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Axonal Patterns and Sites of Termination of Cat Superior Colliculus Neurons Projecting in the Tecto-Bulbo-Spinal Tract

A. Grantyn and R. Grantyn

Carl-Ludwig-Institute of Physiology, Karl-Marx-University, Liebigstr. 27, DDR-7010 Leipzig, German Democratic Republic

Summary. Horseradish peroxidase was injected in the somata or axons of neurons located in the intermediate and deep layers of the superior colliculus. A group of 34 neurons with physiologically identified projection in the predorsal bundle (tecto-bulbo-spinal neurons, TBSNs) and two commissural tecto-tectal neurons were characterized with regard to soma-dendritic profiles, axon trajectories, collateral branching, and terminations.

TBSNs belong to the class of large, multipolar, wide field neurons. They send axons through the deep white layer without generating local collaterals. Prior to decussation, all TBSNs bifurcate into an ascending branch which reaches the caudal diencephalon, and a main axon descending to the medulla or spinal cord. Regularly spaced collaterals supply a variety of structures at all rostro-caudal levels. In the midbrain, preterminal and terminal ramifications are present in the medial and lateral reticular tegmentum, in the central grey (including its supraoculomotor zone), in the nuclei of Cajal and Darkschewitsch and in the medial aspects of the prerubral area and the fields of Forel. Rhombencephalic targets of TBSNs include the medial pontine and bulbar reticular formation, the abducens nucleus, the nucleus reticularis tegmenti pontis and the nucleus prepositus hypoglossi. An increased density of terminal ramifications was found in several brain stem regions related to the control of eye and head movements. The widespread connections of each individual TBSN suggest that neurons of this type may provide a spatio-temporal pattern of facilitation which promotes rapid orientation of eyes, head and body towards the contralateral hemifield but does not specify the details of movement to be executed.

Key words: Colliculus superior – Projection neurons – Intracellular HRP – Axonal architecture – Tecto-reticular connections – Oculomotor control – Eye-head coordination

Offprint requests to: Dr. A. Grantyn (address see above)

Introduction

The superior colliculus (CS) is a favorable model structure for the studies of sensori-motor transformation processes. Current concepts emphasize its role in the integration of multimodal sensory inputs and in the control of eye, head and body movements underlying rapid orientation towards external objects (see recent reviews: Sparks and Pollack 1977; Wurtz and Albano 1980).

The intermediate and deep layers of the CS are regarded as the site of origin of output signals related to the initiation of gaze shifts. This conclusion has been derived from recordings of neuronal discharges correlated to saccades or head movements (Schiller and Stryker 1972; Wurtz and Goldberg 1972; Sparks 1975; Straschill and Schik 1977; Harris 1980; Peck et al. 1980) and from anatomical demonstration of direct connections between the deep division of the CS and the so-called 'preoculomotor' regions of the brain stem (Altman and Carpenter 1961; Kawamura et al. 1974; Graham 1977; Harting 1977; Edwards and Henkel 1978). However, anatomical studies provided an excellent illustration of the multiplicity of tectal efferent pathways, the 'preoculomotor' areas being only one of many targets addressed by fibers from the deeper collicular layers. This prompted the development of a concept which describes the tectal efferent system as composed of morphologically distinct subsets of projection neurons subserving, presumably, different functions (see e.g. Edwards 1980). Recent studies employing the retrograde transport of HRP reinforced the idea of anatomical parcellation by showing that collicular

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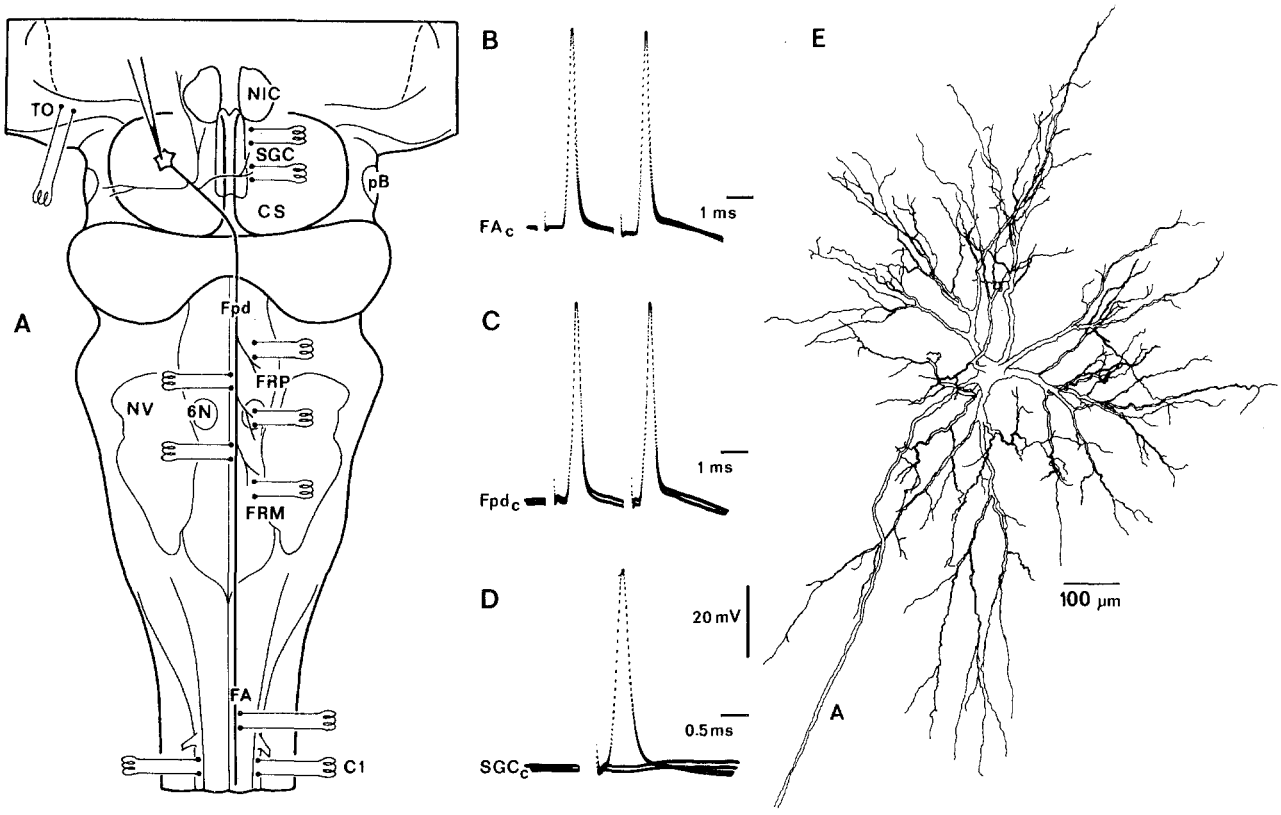

Fig. 1A–E. Electrophysiological identification and soma-dendritic profile of tecto-bulbo-spinal neurons (TBSN). **A** Schematic diagram of experimental arrangement for antidromic activation of TBSNs. **B–D** Specimen recordings of antidromic spikes to show identification of axonal projection into the contralateral predorsal bundle (Fpd_c) and anterior funiculus (FA_c) and collaterals to the perioculomotor zone of the central grey (SGC_c). **E** Soma, dendrites, and axon (A) of a representative TBSN. Complete reconstruction from serial sections after intrasomatic HRP injection

neurons projecting to different brain stem structures can be distinguished according to their size and topographical distribution (Henkel and Edwards 1976; Edwards 1977; Edwards and Henkel 1978; Kawamura and Hashikawa 1978; Weber et al. 1978, 1979). Similarly, models based on physiological experiments have been introduced which incorporate functional subsets of efferent neurons with differential influences on the components of the saccadic pulse generator (Keller 1979, 1980; Wurtz and Albano 1980) or on premotor circuits related to either the coordination of eye movement or head movement (Roucoux et al. 1980).

The available information does not yet allow to match such hypothetical functional subsets with morphologically and electrophysiologically defined classes of tectal neurons. Further elaboration of criteria for classification of efferent neurons is a necessary step in this direction which should contribute to our understanding of the intrinsic organization of the CS and of its role in motor control. In the present study intracellular HRP injections were utilized to characterize the somadendritic profiles and axonal branching patterns of collicular neurons projecting in the predorsal bundle and eventually reaching the spinal cord (tecto-bulbo-spinal neurons – TBSN). It was found that these neurons, all of which are large multipolar cells, take part in most of the known connections of the deeper CS layers. However, a certain degree of specificity can be recognized in their collateral and termination patterns.

