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TYPES OF UNITARY RESPONSE AND CORRELATION WITH THE FIELD POTENTIAL PROFILE DURING ACTIVATION OF THE AVIAN OPTIC TECTUM

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SUMMARY

1. Unitary responses were recorded in the lateral tectum of the pigeon, with electrolyte-filled micropipettes after electrical stimulation of the optic nerve-head.

2. Optic nerve fibre spikes could be recognized by their conformation, fixed latency, brief recovery times, and location in the superficial tectum. Their action potentials were either triphasic with a prominent second phase, or monophasic positive.

3. The optic nerve consists of small myelinated fibres conducting at 5·3–8·0 m/sec. These axons probably have diameters in the order of 1·6–2·2 μ.

4. The fibre spikes were localized to the N-zone and R-zone. None was recorded deeper. Most of the fibre spikes preceded the tectal N-wave.

5. One hundred and fifty-six post-synaptically fired cells were recorded. These had a diphasic positive-negative conformation, and were fired at variable latency.

6. One hundred and forty of these cells fired a single spike to each stimulus to the optic nerve-head. Even the most stably fired cells could be proved to be trans-synaptically activated by the evidence of non-collision.

7. Sixteen of the 156 cells fired repetitively to single stimuli to the optic nerve-head.

8. Evidence could be obtained that afferent inhibition operates upon tectal cells.

9. Cells in the N-zone were fired earliest in the 3 msec interval, corresponding to the rising phase of the tectal N-wave. By comparison, cells in the P-zone were not fired in the 3 msec interval, and the proportion fired in the 4 msec interval was reduced. Cell firing in the P-zone must be produced by tectal interneurones.

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10. Cells were present in the N-zone with recovery times below 5 msec. No cells in the P-zone had recovery times below 5 msec.

11. A clear correlation could be made between the distribution of fibre and cell spikes, and the field potential profile. A correlation could also be made between the timing and recovery time of cells in the N-zone and P-zone. The unitary records show that the tectum is activated radially by the retinotectal pathway.

INTRODUCTION

It has been shown in a previous paper (Holden, 1968) that a simple description can be given of the field potential profiles occurring in the optic tectum when the optic nerve-head is stimulated electrically. This stimulation will excite retinotectal axons; these will be recorded at the lateral tectal surface as unitary axonal spikes fired at fixed latency. Post-synaptically fired cells will be recorded as spikes fired at variable latency. If centrifugal cells project from the tectum to the retina (for which there is no anatomical evidence (Cowan & Powell, 1963)), these would be recorded as somatic spikes fired at fixed latency.

This paper reports the types of unitary activity recorded at the lateral tectum in response to optic nerve-head stimulation. It was intended to develop criteria to distinguish between axonal firing and trans-synaptic cell firing. Many tectal cells would be expected to be fired monosynaptically. It is possible, however, to prove that these stably fired cells are in fact trans-synaptically activated by the evidence of non-collision. The collision argument can be used to prove that a neurone is being activated antidromically (Gordon & Jukes, 1964). Conversely, if it can be shown that a cell fired at a relatively stable latency L is *not* eliminated when preceded by a spontaneous discharge falling within the interval $(2L+R)$ where R is the 'refractory time' for cell discharge, then it is *proved* that the cell is being fired trans-synaptically.

In this study it was hoped to provide data about the fibre composition of retinotectal axons, and to correlate unitary firing with the field potential profile. If the interpretations suggested in a previous paper (Holden, 1968) are correct, it would be expected that axonal spikes bear the same relation to the tectal N-wave as do the spikes of motor terminals to the end-plate potential (cf. Katz & Miledi, 1965). It would also be expected that cell firing follows the rising phase of the tectal N-wave. These expectations are investigated below.

METHODS

The procedure is described in detail elsewhere (Holden, 1968). Micro-electrodes were advanced radially into the lateral tectum to which the fovea projects.

RESULTS

Unitary records were obtained with electrolyte filled pipettes of impedance 3–7 MΩ at 50 c/s.

Axonal spikes

Optic nerve fibre spikes could be recognized by four criteria: their conformation, fixed latency, short recovery-time to paired twice-threshold stimuli, and their anatomical localization to the superficial laminae of the tectum. The first two properties were sufficient to permit confident identification.

