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TONIC AND REFLEX SYNAPTIC ACTIVITY RECORDED IN CILIARY GANGLION CELLS OF ANAESTHETIZED RABBITS

BY DAVID A. JOHNSON AND DALE PURVES

From the Department of Physiology and Biophysics, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, U.S.A.

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SUMMARY

1. We have studied patterns of synaptic activity in rabbit ciliary ganglion cells by intracellular recording *in vivo*, and have examined the morphology of functionally characterized neurones by intracellular injection of horseradish peroxidase.

2. Nearly all of the neurones studied (293 of 300) received tonic synaptic input from preganglionic neurones. This tonic activity was not decreased by darkness or by acute optic nerve section.

3. The rate of tonic synaptic activity recorded in the vast majority of neurones (94%) changed in response to retinal illumination. Most ganglion cells showed an increased rate; some cells, however, showed decreased activity during illumination.

4. The rate of synaptic activity recorded in ciliary neurones tended to be progressively higher in neurones with more complex geometries.

5. Neurones with similar reflex properties included cells that lacked dendrites and cells with complex dendritic arborizations; conversely, neurones with similar geometries often had different reflex characteristics.

6. The synaptic activity arising from different preganglionic axons innervating the same ganglion cell was not temporally linked in any obvious way.

7. The relevance of these results to the regulation of the number of axons that innervate target neurones is discussed.

INTRODUCTION

A general feature of neural organization is the convergence of multiple axons onto individual target neurones. Little is known, however, about the way in which the number of axons that innervate nerve cells is controlled. Some insight into this problem has been provided by the observation that convergence of inputs is affected by patterns of neuronal activity. In the primary visual cortex of cats and monkeys, for example, axons innervating the same neurone evidently compete with one another according to the timing of impulse activity amongst the competitors (Hubel & Wiesel, 1965; see also Wiesel & Hubel, 1965; Hubel, Wiesel & LeVay, 1977). A second clue about the control of convergence arises from studies of the peripheral autonomic nervous system in mammals (see Purves, 1983). In some mammalian autonomic ganglia the number of different axons that innervates each target neurone correlates

closely with the geometrical complexity of the post-synaptic cell. In the rabbit ciliary ganglion, for example, neurones that lack dendrites generally receive innervation from a single preganglionic axon, whereas neurones that have dendrites receive a number of different inputs that increases with the complexity of each cell's dendritic arbor (Purves & Hume, 1981). This suggests that post-synaptic geometry influences convergence by mitigating competition between different axons innervating the same cell (Purves & Hume, 1981; Hume & Purves, 1981, 1983; Purves, 1983).

In the present study we have recorded tonic and reflex synaptic activity in ciliary ganglion cells in anaesthetized rabbits, and determined the geometry of some of the neurones studied physiologically. The purpose of this effort was to examine the relationship between activity, cell shape, and the number of axons that ultimately innervate each ganglion cell.

Some of the results have appeared in abstract form (Johnson & Purves, 1982).

METHODS

Adult New Zealand white rabbits (2·5–3·5 kg) were anaesthetized with ethyl carbamate (1·0–1·5 g/kg, I.V.). This dosage provided a satisfactory level of surgical anesthesia for the duration of the experiment; a brisk pupillary light reflex, however, could still be elicited by retinal illumination. Animals were intubated through a tracheotomy, ventilated with room air, and the head stabilized in the right lateral position with ear bars. The electrocardiogram was monitored continuously and end-expiratory CO_2 concentration maintained at 4–6 % by adjustments of tidal volume (18–25 ml).

A wide incision was made below the right eye to expose the globe and extra-ocular muscles just behind the limbus. The inferior oblique, inferior rectus and a portion of the retractor bulbi muscles were cut and retracted to expose the optic and ciliary nerves (Fig. 1). The ciliary nerve was followed centrally to its disappearance under the edge of the medial rectus muscle which was then partially divided to reveal the ganglion. A ring-shaped metal platform above the incision carried adjustable retractors which were used to pull back the surrounding tissues and fully expose the ciliary ganglion. Particular care was taken to avoid injury or vascular compromise to the optic, oculomotor, and sympathetic nerves in the orbit. After the surgery was complete, animals were paralysed with curare (1·0 mg/kg, I.M.). The ganglion and optic nerve were kept immersed in a pool of oxygenated saline.

In additional experiments one or both optic nerves were severed acutely close to their emergence from the globe.

Intracellular recording

Ganglion cells were impaled with glass micro-electrodes filled with 0·5 M-potassium citrate (50–80 MΩ) or with 5 % horseradish peroxidase (HRP) in potassium acetate, as in previous *in vitro* studies (Johnson & Purves, 1981; Hume & Purves, 1981). Because of arterial pulsation, satisfactory impalements were more difficult to obtain *in vivo* than in isolated ganglia. Our usual criterion of a successful impalement *in vitro* is an action potential amplitude of 60 mV or greater; although many impalements *in vivo* met this criterion, the incidence of damage and reduced action potential amplitude was greater. We therefore included impalements of cells with action potential amplitudes as low as 30 mV in the present study.

Measurement of ganglion cell activity

In preliminary work we found that virtually all ganglion cells impaled at the beginning of an experiment gave responses to retinal illumination of the ipsilateral eye. In some animals, however, there was deterioration of the reflex response to light (but not of tonic synaptic activity), presumably because of compression or other compromise of the retina or optic nerve. In most of these instances we found that if two consecutive cells were unresponsive, many subsequent cells did not respond to retinal illumination. Therefore, we arbitrarily stopped an experiment when two successive impalements failed to show an ipsilateral reflex response; roughly one in ten experiments

was terminated for this reason. If a particular cell failed to respond to retinal illumination but subsequent cells did, the unresponsive cell was included in the study.

