

# SINGLE-CELL RESPONSES OF THE NUCLEUS OF THE TRANSPEDUNCULAR TRACT IN RABBIT TO MONOCHROMATIC LIGHT ON THE RETINA<sup>1</sup>

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## INTRODUCTION

THE ACCESSORY OPTIC SYSTEM which terminates in the nucleus of the transpeduncular tract is the least known of the four pairs of optic pathways from the retina to the brain. It has fewer fibers than any of the other three; to the lateral geniculate nucleus, superior colliculus and pretectal nucleus and its function is perhaps more obscure. It is, however, found in most, if not all, vertebrates, including man.

We have been studying in this laboratory for several years the accessory optic system (21, 11, 12, 13, 7, and manuscript in preparation). The transpeduncular tract, which is its central mesencephalic section, was first described by von Gudden in 1870 and 1881 in rabbit, rat, and man (9, 10), and has since been reported widely throughout the vertebrates from cyclostomes to primates (11, 7).

Despite the finding of a number of physiological characteristics unique in the visual system (12, 13) the precise function of the accessory optic system remains unclear (manuscript in preparation).

As part of a program to attempt to limit or define its possible functions, we have examined the responses of cells in the nucleus of the transpeduncular tract to monochromatic light on the retina of the rabbit. These results have been compared with similarly recorded responses from the lateral geniculate body (14, 16). It will be shown that the nucleus represents only two wavelength peaks, in contrast to as many as seven in the lateral geniculate body. Wavelength coding by facilitation and inhibition of spontaneous activity was also noted as a property of this nucleus.

## METHODS

Pigmented rabbits (Black Dutch and hybrid) of both sexes and 2 kg. average weight were studied under photopic conditions. Five were maintained under urethan anesthesia (ca. 12 ml/kg. body wt. of 25% ethyl carbamate in 0.9% NaCl). Two were free of general anesthesia but restrained as *encéphale isolé* preparations, in which all pressure points and wounds were infiltrated with 2% lidocaine.

The monochromatic source consisted of a high-pressure xenon arc and a grating monochromator with a motor-driven automatic scanning apparatus as described by Hill *et al.* (15), without the complicated servomechanism. Its continuous spectral energy distribution

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is shown in Fig. 1. It gives an absolute energy of  $159 \mu\text{W}/\text{cm}^2$  at  $500 \text{ m}\mu$ . A  $1\text{-cm}^2$  image of the monochromator exit slit ( $10 \text{ m}\mu$  half-amplitude bandwidth) was formed as a  $35^\circ$  cone in Maxwellian view, the pupil being maximally dilated with a drop or two of 10% phenylephrine hydrochloride.

Stainless steel microelectrodes (8) were used to record from single cells in the nucleus of the transpeduncular tract. Their average resistance in physiological saline was 40 megohms.<sup>2</sup> The remote electrode was a chloridized silver wire inserted between the skull and the dura. A conventional cathode follower was used with less than  $10^{-13}$  amp. grid current and an internal capacitance of 0.7 pf. The microelectrode signal was displayed on one channel of a

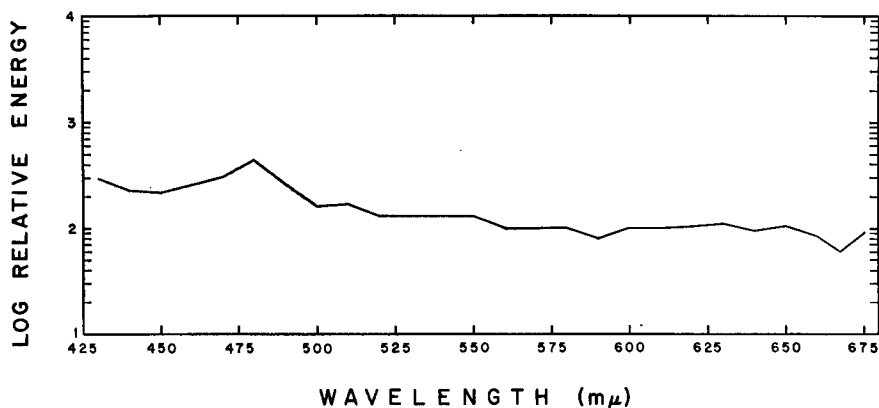


FIG. 1. The relative energy distribution of the high-pressure xenon source monochromator and optical system measured at the plane of the pupil. The absolute value at  $500 \text{ m}\mu$  was  $159 \mu\text{W}/\text{cm}^2$ .

