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Location of the Cell Bodies of the Superior Rectus and Inferior Oblique Motoneurons in the Cat

DON C. BIENFANG[1]

Branch of Electroencephalography and Clinical Neurophysiology, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland 20014

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Stimulation of the inferior oblique and superior rectus branches and of the whole oculomotor nerve was carried out in cats while recording either gross or unit activity within the region of the oculomotor nucleus. On the basis of a number of criteria, unit responses were selected to represent antidromic activation of cell bodies in that nucleus. For the superior rectus, these were found to be located in the contralateral caudal half of the nucleus. For the inferior oblique, antidromically activated cells appeared to be scattered throughout the ipsilateral anteroposterior extent of the nucleus, although predominating in its caudal portion. No evidence was found for a dorsoventral or lateral organization of these subnuclei cells within the main nucleus.

Introduction

The arrangement of the subnuclei of the oculomotor nucleus responsible for motor innervation of the individual extrinsic muscles of the eye has been the subject of many papers (1–6, 10, 18, 19, 21, 24). Conflicting maps have been proposed in a variety of animals using one of two techniques: retrograde degeneration of the oculomotor nerve or electrical stimulation of regions of the nucleus.

A primarily dorsoventral and ipsilateral arrangement of the subnuclei of the inferior rectus, inferior oblique, medial rectus, and a contralateral arrangement of the superior rectus subnucleus was proposed in both cats (2) and monkeys (24) on the basis of retrograde degeneration studies. This arrangement conflicts with the commonly cited Bernheimer (4) representation in monkeys; i.e., an ipsilateral and rostral organization for the superior rectus, a bilateral rostrocaudal arrangement for the medial rectus and inferior oblique, and a contralateral, caudal position for the inferior rectus subnuclei. It also conflicts with the reports of both Danis (10) and Szentogathai (21) who, using focal stimulation to the oculomotor nucleus of the cat, arranged the subnuclei in a rostrocaudal, exclusively

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ipsilateral orientation. In the rabbit, the retrograde degeneration study of Schwabe (18; also 5), would place the superior rectus caudal and controlateral and the other subnuclei (with the questionable exception of the levator palpebrae) ipsilateral in the somatic complex of the nucleus. Abd-El-Malek (1), on the other hand, using retrograde degeneration in the cat, found a bilateral representation of the motor cells for each muscle. He also arranged the subnuclei in a rostrocaudal direction.

Because of these conflicting results, the localization of the subnuclei of the extrinsic eye muscles has been re-investigated in the cat by means of a different technique; i.e., the plotting of the gross and unitary potentials evoked by stimulation of several branches of the oculomotor nerve.

Method

Experiments were carried out in 29 cats weighing 2–3 kg. The animals were anesthetized with pentobarbital sodium (35 mg/kg, ip; initial dose supplemented as necessary during the surgical procedure). General anesthesia was interrupted after decerebration.

With the animal's head placed in a stereotactic apparatus, the cisterna magna was drained and the brain was exposed through a wide craniotomy. Next, the orbital structures were approached from above, through the frontal sinus (Fig. 1). The levator palpebrae muscle was separated from its insertion on the globe and retracted with a suture. The content of the globe was then aspirated through the pupil and the superior rectus (SR) muscle was separated from the globe and pulled vertically by means of a suture in its tendon of insertion on the globe. Following the ligation of the optic nerve as close as possible to the globe, these two structures were separated and the globe was then pulled forward to expose the inferior oblique (IO) muscle and its nerve. The orbit was then loosely packed with saline-moistened cotton.

Next, through the removal (by suction) of the overlying cerebrum, both superior colliculi were exposed from the edge of the tentorium to 4 mm anterior to their rostral margin.

Stimulation was carried out with a Grass S4 stimulator through a stimulus-isolation unit. The IO branch of the oculomotor nerve was hung on a pair of silver hooks and the stimulation pulse was adjusted to yield a twitch isolated to its muscle (about 3v and 0.2 msec). The tips of a pair of silver wires were placed on either side of the SR branch which was identified as it curved around the lateral edge of the muscle to ascend on its inferior surface. Since the nerve could not be isolated from its muscle, the electrode position was adjusted to give a maximum twitch isolated to the SR muscle at a threshold stimulus (about 5v and 0.2 msec) but *no* twitch in the curarized preparation.