Latency. Each response consisted of a single spike at fixed latency. Figure 1 (*a*) shows the two kinds of axonal spike that were recorded. Each is unitary, and precedes the N-wave. Figure 1 (*b*) shows the latency histogram of these axonal spikes, pooled between experiments. Projected below the histogram is a tracing of the N-wave. Most of the fibre spikes precede the N-wave, and cannot therefore be unitary events of which the N-wave is an envelope. The time relations are consistent with the N-wave being a sink of a post-synaptic process following the unitary axonal spikes.

The conduction distance from optic nerve-head to the recording site was approximately 1·6 cm. This value gives the 2 msec interval in the latency histogram a conduction velocity of 8 m/sec, and the 3 msec interval a conduction velocity of 5·3 m/sec. These velocities correspond to the lower range of velocities found in mammalian myelinated fibres. To convert them to an axonal diameter requires the assumption that axonal diameter is constant from optic nerve-head to tectum, and a relationship between conduction velocity and axonal diameter. The conversion factor of 3·2 m/sec/μ found by Ogden & Miller (1966) for the small myelinated fibres of the monkey optic nerve can probably serve as an approximation. It gives the pigeon retinotectal axon diameters from 2·2 to 1·6 μ.

Conformation. The axonal spikes had two typical conformations which are illustrated in Fig. 1. The first was a triphasic positive–negative–positive spike with a prominent negative phase. Spike amplitudes ranged from 100 to 500 μV, most commonly being 150–200 μV. These negative spikes could be held stably for periods up to 30 min, and could not be caused to fire by deliberate electrode movement. The second type was a monophasic positive spike with a linear rise and fall, and amplitude ranging from 1 to 10 mV. The positive spikes were highly unstable, and declined from first contact, often being lost in seconds. This conformation corresponds to impalement and rapid death of the axon. The positive spikes could be distinguished from cell spikes by several criteria. They were not diphasic in conformation, had shorter durations than cell spikes,

and were fired at fixed latency. Two of the positive spikes showed a notching when responding to closely paired stimuli. This behaviour was examined closely to distinguish it from somatic *A–B* blocking. One of these occasions is described here. The spike had an amplitude of 3·0 mV and a latency of 2·0 msec. It was followed by an N-wave. To paired stimuli separated by 6·0 msec the second spike was reduced stepwise to 1·5 mV. The second spike failed completely at a separation of 1·5 msec. With the inter-stimulus

