

Axonal Projection of Prepositus Hypoglossi and Reticular Neurons in the Brainstem of the Cat

O. Hikosaka¹ and Y. Igusa

Department of Neurophysiology, Institute of Brain Research, School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan

Summary. 1. Extracellular unit spikes responsive to horizontal head rotation were recorded in and around the cat nucleus prepositus hypoglossi. Type II neurons (activated by contralateral angular acceleration) were much more common than type I neurons. The axonal projections of single prepositus and reticular neurons to the rostral brainstem were investigated by a systematic survey of antidromic responses using microstimulation.

2. Most type II prepositus and reticular neurons sent axons to the rostral brainstem either ipsilaterally or contralaterally (i-type II or c-type II neurons). The stem axons ran through the reticular formation without forming a discrete fiber tract, usually reaching the midbrain tegmentum.

3. Axons of type II prepositus and reticular neurons were presumed to terminate in some of the following areas: the contralateral prepositus and reticular region, pontine reticular formation, raphe nucleus, pontine tegmental reticular nucleus, midbrain reticular formation, and lateral midbrain tegmentum near the parabigeminal nucleus.

4. The possibility of efferent connections to the abducens and oculomotor nuclei was closely examined. Some of type II prepositus and reticular neurons (24%) sent axonal branches into the abducens nucleus on the ipsi- or contralateral side. The ramification of such axonal branches seemed to be poor. Axonal projection to the oculomotor nuclei was also observed but less commonly (5%).

Key words: Nucleus prepositus hypoglossi - Reticular formation - Abducens nucleus - Oculomotor nucleus

The nucleus prepositus hypoglossi has recently been considered as one of the structures involved in

oculomotor function. Stained cells were found in this nucleus after injection of horseradish peroxidase (HRP) into the oculomotor complex of the cat (Graybiel and Hartweg 1974) and the monkey (Graybiel 1977b; Steiger and Büttner-Ennever 1979). A similar HRP study (Maciewicz et al. 1977) has shown axonal projection to the ipsi- and contralateral abducens nuclei. Electrophysiological studies (Baker and Berthoz 1975; Baker et al. 1977) have suggested monosynaptic connections of prepositus hypoglossi neurons with extraocular motoneurons. Moreover, the nucleus prepositus hypoglossi contains many neurons whose firing patterns are closely coupled with saccades (Baker et al. 1976; Gresty and Baker 1976), optokinetic nystagmus (Baker 1977) or vestibular-induced eye movements (Blanks et al. 1977; Fukushima et al. 1977).

Preoculomotor activity recorded from axons within the abducens nucleus during vestibular nystagmus (Hikosaka et al. 1977) suggested the presence of excitatory neurons behaving like abducens motoneurons. A subsequent study (Hikosaka et al. 1978), however, could not provide evidence that the cellular origin of the excitatory neurons was in nucleus prepositus hypoglossi. Specifically, microstimulation in the abducens nucleus failed to antidromically activate neurons responsive to horizontal head rotation in and around the ipsilateral nucleus prepositus hypoglossi.

This preliminary observation raised a doubt as to whether prepositus hypoglossi neurons send axons mainly to motoneurons of the extraocular muscles. Therefore, we reexamined the axonal projection of horizontal rotation-sensitive neurons located in and around nucleus prepositus hypoglossi. It will be shown that axons of most neurons ascend into the reticular formation and terminate on nearby structures along their courses. Some of these neurons send axonal branches to abducens or oculomotor nuclei.

¹ Present address: Department of Physiology, Toho University School of Medicine, Omori, Ota-ku, Tokyo, Japan

Methods

Forty-one adult cats were used. A tracheal cannula was introduced under ether anesthesia and the animal was mounted on a stereotaxic frame fixed to the turntable, which could be manually rotated in the horizontal plane. For electrical stimulation of the vestibular nerve, bipolar Ag-AgCl electrodes were placed on the round and oval windows of the labyrinths on both sides. The abducens nerve and/or the medial rectus branch of the oculomotor nerve were detached from the muscles they innervated and placed on Ag-AgCl hook electrodes for stimulation or recording of compound action potentials.

In order to trace the axons of single prepositus and reticular neurons as far as possible through successive tracks with a stimulating microelectrode, the dorsal surface of the brainstem was widely exposed from the obex to the pretectum or the posterior thalamus. In most experiments the cerebellar tentorium and the sagittal venous sinus were kept intact, and the microstimulation survey in the pons and midbrain was implemented independently with two stimulating electrodes held by different micromanipulators. In some experiments the venous sinus together with the bony cerebellar tentorium were also removed, and the stimulation survey was done with a single electrode without changing the direction of penetration (Fig. 2).

After the upper cervical cord was transected, the animal was immobilized with gallamine triethiodide (Flaxidil) under artificial respiration. Blood pressure and rectal temperature were maintained within a physiological range. All incision and pressure points were infiltrated with 4% Xylocaine, carefully maintained throughout the experiments. The effectiveness of the local anesthetic was judged by electrocortical patterns as described in detail in a previous paper (Maeda et al. 1972).

Extracellular spike potentials of neurons in and around nucleus prepositus hypoglossi were recorded with 2 M NaCl Fast Green FCF electrodes. Based on several criteria (Hikosaka et al. in press), they were considered to be recorded near cell somata and not from axons. For microstimulation, glass-insulated tungsten electrodes were usually used whose exposed tip was 10–20 μm in length and 5–10 μm in diameter. In some cases glass micropipettes filled with 2 M NaCl Fast Green FCF were used for pontine stimulation. In experiments which aimed exclusively at determining whether or not prepositus and reticular neurons send their axons to the oculomotor nucleus, glass-insulated tungsten electrodes with longer exposed tips were positioned at the center of the medial rectus subdivision of the oculomotor nucleus.

