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## Cells in the Pretectal Olivary Nucleus are in the Pathway for the Direct Light Reflex of the Pupil in the Rat

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**Key words:** tonic-on cells — pretectum — rat — pupillary light reflex — pretectal olivary nucleus — electrical stimulation

Extracellular microelectrode recordings from 148 single cells in the pretectum of the hooded rat were classified according to their temporal response properties to light stimulation of their retinal receptive fields. Fifty-six cells were classified as tonic-on cells, 22 cells were classified as tonic-off cells, and 53 cells were classified as phasic cells. Seventeen cells could not be assigned to one of these 3 groups. The diameters of the receptive field centers of the tonic-on pretectal cell were clustered about a mean of  $31^\circ$  and the temporal response of these cells was sustained. Constriction of the contralateral pupil was produced by electrical stimulation through the recording electrode at sites containing tonic-on pretectal cells, but not at sites containing tonic-off pretectal cells or phasic pretectal cells. For this reason, we argue that tonic-on cells are likely to mediate constriction in the light reflex of the rat's pupil. Receptive field maps together with electrolytic marking lesions at recording and stimulation sites showed that tonic-on pretectal cells are retinotopically organized and are aggregated in a strip running from the dorso-medial tip of the pretectum to the ventro-lateral boundary. The anatomical distribution of these cells is coextensive with the region known as the pretectal olivary nucleus (PO) in the rat<sup>26,27</sup>.

Using fine microelectrodes, recordings were obtained from 27 axons presumed to be of optic origin (fibers). Of these, 14 were tonic-on, 10 were phasic, 2 were tonic-off, and 2 were unclassified. Recordings from tonic-on fibers were obtained near tonic-on pretectal cells, typically in the most dorsal light-responsive region of the pretectum. These fibers were activated by single pulse electrical stimulation of the optic chiasm. The mean receptive field center diameter of 6 tonic-on fibers was  $10.1^\circ$ , or about a factor of 3 less than that of pretectal tonic-on cells. The mean conduction velocity of 14 tonic-on fibers was 3.1 m/s.

We argue that the tonic-on cells of the PO serve to integrate signals from tonic-on center retinal ganglion cells with adjacent receptive fields to provide signals for constriction of the pupil to neurons in the oculomotor nucleus.

### INTRODUCTION

The pathway for the direct light reflex of the pupil in mammals appears to involve at least two central brain nuclei. The optic fibers subserving the light reflex of the pupil are thought to connect with neurons in (1) the contralateral pretectum (ipsilateral for the consensual light reflex), which in turn project to (2) preganglionic pupilloconstrictor neurons in the contralateral nucleus of Edinger–Westphal (EW)<sup>20,21</sup>. The distribution and response properties of the cells in the cat's EW that subserve pupillary constriction have been analyzed in some detail<sup>29</sup>, but in no mammal has the distribution of constriction-related cells in the pretectum been defined, nor have the response properties of these cells to stimulation of the retina

with light been adequately described<sup>1,28,31</sup>.

Recent studies have shown that the rat's pretectum contains multiple representations of the visual field that overlap with optic terminal projection zones defined by orthogradely transported radiolabel and with distinct aggregations of cell bodies<sup>26,27</sup>. One of these aggregations, the pretectal olivary nucleus (PO), has been implicated in the light reflex of the pupil in the rat, and in other mammals, on the basis of its afferent and efferent connections. The PO receives a retinal projection in every mammal which has been examined<sup>2,3,26,27</sup>, and it has been shown to project to EW in the cat<sup>6</sup> and in the monkey<sup>2,23,32</sup>. (The nucleus of the optic tract (NOT), a pretectal region which has been implicated in pupil control in the cat<sup>28</sup>, apparently does not project to EW<sup>3</sup>.) Although

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the efferent connections of the PO in the rat remain to be examined, there have been two reports of a large proportion of tonic-on cells or 'luminance detectors' in the PO of this animal<sup>10,34</sup>. Such cells could provide signals to oculomotor neurons for constriction of the pupil. Studies of experimentally produced lesions have not supported such a discrete localization of pupil constrictor function in the PO<sup>9,18</sup>, but these results are inconclusive because lesions that exclusively involve the PO have not been produced.

In this study, we sought to describe the response properties of cells in the pretectum of the rat and to define the extent of localization of pupil constrictor function in the rat's pretectum. Our approach was to identify regions containing pretectal cells with defined response properties; to electrically stimulate these regions via the recording microelectrode; and to mark the sites where electrical stimulation produced constriction of the contralateral pupil with small electrolytic lesions. Our findings confirm previous results indicating that the cells in constriction-related pretectal regions respond in a sustained (tonic) fashion to stimulation of the retina with light. The results also establish a link between the activity of these pretectal tonic-on cells and constriction of the pupil. We find that constrictor function is well localized and that it is the PO that contains these constriction-related tonic-on cells. We also describe receptive field properties of these and other physiologically distinct pretectal cells and their presumptive input fibers.

## MATERIALS AND METHODS

### *Surgical preparation*

The experiments were performed on 31 black hooded Long-Evans rats (Charles River Breeding Laboratories). An opaque contact lens was placed over the eye to maintain dark adaptation. As a further precaution, all preparations were made under dim illumination. Animals were anesthetized with an initial intraperitoneal injection of urethane (ethyl carbamate, 1200 mg/kg) and maintained with smaller doses (100–200 mg/kg). A small opening was drilled in the skull and the dura mater was reflected to expose the brain. Electrodes were stereotactically guided into the left pretectal area using the atlas coordinates of Pellegrino et al.<sup>22</sup>. Stimulating elec-

trodes, when required, were placed in the optic chiasm and cemented to the skull using bone screws and dental acrylic. The placement in the chiasm was verified in each case by stimulating the right eye with light and recording the mass response of optic fibers, and by post-mortem examination of the brain.

The right eyelids were removed or retracted; the conjunctiva was cut all the way around, and the flap of conjunctiva which remained attached to the globe was sewn to a metal ring at the end of a thin rod which was rigidly mounted on the stereotaxic instrument. This mechanical stabilization restricted eye movements and allowed accurate mapping of receptive field center boundaries. To ensure that single neuron responses were due to stimulation of the right eye, an opaque contact lens was placed on the left cornea and the left eyelids were sewn shut for the duration of the experiment.

The cornea was kept clear and moist by periodic washes with saline and application of artificial tears (Liquifilm Forte) consisting of an isotonic solution of polyvinyl alcohol. The eye media were periodically checked for clarity with an ophthalmoscope.

### *Recording*

When required, recording was biased for neuronal somata by using commercially manufactured (tungsten) microelectrodes with impedances of 9–12 megohms (Frederick Haer, Brunswick, ME). Optic axons terminating in the pretectum were isolated with (Levick-type) electrolytically sharpened tungsten wires in glass micropipettes<sup>19</sup>. Spikes (action potentials) were displayed on an oscilloscope and heard over a loudspeaker. Spikes were also discriminated with a window comparator and counted in bins of selectable width by an on-line computer (Nova III, Data General). Occasionally, as when receptive fields were mapped, auditory criteria were used. Cells were isolated with both types of electrodes. To distinguish cells from fibers when using the Levick-type electrodes, the waveform of the action potential, and receptive field size were used as guides.