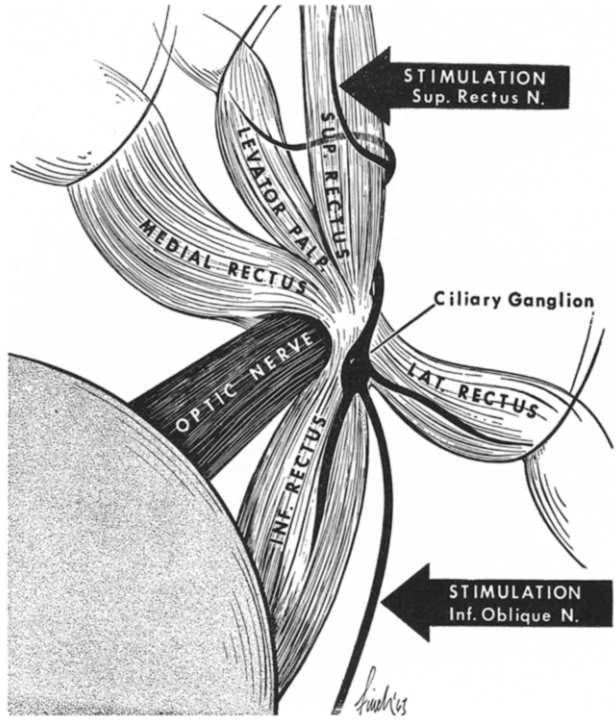

FIG. 1. Diagram of the left orbital structures (from above) to show position of stimulating electrodes. For simplicity, several structures have been omitted. The sensory root to the lateral rectus and the motor roots to the inferior rectus and levator palpebrae are included.

The whole (left) oculomotor nerve was approached at the level of the sella turcica with a pair of insulated needle electrodes (0.5 mm apart; tip exposure about 1 mm) placed stereotactically (20) from above, with a slightly oblique (5 deg) orientation, 4–5 mm below a point 3.75 mm in front of the rostral edge of the superior colliculi and 4.3 mm lateral (left) to the midline. The position of the electrodes was adjusted to give a twitch of all the five appropriate muscles with pulses of minimal voltage and duration (about 3v and 0.2 msec).

After the various nerves were identified and the optimal parameters of stimulation were established, the cat was curarized (tubocurarine chloride, iv) and artificially respirated through a tracheal cannula.

Gross evoked potentials were recorded with low-resistance (about 1 Mohm) glass micropipettes, and unit activity was recorded with glass micropipettes of 10–20-Mohm resistance. The pipettes were mounted on a stereotactically vertical, hydraulic micromanipulator and the signals were

led through a Bak high-impedance unity-gain amplifier, amplified with a Tektronix type 122 preamplifier and displayed on a CRO. The animals were grounded at the scalp muscle and the reference was the frame of the stereotaxic apparatus. The pipettes were filled with 2 M KCl and 0.6 M $K_3Fe(Cn)_6$ to mark the position of the tip. After each experiment, 50 μ sections of the brain stem were made in the frontal stereotactic plane, appropriately fixed and stained (14) and the electrode tip positions identified on the guide of the prussian blue reaction.

In all cats, when looking for single unit activity, one or both SR were stimulated; in 16 of these cats the left IO, and in 10 of these 16 the left oculomotor nerve were also stimulated. Gross evoked responses to stimulation of the left SR, IO and oculomotor nerve were studied in three cats; of both right and left SR in two cats; of the left oculomotor nerve in one cat; of the left IO and SR in one cat and of both right and left IO in one cat. In all cases the nerves were separately stimulated for each advancement of the micromanipulator. Because of the relatively small size of the oculomotor nucleus (only 1 mm from the midline at its widest point) a systematic survey of a larger region was necessary. This included an exploration of about 8 mm in depth from the surface of the superior colliculi on the two sides of the midline within 2–3 laterally and for about 6 mm in the frontal plane [stereotaxic coordinates (20): F−1 to +5].

Results

Gross Evoked Potential. The gross response evoked by unilateral stimulation of the oculomotor nerve, the IO or the SR was recorded in eight cats and its distribution mimicked in a general way that of the unitary spikes (see below) except in the case of the whole oculomotor nerve stimulation.

The response consisted of an early, small positive component followed by a large negative deflection; a third long lasting positive wave was occasionally seen. Actually, with the exception of SR stimulation, this early component was rarely observed (never with IO stimulation) within the ipsilateral somatic part of the nucleus. It was, on the other hand, rather prominent in the regions surrounding the nucleus where the larger negative component was practically absent (17, 19).

With the whole oculomotor nerve stimulation an early positive deflection (latency of 0.2 msec, duration 0.5 msec) was seen, when present, in all but one instance *outside* the ipsilateral oculomotor nucleus; i.e., either below it or in the contralateral nucleus.