Methods

Adult cats weighing 2.0–3.5 kg were anesthetized with Nembutal (35 mg/kg) and paralyzed with gallamine triethiodide. The medial portion of the cerebellum was extirpated to gain access to the rhombencephalon. In some preparations the caudal part of the left hemisphere also was removed to allow visual control of electrode placement in the superior colliculus. The exposed brain surface was continuously superfused with warmed Ringer's solution. Blood pressure, body temperature, and pupil reactions were monitored and were used to adjust the level of anesthesia by repeated injections of small doses of Nembutal. Prednisolut (1.5 mg/kg), atropine sulphate (0.5 mg) and chlorpromazine (0.5 mg/kg) were used for premedication.

The course and ramification patterns of tectal efferent neurons were traced by a combination of electrophysiological and

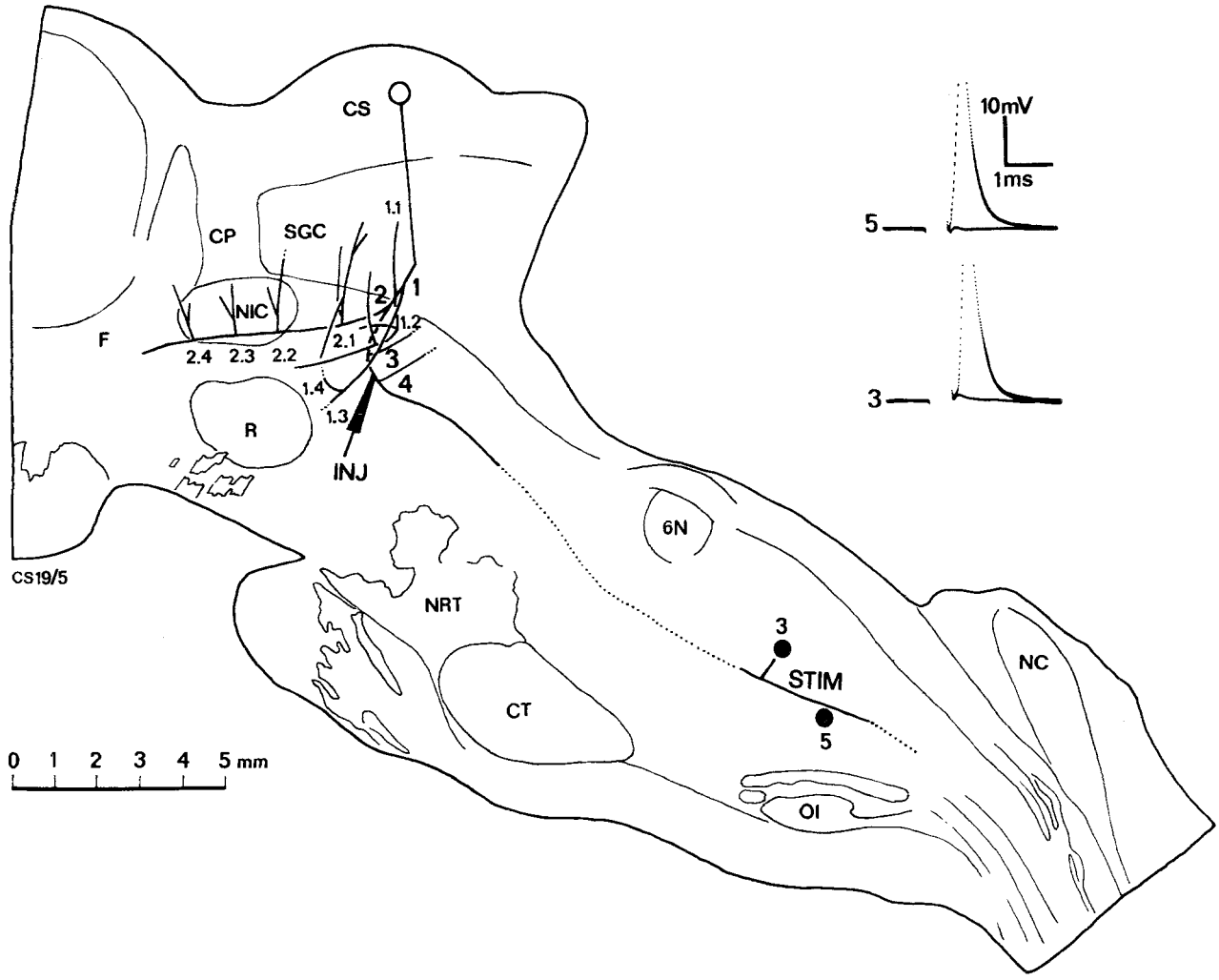

Fig. 2. Axonal pattern of TBSN labeled by HRP injection into the main axon near the dorsal tegmental decussation. Schematic drawing in parasagittal plane. INJ: injection site. *Solid lines:* part of axonal tree reconstructed on the basis of HRP staining. *Dotted line:* extension of the main axon into the medulla as demonstrated by antidromic response to contralateral predorsal bundle stimulation at point 5 (record 5). Presence of collaterals in the bulbar tegmentum proved by antidromic response to stimulation at point 3 located 1.7 mm from midline (record 3, threshold 50 μA). 1, 3, 4: First order collaterals of the main axon within the mesencephalic RF. 2: The main ascending branch. See Figs. 3A and 4 for detailed tracing of collaterals

morphological techniques. For reasons given below (see description of histological procedures), different experimental paradigms had to be applied to achieve a complete delineation of the neurons. Figure 1 illustrates the arrangement of recording and stimulation sites used in a majority of the experiments. Collicular neurons were penetrated intrasomatically and considered for staining only if they displayed antidromic responses to stimulation of the contralateral predorsal bundle at different pontine and bulbar locations and/or of the anterior funiculus of the spinal cord at C2. Separate stimulation sites in the pontobulbar tegmentum and in the supraoculomotor zone of the central grey or in the adjacent paramedian mesencephalic reticular formation served to identify axon collaterals in these areas. A similar arrangement was used in a second set of experiments in which intracellular penetrations were aimed at tectal axons along their course through the midbrain (Fig. 2). Finally, we recorded from and injected axons passing within the ponto-bulbar portion of the predorsal bundle (Fig. 7). Their collicular origin was identified by direct orthodromic responses to stimulation of the deep layers of the contralateral CS. Stimulation was applied through electrolytically sharpened nichrome electrodes with insulation-free tips of 50–80 μm in length. Cathodal pulse duration was set at 0.15 ms and current intensities did not exceed 100 μA. All stimulus sites were verified histologically. Since stimulation served only for crude determination of axonal projections to areas at a large distance from the point of penetration, we did not attempt a systematic mapping of antidromic thresholds. However, excitation of tegmental collaterals could be differentiated from excitation of main axons in the predorsal bundle applying a factor of 125 μm/10 μA for effective current spread. On several occasions we were able to verify the validity of this factor by relating the threshold currents to the distance measured from the electrode tip to the nearest portion of the HRP-labeled axon.

Micropipettes for recording and iontophoretic injections were backfilled with 10 to 25% HRP (Sigma VI) in 0.5 M KCl and Tris buffer (pH 7.6). Electrode tips were beveled to diameters between 1 and 2 μm and had a DC-resistance ranging from 15 to 25 MΩ. HRP was injected with positive current pulses, the total transfer-