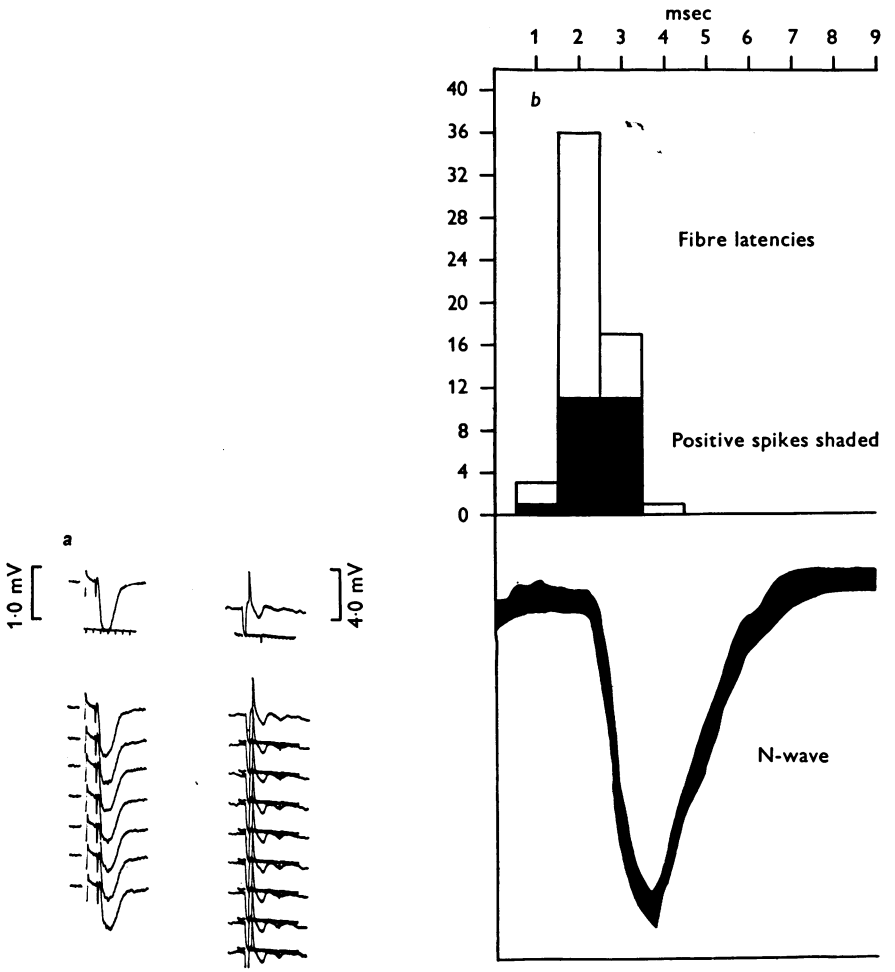

Fig. 1. (*a*) The two types of fibre spike recorded at the lateral tectum in response to stimulation of the optic nerve-head. Each has a fixed latency, and precedes an N-wave. Time markers show msec. In this and succeeding figures, positivity is displayed upwards.

(*b*) The latency histogram of the fibre spikes. Ordinate shows no. of units, abscissa shows latency in msec. An N-wave is included for comparison.

interval straddling 5–7 msec the second spike occasionally displayed a notched rising phase. Notching and stepwise reduction in amplitude also occurred to trains of stimuli.

The following considerations suggest that this behaviour is distinct from *A–B* blocking (cf. Hern, Phillips & Porter, 1962). The two phases of the axonal spike are almost equal in amplitude, and duration. They are both monophasic positive in conformation. In *A–B* blocking the *B* phase usually has a longer duration than the *A* phase, has a larger amplitude, and a differing conformation. Antidromically activated somata in the pigeon isthmo-optic nucleus and retina show typical *A–B* blocking. The notching of the positive axonal spikes was probably due to damage from impalement.

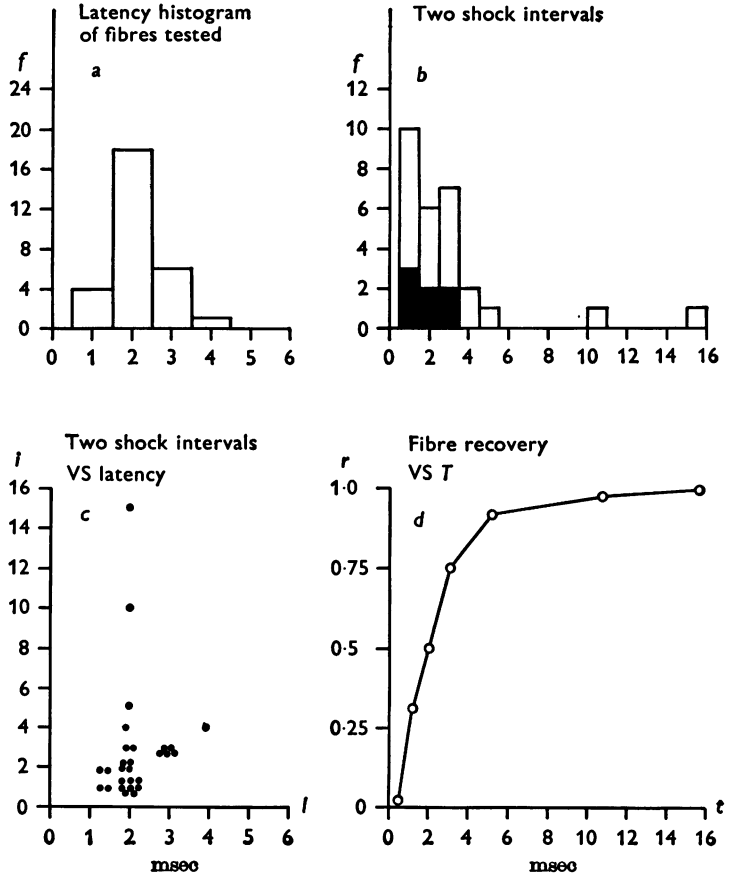

Fig. 2. Recovery times to twice-threshold stimuli for fibre spikes. In (*a*) and (*b*) the ordinate is the number of units. In (*c*) the ordinate is the recovery time, and the abscissa is latency. In (*d*) the ordinate is the proportion of the total population that has recovered; the abscissa is the interval between stimuli.