Experiments were carried out in a dimly lit room (ambient illumination of approximately 0·1 cd/m²). The eyes were stimulated independently by fibre-optic light pipes positioned about 3 cm from each cornea so that illumination of one eye caused little or no direct illumination of the other.

The frequency of tonic synaptic activity was estimated by averaging five determinations of the number of action potentials and subthreshold excitatory post-synaptic potentials (e.p.s.p.s) occurring in one second. For analysis of the interval distributions of synaptic activity, tonic activity

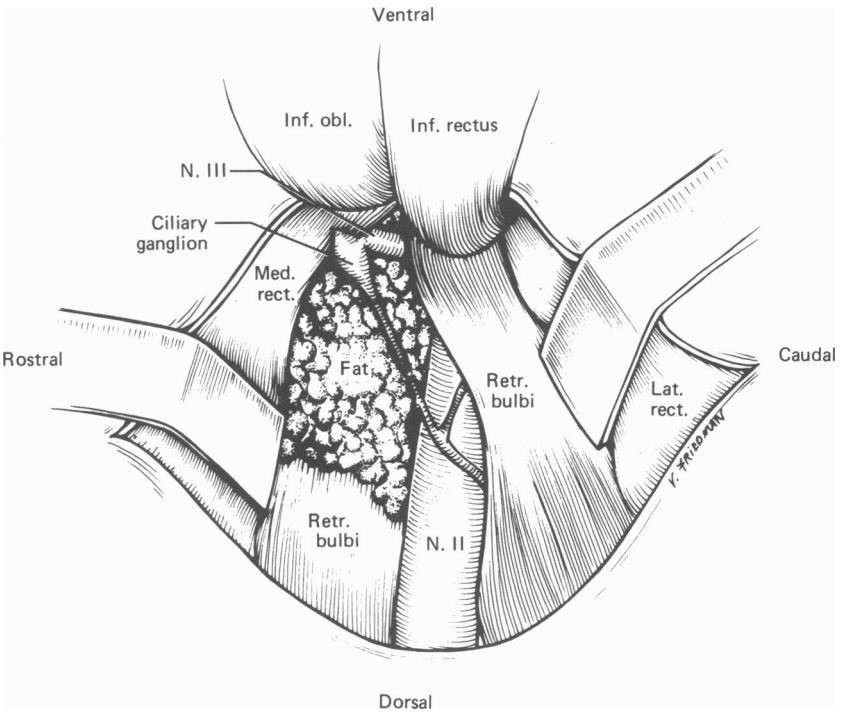

Fig. 1. Diagram of the surgical exposure of the rabbit ciliary ganglion (looking down on an animal in the right lateral position). The inferior oblique (Inf. obl.), inferior rectus (Inf. rectus) as well as some of the retractor bulbi (Retr. bulbi) muscles have been reflected; a portion of the medial rectus (Med. rect.) muscle has also been incised to reveal the ganglion. The major post-ganglionic ciliary nerve is joined by a post-ganglionic sympathetic nerve at the point where it crosses over the optic nerve. N. II = optic nerve; N. III = oculomotor nerve; Lat. rect., lateral rectus muscle.

in some cells was recorded for up to several minutes on moving film or magnetic tape. Although the intensity of the illumination was sometimes varied, it was convenient to use moderately intense illumination for routine testing of impaled ganglion cells (about 600 cd/m²).

In all neurones impaled we estimated the number of innervating axons by counting the number of different classes of synaptic responses discernible on film records (see Fig. 2*C*, *D*). The accuracy of this estimate was limited by at least three factors. First, some preganglionic cells may have been inactive and thus would not be counted. Secondly, if two or more inputs to the same cell elicited identical post-synaptic responses, they would be scored as arising from a single innervating axon. Thirdly, small inputs to a multiply innervated cell might be obscured by larger e.p.s.p.s and/or action potentials. Since previous studies showed that there are few if any connexions between ganglion cells under normal circumstances (Johnson & Purves, 1981), we have assumed that all the synaptic responses arose from activity in preganglionic neurones.

Finally, a small number of experiments was conducted in total darkness to assess tonic activity in the absence of light. In these experiments levels of activity were followed by an audiomonitor and recorded on film using a closed hood over the oscilloscope.

Intracellular injection of horseradish peroxidase

Some cells which had been studied electrophysiologically were subsequently injected with HRP to assess their geometry. The methods of enzyme injection and the processing of ganglia for viewing as whole mounts were the same as those used in previous studies (Johnson & Purves, 1981; Purves & Hume, 1981).

