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Microelectrode study of accessory optic tract in the rabbit

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HAMASAKI, DUCO, AND ELWIN MARG. *Microelectrode study of accessory optic tract in the rabbit.* Am. J. Physiol. 202(3): 480–486. 1962. — Microelectrodes were used to study the physiological properties of single units from the posterior accessory optic tract–transpeduncular tract in the rabbit. Both on and on-off elements were isolated in the nucleus of the transpeduncular tract. Only one off element was isolated. Intermittent photic stimulation was found to evoke a unique sequence of responses from the accessory optic system. The units gave a good response to the low rates of stimulation, decreased or stopped firing at the intermediate rates, and resumed firing at still higher rates of stimulation. The nucleus of the transpeduncular tract was found to receive its activation from elements in the central part of the retina. Recordings from elements of the nucleus of the posterior accessory optic tract showed that their physiological properties were very similar to those of the nucleus of the transpeduncular tract.

THE POSTERIOR ACCESSORY optic tract–transpeduncular tract has been known for a long time, and considerable work has been done on its histological architecture (1). Neuroanatomists have reported that this neural pathway originates from the eye, and decussates to run with the contralateral optic tract. It can be seen as a separate fascicle just rostral to the superior colliculus, whence it runs ventrally and transversely over the cerebral peduncle to end in the base of the midbrain in the region of the substantia nigra, the red nucleus, and the exit of the oculomotor nerve. A schematic drawing of this pathway is shown in Fig. 1.

The physiological properties of this pathway were studied recently with electrophysiological techniques in the rabbit (2). The photic response from the nucleus of the transpeduncular tract was found to be mainly an on response. The on-off type of response was seen occasionally, but with the off response always weaker. A pure off response was never seen in this earlier study.

Electrophysiological findings indicated that a synapse must be situated between the retina and the nucleus of the transpeduncular tract. This synapse has been called the nucleus of the posterior accessory optic tract.

The present investigation of the physiological properties of the posterior accessory optic tract–transpeduncular tract was carried out with both glass micropipette and the metal type of microelectrodes. The results confirmed those obtained earlier with macroelectrodes.

METHODS

Both urethanized and encéphale isolé rabbits were used. The surgical procedures have been described (2). The encéphale isolé preparation was made under ether, with all wounds and pressure points procainized before the animal recovered consciousness. Conventional electrophysiological equipment was used. The photic stimulating system flooded the dilated pupil with a maximum illumination of 2690 lux.

The glass pipette microelectrodes were filled with 3 M KCl, and their resistances ranged from 5 to 50 megohms. Those of the metal type were prepared from stainless steel insect pins according to the technique of Green (3) and were found to give better isolation of single units.

RESULTS

Characteristics of photic responses from nucleus of transpeduncular tract. Typical responses evoked from the nucleus of the transpeduncular tract by photic stimulation are shown in Fig. 2. Both on (2*A*, *B*) and on-off (2*C*, *D*) types of units were located with the microelectrode. Only one pure off unit was ever seen.

The isolation of a single unit from the nucleus of the transpeduncular tract was difficult. In most instances, the recordings were from several units, as seen in 2*B* and 2*D*. When a single unit was isolated, it was usually of the on-off type (2*C*).

The majority of the recordings from the nucleus showed only on responses. Moreover, the number of spikes at on of any on-off unit was always greater than the number at off.

There was topographic segregation of the on and the on-off units. In general, the on-off element was found more dorsolaterally in the nucleus of the transpeduncular tract than was the on type.

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Effect of intensity of photic stimulation on response from nucleus of transpeduncular tract. The effect of increasing stimulus intensity on the response from an on-off unit is shown in Fig. 3. The numbers at the left are the densities of the filters used to reduce the intensity of the light from 2690 lux.

The absolute intensity threshold for units from the lateral geniculate nucleus, the superior colliculus, and the nucleus of the transpeduncular tract were compared. The animals were dark-adapted for at least 1 hr, and for as long as 2 hr, before the recordings were made. In one experiment, the eye of the rabbit was occluded during the surgical procedures (1½ hr), and was stimulated only with low intensity light during the placement of the electrodes. The animal was then left in the dark for an additional hour before any readings were taken.

There was good agreement in all the experiments. For elements in all three structures studied, the threshold for a response was found to be 3.5–4.0 log units below the maximum available stimulus (i.e., 0.19–0.27 lux).

Effect of intermittent photic stimulation. Figure 4 shows the effect of intermittent illumination on a unit from the nucleus of the transpeduncular tract. The numbers at the left of the records are the frequencies of stimulation in cycles per second.