dual-beam cathode-ray oscilloscope, the other beam indicating wavelength by its base-line height, time by negative pulsing, and the presence of the stimulus by positive pulsing. The display was photographed at a constant film velocity of  $10 \text{ cm}/\text{sec}$ . for subsequent analysis.

The eye was flooded with a bright preadapting white light<sup>3</sup> for 10 min. before beginning the search for a single-cell response with the microelectrode. Then while the light was slowly flickering (2 cycles/sec., 50% duty cycle) the electrode was lowered into the contralateral nucleus of the transpeduncular tract in search of a single functional cell. The nucleus and its locus in a transverse section of the midbrain is shown in Fig. 2.

Upon isolating a responding cell the white flickering light was shut off and monochromatic light introduced. The spectrum was scanned in 20 sec. from 400 to  $700 \text{ m}\mu$ , each scan successive through a neutral-density wedge filter from log 0 to log 4.0 in half-log steps, then back to log 0. Each scan was followed by 20 sec. of re-exposure to the adapting source before scanning with the next intensity level. The same shutter rate was maintained as during the searching procedure.

A small deposit of iron from the stainless steel electrode tip was made electrolytically ( $6 \times 10^{-3}$  coul.) after recording a cell. The animal was perfused with a 10% formaldehyde solution containing 1% potassium ferrocyanide. The latter produced a characteristic Prussian blue mark at the deposit site which was readily seen in the frozen sections made of each brain. This provided anatomical verification of the electrode tip recording site.

Determinations of the wavelengths most effective for producing a response from the nucleus and of the spectral range of sensitivity of each cell were based on four criteria: 1)

<sup>2</sup> Electrode resistance was measured to predict its usefulness with an electronic electrometer ( $I_g < 10^{-13}$  amp.). The microelectrode was measured in parallel with a 100-megohm resistor which was in series with a 1.5-V cell. The resistance of the microelectrode was taken to be that of a standard resistor producing an equal voltage measurement.

<sup>3</sup> The preadapting and exploratory light source was a 6-V., 4-amp. tungsten lamp which provided  $2,340 \mu\text{W}/\text{cm}^2$  at the plane of the pupil.

Regularity of the response as stimulus wavelength was varied. This response was considered regular if there was not more than one consecutive failure to discharge. 2) Regularity of the response as stimulus intensity was varied. This response was considered regular if there was not more than one consecutive failure to discharge. 3) Spike rate. This rate was evaluated with regard to intensity of the stimulus as well as wavelength. 4) Consistency and shortness of latency. The most effective wavelength had the shortest latency during a chromatic scan. Consistency of the latency with respect to intensity indicated the validity of the result.

When the sensitivity-wavelength function was plotted, symmetry of the curve relative