With stimulation of the SR the positive component was always seen, either within the nucleus or immediately below it. However, it was seen

both contralaterally and ipsilaterally (where in most cases no negative component existed) to the stimulated SR nerve.

The larger negative wave that had a latency of 0.3–1.25 msec and a duration of 1.00–1.75 msec was very rarely seen outside the cell body area. With IO stimulation, this potential could only be recorded ipsilaterally. It occupied the whole length of the cell body complex but tended to be maximal in its posterior portion. In contrast the SR negative response was almost always contralateral and seemed to be limited to the more posterior portions of the nucleus.

The negative component of the response to oculomotor nerve stimulation was also limited almost entirely to the cell body region. It could be seen contralaterally but always smaller in amplitude than in the case of the ipsilateral response. Only in the most posterior regions of the nucleus was the contralateral response of greater amplitude (thus suggesting the contribution of SR activity).

Unit Activity. Four criteria had to be met for a unit to be accepted as antidromically activated (Fig. 2). These are: (a) latency appropriate on the basis of the mammalian "A" fiber formula for conduction velocity (6 mm/msec/μ) as described by Hursh (15);[2] (b) localization in a region of cell soma; (c) spike duration greater than 0.8 msec (17); and (d) response pattern showing no repetitive spikes nor changes greater than 0.1 msec in latency with increase in stimulus duration or strength (12).

Two other criteria (i.e., presence of an "A-B" break and frequency following) were also applied but they were not consistently met (Fig. 2; Table 1). An inflection on the rising phase of the spikes, with an all-or-none early phase (13), was seen for most of the included units when activated by oculomotor nerve or SR stimulation but only in less than half of those activated by IO stimulation.

The interpretation of the pattern of frequency following, which was systematically tested, was often ambiguous. Spikes that, on the basis of duration and location, seemed to arise from axons, invariably followed high rates (700–800/sec) of stimulation (12). On the other hand, spikes which, on the basis of all other criteria, were thought to be from antidromically activated cells, might fail to follow frequencies as low as 10/sec.

Oculomotor Nerve Units. Spikes from 19 cells were considered to be antidromically activated by stimulation of the whole nerve. The conduction velocity of this group was between 10 and 50 m/sec. Nine of these units

[2] In this series of experiments the approximate length of the conduction path for the IO was 39 mm, for the SR 30 mm, and for the oculomotor nerve 10 mm. The myelinated fiber diameter of the IO nerve in rabbit is 2–14 μ (11); in the medial rectus nerve of cats is 3–17 μ (16). According to Yamanaka and Bach-y-Rita (25) the conduction velocity in the abducens nerve of the cat would be from 6 to 83 msec.

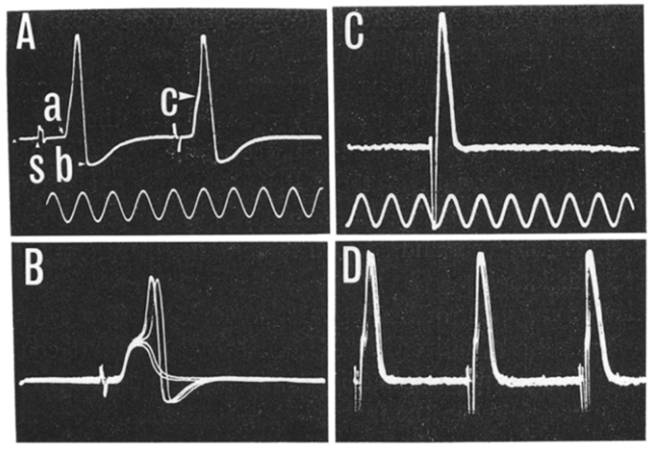

FIG. 2. Examples of Single Units. A. Cellular response to a single pulse stimulation of both IO (s) and the oculomotor nerve. Note "A-B" break (arrow at c) more evident in the spike elicited by the latter stimulation. Latency measured from onset of stimulus artifact (s) to onset of "A" wave (arrow at a). Duration measured from onset of "A" wave to end of "B" wave, (arrow at b). Calibration (for A and B) : 1 kc; 10 mv. B. Same cell responding to a 100/sec stimulation of the oculomotor nerve. Note how the dome shaped "A" wave follows perfectly while the "B" wave is variable in its appearance (five superimposed sweeps). C. Spike, probably a fiber responding to oculomotor nerve stimulation. Note shorter latency and duration. Calibration (for C and D: 1 kc; 2mv.) D. Same element, stimulation at 300/sec. Note perfect following. Inflection on rising phase is stimulation artifact.

were not activated by the stimulation of either the SR or the IO. All cells were located ipsilaterally to the oculomotor nerve and all were within the oculomotor nucleus.