A typical testing sequence for fibers was to first examine the waveform and then stimulate the optic chiasm with single pulses (50  $\mu$ s) of increasing current at a frequency of 1 Hz. When each pulse elicited an action potential, the spike latency was measured

from the face of a storage oscilloscope and the peak stimulus current was noted. Then the receptive field was mapped with small flashing spots of light. Surrounds were mapped with steady adapting spots in the center. Finally, peristimulus time histograms were counted with the computer.

#### *Electrical stimulation*

Stimulating electrodes for implantation in the optic chiasm were made by cutting the tips of tungsten electrodes to produce a flat uninsulated metal surface at the tip. Stimulation in the pretectum was delivered through the unaltered tungsten recording electrodes. Negative-going monophasic pulses of 50  $\mu$ s duration were produced by a constant current stimulator. The range of currents that were effective in producing pupillary constriction was 4  $\mu$ A–30  $\mu$ A in the optic chiasm and 5  $\mu$ A–40  $\mu$ A in the pretectum. The ear bars and incisor bar of the stereotaxic frame served as the reference. Lesions were produced by applying a current of 5  $\mu$ A (anodal DC) for a 5 s period.

#### *Histology*

Rats were anesthetized with sodium pentobarbital and perfused through the heart with phosphate-buffered 10% formalin (pH 7.4). The brains were sunk in 30% sucrose–70% phosphate-buffered formalin, sectioned frozen, mounted on subbed slides and stained with cresyl violet acetate. The mounted slides were projected and aligned onto drawings of stereotaxic atlas sections, and electrode tracks and lesions were traced by hand. When necessary, finer details of lesions and electrode tracks were viewed at a magnification of 100–400 $\times$  and traced free-hand onto the atlas drawings.

#### *Pupillometry*

The diameter of the pupil of the right eye was measured electronically with an infrared television pupillometer modeled after the design of Green and Maaseidvaag<sup>14</sup>. Our instrument was improved by incorporating an optical attachment (allowing the camera to mimic the operation of an ophthalmoscope) that produced a high-contrast, white-on-black image of the pupil on the face of a video monitor. This image was recorded on videotape (to permit calibration) and on-line chart recorder tracings of pupil diameter were also obtained. (A detailed description

of the pupillometer is in preparation.) In all cases the direct light reflex of the right pupil was measured.

#### *Light stimulation*

A xenon arc lamp operated at constant current and a system of lenses, mirrors, electronic shutters, and adjustable apertures were used to project and focus spots of light on a spherical plexiglass screen with a radius of 20 cm positioned such that the surface was roughly concentric with the eye of the rat. We did not measure the accommodative state of the eye; however, it has been shown previously that contrast sensitivity is best and shows no significant changes in the rat's eye for targets within the range of 15–40 cm<sup>4</sup>. Unless otherwise stated, all stimuli were presented in darkness and background illumination was negligible (typically,  $-3 \log E$ , see below). A calibrated neutral density wedge (Kodak Wratten) and calibrated neutral filters (Oriol) were used to attenuate the light intensity. Receptive field centers and surrounds could be selectively stimulated by light spots of various sizes. For diffuse stimulation, light was rear-projected on a section of a ping-pong ball covering the eye (ganzfeld). To permit measurement of the pupil while presenting ganzfeld stimuli, another ping-pong ball sector with a small opening in it was substituted. Stimuli were calibrated with an electronic radiometer (E.G. & G. Model 450, Electro-Optics Corp., Salem, MA). All stimulus intensities are reported as the radiant power measured at the position of the eye of the rat. The units of our intensity metric,  $E$ , are  $\mu$ W/cm<sup>2</sup>/s. To permit comparisons with the results of other studies, we estimated the effective intensity at the retina for our brightest stimulus,  $1.303 \log E$ , in units of quanta absorbed/rod/s. This calculation, the method of which has been previously described<sup>35</sup>, gives a value of  $4.305 \log$  quanta absorbed/rod/s, assuming a pupil diameter of 1.6 mm.

## RESULTS

#### *Response characteristics of pretectal cells*

Following existing nomenclature, based entirely upon temporal response<sup>30</sup>, we distinguished 3 major groups of cells in the pretectum of the rat. The average responses of representative single cells in each of these groups to 10 presentations of a 1.0 s flash of white light just covering the receptive field center, at

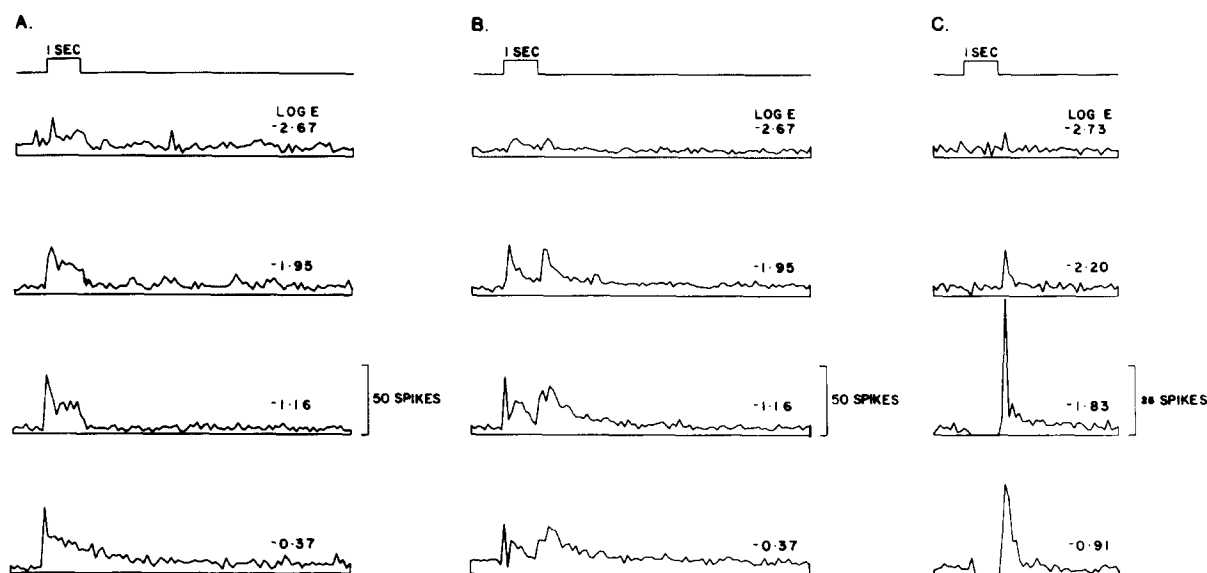


Fig. 1. Pretectal cell groups. A: shown are tonic-on cell responses to 4 intensities of a spot just filling the receptive field center flashed on for 1.0 s. Intensities, in units of log E (see Methods), from top to bottom are  $-2.67$ ,  $-1.95$ ,  $-1.16$  and  $-0.37$ . Peristimulus time histograms are averaged over 10 trials in 50 ms bins. The time marker is the stimulus duration, 1.0 s. The response height marker is 50 spikes/s. B: phasic (on-off) cell responses to 1.0 s flashes of the same intensities as in A are shown. Display parameters are also the same. C: tonic-off response to 4 intensities of flash covering receptive field center are shown as averages of 5 trials. The response scale is magnified ( $2\times$ ). Intensities, in units of log E, from top to bottom are  $-2.73$ ,  $-2.20$ ,  $-1.83$  and  $-0.91$ .