Paired stimuli. Twenty-eight of the fifty-six spikes were tested with closely paired twice-threshold stimuli. As the interval between the stimuli was reduced, so at a critical 'two-shock interval' the second response failed abruptly. Figure 2 summarizes the findings. Figure 2(*a*) shows the latencies of the fibres tested. Figure 2(*b*) is a histogram of the two-shock intervals. The majority lie between 1 and 4 msec. It can be seen in Fig. 2(*c*) that there is no systematic relation between latency and two-shock interval. Figure 2(*d*) shows the cumulated recovery times. There is a steep near-linear recovery of 75% of the axons by 3·5 msec.

Anatomical localization

It has been shown in the previous paper that the N-wave and R-zone can be localized to the superficial shell of tectum containing optic nerve terminals. The localization of the fixed latency fibre spikes can be briefly and accurately described. Most of the fibre spikes were recorded in the N-zone. A small minority was recorded in the R-zone. *None* was recorded deeper. Therefore all the fibre spikes were confined to the superficial tectal laminae in the region where optic nerve fibres and their terminals can be found anatomically. No evidence can be provided as to whether these spikes are recorded from the axon, or from the much branched terminal arborization.

Two complementary observations were made. Spikes with large amplitude and typically somatic conformation were never observed to be fired at fixed latency in tectal tracks. Thus no evidence can be provided that centrifugal cells of origin are located in tectal laminae. Spikes with the fibre conformations described above were never observed responding at variable latency. This provides a clear distinction between the axonal spikes and the 156 units described in the next section.

Each stimulus to the optic nerve-head will activate retinotectal axons orthodromically and antidromically. It will excite antidromically centrifugal axons running from the isthmo-optic nucleus to the retina (Holden, 1966) and also excite centrifugal axons orthodromically. The optic nerve fibres recorded at the lateral tectum were not observed to fire repetitively. Thus the centrifugal fibres to the retina do not cause retinotectal ganglion cells to fire in the conditions of this preparation.

Cell firing

One hundred and fifty-six units were judged to be post-synaptically fired cells by the following criteria: (i) their spike properties; (ii) their variable latency; (iii) their long recovery time to paired twice-threshold stimuli; and (iv) the evidence of non-collision.

Spike properties

The cells spikes had a diphasic positive-negative conformation, a duration of 1–2 msec, and ranged in amplitude from 0·5 to 2·5 mV. The largest spikes tended to have positive and negative phases equal in amplitude. In the superficial tectum units tended to be fleeting and unstable.

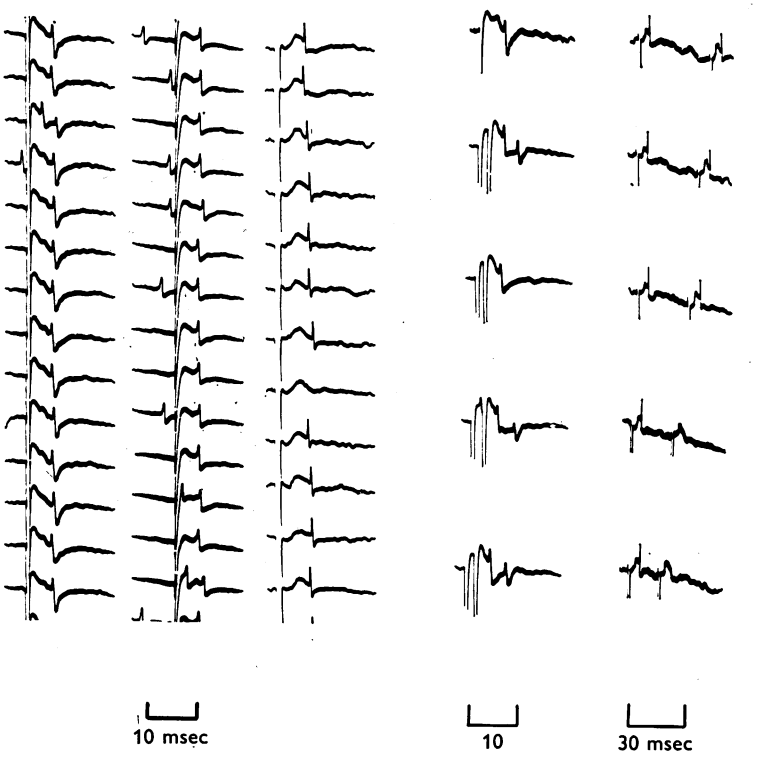

Fig. 3. (*a*) Three trans-synaptically fired cells, in N-zone, R-zone and P-zone. See text for details. (*b*) Response of two cells to paired stimuli.