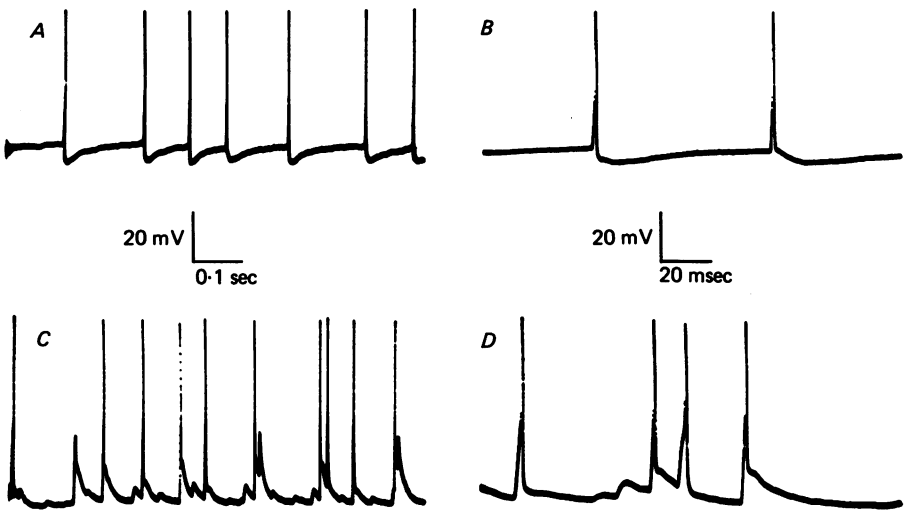

Fig. 2. Tonic synaptic activity recorded in ciliary ganglion cells in ambient light (approximately 0·1 cd/m^2). *A* and *B* are records at two different sweep speeds of a neurone which apparently received input from only a single preganglionic axon. Each synaptic response was suprathreshold, and in this case no responses occurred at intervals of less than about 65 msec (see also Fig. 6). Hyperpolarization of such neurones by current injection blocked action potentials and revealed the underlying e.p.s.p.s (not shown). *C* and *D* are similar records of a ganglion cell that received innervation from several different axons. In addition to suprathreshold responses, subthreshold synaptic activity of several different amplitudes is apparent. Note that, in contrast to the recordings shown in *A*, *B*, responses often occur at intervals of only a few milliseconds.

RESULTS

Tonic synaptic activity in ciliary ganglion cells

Nearly all of the ciliary ganglion cells that we impaled (293 out of 300) showed excitatory post-synaptic potentials in ambient light (Fig. 2). In the majority of cells, one or more of these synaptic potentials exceeded threshold; action potentials occurred at frequencies of 1–20/sec (see also Fig. 4 below).

Many neurones had only suprathreshold responses which occurred at a relatively low frequency (Fig. 2*A*, *B*); this pattern would be expected in ganglion cells that received innervation from only a single axon (see Johnson & Purves, 1981; Hume & Purves, 1983). The number of these apparently singly innervated cells, 83 of 300 neurones studied, is consistent with the observation that about 25–30% of ciliary ganglion neurones receive supratheshold input from only a single preganglionic axon

in isolated ganglia studied *in vitro* (Johnson & Purves, 1981; Purves & Hume, 1981). Neurones judged to be singly innervated *in vivo* were found, after HRP injection, to have few if any dendrites (see below). Since cells lacking dendrites are generally innervated by a single axon when tested *in vitro* (Purves & Hume, 1981), our conclusion that most neurones with these electrophysiological characteristics *in vivo* were contacted by one axon is probably correct.

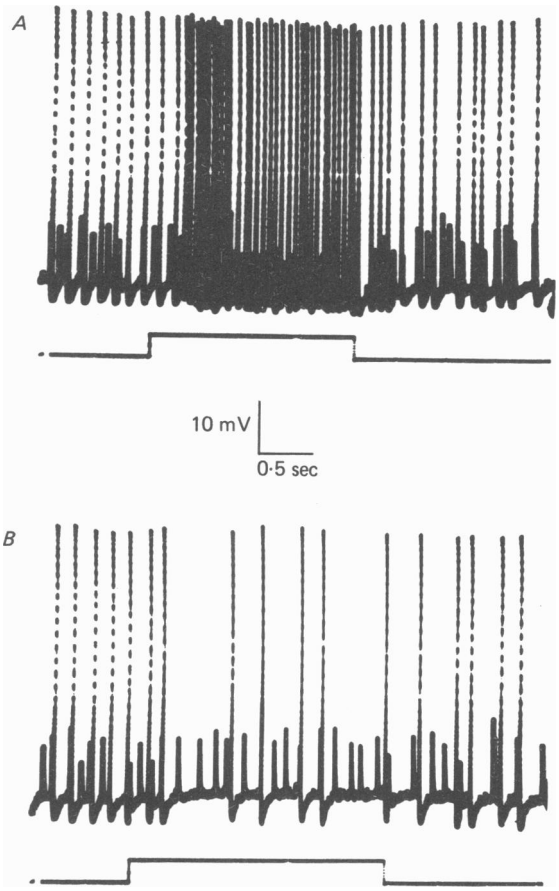

Fig. 3. Example of a ganglion cell that showed increased synaptic activity during illumination of the ipsilateral retina (*A*), and decreased activity during illumination of the contralateral retina (*B*). This reflex pattern was commonly observed (see Table 1). Lower trace indicates onset and duration of the light stimulus.

In most neurones, however, synaptic inputs from more than one preganglionic axon were apparent. In these cells, post-synaptic responses of several different amplitudes were recorded (Fig. 2*C*, *D*), and the over-all frequency of synaptic events was higher than the incidence of synaptic responses in cells apparently innervated by a single axon (cf. Fig. 2*A*, *B* and 2*C*, *D*; see also Fig. 4 below). In contrast to the neurones judged to be singly innervated, synaptic responses in these cells often occurred at short intervals and could sum. Although our estimates of the number of different axons impinging on individual neurones are subject to considerable error (see

Methods), some ganglion cells certainly received at least four or five different inputs. This is within the range of multiple innervation determined *in vitro* (up to seven inputs), where the number of innervating axons can be estimated more accurately (Johnson & Purves, 1981; Purves & Hume, 1981).