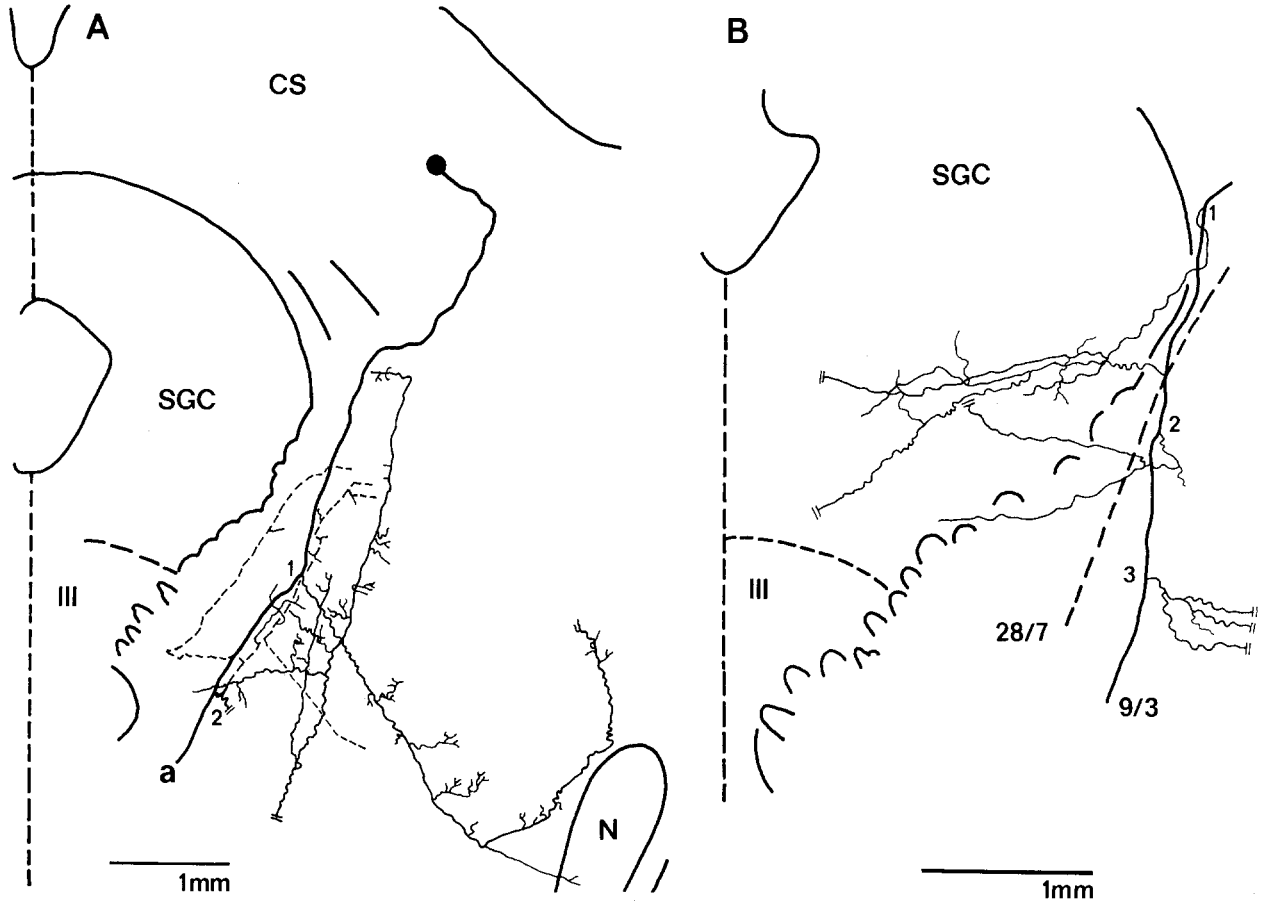

Fig. 3A, B. Collaterals of TBSNs in the mesencephalic reticular formation and the central grey. **A** Thin solid lines: Ramifications of the most proximal first order collateral (Number 1, compare to Fig. 2) in the medial and lateral reticular tegmentum. Dashed lines: First order collateral of the main ascending branch (2) with similar distribution but at more rostral level (see collateral 2.1, Fig. 2). Diameters of caudal and rostral first order collaterals 1.5 and 1.1 μm, respectively. Boutons present at all terminal ramifications. Diameter of main axon (a) 5.5 μm. **B** Collaterals in the central grey dorsal to the III nucleus from two different neurons located in rostro-medial quadrant of the superior colliculus. Collaterals 1 and 2 from axon 9/3 enter the central grey at different rostro-caudal levels. Collateral from axon 28/7 (not numbered) takes similar course. In both cases staining was sufficient to identify terminal branches and few boutons

red charge ranging from 80 to 8,000 nA × min. To insure clean intracellular staining the antidromic spike was monitored during the off-periods of current injection.

Two to twenty hours after HRP injection the animal was perfused intracardially with a mixture of 0.8% paraformaldehyde and 1.2% glutaraldehyde. Blocks of brain tissue were stored in 30% sucrose and then cut on a freezing microtome into 75 or 100 μm thick serial sections. The sections were processed for HRP by incubating them for 40 min in 0.1% diaminobenzidine at 39° C and for 30 min in the same solution after adding 0.06% H_2O_2. Pretreatment of sections in 5% CoCl solution for 20 min was used to intensify the staining. Phosphate buffer (pH 7.4) was employed as the solvent for perfusion and HRP processing.

Labeled neurons were reconstructed using a drawing tube. No corrections were made for shrinkage or swelling. The frontal plane appeared to be more suitable for reconstruction of mesencephalic collaterals, while pontine and medullary collaterals were reconstructed in the sagittal plane. With mesencephalic injections, it was possible to follow the main descending axon to rostral pontine levels and the main ascending branch up to the caudal mesencephalon. After pontine injections, the main axons could be traced rostrally to their exit from the CS and caudally to the first cervical segment. Although numerous collaterals (up to 18) were observed with each injection, usually only three or four of them located close to the injection site appeared to be filled completely. For a detailed reconstruction of collaterals supplying widely separated brain stem areas we had to resort to a piecewise reconstruction of tecto-bulbo-spinal neurons. Therefore, we varied the rostro-caudal level of recording and injection, as described above. Our material includes 16 neurons injected intrasomatically within the CS. The remaining 18 neurons were reconstructed after intraaxonal injections into the main ascending branch in the rostral mesencephalon (n = 4), the main axon on its course towards the decussation of Meynert (n = 8) or the ponto-bulbar portion of the main axon (n = 6).

Results

All tectal neurons injected either intrasomatically or through the mesencephalic portions of their axons (n = 28) responded antidromically to stimulation of the

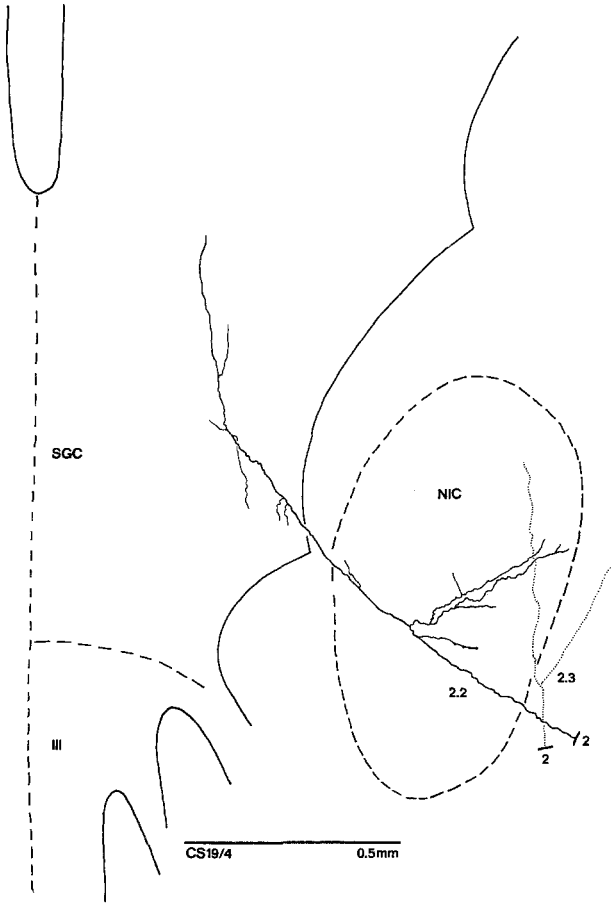

Fig. 4. Collaterals and terminal ramifications within the interstitial nucleus of Cajal and adjacent ventro-lateral central grey. Same neuron as in Fig. 2. (2): Main ascending branch proceeding rostralward to the field of Forel. (2.2) and (2.3): Thin (0.6 μm) first order collaterals entering the interstitial nucleus of Cajal at different rostro-caudal levels (see Fig. 2). Collateral 2.3 continues into the ventro-lateral central grey and nucleus of Darkschewitsch