To reconstruct the axonal projection area of each prepositus and reticular neuron, 3–5 stimulating points were marked after each mapping experiment by passing cathodal current (10 μA , 20 s) through the glass-insulated tungsten electrode or by electrophoretic ejection of Fast Green FCF through the glass micropipette. The site of unit recording was also marked by the dye. At the end of the experiment, the animal was deeply anesthetized with ether and perfused with 10% formalin. For double representation of stimulation and recording points in frontal and sagittal sections, the fixed brain was marked by several needle penetrations perpendicular to the sagittal plane and was then cut at the midline, each half being sectioned frontally (e.g. Fig. 2C–F) or sagittally (e.g. Fig. 2A). This procedure made it possible to translocate a point in a frontal section to the symmetrical point in a sagittal section and vice versa. Based on the locations of stimulation points found in the histological sections, the change in brain size was corrected and the electrode tracks for stimulation were reconstructed. When data obtained for different experiments were summarized in a representative section as in Fig. 3, effects of the difference in size and shape among the brains studied were compensated so that the location of each axon relative to the

surrounding structures was kept unchanged by this summarizing procedure.

Results

Type and Location of Prepositus and Reticular Neurons

In order to investigate the course of efferent fibers of prepositus and reticular neurons, extracellular unit spikes were first recorded in the medial brainstem at the level of the abducens nuclei to the obex rostro-caudally and within 2 mm from the midline. We selected 75 units which responded to horizontal angular acceleration of the turntable by changing their firing rate. The present study has focused mainly on a group of neurons which increased their firing rate during contralateral angular acceleration and decreased it with ipsilateral acceleration (73/75). Two neurons responded in the opposite manner. Following Duensing and Schaefer's (1958) classification scheme, these two neuron types will be called type II and type I.

During extracellular recording from single neurons, a glass-insulated tungsten microelectrode was inserted into the brainstem from the surface of the fourth ventricle for stimulation. Rectangular cathodal current pulses (0.1 ms duration) with an intensity of 30–50 μA , were passed through the stimulating electrode in the course of electrode advancement while searching for antidromic responses. Evoked spikes showing the following properties were regarded as antidromic. First, single shock stimulation of suprathreshold intensity induced single spikes at fixed latency (Fig. 1Aa). Second, suprathreshold double shocks with a short interval (usually less than 1 ms) induced double spikes with fixed latencies (Fig. 1Ab). Third, there was evidence that synaptically evoked spikes collided with the stimulus-locked ones. Single shock stimuli were triggered by spontaneous spikes (Fig. 1Ac). The possibility of anodal blocking due to large inward currents through the axonal membrane (Ranck 1975) was carefully checked by frequently lowering stimulus currents.

Electrode penetrations for antidromic activation were first made for each neuron in a nearly frontal plane through the abducens nuclei (Fig. 1B). Intervals between the electrode tracks were no more than 400 μm . With this procedure, almost all neurons examined (type II: 68/73, type I: 1/2) were activated antidromically from some points along the electrode tracks. Among these, 41 type II neurons and 1 type I neuron were activated antidromically from single or multiple loci in the ipsilateral half-plane of the

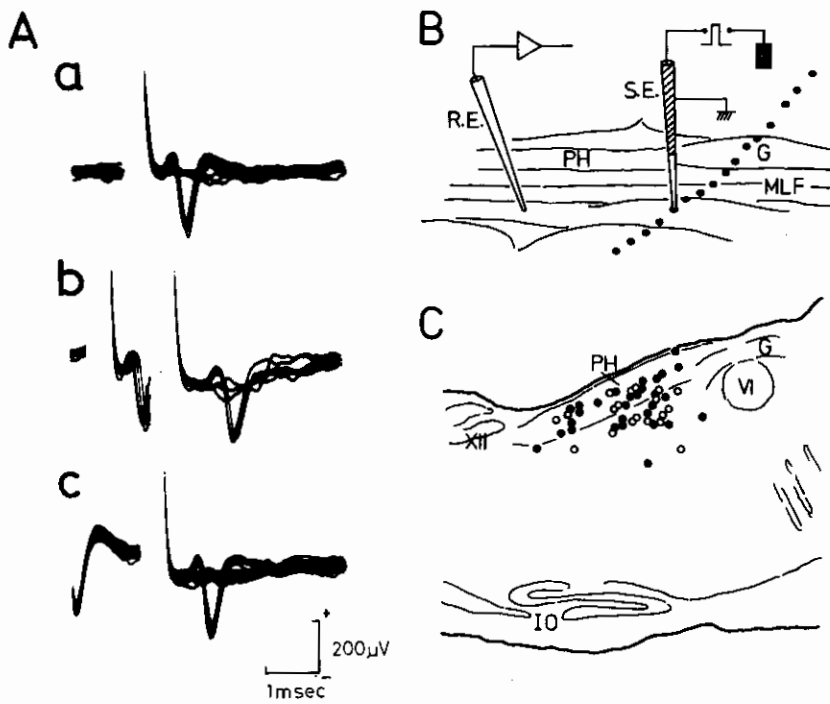


Fig. 1A-C. Type and location of prepositus and reticular neurons. **A** Identification of antidromic spikes of a type II neuron in nucleus prepositus hypoglossi. **a** Responses to single pulse stimuli (0.1 ms, 13 μ A) in a threshold-straddling condition. **b** Responses to double shocks at an intensity near threshold for the second spikes. **c** Collision test between spontaneous and evoked spikes. The sweeps were triggered by spontaneous spikes which were followed by single pulse stimulations in the brainstem. **B** Arrangements of recording electrode (R.E.) and stimulating electrode (S.E.). Dots show points at which the electrode was successively inserted for antidromic activation. Details are described in the text. **C** Recording sites of i-type II neurons (filled circles) and c-type II neurons (open circles) projected on a representative sagittal section. VI Abducens nucleus. XII hypoglossal nucleus. G genu of the facial nerve. IO inferior olive. MLF medial longitudinal fasciculus. PH nucleus prepositus hypoglossi