Units recorded deeper had larger amplitudes. These post-synaptic responses are all classed as somatic spikes. If action potentials were developed upon tectal dendrites, it was not possible to recognize this as a distinctive event. Deliberate electrode movement generally resulted in a rapid growth in spike amplitude, high frequency firing, and cell death.

Types of response

One hundred and forty of the 156 cells discharged a single spike at variable latency to each stimulus to the optic nerve-head. The responses ranged from stable activation which could follow 10/sec and had short recovery-times, to refractory activation which often failed at 10/sec. Figure 3(*a*) illustrates three stably fired cells, recorded in the N-zone,

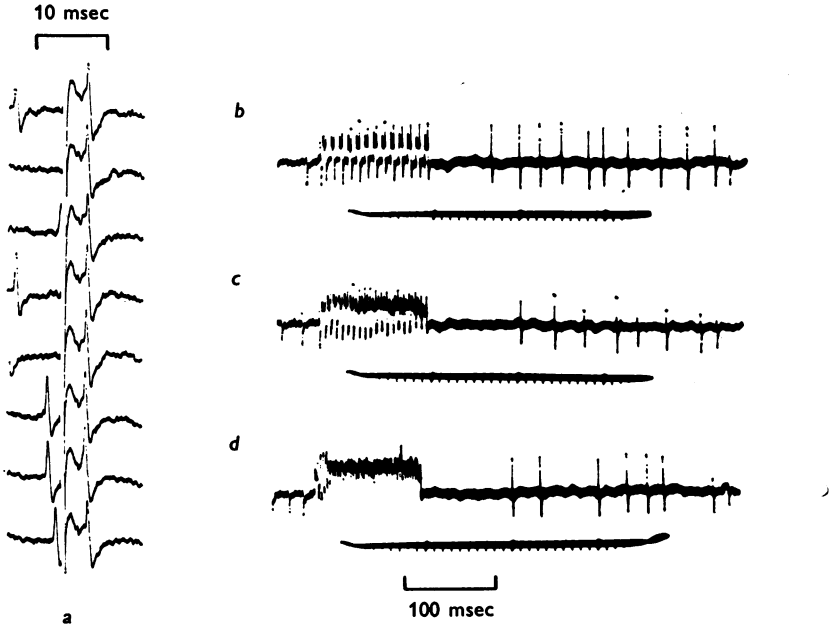

Fig. 4. A cell fired stably in the R-zone. (*a*) Non-collision can be seen in sweeps 3, 6, 7 and 8. (*b*)–(*d*) shows the response to trains of stimuli, with a silent period in the background firing.

R-zone and P-zone respectively. Cell 2, in the R-zone, has been included to illustrate non-collision. In sweeps 2, 4 and 6 a background discharge falls into the interval $2L + R$ yet there is no collision. This proves that the cell is actually being fired trans-synaptically, even though there is relatively little latency variation.

Response to paired stimuli

Figure 3(*b*) shows the response of cells 1 and 3 to paired stimuli. Cell 1, in the N-zone, shows spike inactivation and final failure at separations of 2 msec. Cell 3, recorded in the P-zone, shows that the second response fails at separations of 20 msec, without recovery at shorter intervals.