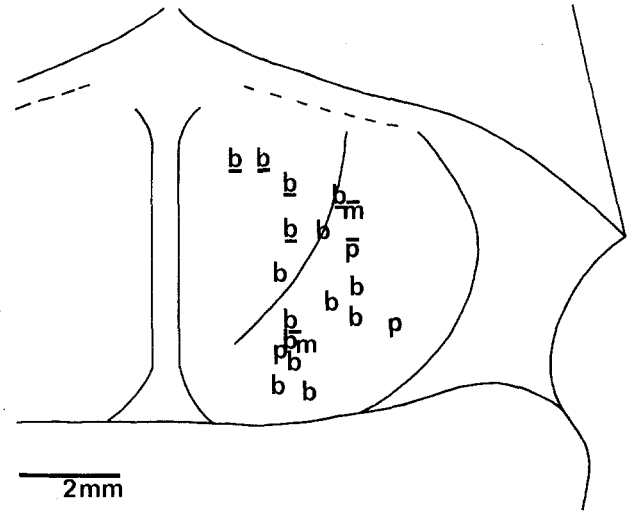

Fig. 5. Locations of tectal neurons with different patterns of axonal projection. Schematic dorsal view of right superior colliculus. Up is rostral. Oblique line indicates approximate location of horizontal meridian (Feldon et al. 1970). The map includes only those neurons for which coordinates could be precisely measured (intrasomatic HRP injections or axonal injections within the caudal midbrain). Location of neurons shown by letters which also symbolize caudal extension of main axon as revealed by antidromic tests: b – projection to bulbar levels; p – projection into the pons; m – mesencephalic projection only. Neurons sending collaterals towards the midline and showing terminal ramifications in the supraoculomotor zone of the central grey are marked by bars below the letters. Neurons devoid of such collaterals bear no mark. Bars above the letters indicate projection into the commissure of the superior colliculus (tecto-tectal neurons)

contralateral predorsal bundle at pontine and bulbar levels (Figs. 1A, C and 2). The remaining six neurons were reconstructed after intraaxonal injections within the pons or medulla (Fig. 7). The axonal projection into the predorsal bundle (the tecto-bulbo-spinal tract) is thus a major common feature of tectal neurons described in the present study. For convenience, we shall call them "tecto-bulbo-spinal neurons" (TBSN), although not all of them project to the spinal cord. Of the 18 labeled neurons tested with spinal stimulation, 12 projected to the first cervical segment. Since we found no differences between electrophysiologically identified "tecto-spinal" and "tecto-bulbar" neurons with respect to soma-dendritic profile, topography and pattern of axonal branching in the brain stem, it is appropriate to describe them as a single class of collicular neurons.

Size, Soma-Dendritic Profiles and Location of Tecto-Bulbo-Spinal Neurons

Figure 1E shows a complete reconstruction of a representative TBSN whose soma was located in the intermediate grey layer. The size of slightly elongated polygonal cell bodies ranged from 30 × 40 to 45 × 65 μm (average 32 × 60 μm, n = 14). All TBSN showed a radial arrangement of the dendritic tree with 7 to 10 stem dendrites (diameters 9–16 μm) which spread in all directions and ramified into secondary branches at a distance of 50–180 μm from the soma. Three dimensional reconstructions indicate a symmetrical distribution of dendrites in anterior-posterior and medio-lateral directions. Thus, the dendritic field occupies a roughly spherical volume with a diameter of 800 to 1,400 μm for different neurons. Axons emerge from the cell bodies and course towards the deep white layer. In no case did we observe local axon collaterals towards the cell of origin or to any other sites within the boundaries of the CS.

TBSN were located at depths between 1.15 and 3.1 mm below the collicular surface. Two-thirds

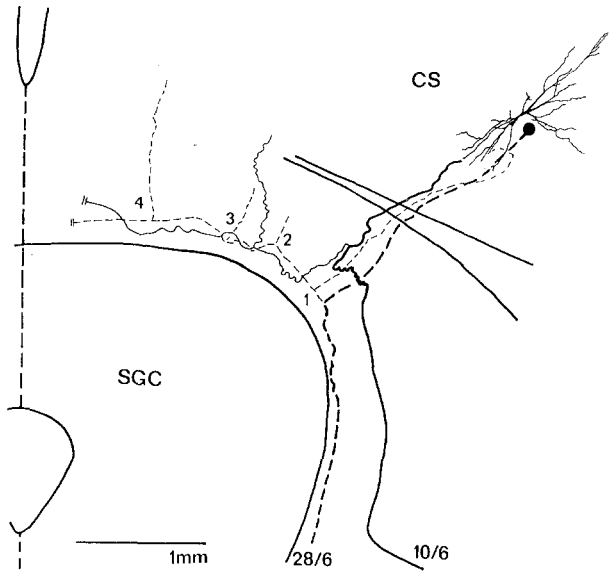

Fig. 6. Commissural neurons of the superior colliculus. Solid and broken lines represent axonal patterns of two different neurons. Soma-dendritic profile is shown for one of the cells (10/6) with complete staining of dendritic tree. In both cases staining was too faint for identification of terminals and for tracing commissural collaterals beyond midline

resided in the intermediate grey layer, one-third in the deep laminae. The distribution of neurons according to depth shows two peaks around 1.8 and 2.7 mm. We penetrated TBSN throughout the entire rostro-caudal extent of the CS (Fig. 5).

Two neurons had axonal projections into the collicular commissure (Fig. 6). They were located in the dorsalmost portion of the intermediate grey layer, in the rostro-caudal quadrant of the CS, where Edwards (1977) observed retrogradely labeled commissural neurons. Comparing them to TBSN confirms the validity of the soma-dendritic profile as a criterion for differentiating between cell groups with different efferent projections. The soma diameters (8.5 × 17 and 13 × 21 μm) were considerably smaller than those of any TBSN. Furthermore, they showed a distinct vertical elongation of cell bodies and dendritic trees. The completely reconstructed neuron of Fig. 6 had a dendritic extension in the vertical direction of 1,000 μm, in the horizontal direction of 500 and 250 μm for dorsal and ventral dendrites, respectively. The axonal branching pattern of commissural neurons was different from TBSN, as will be described below.

Main Axon and First Order Collaterals of Tecto-Bulbo-Spinal Neurons

The schematic diagrams of Figs. 2 and 7 summarize the general features of the axonal tree of TBSN. After traversing the stratum album profundum, the main axon curves around the central grey (SGC) and courses ventromedially towards the decussation of Meynert (Figs. 3 and 7C). Diameters of the axons measured near the decussation ranged from 4.5 to 7.5 μm (average 6.2 μm, n = 17). After crossing the midline, they take up a paramediane position, ventral to the medial longitudinal fasciculus (MLF) and are incorporated into the predorsal bundle. When main axons were labeled by injections within the pons and medulla (Fig. 7) it was observed that at the ponto-medullary junction they shift dorsalward and become intermingled with the fibers of the MLF. The axons showed an increase in diameter at rhombencephalic levels (range 7–10 μm). This was confirmed also by measurements of conduction velocities which for the mesencephalic and rostral pontine portions of axons were on the average lower (range 30–55 m/s, average: 38 m/s) than for the bulbo-spinal portions (range: 39–70 m/s, average: 52 m/s).

TBSN axons give off their first branches only after reaching the most ventral portion of the stratum album profundum (Fig. 3B, collateral 1) or on their course along the ventrolateral margin of the SGC (Figs. 2, 3A, 7B). All TBSN axons bifurcate within the midbrain to form ascending and descending connections. One ascending branch which was present in all TBSN departs from the main axon at the level of or just dorsal to the oculomotor nucleus and takes a rostral course within the fiber plexus surrounding the SGC (Fig. 2, collateral 2). Since, in most cases, this collateral was also the thickest of all the mesencephalic collaterals (average diameter 2.8 μm), we shall call it "the main ascending branch". First order collaterals emanating from the main axon or from the main ascending branch showed more variability in their points of departure and trajectory. Nevertheless, a certain degree of regularity could be recognized in collateral spacing. This is most clearly seen in the parasagittal reconstructions of Fig. 2 (collaterals of the main ascending branch) and Fig. 7 (collaterals of the main axon). The distance between first order collaterals ranged from 500 to 1,500 μm, on the average – about 700 μm (for the ponto-medullary segment).