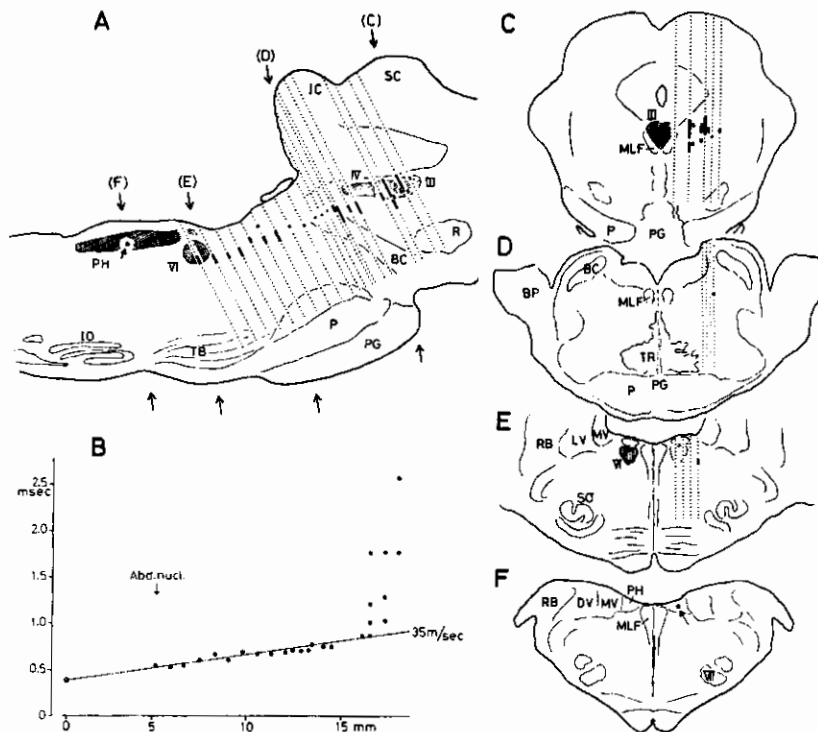


Fig. 2A-F. Axonal trajectory of a type II neuron in nucleus prepositus hypoglossi. **A** Sagittal section showing all electrode tracks (dashed lines). Thickened portions indicate stimulation sites from which antidromic spikes were evoked with cathodal pulse stimuli of 30 μ A or less. The oculomotor (III), trochlear (IV) and abducens (VI) nuclei as well as the nucleus prepositus hypoglossi (PH), located medial to this section, are projected as hatched areas. Recording site of the neuron is projected as a dot with arrow. **B** Latencies of antidromic spikes (ordinate) plotted as a function of the estimated distance over which the action potentials propagated (abscissa). The straight line is drawn by hand. **C-E** Frontal sections at three different rostro-caudal levels showing electrode tracks for stimulation (dashed lines) and effective sites for antidromic activation (thickened portions). The levels are shown in **A** by arrows with capital letters in parentheses. **F** Recording site of the prepositus neuron. Additional abbreviations: VII facial nucleus. BC brachium conjunctivum. IC inferior colliculus. LV lateral vestibular nucleus. MV medial vestibular nucleus. P pyramidal tract. PG pontine gray. R red nucleus. RB restiform body. SC superior colliculus. SO superior olive. TB trapezoid body. TR pontine tegmental reticular nucleus

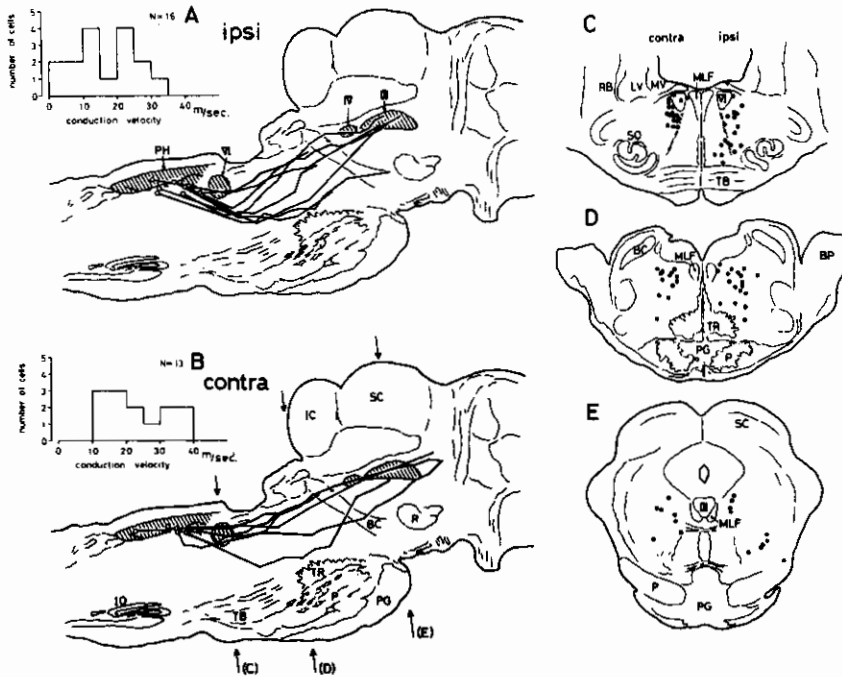


Fig. 3A-E. Summary of axonal trajectories of type II neurons in and just ventral to nucleus prepositus hypoglossi. **A** 8 i-type II neurons. **B** 6 c-type II neurons. Thick lines indicate the estimated course of the ascending stem axons obtained by experiments similar to that in Fig. 2. Open circles indicate the recording sites of the neurons. Note that the cell bodies of c-type II neurons are located in the contralateral brain stem. Inset histograms in **A** and **B** show the distributions of axonal conduction velocities of i-type II and c-type II neurons, respectively. **C-E** Estimated locations of the stem axons (dots) at three different rostro-caudal levels. The levels of the sections are shown in **B** by arrows. Axons of i-type II and c-type II neurons are shown on the right and left sides of the sections, respectively

brainstem (i-type), while the remaining 27 type II neurons were activated antidromically from the contralateral brainstem (c-type). No neurons were found to be activated antidromically from both the ipsi- and the contralateral brainstem throughout the rostro-caudal levels.