Response to trains of stimuli

Figure 4 illustrates a cell fired stably in the R-zone. It can be proved to be trans-synaptically fired by the evidence of non-collision (*a*). Its two-shock interval was 3 msec. It follows a train of stimuli 120 msec in duration to 158/sec. Gradual spike inactivation begins at 108/sec, and is complete by 158/sec. Each train was followed by a silent period in the back-

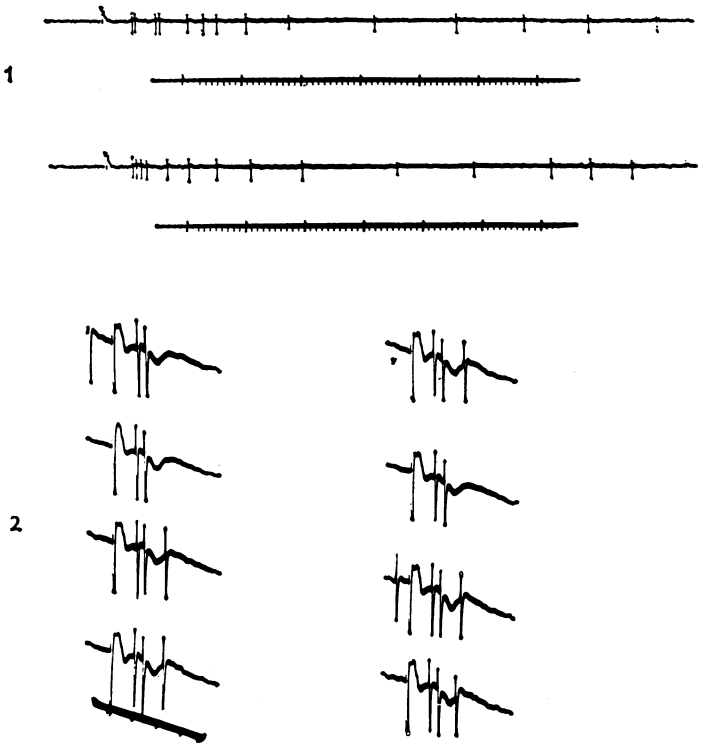

Fig. 5. Repetitive firing of tectal cells. In (1) the time markers show 100 and 10 msec intervals. In (2) the time markers show 10 msec intervals. See text for details.

ground firing. The length of the silent period was greatest when the train was at 125/sec. The mean rate of background firing was 40/sec, with an interspike interval of 25 msec. The longest period of silence was 6 times as long, suggesting that afferent inhibition was involved in addition to the possible effect of after-hyperpolarizations.

Repetitive firing

Sixteen of the 156 cells showed repetitive firing to single retinal stimuli. Since this was not observed in the axonal spikes, it suggests that the firing is the result of intra-tectal circuitry. Two extreme types of repetitive firing are illustrated in Fig. 5. Figure 5(*a*) shows a cell in the N-zone, recorded at first contact with the tectum. It fired a single spike in the trough of the N-wave with the retinal stimulus straddling threshold. As the stimulus was increased, the cell fired a cluster of spikes at a latency of 45 msec, eventually producing a prolonged discharge lasting for 800–1000 msec, and totalling 15-16 spikes. Figure 5(*b*) shows a cell with a spike amplitude of 2·5 mV. It fires three spikes at a latency of 9 msec, in a stereotyped burst discharge.

Afferent inhibition

Evidence suggestive of afferent inhibition could be obtained when the spike triggered from the retina produced a period of silence in the background firing. This was observed in most cells that showed background firing. However, this effect could be due to after-hyperpolarization following the somatic spike. It was possible to demonstrate afferent inhibition more conclusively for some cells by showing that an increase in the amplitude of the retinal stimulus could prolong the duration of the silent period. One example is described here. The cell was fired trans-synaptically at a stable latency of 4 msec, and was located in the R-zone. In the absence of a retinal stimulus there was brisk background discharge. With a 5 mA stimulus to the optic nerve-head the cell fired once only, and the triggered spike was followed by a silent period extending for 35 msec. With a 10 mA-stimulus the cell still fired a single spike, though the silent period was extended to 55 msec. This proves that a trans-synaptic inhibitory constraint was in action, in addition to the possible effects of after-hyperpolarization.

Timing of cell discharge in the N-zone and P-zone

The timing of unitary tectal firing is summarized in Fig. 6. The ordinates represent no. of units. The majority of cells in the N- and R-zones were fired between the latencies of 3 and 8 msec. There is a correspondence between the earliest cell firing and the rising phase of the N-wave. This would be expected if the N-wave is the sink of synaptic potentials which fire these cells monosynaptically. By comparison, firing in the P-zone does not occur in the 3 msec interval, and the proportion of responses in the 4 msec interval is greatly reduced. This difference in timing would be expected from the anatomical localization of the P-zone to depths *below*

the arborizations of optic nerve fibres. Cell firing in the P-zone must be produced through tectal interneurones. Thus there is a striking correlation between the timing of trans-synaptically fired cell discharges and the field potential profile.