Inferior Oblique Units. Spikes from 29 cells were considered as antidromically activated by IO stimuli. The conduction velocity in this group was in the 24–39 m/sec range with minimum and maximum of 10.8 and 80 m/sec. These cells were located within, and scattered through the extent of, the ipsilateral somatic part of the oculomotor nucleus but were chiefly located in the posterior third of the nucleus. One cell of long but acceptable latency (3.45 msec) was located in the ipsilateral Edinger-Westphal nucleus.

Superior Rectus Units. Spikes from 18 cells were considered to be antidromically activated by stimulation of the SR nerve. Conduction velocity varied between 13.6 and 50 m/sec. All cells were located in the posterior half of the somatic portion of the oculomotor nucleus and all but one were contralateral to the stimulated SR nerve.

The preceding data are summarized in Table 1, Figs. 3 and 4.

TABLE 1

SUMMARIZING DATA ON ALL UNIT SPIKES

Nerve stimulated	Accepted as antidromic [b]	Absolute criteria: Latency [c] Appropriate	Latency [c] Inappropriate	Cell Area In	Cell Area Out	Duration [c] in msec. 0.8	Duration [c] in msec. ≤ 0.8	Single spike Yes	Single spike No	Other criteria: "A-B" break Yes	"A-B" break No	Frequency following > 100/sec	Frequency following < 100/sec
Oculomotor [a] nerve	Accepted	19	0	19	0	19	0	19	0	17	0	4	15
	Not accepted	10	13	16	7	16	7	23	0	12	7	5	18
Inferior oblique	Accepted	29	0	29	0	29	0	29	0	12	0	2	27
	Not accepted	4	2	4	2	3	3	3	0	0	4	4	2
Superior rectus	Accepted	18	0	18	0	18	0	18	0	11	0	1	17
	Not accepted	1	0	1	0	0	1	1	0	0	1	1	0

[a] Among the spikes ascribed to the oculomotor nerve are included the spikes from those units that were also "driven" by IO or SR stimulation.

[b] Those spikes accepted as antidromic met all the criteria under "absolute criteria", those not accepted failed one or more of the "absolute criteria."

[c] For measurement criteria see Fig. 2, *A* and text.

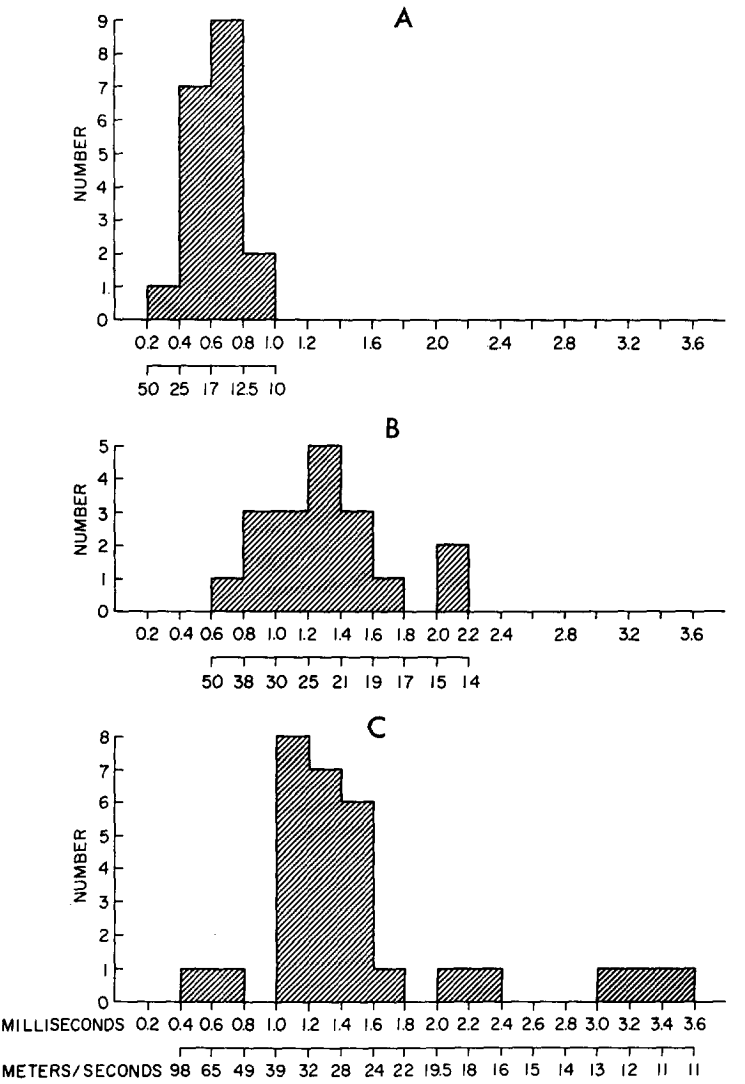

FIG. 3. Histograms of latency and conduction velocity for "Accepted Unit" spikes. Upper set of figures in abscissa refers to latency (in msec), the lower one to conduction velocity in m/sec. A, oculomotor, B, superior rectus; C, inferior oblique nerves.