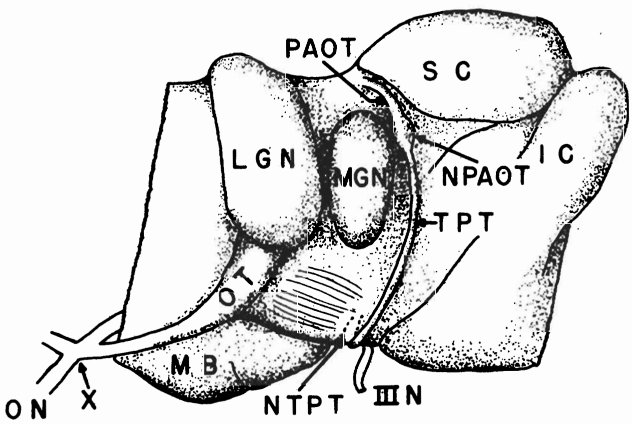


FIG. 1. Drawing of the posterior accessory optic tract–transpeduncular tract. *ON*—optic nerve; *X*—chiasma; *OT*—optic tract; *LGN*—lateral geniculate nucleus; *SC*—superior colliculus; *PAOT*-posterior accessory optic tract; *NPAOT*—nucleus of the posterior accessory optic tract; *TPT*—transpeduncular tract; *NTPT*—nucleus of the transpeduncular tract; *MB*—mammillary body; *III N*—oculomotor nerve; *IC*—inferior colliculus.

Note that a good response was evoked from this unit at the low rate of stimulation (0.8/sec). With increasing rates (2, 5, and 10/sec) the number of spikes per flash, as expected, decreased. Interestingly now, at higher rates of stimulation (15/sec), the response increases and the unit fires twice and sometimes thrice to each flash. With still higher frequencies, the unit follows each flash with two or three spikes. When the light is turned off, the unit stops responding. Measurements of latent periods showed that the last two sets of spikes are on responses to the last two flashes.

This sequence of events was seen in all the recordings from the nucleus of the transpeduncular tract (and also from the nucleus of the posterior accessory optic tract). In fact, this type of reaction was so characteristic that it was used to differentiate a recording from this pathway from recordings from other parts of the visual system.

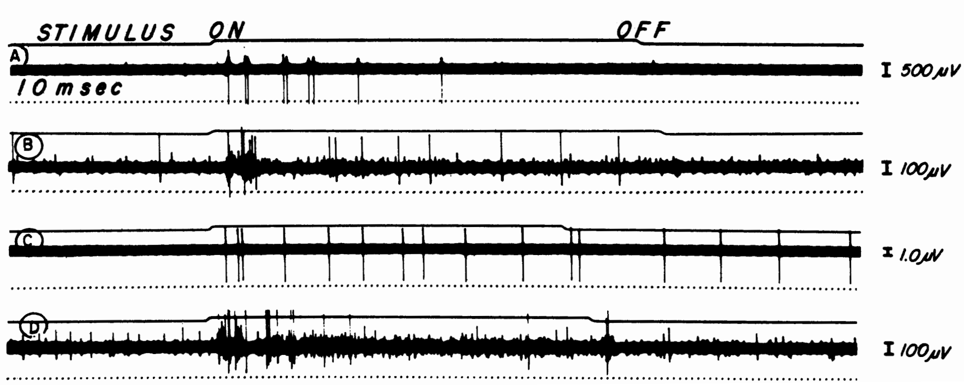
In the lateral geniculate nucleus and in the superior colliculus, a more usual sequence of events occurred with intermittent illumination. The units responded to each flash of light up to a certain frequency, after which they either ceased firing or fired only asynchronously. None of the units examined behaved as did those of the accessory optic system.

Receptive fields of nucleus of transpeduncular tract. The receptive fields of units in the nucleus of the transpeduncular tract were compared with those of the lateral geniculate nucleus and the superior colliculus. The extent of each field was determined by moving an ophthalmoscope bulb in the visual field and noting the area which fired the unit.

The sizes of the receptive fields in the lateral geniculate nucleus and the superior colliculus were small. A given element could be fired only when the light bulb was in a relatively small area of the visual field. Although no systematic plot was made on any one animal, examination of these two structures in different animals showed that all parts of the visual field were represented.

The receptive field of a given unit in the nucleus of the transpeduncular tract was somewhat larger than that in the lateral geniculate nucleus and the superior colliculus. However, it was still localized to one portion of the

FIG. 2. Photic responses from units of the nucleus of the transpeduncular tract. *A* and *B* are on units; *C* and *D* are on-off units. Time constant is 0.001 sec.