4 intensities, are presented in Fig. 1. In Table I, the frequency of occurrence, the mean receptive field center diameter and the range of receptive field center diameters for each group is shown.

A large proportion of the cells we recorded from responded to a light stimulus with a rapid increase followed by a more gradual decline and a sustained elevation of the firing rate. The sustained response lasted for the duration of the stimulus. These features are exemplified by the response of the cell of Fig. 1A. We adopted the convention of calling cells

with these properties tonic-on prepectal cells. Tonic-on cells had roughly circular receptive field centers (mean diameter  $31^\circ$ ) that were excited by focused spots of light and by ganzfeld illumination. The surrounds of these cells were large but weak, and of the silent type. To demonstrate center-surround antagonism it was necessary to illuminate the center continuously, such that a moderately high rate of firing was maintained. Then, flashing a light in the surround inhibited the response of the center. When the surround stimulus was extinguished, no excitatory response occurred at the light levels we tested, which extended well into the photopic range.

Cells were called phasic if and only if a burst of action potentials occurred at the onset and offset of a light stimulus without a maintained elevation of the firing rate during the application of the stimulus. The response of a phasic cell to a 1.0 s flash of white light within the receptive field center is shown in Fig. 1B. The size of the response to light onset as compared to the response to offset varied from one phasic cell to another, but we observed that phasic cells in which the response to light offset exceeded the response to light onset were the most common. For some phasic cells, directional preferences were observed. The

TABLE I

*Pretectal cell statistics*

For each physiologically defined group of prepectal cells, the number recorded from is listed. Also listed are the minimum, maximum and mean receptive field center diameter for the subset of cells in each group with measured receptive fields.

Group	Number	Receptive field center diameter		
		Minimum	Maximum	Mean
Tonic-on	56	17	61	31
Tonic-off	22	16	53	34
Phasic	53	17	124	38
Other	17	—	—	—

mean receptive field center diameter for this group of cells was  $38^\circ$ .

Over all animals we found roughly equal numbers of tonic-on cells and phasic cells. The same was not true for individual electrode penetrations. In many penetrations only tonic-on cells or phasic cells were encountered. When tonic-on cells and phasic cells were encountered in the same penetration, the phasic cells were typically located in the superficial part of the electrode track and tonic-on cells in the deeper part.

A third group of cells, which we called tonic-off, responded with sustained inhibition of firing during the presentation of a light stimulus. Light offset elicited a vigorous burst of action potentials followed by a gradual decline of the firing rate toward baseline. The distinguishing feature of tonic-off cells was the absence of any excitatory response at stimulus onset. This is illustrated by the example in Fig. 1C. Tonic-off

cells had a mean receptive field center diameter of  $34^\circ$ . Eleven of the cells classified as 'Other' in Table I appeared to be off cells but these were not held long enough to make this classification certain. The relative scarcity of tonic-off cells was consistent across animals.

Of the 6 remaining cells classified as 'Other', 4 had unusual properties and two responded to light but were not held long enough to classify. Two of the cells with unusual properties resembled the suppressed-by-contrast cells of the cat's retina<sup>12</sup>.

#### *Concurrent recording of tonic-on cell response and the light reflex*

Fig. 2 shows the response of a tonic-on cell and the direct light reflex of the pupil recorded concurrently. The cell was recorded near the anterior dorsal boundary of the PO. A series of 5.0 s flashes of diffuse light covering most of the retina (ganzfeld), in-

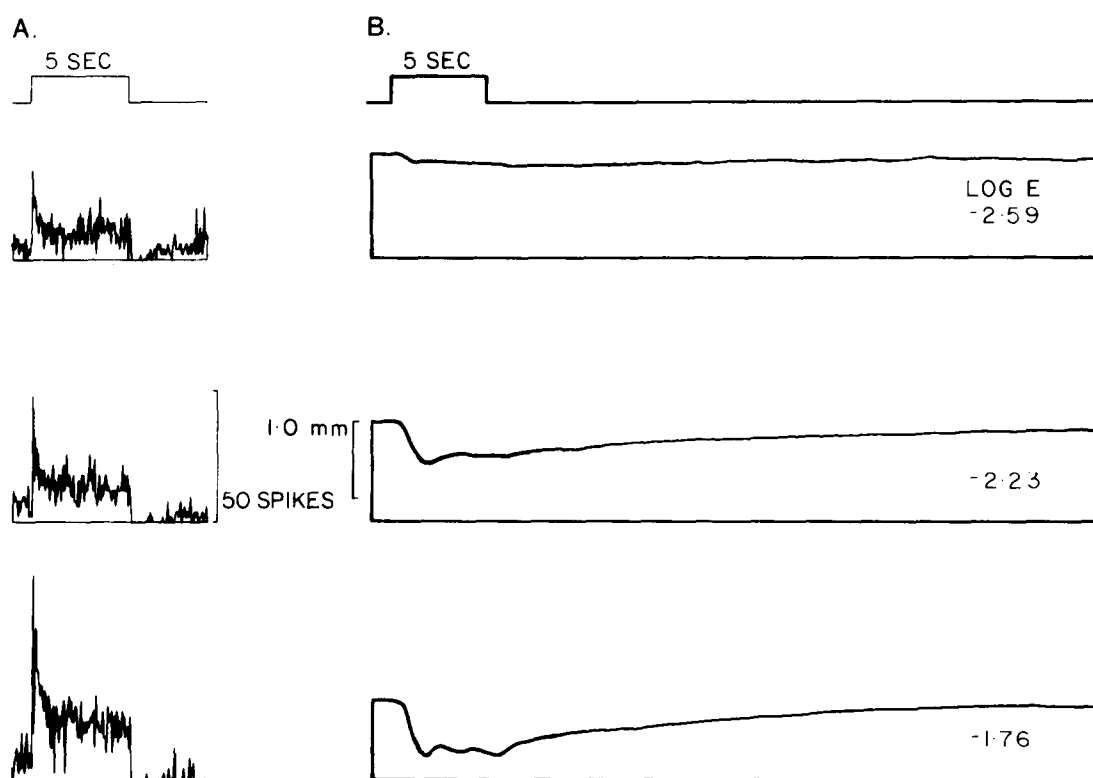


Fig. 2. Responses of a tonic-on cell and the light reflex recorded concurrently. A: tonic-on cell responses to 5.0 s step of ganzfeld illumination are shown (background was dark). The intensities are shown in B. The histograms are averages of 10 trials counted in 50 ms bins. The time marker (stimulus) is 5.0 s and the response marker is 50 spikes/s. B: shown are responses of the pupil to the same stimulus used for the cell at left. The reference level for each response (bottom line) marks a pupil diameter of 4.3 mm. The response marker shows a 1.0 mm change in diameter.