To rule out the possibility that the tonic synaptic drive to ganglion cells might be reflexly induced by retinal illumination in ambient light, some cells were monitored in total darkness. Recordings from twelve neurones in two animals in the absence of light showed no obvious reduction of tonic synaptic responses. A further possibility was that the tonic synaptic drive to ganglion cells might arise from the spontaneous retinal ganglion cell activity known to occur in mammals independently of light stimulation (Kuffler, Fitzhugh & Barlow, 1957). To test this, we cut the ipsilateral optic nerve just before impaling ganglion cells in three animals. Synaptic activity at usual frequencies was again observed in each of twenty-nine ganglion cells tested. Finally, since many ganglion cells are influenced by illumination of the contralateral eye (see below), we cut both optic nerves in two animals. This procedure also failed to reduce tonic activity in all ten ganglion cells studied under these conditions. Thus most preganglionic cells are normally active in the absence of any retinal input.

Reflex activity of ciliary ganglion cells induced by retinal illumination

Of the 300 neurones studied, 281 showed an obvious change in the rate of synaptic activity as a result of illumination of the ipsilateral or, less often, the contralateral retina (Table 1). In general, cells in the anaesthetized animal had a fairly high threshold for this reflex response; thus small changes in ambient illumination (turning on a nearby desk lamp, for example) had no appreciable effect on the synaptic activity recorded in ganglion cells. Responses of individual neurones to retinal illumination, however, appeared to be graded as a function of the strength of illumination (see also Inoue, 1980*b*). Typically, the reflex response began after a brief latency and was maintained with relatively little decrement during the stimulus (Fig. 3).

Most neurones impaled (86%) showed increased synaptic activity in response to illumination of the ipsilateral retina (Fig. 3*A* and Table 1). Many cells, however, showed a decreased rate of synaptic activity when only the contralateral retina was illuminated (41%; Fig. 3*B* and Table 1). About one-third of the neurones studied showed both these responses (Table 1). Simultaneous bilateral stimulation was not applied routinely, but in those cells that were tested in this way, increased activity from ipsilateral stimulation predominated. In no instance were hyperpolarizing synaptic potentials seen in ciliary ganglion cells; neither was there any evidence of a conductance change during decreased activity (i.e. the amplitude of post-synaptic potentials was not affected in any obvious way; see Fig. 3*B*). Thus direct inhibition of ganglion cells does not occur.

In addition, we encountered some cells that were excited by contralateral retinal illumination (about 10% of the neurones studied), and a few neurones (about 3%) that were inhibited by ipsilateral retinal illumination (Table 1). Whereas the vast majority of cells showed an increase in the frequency of synaptic activation with retinal illumination to one eye *or* the other, a small but significant number of cells (about 7%) showed *only* decreased activity in response to light. Although anaesthetic effects or local damage to the oculomotor nerve may have obscured excitatory inputs

to these cells, the fact that over-all synaptic activity was in some cases reduced by light raises the possibility that a minority of ganglion cells are antagonistic to the majority.

Finally, a few ganglion cells (about 6 %) did not respond to light shone into either eye (Table 1). Although again we cannot rule out artifact as a result of anaesthesia or local damage, it is possible that a few neurones in the ciliary ganglion do not subserve the light reflex. Indeed, some gangion cell axons, when stained with HRP, do not emerge in the post-ganglionic nerve but project into small post-ganglionic branches whose destination is not known (Johnson & Purves, 1981; Purves & Hume, 1981); moreover, retrograde labelling of ciliary ganglion cells after injection of HRP into the anterior chamber fails to stain about 6 % of ciliary ganglion neurones (D. A. Johnson, unpublished).

TABLE 1. Proportion of ciliary ganglion cells showing different reflex responses to retinal illumination

Retinal illumination		
Ipsilateral	Contralateral	% of cells showing response
+	+	9·7
+	−	34·0
+	0	42·3
−	+	0
−	−	2·7
−	0	0
0	+	0·3
0	−	4·7
0	0	6·3

Values are percentages based on 300 cells studied; + = increased rate of synaptic activity; − = decrease; 0 = no change. The designations include all the inputs observed in a particular cell; although different inputs to the same neurone often responded independently (see Fig. 8), they did not react oppositely to a stimulus. Only 7 of the 300 cells impaled showed no synaptic activity either tonically or in response to retinal illumination.

Nevertheless, the vast majority of all ciliary ganglion cells (at least 94 %) respond in some way to retinal illumination and in this sense mediate the light reflex.

Responses of ciliary ganglion cells to other stimuli

The tonic activity of some ganglion cells was also influenced by stimuli other than retinal illumination. Of 145 cells tested, 53 responded to noxious and/or auditory stimuli. The effect of such stimuli was not necessarily the same in each responsive cell; for example, pinching the foot increased the frequency of synaptic potentials in some cells, but halted activity in others. Of the 51 cells whose activity was affected by a pinch, about half were excited, whereas the level of tonic activity was decreased in the remaining cells. Only 10 of the 145 cells tested responded to the bell of an ordinary laboratory timer; the activity of three cells was increased while the other seven cells showed decreased activity. In one case, a cell tonically active in ambient light became silent when a nearby telephone rang, but resumed firing during the period between rings. Although we did not study these effects systematically, we

noted that in those instances where non-visual stimuli decreased tonic activity, the inhibitory effect was easily overridden by retinal illumination.