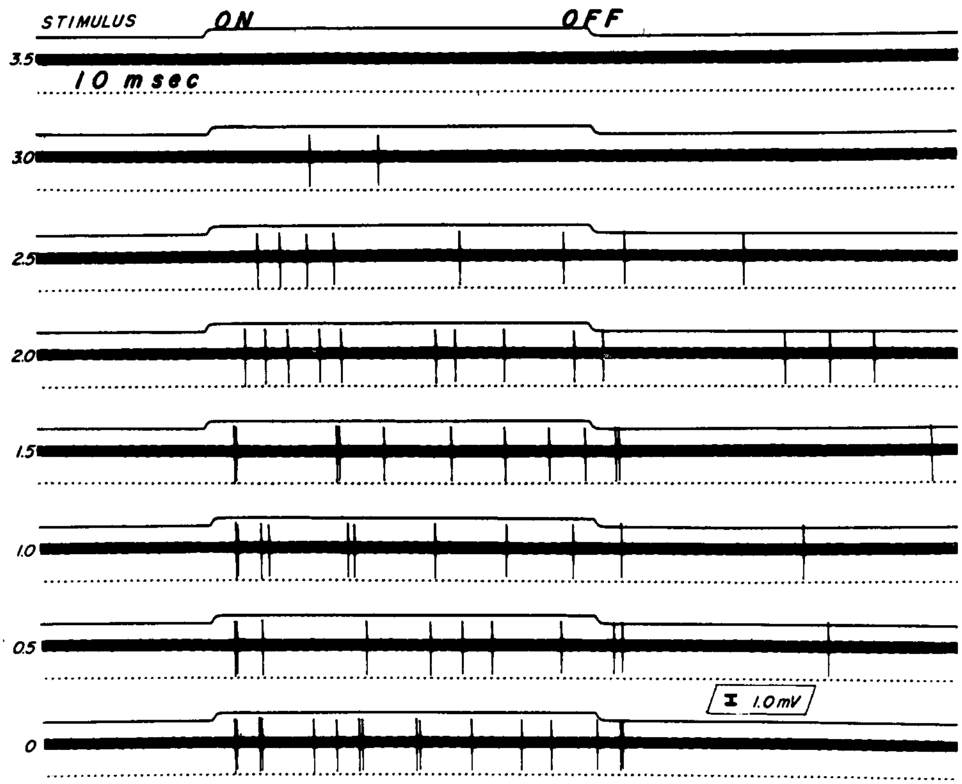


FIG. 3. Effect of increasing intensity of photic stimulus on response of a single unit from nucleus of the transpeduncular tract. Numbers at the left are the densities of the filters used to reduce the intensity of the stimulus. Time constant is 0.001 sec.

visual field, and was fired only when the light was moved in this restricted area.

All the elements of the nucleus of the transpeduncular tract had their receptive fields restricted to the central portion of the visual field, measuring approximately 30 degrees in the vertical meridian and 60 degrees in the horizontal meridian.

Responses to electrical stimulation of optic nerve fibers. Action potentials evoked by electrical stimulation of optic nerve fibers are shown in Fig. 5. The characteristics of the action potentials, as reported by Frank and Fuortes (4) and by Tasaki and others (5), were used to identify the location of the electrode relative to the neuron.

In 5*A* is shown a recording which demonstrated all the characteristics of a presynaptic recording from an axon. Its latency is 0.5 msec with a duration of 0.4 msec. It is monophasic without a slow synaptic potential. This unit gave one spike for each stimulus and was able to follow repetitive stimulation up to 400/sec. Anatomically, the electrode was located among the axons which make up the brachium of the superior colliculus.

In 5*B* is shown a recording from the soma of a cell in the lateral geniculate nucleus. This action potential from the soma can be seen to be made up of the slow synaptic potential from which the fast spike potential arises. This compound nature leads to a notch in the