Mesencephalic Collaterals of Tecto-Bulbo-Spinal Neurons

The example chosen for Figs. 2 and 3A is representative for TBSNs located in the caudal half of the superior colliculus (Fig. 5). A regularly encountered collateral emerges from the main axon proximal to

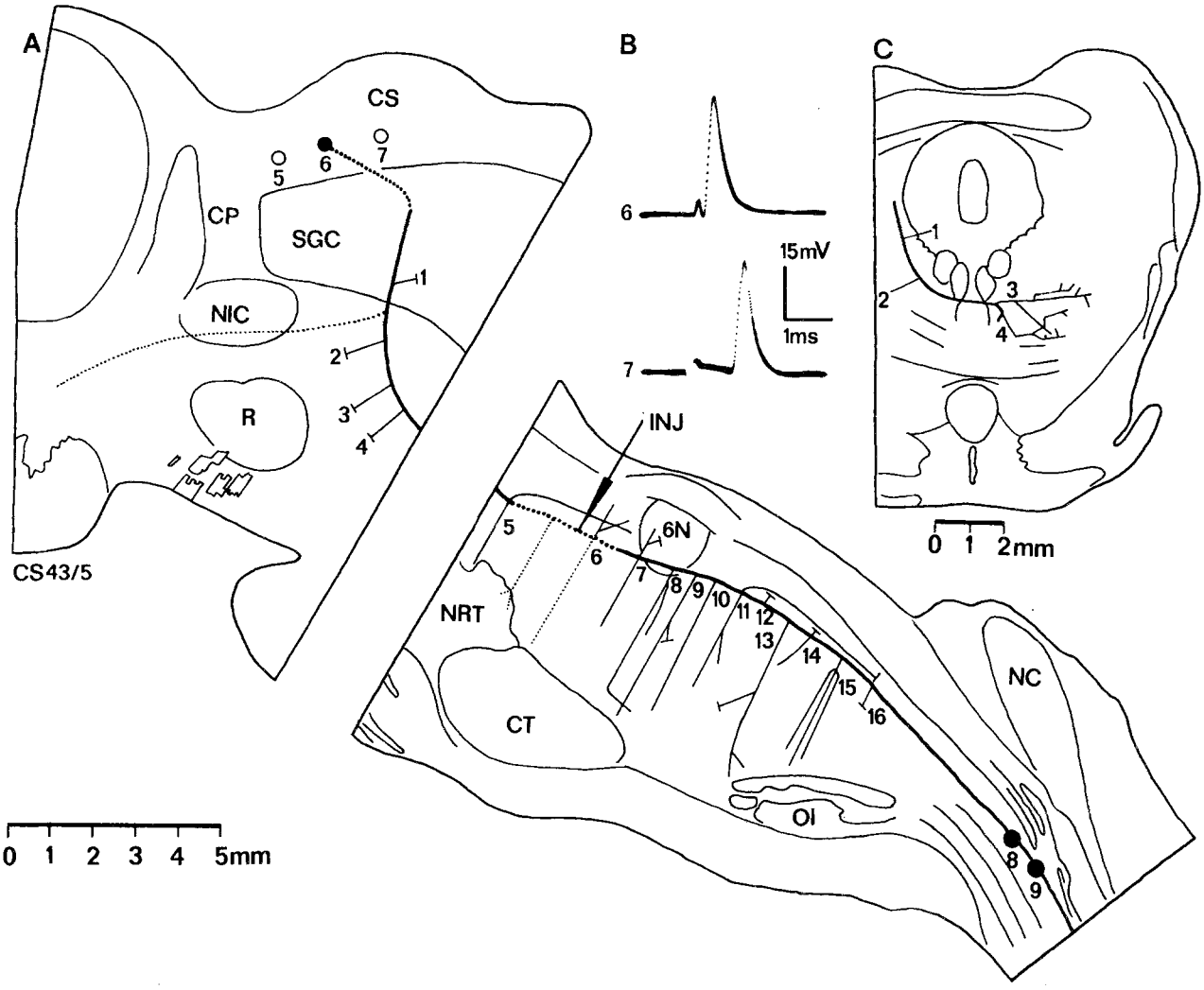

Fig. 7A–C. Axonal pattern of a tecto-bulbo-spinal neuron labeled by HRP injection into the pontine portion of the main axon. **A** Schematic reconstruction in parasagittal plane. Circles indicate stimulus sites used for electrophysiological identification. Location of neuron in the CS proved by direct orthodromic response to stimulation at point 6 (10 μA, record B 6). No response was elicited from site 5 and only long latency transsynaptic response from site 7 (300 μA, record B 7). Cell was activated antidromically from the upper cervical cord (stimulation points 8, 9). Note regular spacing of first order collaterals. Missing portions (*dotted*) of axonal tree at injection site (INJ) are added to the scheme according to observations on four other TBSNs injected at more caudal levels. **C** Reconstruction in frontal plane of caudal mesencephalic collaterals (1–4)

the main ascending branch and takes a straight ventro-lateral course through the reticular tegmentum. At their origin these ventro-lateral collaterals had diameters between 1.5 and 2.6 μm. They reach the lateralmost region of the paralemniscal reticular formation. On their way they give off several secondary collaterals, the most prominent of which supply the medial tegmentum adjacent to the ventro-lateral margin of the SGC. All secondary collaterals represented in Fig. 3A could be traced to terminal ramification bearing synaptic boutons with an average size of 2.0–3.5 μm.

The trajectory of a secondary collateral issued by the main ascending branch is shown by dashed lines in Fig. 3A. Again, extensive ramifications are found in the medial mesencephalic tegmentum in the vicinity of the MLF and the central grey. For other neurons, a similar pattern was observed for secondary collaterals leaving the main ascending branch at different rostro-caudal levels. It can be concluded that the medial mesencephalic reticular formation represents a major target of TBSN, although they also provide a sparce innervation of more lateral reticular areas, including the paralemniscal region.

In the rostral mesencephalon the main ascending branch assumes a position lateral to the interstitial nucleus of Cajal. We paid special attention to possible terminal ramifications within this nucleus in view

of the controversial opinions on this point (see Edwards and Henkel 1978). In four cases, the staining of the rostral portion of the main ascending branch was intense enough to rule out a false negative result. In only two of them were terminals observed in the interstitial nucleus of Cajal and in the nucleus of Darkschewitsch (Fig. 4). It must be noted that collaterals to these nuclei were thin (0.6 μm) and produced sparse ramifications. Other identified targets of the main ascending branch were the rostral interstitial nucleus of MLF (Büttner-Ennever and Büttner 1978) and the field of Forel. Further rostral, the staining became too faint to identify collaterals or their terminal ramifications.

Searching for correlations between the axonal pattern of tectal projection cells and their anatomical coordinates in the CS, we examined the collateralization in the perioculomotor region of the central grey. Our material confirms observations by Edwards and Henkel (1978) who described tectal fibers entering the "cap of the IIIrd nucleus" and crossing the midline to reach contralateral targets. Figure 3B shows the medial collaterals of two different TBSNs (diameters at origin 1.5, 2.0, and 3.7 μm) which arise from the main axon and proceed ventro-medially towards the midline. Here the staining becomes incomplete but the still large diameter suggests their continuation. The collaterals emit terminal branches endowed with boutons. We never detected any terminals within the confines of the IIIrd nucleus, although they were regularly found in the abducens nucleus (see below). The supraoculomotor region of the central grey appears to be another major target in the mesencephalon. However, not all TBSN contribute to this projection. Medially directed collaterals invading the supra-oculomotor zone were found in only six of the 18 neurons with appropriate staining of mesencephalic branches. As shown in Fig. 5, collaterals of this type originated preferentially from TBSN located in the rostral half of the CS, which suggests a topographical segregation of neurons with different patterns of mesencephalic collateralization. On the other hand, our sample does not reveal any correlation between the coordinates of TBSN and the caudal extension of their main axons.

We already mentioned that tectal neurons with identified projections into the collicular commissure are quite different from TBSN in size and soma-dendritic profile. In addition, they differ with regard to their axonal patterns (Fig. 6). Initially, their main axons follow the same trajectory as the main axons of TBSN, curving ventrally along the border of the SGC. However, they did not reach further caudally than the rostral pons or caudal mesencephalon. For both neurons we could not detect collaterals along the tegmental course of the main axon. The proximal commissural collaterals showed a satisfactory staining (diameters 3.75 and 1 μm). They emit several secondary collaterals which depart at intervals of about 500 μm and course upward into the ipsilateral CS. One of such secondary collaterals could be followed almost to its cell of origin in the upper part of the intermediate grey layer.

Pontine and Medullary Collaterals of Tecto-Bulbo-Spinal Neurons

The best staining of pontine collaterals was obtained with injections at the level of abducens nucleus and up to 2 mm further caudally. Relatively strong and long lasting currents had to be applied to fill the axon as much as possible. This caused injury and poor collateral staining near the site of penetration. For the neuron represented in Fig. 7 collaterals corresponding to the damaged segment are introduced basing on observations on other four neurons which were labeled at more caudal levels.