Geometry of ganglion cells whose activity patterns had been characterized

To examine the shape of neurones with particular tonic and reflex behaviours, fifty neurones were labelled with horseradish peroxidase after electrophysiological characterization *in vivo*. As in a previous study (Purves & Hume, 1981), the complexity of ciliary neurones ranged from cells without dendrites to cells with as

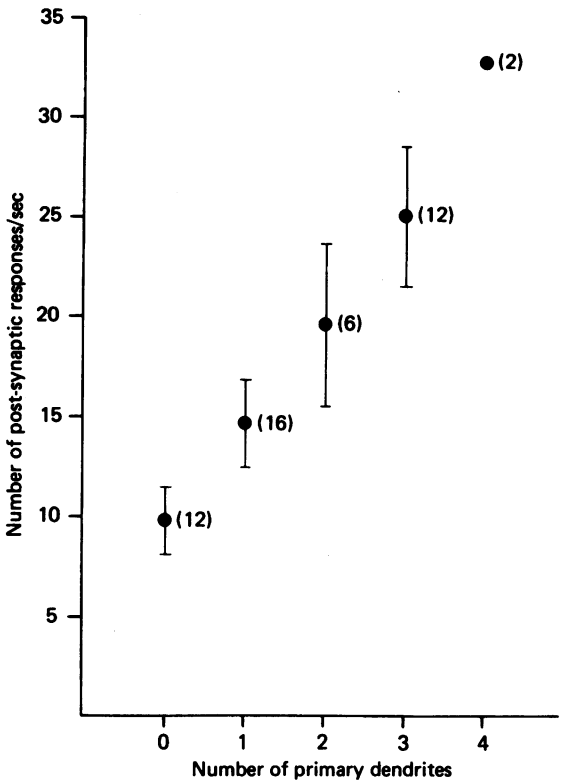

Fig. 4. Frequency of tonic synaptic potentials recorded in neurones with different numbers of primary dendrites. The frequency of synaptic responses increased progressively as a function of dendritic complexity. Number of cells in each category is shown in parentheses. Primary dendrites were defined as processes other than the axon which extended radially from the cell body for at least 15 μm; most dendrites are of course much longer (see Fig. 5).

many as eight primary dendrites. As expected, neurones judged to be innervated by a single axon had relatively simple geometries whereas multiply innervated cells were more complex (see Fig. 5).

Interestingly, the over-all frequency of tonic synaptic activity observed in ganglion cells increased progressively with the complexity of the dendritic arbor (Fig. 4). There was also a tendency for neurones having more complex geometries (and therefore receiving innervation from several different axons) to respond not only to light but

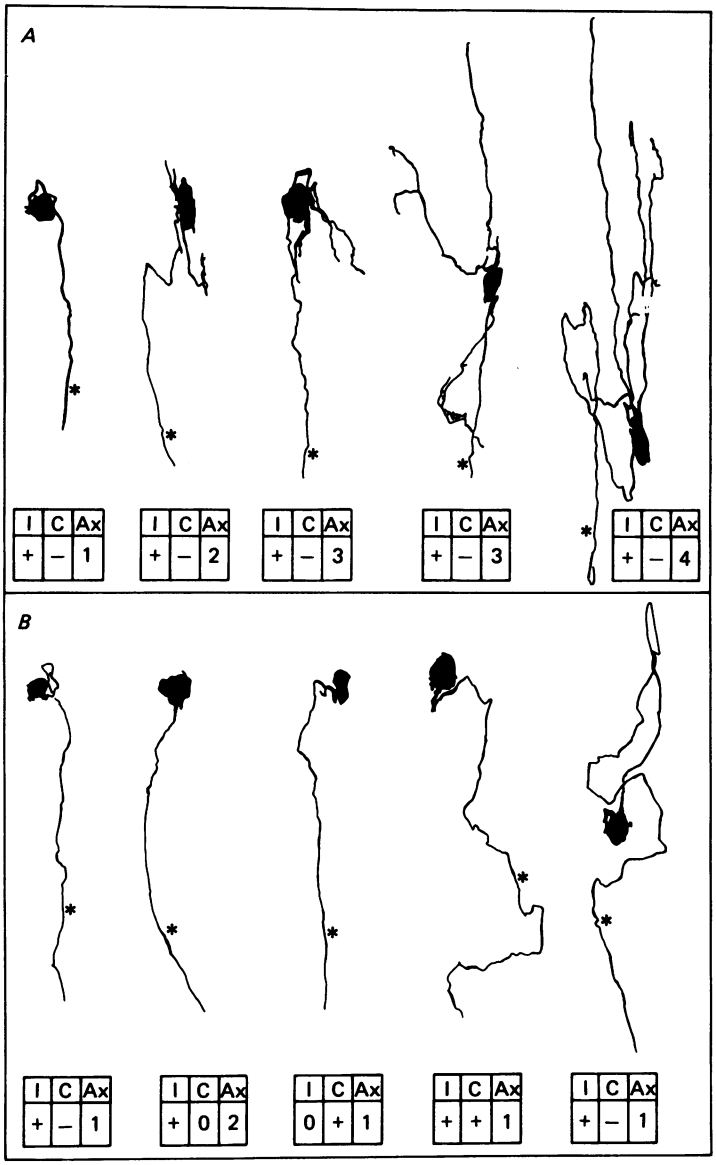

Fig. 5. Camera lucida drawings of neurones whose reflex behaviour to retinal illumination had been characterized. The box below each neurone shows the cell's reflex response and the number of innervating axons (I = response to ipsilateral illumination; C = response to contralateral illumination; Ax = estimated number of innervating axons; +, −, 0 = increase, decrease, or no effect, respectively, in the rate of synaptic activity). *A*, five neurones which exhibited the same reflex behaviour. Cell shapes span the full range of geometrical complexity found in the ganglion as a whole. *B*, five neurones of similar geometry (in this case, neurones lacking dendrites). Note that most of these cells have diverse reflex behaviours. Asterisk denotes the axon; calibration can be taken from the width of each box which corresponds to 0·1 mm.

to stimuli of other modalities. For example, amongst neurones judged to receive only one innervating axon (with, on average, less than one primary dendrite), only one of six cells responded to non-visual stimuli (a pinch on the foot or a loud noise); in contrast, six of seven neurones judged to receive four axons (with an average of three primary dendrites) were responsive to pinch or noise. The fact that cells with relatively complex dendritic arbors show more tonic activity and a greater diversity of reflex responsiveness is presumably explained by their innervation from a proportionally greater number of the approximately forty preganglionic neurones in the ipsilateral brain stem (Johnson & Purves, 1981).