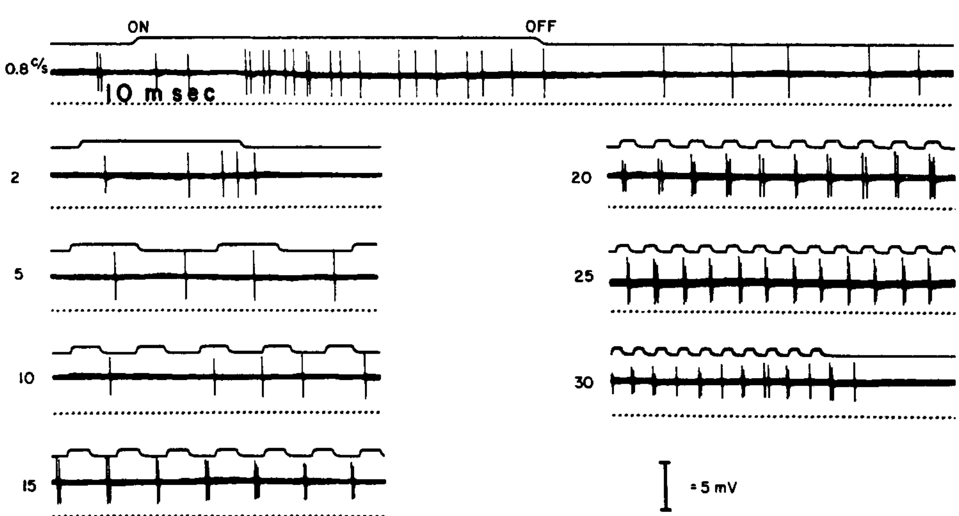
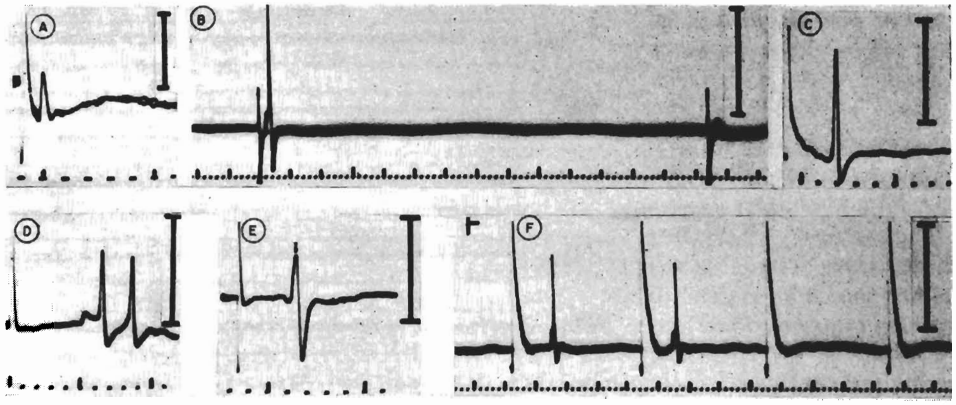


FIG. 4. Effect of intermittent photic stimulation on a unit from the nucleus of the transpeduncular tract. Numbers at the left represent the rate of stimulation in cycles/sec.; d-c recording.

FIG. 5. Recordings of action potentials evoked by electrical stimulation of optic nerve fibers. *A:* axon in the optic tract; *B:* soma of neuron in lateral geniculate nucleus; *C:* axon in nucleus of transpeduncular tract (retouched). *D, E.* and *F:* Soma of neurons in nucleus of the transpeduncular tract; d-c recordings. Calibration is 2 mv in *A*, 5 mv in *B–F*. Time = 1 msec (retouched).