Recording sites of 29 i-type II neurons and 19 c-type II neurons were projected on a parasagittal section (filled and open circles in Fig. 1C). They were located by dye marks in histological sections or estimated by measuring distances from appropriate landmarks, such as the genu of the facial nerve, the midline and surface depths, with the aid of the micromanipulator scale. They are illustrated together, since the results of the two methods corresponded well after correcting for the change in brain size. About half of the neurons were in nucleus prepositus hypoglossi and the other half in the dorsal reticular formation. i-type II neurons and c-type II neurons were intermingled.

Axonal Trajectory of Single Prepositus and Reticular Neurons

Figure 2 shows an experiment which aimed at determining the whole axonal trajectory of a single type II neuron in the right nucleus prepositus hypoglossi (Fig. 2A and F). Antidromic responses were obtained in the course of an electrode track 2.0 mm from the midline (Fig. 2A and E). The stimulating electrode was then moved 800 μ m to reveal the

rostral extension of effective sites for antidromic activation. In this way effective sites for antidromic activation were traced step by step up to the level of the oculomotor nuclei (Fig. 2A). In the ascending course, they gradually shifted lateralward (Fig. 2D), reaching 2.8 mm from the midline at the level of the trochlear nuclei. In the midbrain, antidromic responses were obtained from several isolated regions in the tegmentum lateral to the oculomotor complex (Fig. 2C). The most medial penetrations through the lateral part of the oculomotor complex, however, were not effective in evoking antidromic responses.

In Fig. 2B, latencies of antidromic spikes were plotted against distance from the cell soma. The proximal portion of the stem axon was tentatively assumed to be straight from the soma to the most caudal of the effective sites. Within the pontine tegmentum, the antidromic latencies lengthened almost linearly with the increase in distance (straight line in Fig. 2B) except for one later response which was evoked from an isolated spot (see Fig. 2A). This suggests that the type II neuron had a stem axon in the dorsolateral pontine tegmentum. The conduction velocity of the stem axon calculated from the slope of the straight line in Fig. 2B was 35 m/s. The latent period for spike initiation in the stem axon was estimated as the time obtained by extrapolation of the straight line to distance 0, yielding 0.37 ms. Antidromic spikes evoked from most of the multiple loci in the midbrain tegmentum, particularly the

more medial ones, had significantly longer latencies than those expected as a simple continuation of the stem axon. It is suggested, therefore, that many fine axonal branches having slow conduction velocities are emitted from the stem axon. The rostral extent of the axon was not determined, since the most rostral electrode track examined was also effective in evoking antidromic responses.

Similar experiments were undertaken for 32 type II neurons. Axonal trajectories of 8 i-type II neurons and 6 c-type II neurons are illustrated as thick lines in Fig. 3A and B. Those neurons have been selected whose antidromic latencies measured at serially ordered effective loci showed a gradual, almost linear, change as in Fig. 2B. Estimated axonal branches diverging from the stem axon are not shown in these figures. Since in most experiments the level of the abducens nuclei was the most caudal limit of electrode penetration for stimulation, the proximal portion of each axonal trajectory is tentatively illustrated by connecting the recording site of each neuron and the most caudally located effective site. The rostral termination of the thick lines does not necessarily indicate the termination of the stem axons, for in some cases the experiments had to be terminated when unitary spikes were lost or because of other inappropriate experimental conditions.

Some features of the axonal trajectories can be drawn from the data in Fig. 3A-E. First, we could not find any axons ascending in the medial longitudinal fasciculus (MLF). Second, the stem axons of i-type II neurons, shortly after leaving the cell somata, scattered out widely in the relatively deeper part of the pontine tegmentum, and appeared to diverge into two groups in the midbrain: one situated in the tegmentum lateral to the oculomotor complex, the other situated in the ventrolateral part of the tegmentum. Third, the stem axons of c-type II neurons, after crossing the midline, were localized just lateral or ventral to the abducens nucleus, thereafter becoming scattered at the rostral pontine and midbrain levels. Axons of 3 c-type II neurons were shown to reach the levels rostral to the oculomotor nuclei near the caudal end of the thalamus. The level of midline crossing was investigated in detail for 3 c-type II neurons, revealing that all of their stem axons crossed the midline nearly at the level of their cell somata. In the insets of Fig. 3A and B are shown histograms of axonal conduction velocities of i- and c-type II neurons. Both of them had a relatively wide distribution. There was no significant difference between the conduction velocities of these two axon groups. No correlation was noted between the location of the cell soma and either the axonal projection pattern or the axonal conduction velocity.

Target Areas of Rotation-sensitive Prepositus and Reticular Neurons

In the course of tracing single stem axons we frequently noticed additional effective loci for antidromic activation which were separated from the trajectories of the stem axons and had significantly longer latencies (see Fig. 2), suggesting the presence of axonal branches. We investigated axonal branching patterns in more detail at several rostro-caudal levels. For this purpose, effective sites for antidromic activation were surveyed with closely spaced penetrations and threshold and latency were measured at depths differing by 100 μm . Considering that most type II neurons we sampled had firing patterns quite similar to that of ipsilateral abducens motoneurons (Hikosaka et al. 1978), and consequently to that of contralateral medial rectus motoneurons, we took particular care to see whether type II neurons send axonal branches into the oculomotor complex or the abducens nuclei.