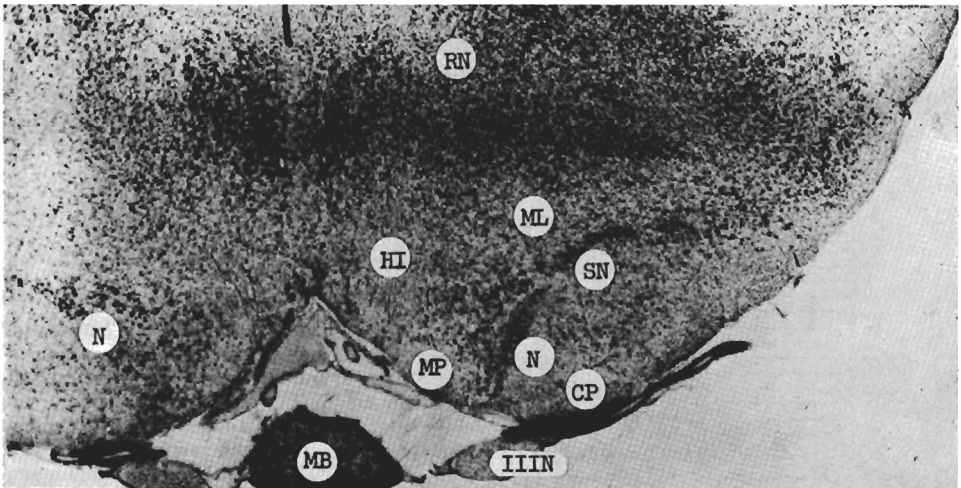


FIG. 2. A transverse section through the midbrain at the level of the nucleus of the transpeduncular tract, N. Also indicated in relation to this nucleus are the third nerves, III N; the red nucleus, RN; the cerebral peduncles, CP; the mammillary body, MB; the substantia nigra, SN; mammillary peduncle, MP; the habenulo-interpeduncular tract, HI; and the medial lemniscus (after Giolli, ref. 7).

to the wavelength of maximum sensitivity was not used as a criterion because large asymmetries were often observed.

The rate of flicker stimulation was varied in preliminary, exploratory experiments in which it was found that some cells would not respond to rates faster than 2 cycles/sec. This rate was used subsequently so that slow-responding cells would not be excluded.

## RESULTS

*Response criteria and localization.* It was our intention to record the responses of the cell bodies of the nucleus of the transpeduncular tract as opposed to the axons which may be merely passing through the nucleus. Two general response forms were commonly seen. One was of short duration, low amplitude, poorly isolated discharges, often as a triphasic waveform. The second response type consisted of a longer duration spike of greater amplitude, commonly biphasic, occasionally displaying a prepotential. Responses of the second type are the ones presented here and are assumed to be post-synaptic discharges from or proximal to the soma (6, 23).

Since the nucleus of the transpeduncular tract is a long and somewhat

diffuse structure, we have noted where within the nucleus each response was recorded (Fig. 3). The geometric center of the nucleus is designated O for origin and all other position designations are relative to it.

*Wavelengths of maximum response and spectral limits.* Figure 3 shows 37 responses to wavelength by cells of the nucleus by maximum response (triangles) and range or spectral limits (horizontal lines). Occasionally, a single cell displayed more than one maximum. Each maximum was considered individually, however, since its combination of latency, spectral range of response mode (on-phase vs. off-phase of the stimulus cycle) was usually unique.

Two spectral maxima were consistently associated with this nucleus:

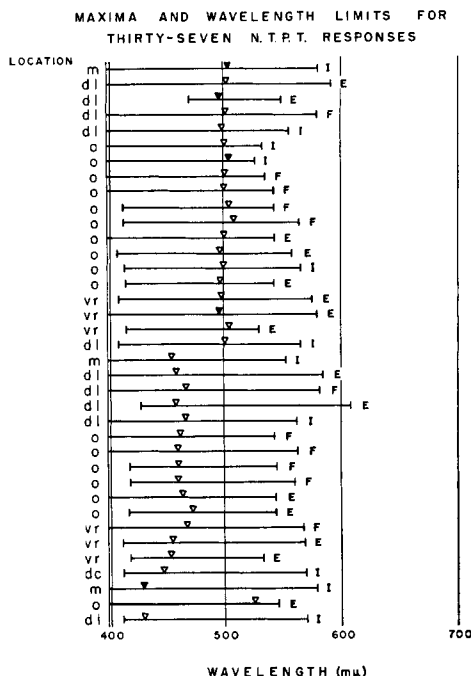


FIG. 3. The distribution of 37 maximal responses to wavelength by the nucleus of the transpeduncular tract, with their spectral ranges. E (evoked), F (facilitory), and I (inhibitory), refer to the background activity and form of each response (see text). Solid triangles represent the off-responses; open triangles the on-responses. The aspect of the nucleus from which each response was recorded is indicated by r, rostral; c, caudal; d, dorsal; v, ventral; m, medial; l, lateral; o, center.