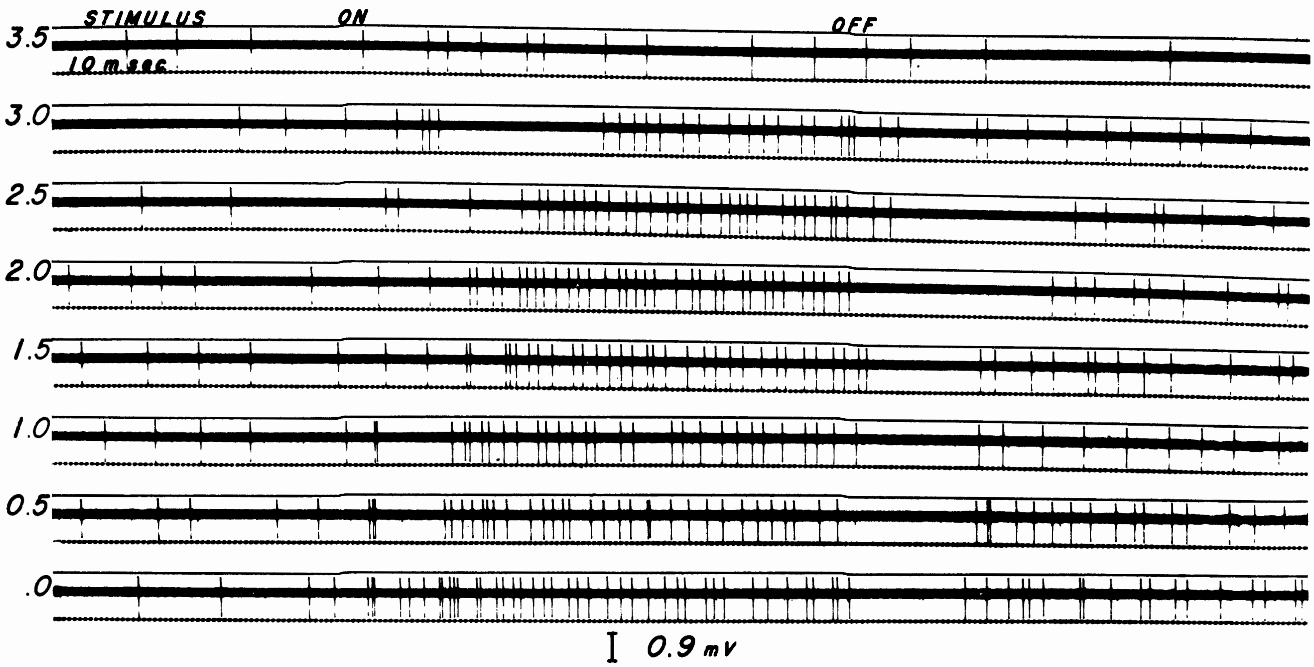


FIG. 6. Effect of increasing intensity of photic stimulus on response of a single unit from the nucleus of the posterior accessory optic tract. Numbers at the left are the densities of the filters used to reduce the intensity of the stimulus from 2690 lux. Time constant is 0.001 sec.

ascending limb of the action potential. The latency of the action potential is 1.2 msec with a duration of 1.0 msec. This unit was able to follow repetitive stimulation up to 14/sec. With higher frequencies, the fast spike potential was absent but the slow synaptic potential was still present.

In 5*C* is shown a recording from the nucleus of the transpeduncular tract. Because of its short duration, 0.3 msec, and the absence of a slow synaptic potential, this is probably a recording from an axon in this nucleus. Its latency is 2.2 msec. Following the spike, there is a small after-hyperpolarization which Frank and Fuortes have reported to be frequently seen in recordings from axons.

In 5*D*, 5*E*, and 5*F* are shown recordings from the somas of neurons in the nucleus of the transpeduncular tract. All these recordings show the slow synaptic potential and the fast spike potential which give rise to a notch on the ascending limb of the action potential. The slow synaptic potential can be seen best in 5*D* and 5*F*.

The recording in 5*D* illustrates the multiple firing of postsynaptic units. Its latency is 4.5 msec with a duration of 1.5 msec. The recording in 5*E* illustrates the biphasic type of action potential. The latency is 2.8 msec and the duration 1.1 msec.

In 5*F* is shown the effect of repetitive stimulation of the optic nerve fibers on the action potential evoked from the soma of a neuron in the nucleus of the transpeduncular tract. This unit was able to follow frequencies up to 50/sec. With higher frequencies, there is no spike response. Unlike the recording from the lateral geniculate nucleus, the slow synaptic potential is also absent.

Nucleus of posterior accessory optic tract. Figure 6 shows the effect of increasing the intensity of the stimulus on the