1. Axonal Branches in or Around the Abducens Nucleus

Forty-two type II neurons (22 i-type II and 20 c-type II) were examined at this level. Thirteen i-type II and 14 c-type II neurons showed no signs of axonal branching. An example is shown in Fig. 4A-C. Antidromic spikes were evoked in the course of four electrode penetrations (thickened portions along tracks No. 1-4 in Fig. 4A) with stimuli of about 20 μA or less. The contour obtained by encircling these effective portions is nearly circular. Antidromic latencies were between 0.60-0.64 ms. Threshold currents for antidromic activation were plotted against depths of the stimulating electrode and interconnected for each penetration (Fig. 4B). With downward electrode advancement, in each penetration the threshold decreased monotonically to the minimum and then increased monotonically. Then, the thresholds were replotted against lateralities (or track No.) and the points obtained at the same depths interconnected (Fig. 4C). This figure shows monophasic threshold-distance relationships in the medio-lateral direction. These data did not suggest the presence of axonal branches, because of the above threshold-distance relationships as well as relatively constant antidromic latencies. Similar threshold-distance (depth) relationships obtained from three other type II neurons with conduction velocities ranging from 13 to 20 m/s are shown in Fig. 4D. A cathodal current pulse of 20 μA appeared to activate axons at most 500 μm away from the electrode tip.

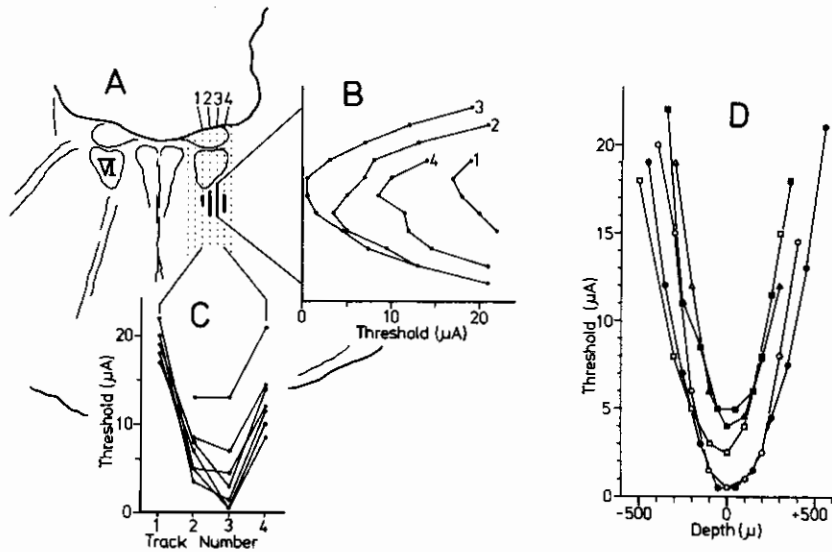


Fig. 4A–D. Antidromic activation without axonal branching. **A–C** Data obtained from a type II neuron recorded in the reticular formation just ventral to the left nucleus prepositus hypoglossi. **A** Frontal section through the abducens nuclei showing electrode tracks for stimulation at 200 μm intervals (dotted lines) and sites from which the neuron was activated antidromically with stimulus currents of less than 20 μA (thick solid lines). **B** Threshold currents of antidromic activation (abscissa) as a function of the depths of the electrode tip for stimulation (ordinate). Thresholds were measured every 100 μm . **C** Threshold currents of antidromic activation (ordinate) as a function of lateralities of the electrode tip (abscissa). Data replotted from **B**. Points obtained at the same depths are interconnected. **D** Depth-threshold relationship obtained from three c-type II neurons (2 in the nucleus prepositus hypoglossi and 1 in the reticular formation). They are arranged so that the point of the minimum threshold is at depth 0, upper points being on the left side (negative values) and lower points on the right side (positive values)

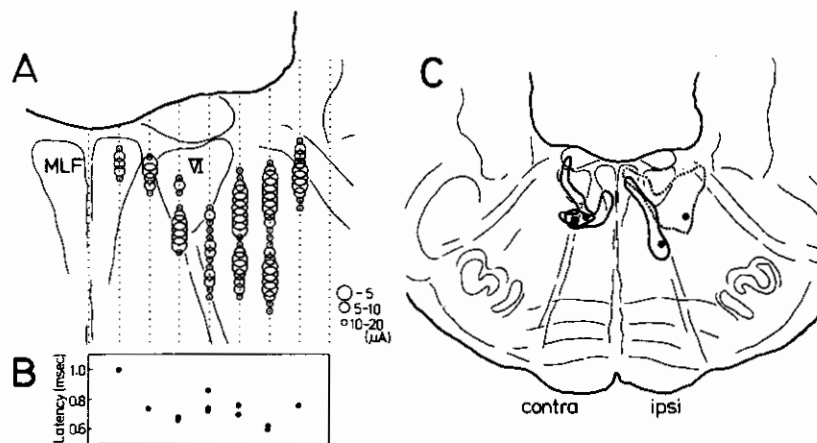


Fig. 5A–C. Antidromic activation suggesting axonal branches in and around the abducens nucleus. **A** and **B** Data obtained from a type II neuron in the reticular formation ventral to the right nucleus prepositus hypoglossi. **A** Distribution of effective sites for antidromic activation. Threshold currents are denoted by the size of circles. Dots without circles indicate ineffective sites for antidromic activation with stimulus currents of less than 20 μA . **B** Latencies of antidromic spikes (ordinate) plotted against laterality of effective sites (abscissa). Dots are positioned relative to the abscissa so as to correspond to the lateralities of the stimulating points in **A**. **C** Contours of axonal branching estimated on the basis of experiments similar to **A** and **B**. The area encircled by the dotted line is from the data in **A** and **B**. Axons of 2 i-type II neurons and 2 c-type II neurons are shown on the right and left sides, respectively. They were recorded invariably in the reticular formation ventral to nucleus prepositus hypoglossi. A dot in each encircled area indicates the estimated position of the stem axon

Nine i-type II and 6 c-type II neurons showed signs of axonal branching at the rostro-caudal level of the abducens nuclei. Of these, 6 i-type II and 4 c-type II neurons sent axonal branches into the abducens nuclei. An example is shown in Fig. 5A and B. This i-type II neuron, located beneath the nucleus pre-

positus hypoglossi, had the most extensive axonal branching pattern at this level. Several low threshold foci appeared to be distributed in the tegmentum, abducens nucleus and MLF. Judging from the latency distribution (Fig. 5B), it seems likely that the stem axon was located in the tegmentum lateral and