a)  $461 m\mu$  based on the mean of 15 responses, with a standard deviation of  $\pm 3.7 m\mu$ . Only on-phase maxima appeared. b)  $501 m\mu$ , the mean of 19 responses, with a standard deviation of  $\pm 3.9 m\mu$ . Both on- and off-phase maxima exhibited. Both of these distributions were skewed slightly toward the long wavelengths.

Single examples were observed of five other maxima; which were therefore of questionable validity. On-phase responses occurred at 425, 470, 510, and 520  $m\mu$  and one off-phase response at 425  $m\mu$ .

The spectral limits or range of response, shown in Fig. 3, is for the log 0 intensity level of the energy distribution shown in Fig. 2. The mean long wavelength response limit was  $565 m\mu$ , with a standard deviation of  $\pm 24$

m $\mu$ , with only 1 response of the 37 continuing beyond 600 m $\mu$ . At the other end of the spectrum, every response with two exceptions was present for wavelengths longer than 415 m $\mu$ .

*Response latencies.* A bimodal distribution of latencies is found for cells of the nucleus upon stimulation with monochromatic light, as illustrated in Fig. 4. Each latency was measured at the wavelength of maximum response and at the log 0 intensity level. The mode value for the shorter latency group was 40 msec.; for the longer, 130 msec. The shortest latency evident from the nucleus under these stimulus conditions was 20 msec.

*Regular spontaneous background activity.* Sixty per cent of the cells re-

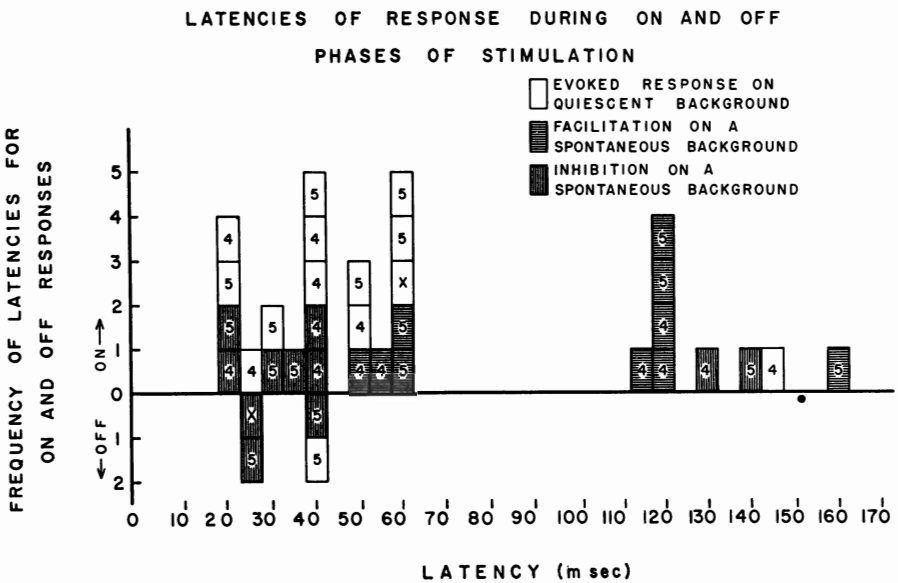


FIG. 4. The distribution of response latencies recorded from the nucleus of the transpeduncular tract. The wavelength of maximum response is designated by 5, for 501 m $\mu$ ; 4 for 461 m $\mu$ ; and x for an unrepeated response. The background activity and form of each response is designated by the coded shading.

corded displayed regular spontaneous background activity and were readily facilitated or inhibited by monochromatic retinal stimulation. These responses are designated by F and I, respectively, in Fig. 3. The regular background discharge rates for these cells ranged from 14 to 55/sec. The remaining cells, labeled E, had essentially quiescent backgrounds, no regular spontaneous discharge being evident. Only a transient on- or off-stimulus would cause these cells to respond. Samples are shown in Fig. 5.