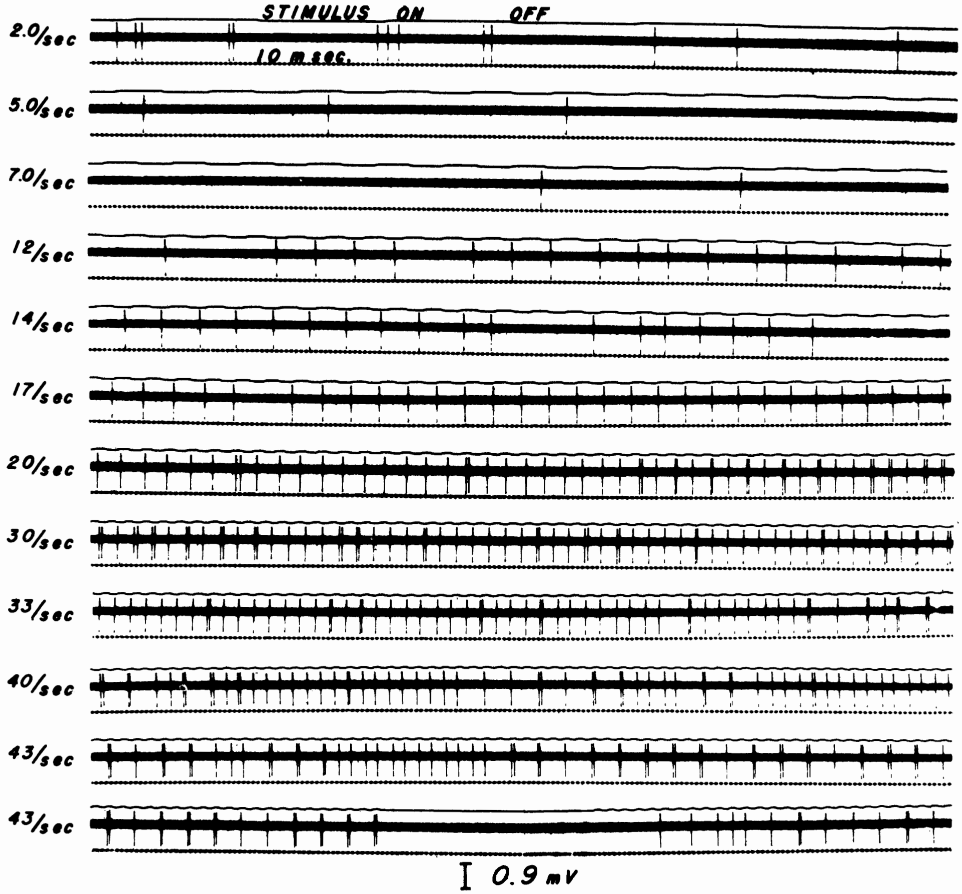


FIG. 7. Effect of intermittent photic stimulation on a unit from the nucleus of posterior accessory optic tract. Numbers at the left represent rate of stimulation in cycles/sec. Time constant is 0.001 sec.

response of a single unit in the nucleus of the posterior accessory optic tract. The numbers at the left are the densities of the filters used to reduce the intensity of the light from 2690 lux.

The threshold for this on-off unit was found with a density 3.0 filter. With increasing intensity, there was a decrease in the latency of the on response and an increase in the number of spikes.

Note that following off there is a finite interval which appears to be more than the latent period for the off response. Even spontaneous activity is absent here. The duration of this inhibitory period decreases with increasing intensity. The number of spikes also increases with increasing intensity.

Figure 7 shows the effect of intermittent illumination on this same unit. The sequence of events noted for the units in the nucleus of the transpeduncular tract can also be seen here. A good response is evoked with the low rates of stimulation (see Fig. 6). The unit stops firing at stimulation rates of 5 and 7/sec. At higher rates (12/sec and up) the unit fires once for each flash of light. At 20 and 30/sec, the unit fires at least once and sometimes twice to each flash of light. At 43/sec, the unit responds to every other flash, but it does so with two spikes. When the flashing light is stopped, the response promptly ceases and reappears when the stimulation is resumed.

Histology. The brain from each experimental animal was sectioned and the appropriate sections studied. Figure 8*A* shows a brain section with the Prussian-blue mark in the nucleus of the transpeduncular tract. Figure 8*B* shows the Prussian-blue mark in the nucleus of the posterior accessory optic tract.

DISCUSSION

The recordings obtained with microelectrodes confirmed our earlier findings (2) with macroelectrodes. The characteristics noted for the summated responses were found to be exhibited by the individual elements in the nucleus of the transpeduncular tract.

As stated, there was some difficulty in isolating single units in this nucleus. The relative ease of isolating a single unit from the lateral geniculate nucleus or the superior colliculus indicated that our techniques were adequate. The difficulty was probably due to the size and number of cells in the nucleus of the transpeduncular tract. Koelliker (6), from Golgi preparations, described cells of 20–30 μ, in the nucleus of the rabbit. Giolli (7), in Nissl preparations, has reported that the cells of this nucleus range from 4 to 25 μ with very few of the cells large. From their study of single units in the rabbit lateral geniculate nucleus, Arden and Liu (8) considered that there were clusters of cells associated with the small spikes, and that the larger cells associated with the big spikes were scattered among them.

The majority of the units in the nucleus of the transpeduncular tract were of the on type. In addition, the number of spikes at on of any on-off unit was always greater than that at off. These findings account for the earlier (2) observation that the summated response was mainly an on response.

The present study has shown that the on-off unit is not as rare as we had believed. The on-off units were found to lie more dorsolaterally in the nucleus, and probably relate mainly to the larger cells.