cluding the receptive field of the cell, was presented. The responses of the cell to stimuli with radiances of  $-2.59$ ,  $-2.23$  and  $-1.76$  log E are plotted in Fig. 2A. Each response, shown as an outlined histogram, was averaged in 50 ms bins over 10 trials. The cell maintained an elevated firing rate during the entire 5 s stimulus presentation. Individual responses of the pupil measured concurrently for the same 3 stimuli are shown next to the corresponding cell responses in Fig. 2B. For this cell, as for all tonic-on cells, the response increased in a monotonic fashion with the logarithm of the light intensity. Within this intensity range, the peak constriction of the pupil also increased monotonically.

#### *Electrical stimulation of tonic-on cells*

We found that electrical stimulation of spaces containing tonic-on pretectal cells caused the pupil to

constrict, whereas electrical stimulation of spaces containing only phasic or tonic-off cells did not cause the pupil to constrict. Although single pulses were effective in producing constriction, pulse trains required lower current thresholds for constriction and produced larger responses.

In 9 cases, electrical stimulation was delivered to the brain through a microelectrode that had just recorded the activity of a pretectal tonic-on cell. In each of these cases, the diameter of the pupil was continuously recorded, and we verified that the response of the pupil to a light stimulus was within normal limits. The results obtained in one of these cases are shown in Fig. 3. Following the identification of the response to light of a tonic-on cell and mapping of its receptive field, a 1.0 s long train of brief rectangular pulses ( $50 \mu\text{s}$  cathodal, pulse frequency 100 Hz) was delivered. The effect of this stimulus on the di-

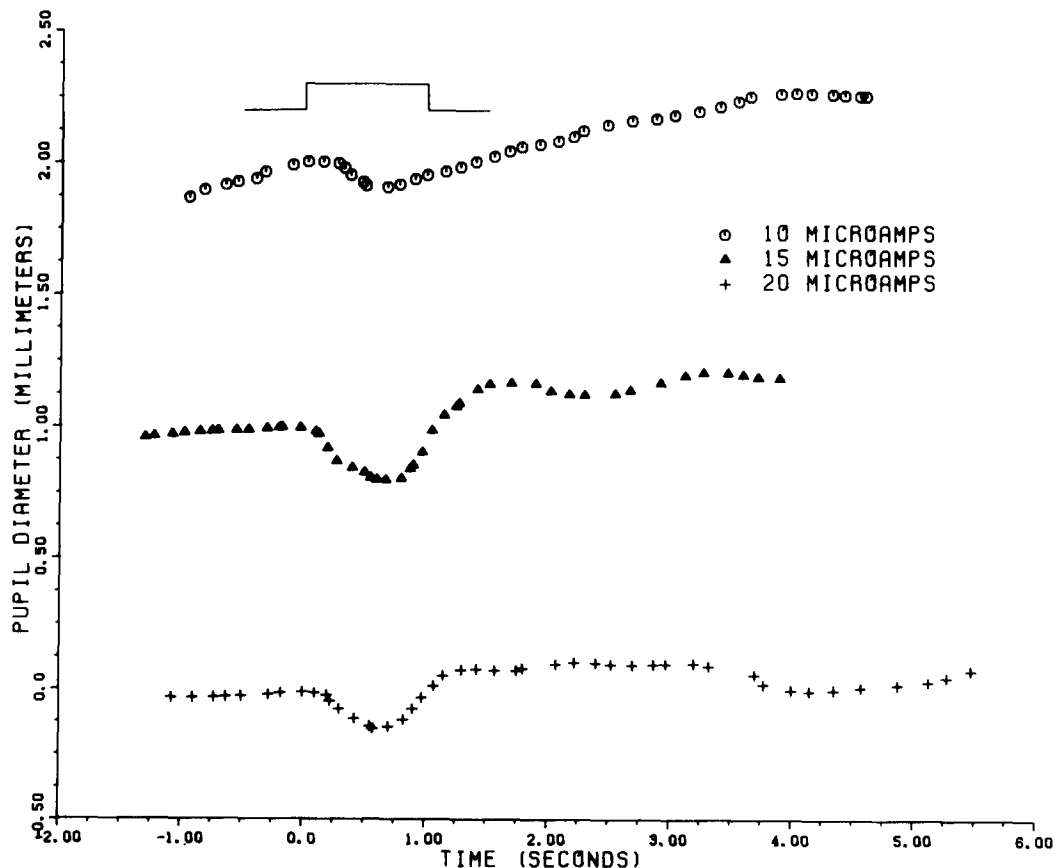


Fig. 3. Response of the pupil to electrical stimulation at the recording site for a tonic-on cell. Pupil responses were digitized from chart recorder tracings and replotted. Circles show the response at  $10 \mu\text{A}$ , triangles at  $15 \mu\text{A}$ , crosses at  $20 \mu\text{A}$ . Stimulation was cathodal (monopolar) with 1.0 s trains of  $50 \mu\text{s}$  pulses at a frequency of 100 Hz. The curves have been displaced vertically such that the pupil diameter at time zero in each trace falls on an integral value on the ordinate.

ameter of the pupil is plotted as a function of time for current intensities of  $10\ \mu\text{A}$ ,  $15\ \mu\text{A}$ , and  $20\ \mu\text{A}$ . These values represent the absolute value of the peak current for each pulse of the train. Below  $10\ \mu\text{A}$  the pulse trains failed to produce a measurable constriction. At  $10\ \mu\text{A}$ , the pupil responded with a constriction. A larger constriction occurred at  $15\ \mu\text{A}$  but further increases in current intensity did not increase the size of the constriction. These responses were not accompanied by eye movements. When the electrode was moved  $150\text{--}200\ \mu\text{m}$  away from the constriction site, constriction was either abolished or other effects, such as dilatation of the pupil, were observed.

The electrode penetration that yielded the constrictions described in Fig. 3 was marked with a small electrolytic lesion which was clearly visible at the dorso-medial border of the PO. This is the zone marked by the arrow in Fig. 4, which is a photomicrograph of a section through the electrode track.

The dashed lines show the outline of the PO. In this case the section was cut at an angle of  $1.2^\circ$  to the true coronal plane such that the plane of the section was  $2.6\ \text{mm}$  anterior to the interaural line at the medial end of the PO.