Few, if any, spontaneous cells were uninfluenced by the light stimulation of the retina. Since this influence was used as one of the criteria to indicate the locus of the electrode tip in the nucleus, some bias may have been exercised in this finding. Nevertheless the conclusion seems generally valid.

## DISCUSSION

Two wavelengths were found most effective for producing maximum responses from cells of the nucleus of the transpeduncular tract under photopic conditions: 461 and 501  $m\mu$ . These maxima, along with others, were also found in the lateral geniculate body by Hill (14, 16) and agree closely with those reported by Dodt and Elenius (5) for single-unit recordings from the light-adapted rabbit retina. They suggested that the 500- $m\mu$  response must be associated with the visual purple mechanism of this retina. Wald (24) has already shown that rabbit rhodopsin has an absorption maximum at  $500 \pm 2 m\mu$ . The possible origin of the 461- $m\mu$  response, however, is less clear.

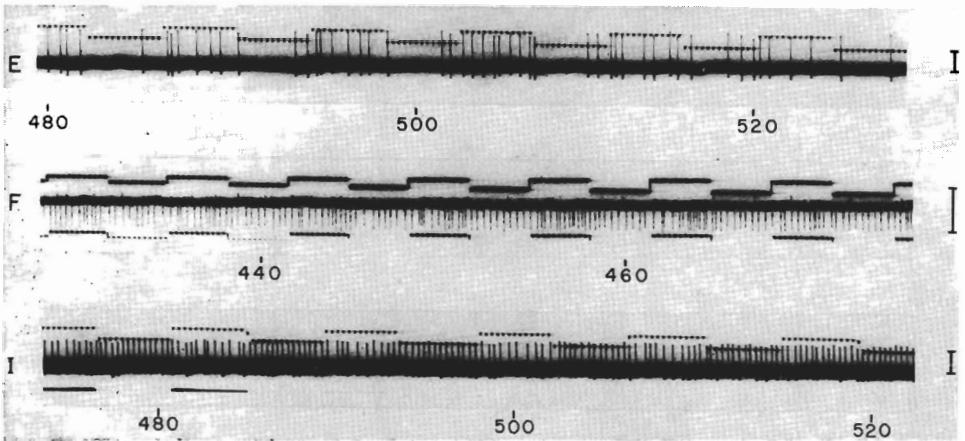


FIG. 5. Examples of the three types of cell responses from the nucleus of the transpeduncular tract: E is a quiescent background cell that produced predominantly on-spike discharge (small off-response also) only when the stimulus was introduced or removed. The wavelength of maximum response is 505  $m\mu$ . F and I are records of cells that maintained regular spontaneous activity under stable stimulus conditions. On introducing the stimulus, facilitation (maximum response, 461  $m\mu$ ) and inhibition (maximum response, 501  $m\mu$ ) occurred respectively for these two cells. No off-response was evident. Amplitude scale, .5 mV.; dots up indicate stimulus on; time, 20 msec.

although consideration may be given to the cone population of the rabbit retina which is now well established (19, 3, 22).

When both maximum response wavelengths are present simultaneously in a single cell they retain their identity through various stimulus parameters indicating two separate and distinct retinal origins. Aside from separate wavelength maxima, each response has its own distinctive spectral response range and latency distribution.

The absence of cell responses in the nucleus to wavelengths longer than 600  $m\mu$  is not an unexpected one since similar results are found in parallel experiments on the lateral geniculate body (14, 16). An absence of response to long wavelength was also reported by Laue (20) who recorded throughout the visual system of the rabbit with gross electrodes.