Our neurophysiological findings parallel the anatomi-

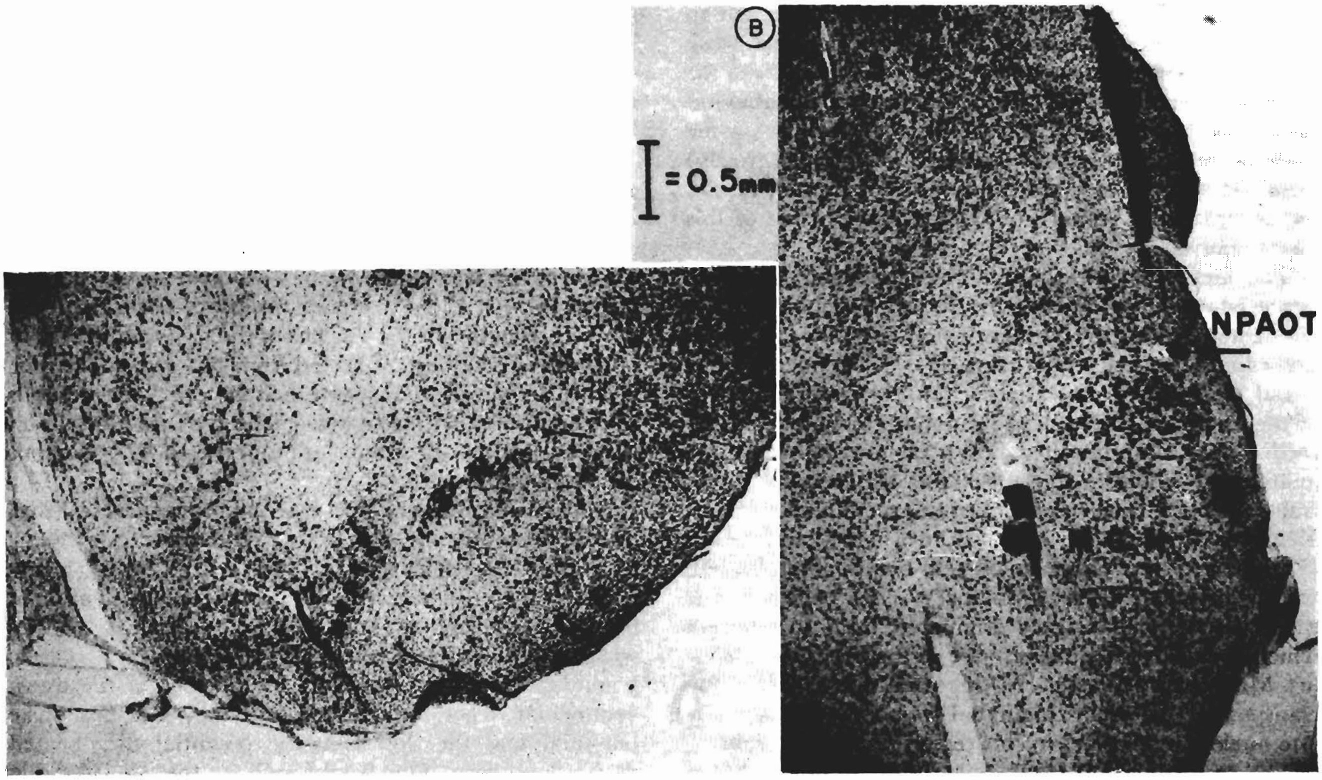


FIG. 8. Photomicrographs of rabbit midbrain stained by Nissl method. *A:* Prussian-blue mark (*arrow*) in nucleus of transpeduncular tract; *B:* Prussian-blue mark in nucleus of the posterior accessory optic tract; *CP*—cerebral peduncle; *MGN*—medial geniculate nucleus; *NPAOT*—nucleus of the posterior accessory optic tract; *NTPT*—nucleus of the transpeduncular tract; *SC*—superior colliculus; *SN*—substantia nigra.

cal descriptions of the nucleus of the transpeduncular tract. Several neuroanatomists have reported that the nucleus is subdivided into a dorsal and a ventral group of cells: Wallenberg (9) in guinea pig; Kosaka and Hiraiwa (10) in rat, guinea pig, dog, and cat; Gillilan (11) in fruit bat, guinea pig, and cat; Hayhow et al (12, 13) in cat and rat. The dorsal subdivision is commonly described as a diffuse group of cells. The ventral portion is larger, more compact, and contains more cells; but the largest cells are in the dorsal subdivision of the nucleus.

Thus, we can infer that the on-off responses come mainly from the larger cells of the dorsal part of the nucleus. Because the cells here are larger and more dispersed, it is easier to isolate single units. On the other hand, pure on responses arise mainly from cells of the ventral group. The difficulty in isolating single on elements is due to their smaller size, and to the compact nature of this group.