All ventral collaterals in the pons and medulla (10–12) spread from dorso-medial to ventro-lateral. Two to three dorsal collaterals run parallel to the trajectory of the main axon rostral and caudal to the abducens nucleus, giving in turn second order collaterals which take a medial or lateral course. In addition, there are short collaterals leaving the main axon in the dorsal direction. Their terminal fields overlap those of longitudinal collaterals (Fig. 8). Similar axonal patterns were found in all six cases analyzed so far and appeared to be the basis for a higher density of the tectal projection onto the dorsomedial reticular formation rostral and caudal to the VI nucleus.

Figure 8 shows the caudal pontine and medullary collaterals of a TBSN (same as in Fig. 7) in more detail. The diameters of the first order collaterals ranged from 1.5 to 3 μm (main axon 9 μm). In all six neurons the thickest collateral diverged from the main axon at the level of the facial nucleus and coursed ventro-laterally to reach the area just dorsal to the rostral pole of the inferior olive (collateral 13 in Figs. 7 and 8). Unfortunately, staining at the ventral end has always been too faint to determine the most distal sites of termination.

Identified targets of TBSNs in the paramedian pons and medulla were: nucleus reticularis tegmenti, pars centralis (its dorsomedial tip), abducens nucleus (ventromedial aspect), nucleus prepositus hypoglossi (very few terminals). The main destination of TBSN-collaterals was certainly the paramedian reticular formation, with the highest density of termination in

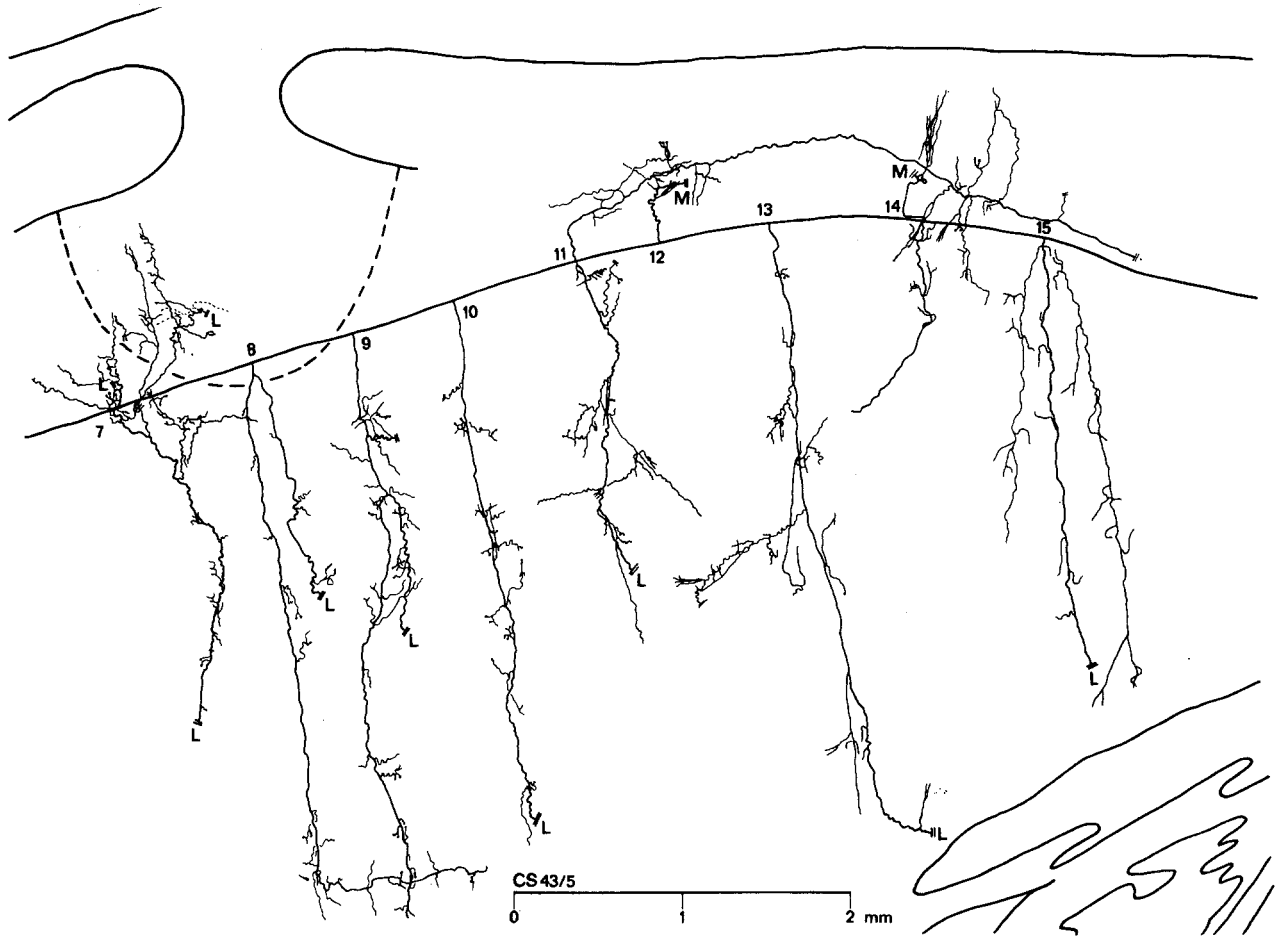

Fig. 8. Detailed reconstruction of TBSN-collaterals within the caudal pons and rostral medulla. Same neuron and same numbering of collaterals as in Fig. 7. Interrupted line: border of the VI nucleus; bottom right – inferior olive. Symbols L and M indicate ventro-lateral and dorso-medial direction of collaterals, respectively. Note overlap of transverse (12, 14) and longitudinal (11) collaterals in dorso-medial bulbar reticular formation

its dorsal portions adjacent to the VI nucleus. Laterally directed branches originating from the dorsal collaterals at caudal pontine levels were difficult to follow in parasagittal sections but they seemed to reach the dorsolateral reticular areas at the ventral border of the vestibular complex.

Morphology of Terminal Structures

The above results characterize TBSNs as distantly projecting cells with a complex and extensive axonal system. Therefore, it is important to enquire, whether the patterns of termination in different target areas are uniform. Since a preference for certain targets might express itself at the terminal or synaptic level, we examined more closely the accessible details of terminal structures to compare different regions with respect to bouton size and configuration and the density of terminal branches.

For this purpose three target regions were chosen: the paramedian reticular formation ventral and rostral to the VI nucleus, the dorsomedial reticular formation caudal to the VI nucleus and the abducens nucleus itself. Representative examples selected for Fig. 9 show that the shape and size of individual TBSN-boutons vary a great deal, even if they belong to the same neuron. Nevertheless, the boutons in the three target zones had similar average major and minor diameters (5.1 × 3.4 μm, 5.05 × 3.5 μm, and 5.2 × 3.5 μm for rostral and caudal reticular zones and for the abducens nucleus, respectively; n = 30 for each zone).

Preterminal branches encountered in the reticular core ventral and rostral to the VI nucleus were remarkably long and widely separated. They spread over considerable distances without ramification to form a few terminals bearing single or serial terminal swellings. The latter correspond, presumably, to synaptic contacts on target neurons (boutons ter-