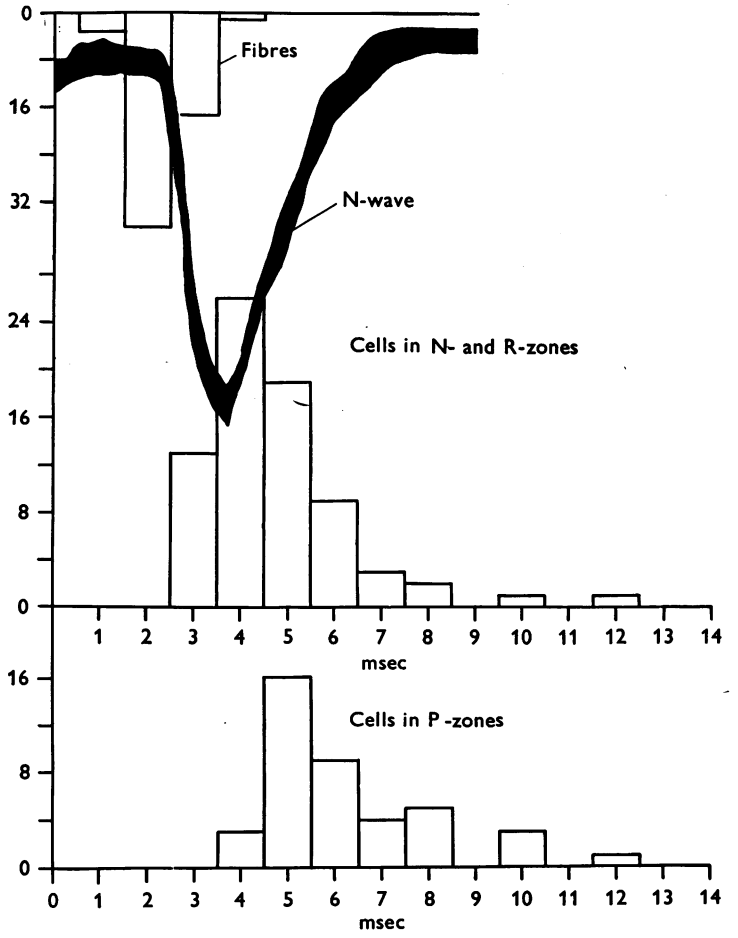

Fig. 6. Ordinates represent number of units. The figure summarizes the time relations of unitary firing. An N-wave is included for comparison. Fibre firing precedes cell firing, while cell firing in the N- and R-zones precedes cell firing in the P-zone.

Recovery times in N-zone and P-zone

Figure 7 summarizes observations on recovery times. The two histograms show recovery times in the N-zone and P-zone. Below them the cumulated recovery curves show how the group of fibre spikes, cells in

N-zone, and cells in P-zone recover. These three groups show progressively greater constraints in action: the cell groups show slower recovery than the fibre group, while the cells in the P-zone recover more slowly than cells in the N-zone.