There is good agreement in the absolute photic intensity threshold for units in the lateral geniculate nucleus, the superior colliculus, and the nucleus of the transpeduncular tract. The value found ranged from 0.19 to 0.27 lux. Elenius (14) had reported that after intense light adaptation (8000 lux for 30 min) it took 3–4 hr for the ERG to return to 90% of its maximum level. With shorter durations of light adaptation, the ERG returned to criterion level in 45–60 min. Rushton and co-workers (15) made in vivo measurements of the bleaching and regeneration of rhodopsin in the retina of a rabbit. They found that it took 60–90 min for the rhodopsin concentration to return to the prebleaching level. Our findings showed that threshold responses could be obtained after relatively short periods of dark-adaptation. However, in none of our experiments was the animal light-adapted as thoroughly as by the other investigators.

The reaction of the elements in the lateral geniculate nucleus and the superior colliculus to intermittent stimulation was similar to that reported by Arden and Liu (16). On the other hand, the type of response from the nuclei of the accessory optic tract was most unexpected. The greater rate of firing at higher frequencies of stimulation was not seen in the other parts of the visual system. Arden and Liu did not report any units in the rabbit's lateral geniculate nucleus which behaved thus. Neither has there been any report in the literature of any elements in the retina which respond to intermittent stimulation as did those of the accessory optic system. This sequence of events was not seen when the response was evoked by repetitive electrical stimulation of the optic nerve fibers. This indicates that the basis for this phenomenon lies in the retina.

The study of receptive fields of units in the nucleus of the transpeduncular tract has shown that a horizontally oblong area of the central visual field is represented in this nucleus. Unfortunately, no neuroanatomical work has been reported on the projection of the retina into the nucleus. However, Chievitz (17) did describe a horizontal band-shaped area, 3.0 × 0.5 mm, immediately below the optic-nerve head, which appeared different (grossly) from the rest of the rabbit's fundus. Apparently, he did not ascertain rod:cone ratios inside and outside of this "area centralis."

The lack of an antidromic response from the optic nerve when the nucleus of the transpeduncular tract is stimulated led to the postulation of the nucleus of the posterior accessory optic tract (2). This nucleus was found electrophysiologically on the dorsolateral surface of the midbrain, between the superior colliculus and the medial geniculate nucleus. There has always been some anatomical evidence for a synapse between the optic tract and the nucleus of the transpeduncular tract. Koelliker (6), Kosaka and Hiraiwa (10), and Giolli (7) reported the presence of cell bodies lying in the transpeduncular tract. Tümianzew (18) believed that the transpeduncular tract originated from a ganglion which lay near the anterior edge of the superior colliculus. Münzer and Wiener (19) reported that a part of the transpeduncular tract originated from the suprageniculate nucleus, which lies in this region of the midbrain.

The recent work of Hayhow et al. (12, 13) has shown conclusively that the fibers of the transpeduncular tract are in synaptic relationship with the cells which lie in the tract. In both the cat and the rat, they have found two collections of cells: a dorsal terminal cell group which lies at the anterolateral edge of the superior colliculus, and a lateral terminal cell group which is located on the dorsolateral surface of the cerebral peduncle. The fine, preterminal degeneration seen among the cells after enucleation indicate that the fibers are in synaptic relationship with these cells. Hayhow believes that these cells are innervated by collaterals of the main fibers which end in the nucleus of the transpeduncular tract (his medial terminal nucleus).

In the rabbit, Giolli (7) has observed preterminal degeneration among the cells lying in the transpeduncular tract.

The location of the dorsal terminal cell group corresponds to the area which we had reported as the location of the nucleus of the posterior accessory optic tract. All our recordings from the nucleus of the posterior accessory optic tract have been obtained from this cell group. The lack of an antidromic response from the optic nerve is not compatible with Hayhow's idea that the dorsal and lateral terminal cell groups are innervated by collaterals of the main fibers.

Additional evidence for a synapse can be derived from the results of the action potentials evoked by repetitive electrical stimulation of the optic nerve fibers. The recording from the soma of the neuron in the lateral geniculate nucleus showed that at frequencies which did not evoke a spike potential, the synaptic potential was still present. In the nucleus of the transpeduncular tract, on the other hand, the fast spike potential and the slow synaptic potential disappeared at the same time. This would indicate that the blocking is not at this synapse but somewhere earlier. The blocking may be in the axon itself. Or, it may be at a synapse which is located between the optic nerve and the nucleus of the transpeduncular tract, i.e., the nucleus of the posterior accessory optic tract. The latter explanation is more likely.

This still leaves unsolved the problem of the rapid appearance of transynaptic degeneration in the pathway of the rabbit.

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