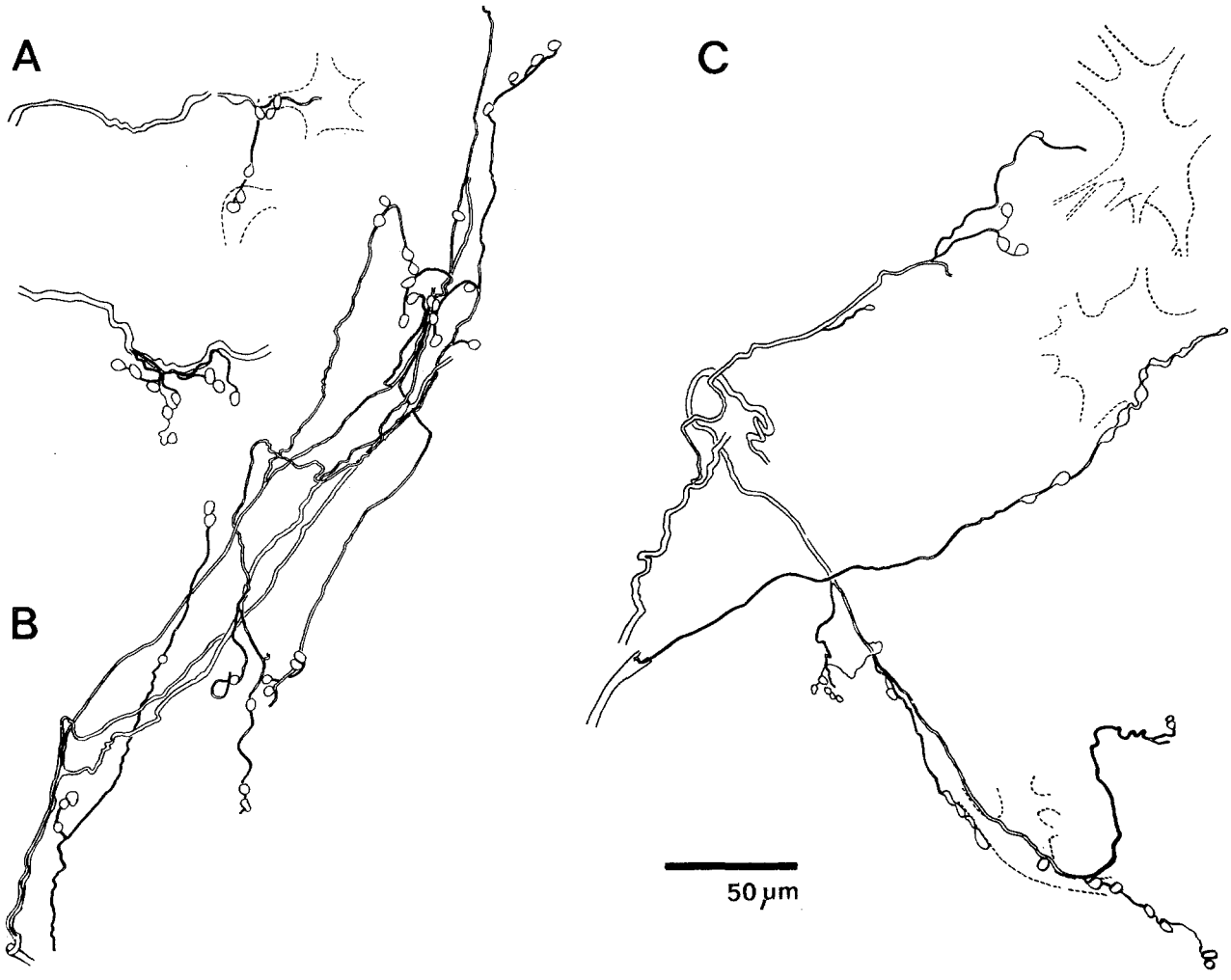

Fig. 9A–C. Camera lucida drawings of terminal arborizations and boutons in different regions of the ponto-bulbar tegmentum. Same neuron as in Figs. 7 and 8. **A** Terminals in ventro-medial sector of the abducens nucleus. **B** Terminals in the dorso-medial bulbar reticular formation originating from the dorsal longitudinal collateral (Number 11, see Fig. 8). **C** Terminals in the pontine reticular formation ventral to the abducens nucleus. Counterstained cell bodies shown by dotted lines

minaux and en passant). Although the average bouton density was very low in this reticular region, this does not necessarily indicate a low synaptic efficacy of the tectal input to individual reticular neurons. Even a weak counterstaining revealed many examples of close apposition of multiple terminal swellings to cell bodies and proximal dendrites (Fig. 9C), in agreement with electrophysiological studies (Grantyn and Grantyn 1976, 1980) which reported large monosynaptic EPSPs in reticular neurons to weak single shock stimulation of the contralateral CS.

Quite different pattern of termination was observed in the dorsomedial reticular zone posterior to the VI nucleus, where fibers tended to concentrate in the form of axonal bundles (Fig. 9B). Such arrangements cause a high local density of boutons from a single collateral. Considering the substantial overlap of longitudinal and transverse collaterals from individual TBSNs one should expect a high percentage of neurons in this area to receive direct tectal influences and/or a high efficacy of synaptic input.

The terminal fibers in the abducens nucleus were shorter than those in the reticular formation and often built irregular clusters of boutons in apposition to counterstained cell bodies and proximal dendrites (Fig. 9A). The average bouton density was intermediate between the two reticular regions described above. TBSN-terminals were found only in the ventro-medial segment of the VI nucleus. As shown by Baker and McCrea (1979), the dendritic field of every abducens motoneuron extends over the entire volume of the nucleus. Consequently, all motoneurons may, potentially, be reached by TBSN-terminals through axodendritic contacts. However, only a small fraction of abducens motoneurons

generates monosynaptic EPSPs in response to CS-stimulation (Grantyn and Grantyn 1976). This suggests either a low efficacy of synaptic transmission or that motoneurons are not the main target of TBSNs in the VI nucleus.

Discussion

The initial objective of this study was to search for morphological distinctions between superior colliculus neurons projecting to different 'pre-oculomotor' areas in the brain stem (see Keller 1980 for a recent review) and to the spinal cord. Accordingly, we selected for intracellular HRP injections those neurons which generated antidromic spikes in response to stimulation of the following regions: (1) the paramedian ponto-bulbar RF surrounding the VI nucleus, i.e., the sites of excitatory and inhibitory premotor neurons subserving the generation of horizontal eye saccades (Hikosaka and Kawakami 1977; Hikosaka et al. 1978; Kaneko et al. 1981); (2) the central grey overlying the III nucleus and/or the rostral paramedian mesencephalic RF, i.e., regions containing premotor circuits linked to oculomotoneurons acting in the vertical plane (Büttner et al. 1977; King and Fuchs 1977; King et al. 1980; (3) the tecto-spinal tract at bulbar levels and/or in the first cervical segment of the spinal cord.

Early in the course of the study it became evident that neurons selected according to antidromic responses have widespread connections. Using the projection into the predorsal bundle as a unifying criterion we obtained a sample of efferent neurons (TBSN) which showed remarkable uniformity of soma-dendritic and axonal architecture. (1) All TBSNs belong to the class of large, multipolar, wide field neurons according to the descriptions given in the Golgi studies of the cat CS (Viktorov 1968; Langer 1976; Norita 1980). (2) They send their main axons in the stratum album profundum without giving local collaterals within the CS. (3) The main axon crosses the midline in the dorsal tegmental decussation and descends within the contralateral predorsal bundle to bulbar levels. At least two thirds of them reach the spinal cord, and only in a few cases was there no projection below the pons, as judged by antidromic stimulation. (4) All TBSNs send an ascending branch reaching beyond the meso-diencephalic junction. (5) First order collaterals are generated at regular intervals along the course of the main axon and the main ascending branch at all brain stem levels from rostral mesencephalon to caudal medulla. (6) TBSNs are located in the intermediate and deep collicular layers, with a clear preference for the former. It can be concluded that TBSNs represent a morphological entity characterized by common soma size, type of dendritic tree and general pattern of axonal projections.

It has been well established by studies using anterograde transport of radioactive aminoacids that intermediate and deep collicular layers project to a large number of brain stem structures (Graham 1977; Harting 1977; Harting et al. 1980). Usually these connections are subdivided into separate fiber systems with different trajectories and destinations. According to the course of their main axons TBSNs represent the cells of origin of the 'medial' descending fiber contingent (Altman and Carpenter 1961; Graham 1977) which is directed mainly to the medial pontine and bulbar reticular formation but terminates also in the precerebellar reticular nuclei (Kawamura et al. 1974), the abducens nucleus (Edwards and Henkel 1978), the inferior olive (Graham 1977; Weber et al. 1978), and the spinal cord (Altman and Carpenter 1961; Graham 1977). Although we did not observe terminal ramifications of TBSNs in the lateral reticular nucleus and the inferior olive, this negative result is inconclusive, since the intensity of collateral staining at the periphery of the bulbar tegmentum was low. All other connections of the 'medial' contingent can be replicated by a single TBSN. Furthermore, projections of TBSNs in the mesencephalon also supply the target areas of 'middle' and 'lateral' (Altman and Carpenter 1961; Graham 1977) contingents of collicular efferent fibers, i.e., the cuneiform nucleus and the lateral (paralemniscal) reticular regions, respectively. At these levels some TBSNs contribute collaterals to the supraoculomotor region of the central grey. Most noteworthy is the participation of TBSNs in the 'ascending' projections of the CS (Niimi et al. 1970; Graham 1977; Harting et al. 1980). We followed the main ascending branches through the prerubral area to the fields of Forel and observed collaterals and terminal ramifications in the paramedian structures of the rostral midbrain (central grey, interstitial nucleus of the MLF, Büttner-Ennever and Büttner 1978). Due to limited resolution power of intracellular HRP technique in reconstruction of extremely widespread axonal trees, the present study provides a still incomplete list of target areas supplied by single TBSNs. Even so, it is evident that neurons of this type participate in most of the connections previously described for intermediate and deep CS layers.