The bimodal aspect and extended range of the latency distribution were unexpected except for somewhat similar results in the lateral geniculate body (14, 16). In the latter the 39 on-responses had a similar latency distribution, the modes being 43 and 130 msec. while the 32 off-responses, which were considerably more common than those of the nucleus of the transpeduncular tract, were not distributed bimodally but became less common at longer latencies. Only five off-responses were recorded from the nucleus, hence no good comparison could be made with off-responses from the lateral geniculate body.

The latency range and distribution is interesting but of uncertain origin. The resemblance between the latencies of the nucleus of the transpeduncular tract and those of the lateral geniculate body points to a retinal origin. Repetitive firing is plausible within the retinal network. Less likely is an origin in the accessory optic pathway, especially in the nucleus of the posterior accessory optic tract (12, 13), or in the nucleus itself. However, extraretinal mechanisms cannot be excluded. Closed loop pathways perhaps involving axons of passage, both of which, according to Giolli (personal communication, 1962) are found in the nucleus, could be involved. The recording criteria established were designed to eliminate responses of axonal origin and the assumption was made that the latency distribution was not directly influenced by them. Closed loop pathways could account for the longer latency second mode.

It is curious that such a large percentage of the cells of the nucleus of the transpeduncular tract exhibited regular spontaneous activity, facilitated or inhibited by specific wavelengths. As we have reported (17) it is commonly found in the nucleus of the transpeduncular tract in the rabbit, but rarely seen in its lateral geniculate body. Independently, de Valois *et al.* (4) have found frequency modulated spontaneous activity in units of the lateral geniculate body of the monkey. A somewhat similar phenomenon showing a decrease of spontaneous activity with white-light stimulation has been noted in the ganglion cells of the cat retina by Brown and Wiesel (2) and termed "pure inhibition" as well as in the visual cortex by Jung *et al.* (18) and called a type-C neuron.

It is unlikely that injury can be responsible for the discharge since identical experiments in the lateral geniculate body (14, 16) produced only one such cell out of 53 studied. Furthermore, cells injured by an electrode in both the nucleus and the lateral geniculate body typically and rapidly increase their spontaneous frequency. In addition, they did not respond to wavelength stimulation and continued to fire only a short time. The possible effect of urethan anesthesia on the responses of the nucleus was determined by comparing them with anesthesia-free *encéphale isolé* preparations. The same kinds of responses, both spontaneous and the well-known on-off were found in both kinds of preparations.

It appears a curious arrangement that most of the cells of the nucleus apparently function by modulation of a carrier frequency. Information thus

transmitted seems to be subtly coded in this fashion in addition to the usual and common on-off responses on a quiescent background.

One conclusion appears justified, that there is much less information about hue found in the nucleus than in the lateral geniculate body. There is both behavioral and electrophysiological evidence that the rabbit has a hue discrimination system (1, 14, 16). The nucleus, however, can be ruled out as a primary color vision station and the search for the functions of the accessory optic system may be continued in other aspects of the visual system.

#### SUMMARY

1. Two wavelengths of maximum sensitivity predominate in the cells of the nucleus of the transpeduncular tract in rabbits, 461 and 501  $m\mu$ . This is distinct from the multiple wavelengths represented in the lateral geniculate body. The nucleus of the transpeduncular tract does not appear to be primarily a color vision center.

2. Rarely did any cell from the nucleus of the transpeduncular tract respond to wavelengths longer than 600  $m\mu$ .

3. The latency distribution for on-responses from the nucleus of the transpeduncular tract was found to be extended and bimodal. Parallel findings from the lateral geniculate body suggest a retinal origin for these characteristics.

4. The occasional presence of more than one wavelength peak response from a single cell in the nucleus of the transpeduncular tract, each having its own maximum latency and spectral range, suggests that at least two distinctive spectral response curves are represented and perhaps integrated.

5. The presence of a majority of cells of the nucleus showing facilitation or inhibition of steady spontaneous background activity by monochromatic stimulation is compared with cellular activity in the lateral geniculate body. The possible significance of this means of coding is discussed.

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