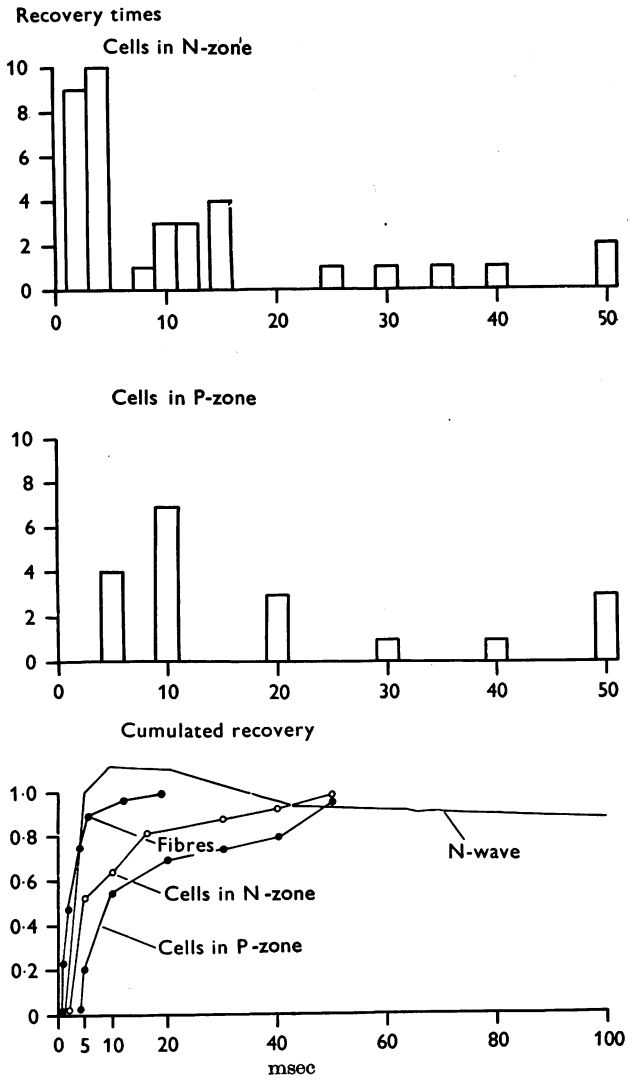

Fig. 7. Recovery times of unitary firing. In the upper two histograms the ordinate is the number of units, and the abscissa is recovery time in msec. In the lower graph the ordinate is the proportion of the total population that has recovered, and the abscissa is the inter-stimulus interval in msec. Recovery of an N-wave has been included schematically.

The histograms allow comparison of recovery times of cells in the N-zone and P-zone. There is a group of cells in the N-zone with recovery times below 5 msec. This group is absent from the P-zone. This suggests that cells in the P-zone are subject to greater constraints than cells in the N-zone. These may be related to the lack of monosynaptic activation in the P-zone, introducing an extra relay and the possibility of further inhibitory actions.

Correlation of unitary firing with the field potential profile

A very distinctive pattern of responses results from radial penetrations into the tectum. In the superficial laminae which contain optic nerve terminals there is a negative-going field potential, with a second sink in the R-zone. Fixed latency fibre spikes, and monosynaptically fired cell spikes, often showing brief recovery times, can be recorded from this zone. There is a sudden polarity reversal, giving the P-wave. Trans-synaptically fired cells continue to be recorded in this zone, though the timing of the earliest firing is delayed when compared with the N-zone. The firing cannot be monosynaptically produced. There is an absence of fibre spikes in the P-zone. There is also an absence of antidromically activated somata in tectal laminae. Many of the monosynaptically fired cells are discharged very stably, but can be proved to be synaptically activated by the evidence of non-collision.

DISCUSSION

The trans-synaptic cell firing found in this study is of the same kind as has been found in the mammalian lateral geniculate nucleus in response to optic nerve stimulation (Arden & Liu, 1960; Suzuki & Kato, 1966). Single volleys in the retinogeniculate and retinotectal pathways can cause stable cell discharge at short latency. Evidence can also be provided for inhibitory phenomena, such as the blocking of spontaneous firing (Arden & Liu, 1960) and post-synaptic inhibition (Suzuki & Kato, 1966). Evidence has been presented in this study showing the operation of afferent inhibition. It would be of interest to investigate whether this underlies the elaboration of receptive field properties in the tectum.

The correlation between unitary responses and the field potential profile supports the suggestion that the N-wave is the sink of excitatory post-synaptic potentials developed by tectal dendrites. The N-wave bears the same temporal relation to the unitary fibre spikes as does the end-plate potential to unitary motor nerve spikes (cf. Katz & Miledi, 1965). This study has shown with unitary responses that the tectum is activated radially from the retinotectal pathway. Optic nerve fibre spikes occur most superficially, at the shortest latencies. Cell discharge in the N-zone

occurs later than fibre firing, and earlier than cell discharge in the deeper P-zone.

It has been shown previously that radial penetrations into the pigeon tectum (Hamdi & Whitteridge, 1954; Wylie, 1962) encounter cells serving a restricted retinal area. It seems possible therefore that the tectum may be composed of columnar subunits. The results of this study are compatible with the possibility that these units process visual inputs radially. It would be of interest to investigate the receptive field organization of cells in the N-zone and P-zone with this possibility in view.

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