A number of experimental anatomical studies have been aimed at the delineation of neuronal subsets which might project specifically to different target areas and, by implication, subserve different functions. We shall mention only a few examples

more closely related to our present findings. In the cat, the majority of tecto-reticular and tecto-spinal cells are large or medium-size and they are more numerous in the stratum griseum intermediale, as compared to deeper layers. The percentage of large and giant neurons is higher in the tecto-spinal population (Edwards and Henkel 1978; Kawamura and Hashikawa 1978; Weber et al. 1979). Unfortunately, these reports disagree considerably with regard to the rostro-caudal and medio-lateral distribution of these two cell contingents. Neurons establishing commissural tecto-tectal connections are of small size and occupy only the rostral half of the CS (Edwards 1977; Magalhaes-Castro et al. 1978). Our data emphasize a distinction between tecto-reticulo-spinal and tecto-tectal neurons and add a new detail by showing that the latter contribute not only to the collicular commissure but also supply intermediate layers of the ipsilateral CS with local collaterals and send axons to as yet undetermined extracollicular targets. However, in our sample we could not detect any distinguishing features between tecto-spinal and tecto-reticular neurons. Another example concerns the distinction between cell groups projecting to the two brain stem regions implicated in different aspects of oculomotor control. According to Edwards and Henkel (1978), the supraoculomotor zone of the central grey receives fibers exclusively from small cells situated in the rostral part of the CS, whereas large and medium-size neurons provide terminations within and in vicinity of the abducens nucleus. Although we confirm the rostral location of neurons sending collaterals to the supraoculomotor grey, the present results clearly show that this projection originates, at least in part, from large cells with axons descending into the ponto-bulbar tegmentum.

Remarkable divergence of individual TBSNs points to the difficulties which may be encountered when retrograde transport of HRP is used to characterize specific subsets of collicular neurons projecting to particular target areas. However, the idea of such compartmentalization may still have merit. Our sample was obviously biased in favor of large cell bodies and thick axons. Possibly, a similar approach to the morphology of smaller neurons would reveal a sharper focusing of terminal fields, while the widely projecting type (TBSN) represents only one of many other neuronal subsets.

It is legitimate to ask about a possible function of TBSNs, even though this question can be discussed only in speculative terms. There is no doubt that TBSNs exert excitatory action on their target neurons, since monosynaptic responses induced by collicular stimulation are always EPSPs. This has been demonstrated for abducens motoneurons (Grantyn and Grantyn 1976), for different contingents of pontine and bulbar reticular neurons (Peterson et al. 1974; Grantyn and Grantyn 1976, 1980) and for neurons in and around the interstitial nucleus of Cajal (our unpublished observations). Another common feature of TBSNs is the high conduction velocity of their main axon which can ensure a short latency and practically simultaneous facilitation of neuronal populations separated by large distances along the neuraxis. Axonal ramifications of TBSNs are distributed within a variety of structures intimately related to eye and head movement. In the contralateral pontine RF they overlap the locations of excitatory (Kaneko et al. 1981) and inhibitory (Hikosaka and Kawakami 1977; Hikosaka et al. 1978) burst neurons. If activated directly or indirectly by collicular input through TBSNs, these groups would provide synaptic drive for motoneurons acting in contraversive horizontal eye movement and induce reciprocal inhibition of antagonistic motor nuclei. TBSN-collaterals in the caudal pontine and bulbar tegmentum have ample opportunities to contact different groups of reticulo-spinal neurons (Peterson 1977), including those which exert excitatory influences on ipsilateral neck extensor motoneurons (Anderson et al. 1971). It is not known what kind of signal is transmitted by TBSNs during execution of eye and head movements. The relative efficacy of their excitatory actions at different targets still remains a subject of speculation. However, even assuming an equal efficacy of all collaterals, the pattern of axonal ramifications in the rhombencephalon would ensure a facilitation (or triggering) of contraversive eye and/or head movements and, hence, a very coarse orientation towards the contralateral hemifield.

Some targets of TBSNs in the mesencephalon coincide with regions containing 'premotor' circuits related to the generation of vertical eye movements (Büttner et al. 1977; King and Fuchs 1977; King et al. 1980) or, more generally, to vertical and torsional gaze shifts (interstitial nucleus of Cajal, prerubral area and fields of Forel) (Szentágothai 1943; Hess et al. 1946; Hyde and Toczek 1962). By way of these connections TBSNs may be able to specify not only the hemifield but also the quadrant to which the movement must be directed. This would require different connections of medial and lateral portions of the CS with neurons related to upward and downward movements, respectively. Our unpublished observations indicate that inferior rectus motoneurons indeed receive disynaptic excitation from the lateral CS and are inhibited by stimulation of its medial half. Further studies are required to disclose possible differences in efferent connections of

upward and downward sectors of the CS. In summary, by virtue of their connectivity pattern, TBSNs appear to be suited for a rapid and broad distribution of facilitatory influences which may ensure some degree of directionality of the motor response but specify neither the movement parameters nor the relative involvement of different muscle groups in its execution. Their action may be described as conveying a coarse movement image.

The execution of actual movement could then be achieved by superposition of other more specific inputs on this "image", possibly including also those originating from the subsets of collicular neurons with less widespread connections. An interesting problem requiring consideration in this context is the coordination of eye and head movement during gaze shifts triggered by collicular stimulation. Unitary recordings in the cat CS have shown that the neurons related to eye and to head movement represent different populations (Straschill and Schik 1977; Harris 1980). Analysis of eye and head movements induced by collicular stimulation led to the conclusion that not only different populations of neurons subserve gaze shifts of small and large eccentricities but also that the connections of these subsets with premotor brain stem structures should be different (Guitton et al. 1980; Roucoux et al. 1980). Our interpretation of TBSN-function does not contradict these conclusions so far as it suggests a facilitatory and not a triggering role for these neurons. Depending on the strategy of gaze shift (Roucoux et al. 1981), actions of eye and head related neuronal subsets may specify the characteristics of movement using as a background the spatio-temporal pattern of facilitation produced by TBSNs.

For simplicity, we assumed above that TBSNs exert uniform actions at all their target areas. However, comparing their patterns of termination in different brain stem regions we obtained several indications of selectivity. (1) The average density of boutons was definitely higher in the dorsal pontobulbar RF compared to other regions of reticular core. The sites of higher density correspond to the locations of excitatory and inhibitory preoculomotor neurons and to the origin of direct reticulo-motoneuronal projections to the spinal cord. (2) Only particular neurons within the pontine RF seem to be selected as TBSN targets. This is suggested by the presence of long unbranched preterminal collaterals often giving rise to a restricted terminal ramification and multiple boutons apparently terminating on a few neighbouring neurons. (3) Within the abducens nucleus terminals are present only in the ventromedial sector indicating selective distribution to particular subsets of motoneurons or interneurons. It can be added that according to present knowledge axonal trees of complex architecture may fulfill selective channeling and temporal transformation of impulse trains (see for references Swadlow et al. 1980).

In conclusion, arguments in favor of 'diffuse' as opposed to selectively distributed, 'specific' action of TBSNs remain equally weighted until more is known about the signals they transmit during naturally occurring movements and the synaptic effects which they exert on neurons in different target areas. Not less important for understanding of TBSN-function is the morpho-physiological characterization of other subsets of efferent neurons in the deep collicular layers.

Abbreviations. c – contralateral; CP – commissura posterior; CS – colliculus superior; CT – corpus trapezoideum; F – campus Foreli; FA – funiculus anterior; FRM – formatio reticularis medullae; FRP – formatio reticularis pontis; Fpd – fasciculus predorsalis; MLF – fasciculus longitudinalis medialis; N – substantia nigra; NC – nucl. cuneatus; NIC – nucl. interstitialis Cajal; NRT – nucl. reticularis tegmenti pontis; NV – nucl. vestibularis; pB – nucl. parabigeminalis; R – nucl. ruber; SGC – substantia grisea centralis; TO – tractus opticus; 6N – nucl. nervi VI.

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