likely, therefore, that there is a fairly steep gradient of synaptic effectiveness on the part of those t_1 fibres associated with the neurones that are close to and in the subliminal fringe. During the first half or more of the phase of recovery from the refractory state the geniculate response behaves both in respect to amplitude and time relations as though it had not been conditioned and was simply responding to increasing afferent bombardment. Even during the later stages of the recovery to normal amplitude on the part of the tract spike, the r_1 response does not deviate very much from the response that would be expected from such a t_1 volley without conditioning. These observations mean that, in the case of the unconditioned response, the greater part of the tract volley must provide a stimulus which is well above threshold for the corresponding geniculate neurones because even during the recovery from refractoriness, at a time when those tract fibres which have commenced to respond again require a stimulus much greater than normal, the t_1 volley

nevertheless discharges the usual number of geniculate neurones. Such a comparison between recovery of tract fibres and geniculate neurones cannot be pressed too far, however, not only because the nature of the stimulus in each case is so different but also because delayed conduction in relatively refractory tract fibres allows the corresponding geniculate neurones somewhat longer to recover. Nevertheless, the recovery of the geniculate neurones must have been well advanced by the time the first tract volley is able to reach the nucleus, because a synaptic potential set up during the refractory period of the geniculate neurones would have the effect of prolonging their refractoriness. Eccles & Kuffler (1941) and Kuffler (1942) showed that the end-plate potential set up by a conditioning nerve impulse at the striated neuro-muscular junction lengthens the muscle refractory period. Blair & Erlanger (1933) had earlier found that a nerve's refractory period is lengthened by a brief intercurrent cathodal polarization. The end-plate potential and the synaptic potential represent cathodal polarizations at their respective junctional regions.

A further indication of the powerful nature of the geniculate relay is revealed by the fact that, in the case of spinal motoneurones (Coombs, Eccles & Fatt, 1955), a presynaptic volley only begins to produce a synaptic potential in an antidromically activated motoneurone at the end of the cell body spike potential and even after 5 msec from the start of this spike the synaptic potential produced by the orthodromic volley is still only 70% recovered.

SUMMARY

1. An upper value of 0·85 msec has been set for the absolutely refractory period of the lateral geniculate synapses using conditioning and testing volleys applied to the presynaptic pathway (optic tract) about 10 mm from the lateral geniculate nucleus. The geniculate neurones were always capable of responding as soon as conduction became possible in optic tract fibres.

2. The relatively refractory period (functional recovery cycle) was studied by stimulating the optic nerve. The post-synaptic spike (r_1) recovered its amplitude at a conditioning shock–test–shock interval of from 1·35 to 1·85 msec (mean 1·6 msec), while the presynaptic spike (t_1) took from 1·4 to 2·0 msec (mean 1·7 msec). Supernormal excitability on the part of the geniculate neurones commences while the optic nerve fibres are still relatively refractory.

3. With increasing afferent volleys the unconditioned r_1 spike bears a constant and usually linear relationship to the t_1 spike. A similar relationship also occurs during the recovery from refractoriness. Except for a small degree of supernormal excitability in the later stages, throughout this recovery period the geniculate neurones respond to recovering afferent volleys as though they had not previously been discharged by the conditioning t_1 volley: i.e. the t_1/r_1 relationship is unaltered.

4. Repetitive stimulation is used to illustrate the brief duration of refractoriness on the part of the geniculate synapses. The amplitude of the r_1 spike is well maintained for at least three successive afferent volleys at a stimulation frequency of about 1000/sec.

5. The normal synaptic delay of about 0·3 msec is not affected by refractoriness. There is a slight lengthening of the peak(t_1)-to-peak(r_1) time which is attributed to an increase in the utilization time of the synaptic potential.

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