Stimulation in spaces containing phasic cells typically produced dilatation of the pupil or had no effect on the pupil. Dilatation of the pupil appeared to be a ubiquitous response property, appearing at many stimulation sites. This dilatation had a much slower time course than that of the constriction produced by electrical stimulation. Although in some cases the pupil dilated rapidly, time constants on the order of minutes were observed for the return of the pupil diameter to prestimulus baselines. Dilatation was also observed in response to stimulation of spaces in which tonic-off cells were recorded. Eye movements were also observed in connection with stimulation in spaces containing phasic cells. Movements with hori-



Fig. 4. Photomicrograph of a section through the pretectum which bisected the electrode track left after stimulating the tonic-on cell site which produced the constriction responses in Fig. 3. The light region marked by the arrow is an electrolytic lesion produced by  $5.0\ \text{s}$  of anodal DC current ( $5\ \mu\text{coulombs}$ ). The electrode track can be seen in the overlying neocortex. The tip of electrode was at the dorso-medial surface of the olivary nucleus. Scale bar:  $1\ \text{mm}$ ; section thickness:  $60\ \mu\text{m}$ .



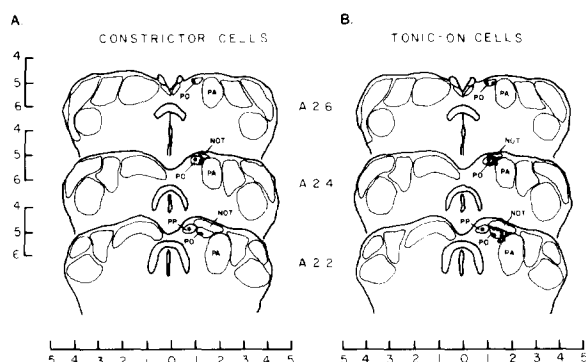


Fig. 5. A: locations in the coronal plane of 6 electrical stimulation sites that produced constriction of the pupil. Abbreviations: nucleus of the optic tract (NOT), anterior pretectal nucleus (PA), pretectal olivary nucleus (PO), posterior pretectal nucleus (PP). Prior to stimulation, a tonic-on cell was recorded at each of these sites. Five of these sites lie within the PO. One site lies in the anterior portion of PP adjoining the PO. Tracings are adapted from the atlas of Pellegrino et al.<sup>22</sup> with nuclear boundaries as described by Scalia<sup>26</sup>. Each division represents 1.0 mm. B: recording sites of 18 tonic-on cells. Two more sites, which are located in PO at A2.4 have been omitted from the plot for clarity. The constriction sites in A. are a subset of the tonic-on cell sites shown in B.

zontal, vertical, and torsional components were observed. Eye movement responses were recorded on videotape but were not quantified.

#### Distributions of constriction sites and tonic-on cells

Histological verification was obtained for 6 of the constriction sites. Fig. 5A shows these locations on tracings of brain sections derived from the atlas of Pellegrino et al.<sup>22</sup> and modified on the right half to show the boundaries of the pretectal cell masses as delimited by Scalia<sup>26</sup>.

Five sites were located within the boundaries of the PO. Electrical stimulation at these sites produced results similar to those shown in Fig. 3. One site was located in the posterior pretectal nucleus (PP), near the anterior margin adjoining the PO. At this site the activity of a tonic-on cell and the activity of a nearby tonic-off cell were recorded simultaneously. Electrical stimulation at this site produced mixed effects. Weak constrictions occurred for 1.0 s trains of pulses at 20 Hz and 10  $\mu$ A. At 50 Hz and 15  $\mu$ A, the pulse trains produced dilatation of the pupil.

The location of every recorded tonic-on cell for which the electrode position was verified is shown in Fig. 5B for comparison. The distribution of tonic-on cell sites was generally coextensive with that of con-

striction sites (Fig. 5A) and with the boundaries of the PO. More laterally, only phasic cells were found in the regions corresponding to the NOT. Medial to the distribution of tonic-on cells in the anterior portion of the PO we found no visually driven activity. Medial to the posterior portion of the PO, in the zone corresponding to the anterior end of the PP, we found tonic-off cells and phasic cells, and one tonic-on cell.

#### Receptive field distribution of tonic-on cells

The locations (relative to the blind spot) in the visual field of the receptive field centers of 28 tonic-on cells are plotted in Fig. 6. Cells were found in each quadrant of the visual field, but more cells were found with receptive fields in the superior hemi-field than in the inferior hemi-field.

The cells labeled 1–9 in Fig. 6 form a subset of tonic-on cells for which the receptive field location as well as histological confirmation of the electrode placement were available. The intersections of the horizontal plane with the electrode penetrations in which these cells were found are plotted in Fig. 7.

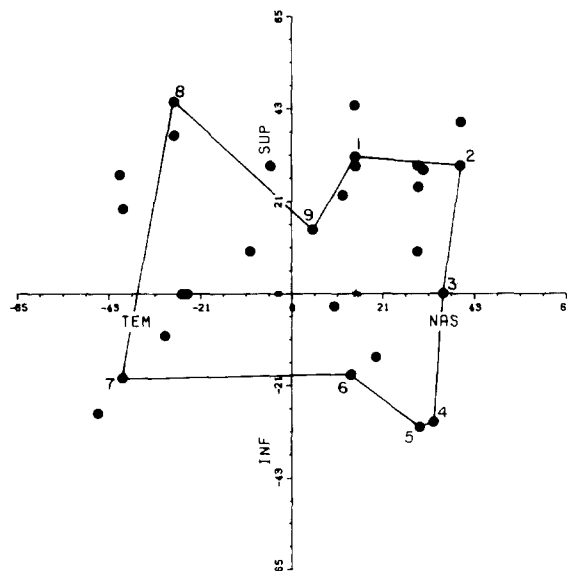


Fig. 6. Scatterplot of receptive field locations of the 28 tonic-on cells with identified receptive fields. Only the geometric centers of the fields are plotted. The origin corresponds to the blind spot and the estimated location of the posterior pole of the retina is shown by the star. The vertical axis is a representation of the vertical meridian of the visual field; the horizontal axis represents the zero parallel of the visual field. For the numbered cells (1–9) connected by lines, we had histologically verified the recording sites in the pretectum (see Fig. 7).