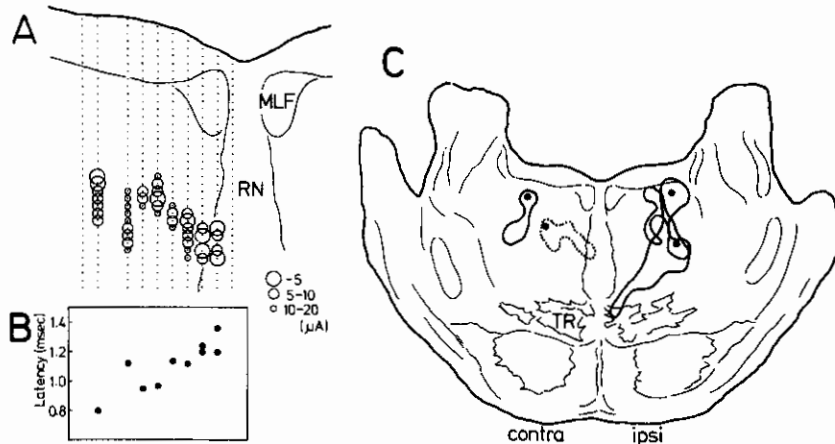


Fig. 6A–C. Antidromic activation suggesting axonal branching in the pontine tegmentum. Arrangements similar to Fig. 5. **A** and **B** Data obtained from a type II neuron in the right nucleus prepositus hypoglossi. **A** Distribution of effective sites and threshold currents for antidromic activation of the neuron. **B** Latencies of antidromic spikes plotted against laterality of effective sites. **C** Contours of axonal branching of the neuron in **A** and **B** (dotted line) and another 3 (2 i-type prepositus and 1 c-type reticular) type II neurons (solid line)

ventral to the abducens nucleus. Other low threshold foci may thus indicate axonal branches. Since a similar examination at a pontine level 4 mm rostral to the abducens nuclei revealed a single antidromic effective locus (latency: 1.02 ms), the axonal branches shown at the abducens nucleus level may terminate on neurons at this level.

The contours of axonal branches of 4 type II neurons obtained in similar experiments are shown in Fig. 5C. In general, antidromic effective loci in the abducens nucleus, when present, were sparse and usually deviated from the center of the nucleus. Taking antidromic latencies into account, it was presumed that a stem axon situated ventrally or laterally to the abducens nucleus sent out a collateral branch toward the abducens nucleus. The branch took its course near the medial or lateral border of the nucleus, finally leaving it. The conduction velocity of such an axonal branch was estimated for 5 type II neurons. It ranged from 3.0 to 7.9 m/s (mean: 5.3 m/s), indicating that these axonal branches were relatively fine. Axonal branches seen in the MLF never ascended nor descended more than 1 mm. When the antidromic activation pattern was investigated at levels caudal to the abducens nuclei, particularly for c-type II neurons, features suggesting the presence of axonal branches in the contralateral nucleus prepositus hypoglossi were occasionally seen.

2. Axonal Branches in the Pontine Tegmentum

Experiments were usually carried out at a level 4 or 5 mm rostral to the abducens nuclei. Twelve out of 16 i-type II and 10 out of 15 c-type II neurons sampled had axonal branches in this area. An example of a c-type II neuron recorded in nucleus prepositus hypoglossi is shown in Fig. 6A and B. Judging from the

antidromic effective sites and their latencies, the stem axon sent out an axonal branch, which soon emitted a small branch ventrally, running medialward and bifurcating into two branches near the raphe nucleus. The conduction velocity of this axonal branch was about 4.6 m/s, assuming that it was located entirely in this plane. The conduction velocity of the stem axon, calculated as in Fig. 2, was 13 m/s.

Axonal branching contours selected from 4 type II neurons are shown in Fig. 6C. The projection area was variable for different neurons, i.e. different parts in the pontine reticular formation, the raphe nuclei or the pontine tegmental reticular nucleus. Several experiments which surveyed other rostro-caudal levels (from the abducens nuclei to the inferior colliculus) also suggested relatively frequent emission of axonal branches.

3. Axonal Branches in or Around the Oculomotor Nuclei

In order to examine in detail the possibility of efferent connections with medial rectus motoneurons, the tip of the stimulating microelectrode was located at the center of the medial rectus subdivision as identified by an antidromic field potential in response to weak stimulation of the medial rectus branch of the oculomotor nerve as well as by type I-like multi-unit discharges in response to manual rotation of the turntable. Each time a rotation-sensitive unit spike was isolated in and around the nucleus prepositus hypoglossi, cathodal current pulses of 100 μ A (0.1 ms duration) were passed through the stimulating electrode to test for the presence of antidromic responses. In the prepositus and reticular region ipsilateral to the stimulated medial rectus subdivision, 8/119 type II and 1/14 type