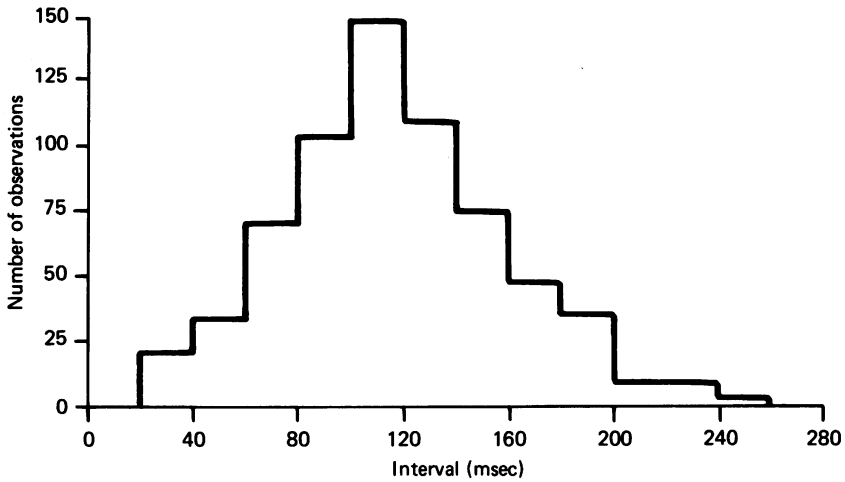

Fig. 6. Interval distribution of synaptic responses in a neurone judged to be innervated by a single axon (see Fig. 2*A*, *B*). In five such cells that were analysed, responses occurred with a preferred interval of preganglionic firing, in this case about 116 msec ($n = 669$ intervals).

Aside from this, we found no obvious correlation between the dendritic configuration of ganglion cells (or the number of axons that innervated them) and the reflex properties that were examined. Thus cells showing a particular response (e.g. ipsilateral excitation/contralateral inhibition) had a variety of geometries (Fig. 5*A*). Conversely, neurones with similar geometries did not necessarily share reflex characteristics: cells that lacked dendrites often behaved differently from one another (Fig. 5*B*), as did neurones with complex dendritic arbors.

Patterns of synaptic activity in singly and multiply innervated ganglion cells

To look explicitly at temporal patterns of synaptic activity in individual ganglion cells, we examined the interval distribution of the synaptic responses in five neurones judged to be innervated by a single axon, and five multiply innervated cells (see Fig. 2). In the five singly innervated cells (see Fig. 2*A*), post-synaptic events did not simply occur at random, but showed some degree of interval preference (Fig. 6). In each of the multiply innervated cells, however, events occurred with little or no interval preference (Fig. 7). Evidently the occurrence of a particular synaptic response in a multiply innervated cell is largely or wholly unrelated to preceding synaptic events arising from other inputs to that neurone. There is, therefore, no obligate temporal

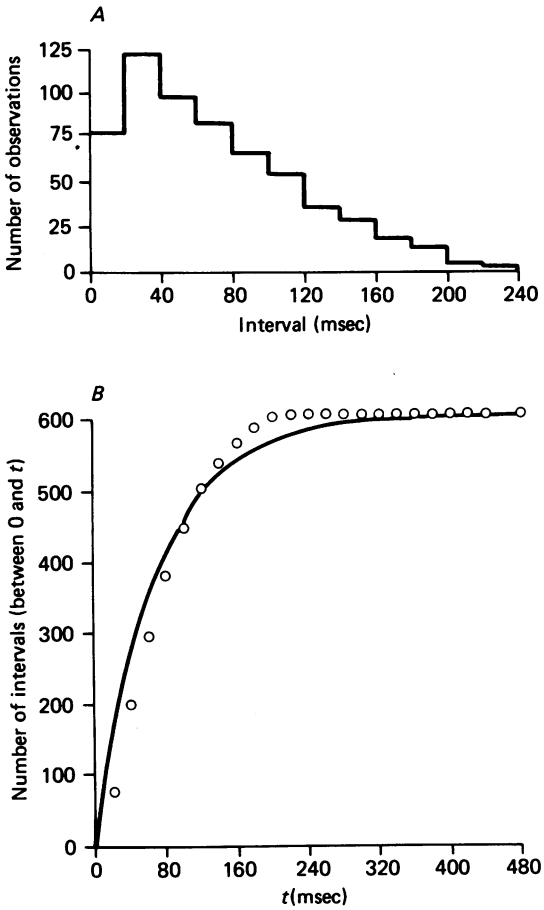

Fig. 7. Synaptic activity of different axons innervating the same ganglion cell. *A*, distribution of the intervals between synaptic responses in a neurone receiving innervation from several different axons ($n = 609$ intervals). In contrast to neurones innervated by a single axon (see Fig. 6), there is little or no interval preference. The somewhat diminished number of observations at very short intervals may represent our inability to distinguish events occurring within a few milliseconds (i.e. the limit of accuracy of the measurements). *B*, the number of intervals observed between 0 and t is plotted against the summed interval duration, t. These values (open circles) fall close to the theoretical distribution (continuous line) for random occurrence given by the equation $y = N[1-\exp(-t/T)]$, where N = total number of observations, and T = mean interval (see Fatt & Katz, 1952). All five cells analysed in this manner showed a similar pattern. This indicates that there is little or no concurrence of activity in different preganglionic axons innervating the same ganglion cell.

link between the activity of different inputs to the same ganglion cell, at least on the time scale of a few hundred milliseconds.