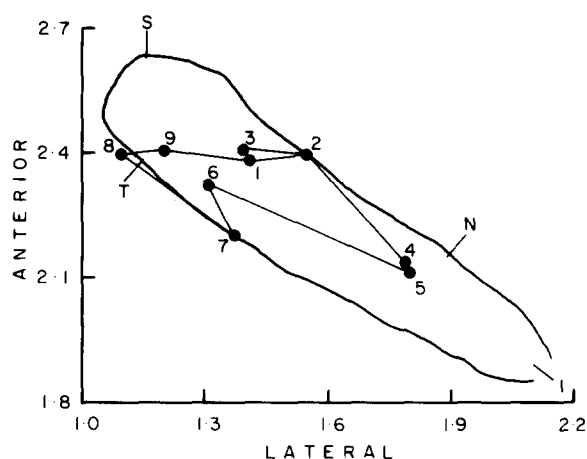


Fig. 7. Histologically verified locations of tonic-on cells with mapped receptive fields are shown projected onto the horizontal plane. The ordinate represents distance along the midline in mm and the abscissa represents the distance in the transverse direction in mm. The locations of the cells, shown by points, are connected by lines drawn in the same order as the lines which connect the receptive field locations of these cells in Fig. 6. The solid curve is the outline of the PO as estimated from the data presented by Scalia and Arango<sup>27</sup>.

The projection of the PO onto the horizontal plane as described by Scalia and Arango<sup>27</sup> was reproduced and superimposed upon this plot (solid outline). The 4 poles of the visual field in this projection are denoted by the labels S, N, I and T. The lines which connect the positions of the cells correspond to the lines which connect the receptive field center locations of the cells in Fig. 6. The locations of the receptive field centers of our physiologically assayed sample of cells roughly agree with this estimate of the visual field projection onto the PO.

#### *Response of fibers recorded near constrictor cells*

In 8 rats, we used fine Levick-type electrodes to record from fibers (axons) terminating near the PO. Fibers were distinguished from cells by: (1) the waveform of the action potential; (2) the depth of the electrode tip from the surface (fibers were usually encountered at the surface of the midbrain); and (3) the size of the receptive field center. The number of fibers isolated in any given penetration was small, presumably owing to the thinness of the fiber layer in the dorso-ventral direction (0.1–0.2 mm).

We estimated the conduction delay after orthodromic activation from the optic chiasm, and meas-

ured the receptive field center diameter and the temporal response of 4 tonic-on fibers, 2 phasic fibers and 2 tonic-off fibers. In addition to this sample, partial characterizations were obtained for 10 tonic-on and 8 phasic fibers. One other fiber, which could not be driven from the optic chiasm, responded reliably with a transient inhibition at both light onset and offset.

Single responses of a representative tonic-on fiber to 5 s flashes of a light spot just covering the receptive field center at radiances of  $-2.67$ ,  $-1.95$ , and  $-1.16 \log E$  are shown in Fig. 8A. Fig. 8B shows the results of successive applications of stimulating pulses through an electrode implanted in the optic chiasm. Each pulse elicited a single action potential. The range of conduction delays was from 2.1 to 7 ms. Although the conduction delay of this fiber is variable, these spikes did not occur at random, as we observed their regular occurrence in the midst of spontaneous activity. (The variability of the conduction delay of other fibers for which conduction delays were measured was considerably smaller, showing jitter of 2% or less.)

Fig. 8C is a schematic drawing of the receptive field of this fiber as mapped with small spots of light ( $<1^\circ$ ). The center of the receptive field subtended  $14^\circ$  and was located in the inferior nasal quadrant of the visual field. The surround was very large, as mapped with the center continuously exposed to an adapting light of radiance  $-1.109 \log E$ . The larger dashed circle in Fig. 8C represents an estimate of the limit of the surround, which measured  $111.2^\circ$ . We repeatedly observed in this fiber and in other tonic-on fibers, that the surround response was expressed as a suppression of the center response, as in the tonic-on cells. Tonic-on fibers did not respond with increased firing to the extinction of a light stimulus in the receptive field surround.

The mean receptive field diameter of 6 tonic-on fibers was  $10.1^\circ$  over a range of  $6.6^\circ$  to  $14.2^\circ$ . The mean conduction delay of 14 tonic-on fibers was 2.42 ms with averages for individual fibers in the range between 0.7 ms and 4.7 ms.

Phasic fibers responded to light flashes after the fashion of phasic cells. The mean receptive field center size of 8 phasic fibers was  $12.5^\circ$  over a range of  $7.0$ – $22.6^\circ$ . Conduction delays ranged from 1.7 to 3.1 ms with a mean of 2.42 ms. The 2 tonic-off fibers in