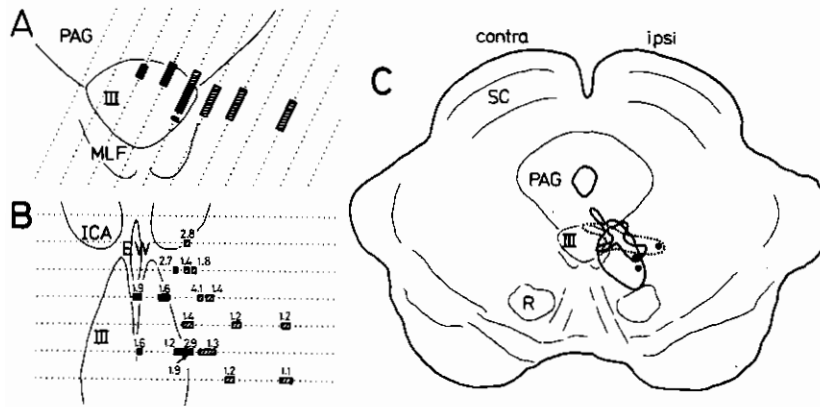


Fig. 7A–C. Antidromic activation suggesting axonal branching in and around the oculomotor nuclei. **A** and **B** Distribution of effective sites for antidromic activation of a type II neuron at the ventral border of the right nucleus prepositus hypoglossi. Stimulus currents were less than $50 \mu\text{A}$. They are illustrated as projections onto a frontal section (**A**) and a horizontal section (**B**). Effective sites in and outside the oculomotor nuclei are denoted, for convenience, by filled and hatched bars, respectively. Data were obtained from 25 oblique electrode tracks for antidromic activation. Figure above each bar in **B** indicates the latency of antidromic spikes evoked from the site. **C** Contours of axonal branching of the neuron in **A** and **B** (dotted line area) and another 2 neurons in the reticular formation ventral to the right nucleus prepositus hypoglossi (solid line area). Additional abbreviations: EW nucleus of Edinger-Westphal. ICA interstitial nucleus of Cajal. PAG periaqueductal gray

I neurons were antidromically activated. In the prepositus and reticular regions contralateral to the stimulation site, only 1/85 type II and 0/4 type I neurons were activated antidromically. The location of the electrode tip was marked in each experiment and those points were invariably found in the lateral and somewhat dorsal part of the oculomotor nuclei, in agreement with previous anatomical (Tarlov and Tarlov 1971; Gacek, 1974) and physiological (Baker and Highstein 1978) studies.

Some of the neurons which exhibited antidromic responses were studied in further experiments so as to give an outline of the axonal projection area. An example of a neuron recorded at the ventral border of nucleus prepositus hypoglossi is shown in Fig. 7A and B. The stimulus intensity was fixed at $50 \mu\text{A}$ and all of the 25 tracks were made in an oblique direction traversing or bypassing the oculomotor nuclei. Electrode tracks (dotted lines) and effective antidromic sites (thickened, filled and hatched portions) are projected onto a frontal section (Fig. 7A) and a horizontal one (Fig. 7B). This figure suggests that the axon, which was at first laterally situated, proceeded to the oculomotor complex, emitting collaterals to its dorsal and lateral divisions. In addition, several isolated effective sites presumably indicating small axonal branches were seen just lateral to the rostral oculomotor complex up to the interstitial nucleus of Cajal. This axonal projection pattern was to some extent common to other neurons (Fig. 7C). Effective sites for antidromic activation were scattered sparsely in the lateral or dorsal part of the oculomotor

complex. No axonal branches were found to cross the midline to the other side of the oculomotor complex.

4. Axonal Branches in the Lateral Tegmentum of the Midbrain

With electrode tracks almost perpendicular to the stereotaxically horizontal plane, a relatively discreet area was found in the lateral part of the midbrain in which some type II neuron axons terminated (Fig. 8). Figure 8A and B shows a detailed map of antidromic thresholds and latencies obtained for a type II neuron in the reticular formation just beneath the right nucleus prepositus hypoglossi. The effective sites occupied a discreet area in the lateral tegmentum just medial to the medial lemniscus. Many low threshold foci are seen in this area (Fig. 8A). The more lateral stimulating points are, the longer antidromic latencies tend to be (Fig. 8B). It seems likely, therefore, that at this rostro-caudal level the stem axon was situated at the most medial part and had laterally projecting axonal branches which arborized profusely.

With cathodal current pulse stimuli of $100 \mu\text{A}$ delivered at a fixed point in the lateral tegmentum just medial to the medial lemniscus, the presence of antidromic responses was examined for 40 type II and 4 type I neurons in the ipsilateral prepositus and reticular regions and 14 type II and 3 type I neurons in the contralateral prepositus and reticular regions. Of these, 7 type II neurons in the ipsilateral prepositus and reticular regions were activated anti-

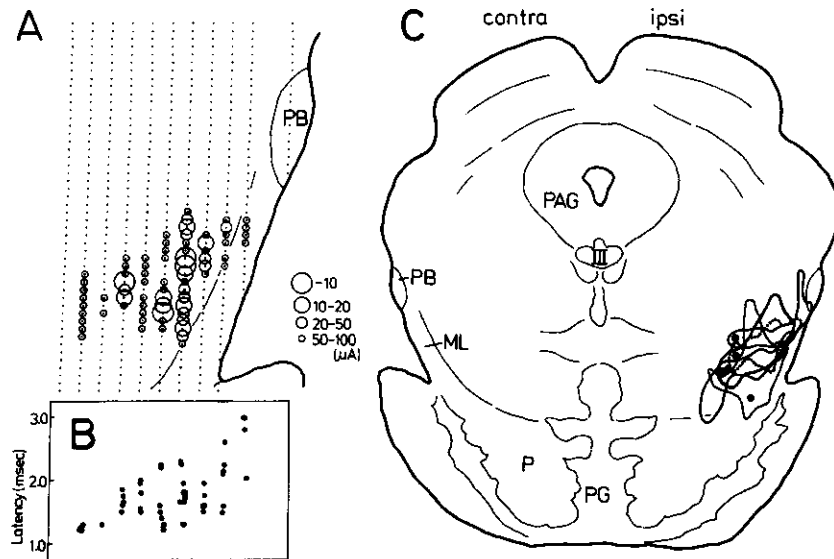


Fig. 8A-C. Antidromic activation suggesting axonal branching in the lateral tegmentum of the midbrain. **A** and **B** Data obtained from a type II neuron in the reticular formation ventral to the right nucleus prepositus hypoglossi. **A** Distribution of effective sites and threshold currents for antidromic activation of the neuron. **B** Latencies of antidromic spikes plotted against laterality of effective sites. **C** Contours of the axonal branching of the neuron in **A** and **B** (dotted line area) and another 4 (3 prepositus and 1 reticular) type II neurons (solid line areas). Additional abbreviations: ML medial lemniscus. PB parabigeminal nucleus