In many multiply innervated neurones we could follow the behaviour of two different inputs to the same cell during reflex activation. In such cases, we could often confirm directly that different inputs to the same cell responded independently (Fig. 8; see also Fig. 3*B*). For example, one input might increase or decrease its rate of

firing quite markedly upon ipsilateral retinal stimulation, whereas another input to that cell might be unaffected. These observations provide further evidence that the several inputs to multiply innervated ciliary ganglion cells are independently active.

DISCUSSION

Organization of the light reflex

Although the aim of this study was to explore the relationship of activity and cell shape to the innervation of individual ganglion cells (see below), the results are also relevant to the way in which the light reflex is organized. Intracellular recording from the neurones in the final common pathway for the pupillary light reflex make several points in this regard. First, virtually all ciliary ganglion cells in the anaesthetized rabbit receive ongoing synaptic input which is independent of retinal activity. Secondly, illumination can either increase or decrease the level of activity of particular preganglionic axons, an observation which adds considerable complexity to the way in which this system must be regarded. One might have imagined that increased illumination would simply increase the activity of ganglion cells, since the ultimate response is pupillary constriction. In fact, intracellular recordings from the cat ciliary ganglion in an earlier study showed some cells to be inhibited by light (Melnitchenko & Skok, 1970); inhibition of some rabbit ciliary ganglion cells had also been suggested by extracellular recordings from the ciliary nerve (Inoue, 1980*a*). The present study confirms these findings.

The significance of the inhibition of tonic activity in some ciliary ganglion cells by retinal illumination is not clear. Observation of normal rabbits under varying conditions of retinal illumination shows that there is little or no consensual light reflex (Inoue, 1980*a*; P. C. Jackson, unpublished observations). The fact that contralateral retinal illumination has a detectable inhibitory effect on the rate of synaptic activity in about 40% of ganglion cells (Table 1) presumably contributes to the lack of a consensual response. Perhaps this is useful in a wall-eyed animal like the rabbit where the two eyes may often be exposed to different light levels.

Neuronal geometry and ganglion cell innervation

In maturity, the number of inputs that rabbit ciliary ganglion cells receive is closely correlated with their geometry (Purves & Hume, 1981). This correlation probably reflects a modulation of axonal competition by post-synaptic cell shape during development (see Hume & Purves, 1981; 1983; Purves, 1983). On the other hand, different geometries and numbers of inputs might be specifically related to reflex function. The present results argue against this. The vast majority of these neurones (at least 94%) are responsive to light and thus, by definition, participate in the light reflex. In this limited sense, the ganglion is relatively homogeneous from a functional point of view. Furthermore, when the detailed reflex behaviour of individual neurones that responded to light was examined with respect to neuronal shape, no clear relationship was apparent. Cells which responded in different ways to retinal illumination often had similar geometries, whereas cells with the same reflex properties often had different geometries. Thus the correlation between geometry and the number of axons that innervate a ganglion cell is not associated with

qualitatively different reflex behaviour. However, cells with complex geometries do have higher rates of tonic synaptic activity (see Fig. 4) and an increased probability of responding to non-visual stimuli. Perhaps multiple innervation in the system provides a means of regulating the level of activity and/or the breadth of reflex responsiveness of individual neurones.

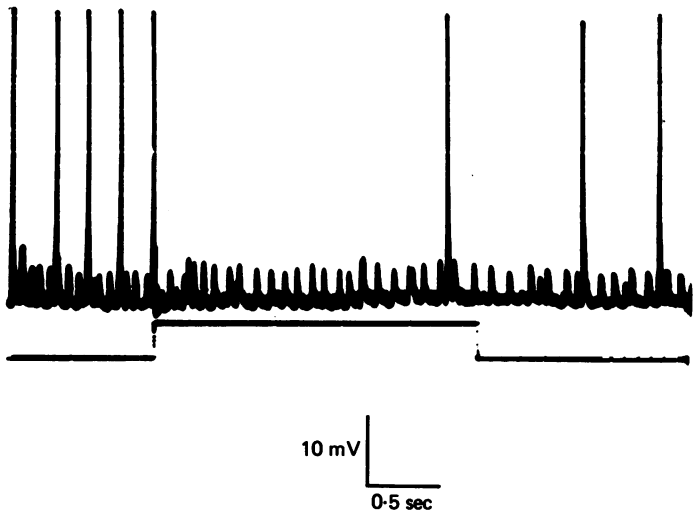

Fig. 8. Independent reflex responsiveness of inputs to the same ganglion cell during retinal illumination. This ganglion cell was innervated by at least two different axons, one of which consistently elicited a subthreshold response. Light stimulation stopped the activity arising from the suprathreshold input, but had little or no effect on the subthreshold inputs. A somewhat less striking example of differential responsiveness is shown in Fig. 3*B*. Such observations provide further evidence that the activity of different preganglionic neurones innervating the same ganglion cell is not closely linked.

Patterns of synaptic activity elicited by different axons innervating the same ganglion cell

Evidence from neural systems as diverse as the mammalian visual pathway (Hubel & Wiesel, 1965; Hubel *et al.* 1977) and the mammalian neuromuscular junction (Jansen, Thompson & Kuffler, 1978; Brown, Holland & Hopkins, 1981) suggests that the activity of neural pathways is involved in the formation and maintenance of connexions between nerve cells and their targets. In particular, activity-related interactions between neurones during early post-natal life probably play an important role in the competitive rearrangement of synaptic connexions that leads to adult patterns of connectivity in various parts of the nervous system (see Purves & Lichtman, 1980; Lichtman & Purves, 1981; Purves, 1983; see also Jackson, 1982).