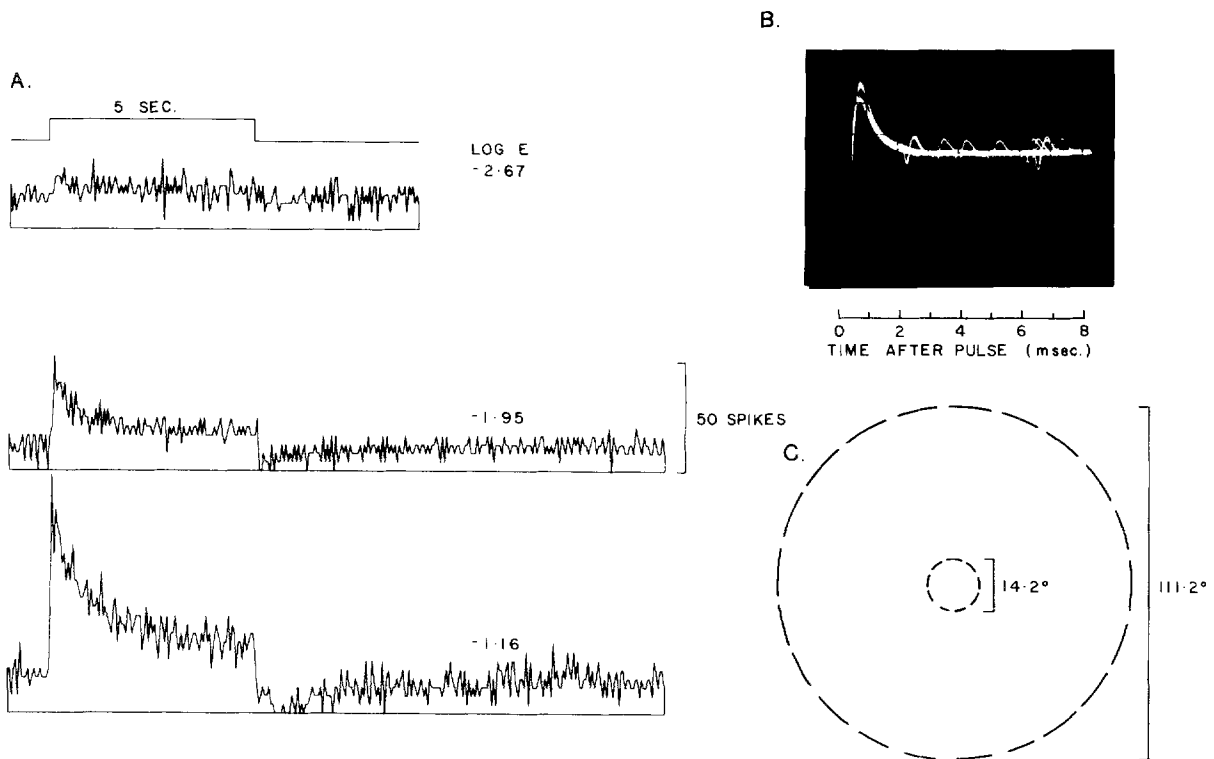


Fig. 8. A: shown is the response of a tonic-on fiber to a 5.0 s flash of a light spot which just covered the receptive field center at intensities of  $\log E$   $-2.67$ ,  $-1.95$  and  $-1.16$ . The time marker (stimulus) is 5.0 s and the response marker is 50 spikes/s. B: shown are action potentials evoked by a stimulating electrode in the optic chiasm. The large peak is the stimulus artifact; small spikes are fiber action potentials. The delay from stimulus onset, measured on the abscissa, ranges from 2.1 to 7 ms. The action potential waveform that occurs at 2.1 ms has a heavier trace because several action potentials were superimposed oscillographically at this point. C: the receptive field center and surround boundaries of the fiber as mapped with flashing spots of light are shown. The center subtended  $14.2^\circ$  and the surround subtended  $111.2^\circ$ .

our sample had receptive field diameters of  $6.6^\circ$  and  $17.0^\circ$  and conduction delays of 0.82 and 2.5 ms.

#### DISCUSSION

##### *Do tonic-on cells in the PO mediate the light reflex of the pupil?*

That the tonic-on cells, as shown in Figs. 1A and 2A, have temporal response properties that could account for the constriction of the pupil to light is not sufficient proof of their involvement in the light reflex, for tonically responding cells are known to project to the lateral geniculate nucleus and to the superior colliculus – structures which are not thought to have any connection with the light reflex. In order to link the activity of the tonic-on cells with the light reflex of the pupil, a more direct method is necessary. The method we used was electrical stimulation with small currents ( $< 40 \mu A$ ) applied to spaces in the pre-

tectum containing tonic-on cells. This stimulation produced pupillary constriction with a time course similar to that of the light reflex (Fig. 2B). Nine constriction sites, each defined by the presence of a tonic-on cell, were found in the pretectum. Six of these sites were marked by small electrolytic lesions, and the lesion centers of 5 of these were in the PO (Fig. 5A). The one site which was not within the PO was very near to its posterior boundary, within the adjacent PP (Fig. 5A,B). The distribution of all tonic-on cells whose locations were histologically verified, including those which we did not stimulate electrically, was also contained in the PO (Fig. 5B). These observations lead us to suggest that the PO consists largely, if not exclusively, of tonic-on cells concerned with the constriction of the pupil in response to photic stimulation of the retina.

It is unlikely that our recording electrodes were selective for tonic-on cells in the PO. Recording

massed activity from this area revealed that the overall temporal response of cells in this nucleus was tonic-on. Using fine LeVick-type electrodes, capable of isolating the activity of single fibers, most of the single cells we recorded in this region were in the tonic-on group. Outside the PO, the same electrodes isolated the activity of many phasic cells and tonic-off cells.

It is also unlikely that the constriction responses to electrical stimulation that we observed were due to activation of fibers of passage through the PO or to activation of cells far removed from the electrode tip. When we moved the electrode away from a constriction site by as little as 150–200  $\mu\text{m}$ , constriction was either abolished or other effects, including eye movements and pupillary dilatation were produced. Also, when a response is produced by stimulation of a fiber bundle with a nearby monopolar cathode, increases in current may abolish the response via the anodal surround effect<sup>24</sup>, but this did not occur. Furthermore, because the stimulation was produced by a monopolar cathode, the region of greatest depolarization would be expected to surround the tip of the electrode and to decrease radially from this point. If the cells producing the response were removed some distance from the electrode, then increases in the current intensity would be expected to recruit greater numbers of cells and so increase the constriction, but this did not occur either. Instead, we found that weak currents produced constriction, and moderate increases in current intensity neither abolished nor markedly increased constriction. The disappearance of the constriction response with movement of the electrode and the appearance of other effects with increases in current intensity strongly suggest that the PO is a discrete region concerned with pupillary constriction, surrounded by regions concerned with eye movements and, possibly, pupillary dilatation.

As a separate issue, the dilatation of the pupil that we produced with the stimulation of tonic-off and phasic cells suggests that a separate pretectal region may control this function. However, it is difficult to interpret pupillary dilatation in response to stimulation of pretectal cells because many areas of the brain give rise to dilatation when stimulated<sup>16,29,31,36</sup>. Under the conditions of our experiments, the possibility of sympathetic arousal effects contributing to this dilatation cannot be discounted<sup>20</sup>.

We also observed that whereas stimulation with single shocks in the pretectum was effective in producing constriction, single shocks delivered to fibers in the optic chiasm did not produce constriction. Similarly, Hultborn et al.<sup>16</sup> observed that single shocks in the posterior commissure or in the EW nucleus elicited a compound action potential in the short ciliary nerves of the cat but that at least two closely spaced shocks were required in the optic nerve or tract. Either synaptic temporal facilitation<sup>7</sup> or temporal summation could account for this difference in the excitability of points prior to, and at or beyond the pretectum. Regardless of the mechanism involved, this change in excitability is evidence for a synaptic connection between optic fibers and cells in the PO.

It is tempting to speculate that constrictor impulses are transmitted directly via synapses between optic fibers and pretectal tonic-on cells. However, ultrastructural analysis of the PO in the rat has shown that this need not be so<sup>6</sup>. Although a large fraction of the presynaptic elements in the PO are terminals of optic axons, many of these appear to be involved in serial synapses with a process of an interneuron interposed between the axon terminal and the dendrite of a PO projection cell. Campbell and Lieberman<sup>8</sup> suggested that these complex synaptic interactions may mediate feed-forward inhibition onto the projection cells of the PO. Comparisons of the temporal responses of tonic-on pretectal cells and tonic-on fibers suggest, however, that very little temporal filtering occurs between tonic-on fibers and tonic-on cells. If complex synapses are involved in the transmission of constrictor impulses, then it seems more likely that they serve a regulatory function, perhaps for other inputs to the PO, such as that known to come from the ventral lateral geniculate nucleus (vLGN)<sup>33</sup>.

A recent review<sup>25</sup> examined the evidence for localization of constrictor function in the pigeon, for which the medially situated area pretectalis (AP) is known to receive retinal inputs and to project to the EW nucleus. Ablation of the AP in pigeons results in fixed dilatation of the contralateral pupil, and electrical stimulation of its cells produces constriction of the contralateral pupil. Thus there appears to be a degree of homology between the pathways for the direct pupillary light reflex in avian and mammalian brains.