dromically and none of the cells in the contralateral region were activated. Axonal projection areas investigated as in Fig. 8A are shown for 5 neurons in Fig. 8C. Their contours as well as the sites of the stem axons are similar. All showed signs of profuse ramification as in A. Some branches were seen to sweep over the parabigeminal nucleus or to reach out toward the pontine nucleus. The three-dimensional extent of the axonal projection area was roughly examined for 3 type II neurons by changing the rostro-caudal level of stimulating electrode tracks. The antidromic activation pattern suggesting axonal ramifications was found within about 1 mm rostrally as well as caudally from the level shown in Fig. 8. The axonal projection area seems to correspond to or, at least, overlap with the retrorubral nucleus (Berman 1968) or the paralemniscal region (Henkel and Edwards 1978). In one type II neuron, a single axon was found to extend further rostrally up to the lateral tegmentum medial to the magnocellular part of the medial geniculate nucleus. This rostralward axon, however, showed no sign of branching.

Discussion

The present study has investigated axonal projections of a particular functional group of neurons in the medial part of the caudal brainstem sensitive to horizontal head rotation. In agreement with previous studies (Blanks et al. 1977; Fukushima et al. 1977; Hikosaka et al. 1978), these horizontal rotation-sensitive neurons were found primarily in or just beneath nucleus prepositus hypoglossi. In the present study no conspicuous relationship was implicated between locations of cell bodies and axonal projection patterns.

Extra MLF Fiber Tract of Prepositus and Reticular Neurons

The MLF has been considered to be a most important fiber tract among those conveying vestibular or oculomotor signals. Most efferent fibers which originate from the vestibular nuclei and project to the ocular motor nuclei ascend within the MLF (McMasters et al. 1966; Tarlov 1970; Gacek 1971). Axons of interneurons in the abducens nucleus which eventually terminate on medial rectus motoneurons (Highstein and Baker 1978) are confined to the MLF (Bienfang 1978). Several groups of vestibular neurons, however, have recently been shown to send axons outside the MLF, for example, into the brachium conjunctivum (Highstein 1973; Graybiel and Hartweg 1974; Yamamoto et al. 1978) and the ascending Deiters tract (Gacek 1971; Baker and Highstein 1978; Reisine and Highstein 1979).

Ascending axons of prepositus and reticular neurons in the present study, therefore, may provide another extra-MLF fiber tract conveying vestibular and/or eye movement signals. It may be difficult to relate them to any well identified fiber tract.

Problems of the Nucleus Prepositus Hypoglossi Preoculomotor Role

Recent anatomical and electrophysiological studies have shown the efferent projection of neurons in the nucleus prepositus hypoglossi to oculomotor nuclei (Graybiel and Hartweg 1974; Baker and Berthoz 1975; Graybiel 1977b; Steiger and Büttner-Ennever 1979). The present study has shown that a small portion (5%) of horizontal rotation-sensitive neurons

in and around nucleus prepositus hypoglossi project to the medial rectus subdivision of the oculomotor nucleus. Since nucleus prepositus hypoglossi contains neurons related to vertical eye movements as well (Baker et al. 1976; Blanks et al. 1977), it seems likely that some neurons activated antidromically or back-filled with HRP from the oculomotor nucleus could be related to vestibular sensitivity and/or eye movements other than horizontal. In addition, other types of neurons, such as those having visual receptive fields or neck displacement sensitivity, have been found in and around the nucleus prepositus hypoglossi (Gresty and Baker 1976).

Efferent projections of prepositus hypoglossi neurons to the abducens nuclei have also been shown in HRP studies (Maciewicz et al. 1977). The present study shows that some (24%) of the horizontal rotation-sensitive neurons in and around nucleus prepositus hypoglossi send axonal branches to the ipsi- or contralateral abducens nucleus. Such axonal branches, however, were sparse and avoided the center of the nucleus, with no indication of profuse ramification.

These data would reduce the possibility that the major function of prepositus hypoglossi neurons is the generation of horizontal eye movements by their direct action on motoneurons. Yet, some problems remain to be solved. We might have overlooked axons of small caliber, since larger current densities may be required to activate such fine axons. Other factors should be considered in interpreting the present results. Considering that the dendritic motoneuron fields in the oculomotor nuclei (Ramón y Cajal 1911; Edwards and Henkel 1978) and the abducens nuclei (Lorente de Nó 1938) are not confined within the ordinary anatomical border of these nuclei, axons of prepositus and reticular neurons might have synaptic contacts with these motoneurons mostly at their distal dendrites and, therefore, stimulation within them might often have been ineffective in evoking spikes in those axons.

Target Areas of Rotation-sensitive Prepositus and Reticular Neurons

The present study suggests target areas of prepositus and reticular type II neurons. They seem to contain several structures related to eye or head movements: paramedian pontine reticular formation (Bender and Schanzer 1964; Cohen et al. 1968), midline omnipause area (Keller 1974; Evinger et al. 1977; Keller 1977), midbrain reticular formation (Büttner et al. 1977; Graybiel 1977b; Büttner-Ennever and Büttner 1978; King and Fuchs 1979) and interstitial

nucleus of Cajal (Hassler 1972; Fukushima et al. 1978). The axonal projection to the lateral midbrain tegmentum has been shown in anatomical studies (Graybiel 1977a; Baleyrier and Magnin 1979). This area, together with the parabigeminal nucleus, has recently drawn attention as one of the satellite structures of the superior colliculus (Graybiel 1977a; Graybiel 1978; Henkel and Edwards 1978; Edwards et al. 1979). Moreover, Henkel and Edwards (1978) have suggested the role of this area in the control of pinna movements.

Some functions of prepositus and reticular type II neurons would thus be implied besides possible roles in oculomotor control. They might, for instance, subserve the coordination of eye, head and pinna movements, all of which are expected to work conjointly, say, during orienting response to novel stimuli. Or they might transmit vestibular and/or eye movement signals to the higher-order visual or oculomotor systems instead.

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