The temporal ordering of activity, rather than simply the level of activity, appears to be a critical determinant of competition between axons innervating the same cell during the development of synaptic connections in the primary visual cortex (Hubel & Wiesel, 1965). Asynchronous activity evidently enhances competitive interactions among axons innervating the same cortical neurone, whereas synchronously active inputs may compete less vigorously. The evidence for this is that the binocular inputs that normally remain on some classes of cortical neurones are more or less

synchronously activated by stimulation of corresponding points on the two retinas; on the other hand, desynchronization of corresponding inputs produced by squint leads to increased monocularity of cortical neurones (Hubel & Wiesel, 1965).

In the rabbit ciliary ganglion, several different preganglionic axons often persist in innervating the same target neurone, having survived the post-natal period of competitive rearrangement that occurs in this ganglion (Johnson & Purves, 1981). Yet the present study shows that activity in the several axons that ultimately innervate the same target neurone in maturity is not linked in any obvious temporal way. Indeed, the reflex responses of different inputs to the same ganglion cell are often independent. Of course, a light shown in the eye will increase the activity of most preganglionic axons; in this weak sense, the activity of inputs must have some synchronization. However, we have found no evidence to suggest that the persistent multiple innervation of many of these neurones is a specific consequence of a temporal coordination of their several inputs.

An alternative explanation of persistent multiple innervation is that post-synaptic geometry modulates competition by allowing inputs from different axons to be relatively separated from one another on the dendritic arbor (Purves & Lichtman, 1980; Hume & Purves, 1981, 1983; Purves, 1983). The basis for this view is that, (1) the number of different axons that innervate ciliary ganglion cells is closely tied to dendritic complexity (Purves & Hume, 1981), and (2) that light and electron microscopical observations of terminals from individual HRP-labelled preganglionic axons on post-synaptic cells indicate some degree of segregation from other unlabelled endings on the same cell (Hume & Purves, 1983; Purves, 1983; C. J. Forehand & D. Purves, unpublished). Perhaps modulation of competition by synchronous activity and by post-synaptic geometry are complementary strategies that neural systems use to achieve appropriate numbers (and types) of inputs to post-synaptic nerve cells.

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REFERENCES

Brown, M. C., Holland, R. L. & Hopkins, W. G. (1981). Excess neuronal inputs during development. In *Development in the Nervous System*. ed. Garrod, D. R. & Feldman, J. D., pp. 245–262. Cambridge: Cambridge University Press.

Fatt, P. & Katz, B. (1952). Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* **117**, 109–128.

Hubel, D. H. & Wiesel, T. N. (1965). Binocular interaction in striate cortex of kittens reared with artificial squint. *J. Neurophysiol.* **28**, 1041–1059.

Hubel, D. H., Wiesel, T. N. & LeVay, S. (1977). Plasticity of ocular dominance columns in monkey striate cortex. *Phil. Trans. R. Soc. B* **278**, 377–409.

Hume, R. I. & Purves, D. (1981). Geometry of neonatal neurones and the regulation of synapse elimination. *Nature, Lond.* **293**, 469–471.

Hume, R. I. & Purves, D. (1983). Apportionment of the terminals from single preganglionic axons to target neurones in the rabbit ciliary ganglion. *J. Physiol.* **338**, 259–275.

Inoue, T. (1980*a*). Efferent discharge patterns in the ciliary nerve of rabbits and the pupillary light reflex. *Brain Res.* **186**, 43–53.

Inoue, T. (1980*b*). The response of rabbit ciliary nerve to luminance intensity. *Brain Res.* **201**, 206–209.

JACKSON, P. C. (1982). Optic nerve section delays synaptic rearrangement in neonatal rabbit ciliary ganglia. *Soc. Neurosci. Abstr.* **8**, 709.

JANSEN, J. K. S., THOMPSON, W. & KUFFLER, D. P. (1978). The formation and maintenance of synaptic connections as illustrated by studies of the neuromuscular junction. *Prog. Brain Res.* **48**, 3–18.

JOHNSON, D. A. & PURVES, D. (1981). Post-natal reduction of neural unit size in the rabbit ciliary ganglion. *J. Physiol.* **318**, 143–159.

JOHNSON, D. A. & PURVES, D. (1982). Neuronal geometry, reflex function, and activity patterns of rabbit ciliary ganglion cells. *Soc. Neurosci. Abstr.* **8**, 709.

KUFFLER, S. W., FITZHUGH, R. & BARLOW, H. B. (1957). Maintained activity in the cat's retina in light and darkness. *J. Neurophysiol.* **40**, 683–702.

LICHTMAN, J. W. & PURVES, D. (1981). Regulation of the number of axons that innervate target cells. In *Development in the Nervous System.* ed. GARROD, D. R. & FELDMAN, J. D., pp. 233–243. Cambridge: Cambridge University Press.

MELNITCHENKO, L. V. & SKOK, V. I. (1970). Natural electrical activity in mammalian parasympathetic ganglion neurones. *Brain Res.* **23**, 277–279.

PURVES, D. (1983). Modulation of neuronal competition by postsynaptic geometry in autonomic ganglia. *Trends in Neurosci.* **6**, 10–16.

PURVES, D. & HUME, R. I. (1981). The relation of postsynaptic geometry to the number of presynaptic axons that innervate autonomic ganglion cells. *J. Neurosci.* **1**, 441–452.

PURVES, D. & LICHTMAN, J. W. (1980). Elimination of synapses in the developing nervous system. *Science, N.Y.* **210**, 153–157.

WIESEL, T. N. & HUBEL, D. H. (1965). Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. *J. Neurophysiol.* **28**, 1029–1040.