### *Temporal response of tonic-on cells*

Cells that transmit impulses for the light reflex of the pupil must respond to increments of light and are likely to maintain firing rates that increase monotonically with the rate of photon absorption in the retinal photoreceptors. Our recordings show that these properties are present in the tonic-on cells of the PO (Figs. 1A, 2A). Clarke and Ikeda<sup>10</sup> found that the curve which relates the steady state consensual response of the rat's sympathectomized pupil to light and the curve which relates the maintained firing rates of 'luminance detectors' in the rat's PO to light have a similar form. Our results show that the responses of the rat's pupil and of tonic-on units in the PO to flashes of light grow in a similar fashion with increasing light intensity. Furthermore, we find that the temporal response of the tonic-on cells is similar to the response of presumed afferent optic fibers recorded nearby (compare Figs. 2A and 8A). Note, however, that the response of the pupil (Fig. 2B) is considerably slower than the response of the tonic-on cells. In the cat<sup>16</sup> and in the rabbit<sup>17</sup> the response of constrictor fibers in the ciliary nerve to light also appears to be more brisk than the response of the pupil to light. This suggests to us that the temporal properties of the neural and muscular elements in the light reflex are different, and that some low pass filtering may occur in the sphincter pupillae.

### *Fiber inputs to tonic-on cells*

Our findings confirm previous results indicating that the optic fibers concerned with pupillary constriction are small. By measuring latencies of electrically evoked potentials in the ciliary nerves of cats in response to stimulation at various points along the pathway for pupil constriction, Hultborn et al.<sup>16</sup> estimated that the conduction velocity of the optic fibers subserving constriction is about 8 m/s. Using 7.5 mm as the distance between the optic chiasm and the PO and our measured mean conduction delay of 2.4 ms, we estimate that the mean conduction velocity of tonic-on fibers recorded near the rat's PO is 3.1 m/s, somewhat slower than in the cat. If it is assumed that the activation of these cells is monosynaptic, then the mean conduction velocity falls into the lower third of conduction velocities of fibers measured in the optic nerve and tract of the rat<sup>15</sup>. In addition, we have used a method of interleaved pulse trains<sup>11,13</sup> to estimate

that the mean refractory period of the optic fibers mediating pupillary constriction is 2.2 ms (unpublished observations). Both the mean conduction velocity estimate and the refractory period estimate indicate that small optic fibers innervate the constrictor cells of the PO.

Ganglion cells with tonic-on centers have been described in the cat<sup>12</sup>, but the receptive fields of optic fibers concerned specifically with constriction of the pupil have not previously been described. The tonic-on fibers we recorded near the PO had receptive field center diameters which ranged between 7° and 14° with a mean of 10.1°. As in the tonic-on cells of the PO, the surrounds of these tonic-on optic fibers were of the silent type, and light stimulation of the receptive field surround alone never elicited an excitatory response.

There is controversy about the origin of constriction-related inputs to the pretectum. In the rat, the PO is known to receive a retinal projection<sup>27</sup> as well as a projection from the vLGN<sup>33</sup>. Legg<sup>18</sup> reported that bilateral lesions in the vLGN of rats produced mydriasis. He also reported that lesions designed to be restricted to those pretectal areas receiving direct retinal input did not produce mydriasis, whereas lesions to other regions of the pretectum did. He concluded that the light reflex is mediated by the pretectum via the indirect projection from the vLGN. However, since the majority of presynaptic elements in the PO are of retinal origin<sup>6,27</sup>, it may be difficult to produce PO lesions that are free of optic involvement. Our data can neither reject nor support Legg's hypothesis. Our estimates of conduction velocities for fibers recorded near the PO fall into the lower third of the range of conduction velocities measured in the optic nerve and tract of the rat<sup>15</sup>. Thus, we cannot exclude the possibility that synaptic delays were involved in some of our measurements.

### *Spatial integration by tonic-on cells*

Although it is not known if the entire retina is 'covered' by constrictor cells, it is known that the response of the pupil is unitary; that is, the entire pupil constricts regardless of the spatial distribution of light on the retina. If each motor unit in the iris received signals from only a subset of tonic-on retinal ganglion cells, then isolated constriction of a sector of the iris could occur when a discrete region of

the retina is illuminated, but this is not observed in normal eyes. So it seems that each pupillomotor unit receives a pooled signal such that it can be driven by any effective part of the retina. Using the mean diameters of the tonic-on cells ( $31^\circ$ ) and the tonic-on fibers ( $10.1^\circ$ ) we compute a 9-fold increase in the area of receptive field centers from retina to pretectum, assuming no overlap. This increase in receptive field size shows that part of the spatial pooling of photon catch information is performed by the tonic-on cells of the PO. Assuming that the entire retina is effective for the light reflex, as it appears to be in humans<sup>5</sup>, we estimate that the signals of a minimum of 20 pretectal tonic-on cells must be integrated to obtain complete retinal coverage. Furthermore, the tonic-on cells whose locations were identified as constrictor sites were shown to have receptive fields in all 4 quadrants of the visual field. In principle, there is no need for a 'relay' in the pretectum for fibers concerned with pupillary constriction — the necessary spatial summation required to produce smooth constriction of the entire pupil in response to light could occur in the EW nucleus. But the results reported here show that this function is delegated to the PO. One reason for this is suggested by the extraretinally determined behavior of the pupil. The pupil is sensitive to many influences other than light<sup>20</sup>. In particular, it is known that the EW nucleus is subjected to inhibitory influences from the mesencephalon<sup>37</sup> and from the perifornical area of the hypothalamus<sup>29</sup>. In addition, the pupil constrictor neurons in EW probably receive signals related to lens accommodation when the pupil participates in the near reaction<sup>20</sup>. Thus the preganglionic motor neurons of EW may serve to integrate pupillary influences converging from several sources, not just those of the PO, which appear to be exclusively concerned with the light reflex.

## CONCLUSION

We have described the receptive field properties

and the time course of response of tonic-on cells in the rat's pretectum. We have linked the activity of these tonic-on cells to the light reflex of the pupil by producing pupillary constriction with electrical stimulation through our recording electrodes. These tonic-on cells were localized within the pretectal olivary nucleus and showed a degree of retinotopic organization. The small size of the stimulating electrode, the small currents used, and the disappearance of the constriction when the electrode was moved argue against the possibility that the electrically produced constrictions were due to the activation of cells distant from the electrode tip. Also, it is likely that the constrictions were due to the activation of pretectal cells rather than nearby optic fibers of passage because constriction could be produced by single shocks in the pretectum, but not by single shocks in the optic chiasm. The similarity in the time courses of the responses to light flashes of these tonic-on cells as compared to that of presumed input fibers suggests that the pretectal tonic-on cells do not play a major role in shaping the time course of the response of the pupil to light. The ratio of the receptive field diameters of pretectal tonic-on cells to tonic-on input fibers was 3 to 1, indicating that these cells in the pretectal olivary nucleus perform spatial pooling of signals from the retina. The input fibers are likely to have small diameters since they conduct action potentials slowly (about 3 m/s) and their absolutely refractory periods are large (about 2 ms). Our results are consistent with the hypothesis that the tonic-on cells in the PO are the first central neurons in the pathway for the direct light reflex of the pupil in the rat.

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