Discussion

The results of the evoked potentials (population activity) agree in general with those of Shimo-Oku and Jampel (19) although the sequence of the potentials in depth are in closer agreement with those reported by Sasaki (17). The results derived from the analysis of unitary activity con-

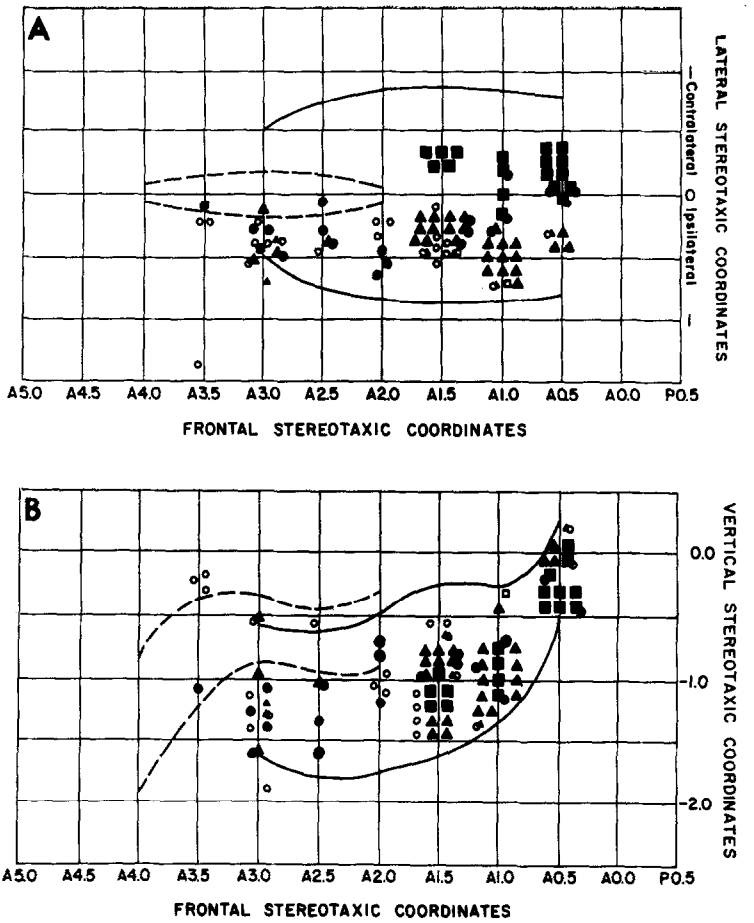

FIG. 4. A, horizontal (about vertical-1), and B, sagittal (about lateral 0.25) sections of the oculomotor nucleus to show locations of recorded units. Solid line outlines "somatic" and dashed the "visceral" nucleus. Nuclear outline was constructed from three typical cat brains in conjunction with Snider and Niemer's atlas (20). Each prussian blue spot was measured as to its location in the nucleus and the stereotaxic frontal plane in which it occurred and then transferred to a general map. Solid figures refer to "accepted" units; open figures to "not accepted". Squares:—superior rectus. Triangles: inferior obliques. Circles:—whole oculomotor nerve including superior rectus and inferior oblique. Divisions in increments of 0.5 mm.

firm the predominantly contralateral and caudal position of the SR subnucleus. In the case of the IO (from which the greatest amount of data on unity activity was collected), it appears that the cellular elements of this ipsilateral subnucleus are scattered throughout the whole rostrocaudal length of the somatic portion of the nucleus. Those cells "driven" by the stimulation of the whole oculomotor nerve, and not by that of either IO

or SR branches, are presumably members of the medial rectus, inferior rectus and levator palpebrae subnuclei. These cells were potentially located anteriorly in the ipsilateral nucleus. On the basis of their small number, however, it is not possible to draw any conclusion as to the actual topography of these three subnuclei.

The present experimental approach has a statistical feature to it, in that the likelihood of recording from a given subnucleus is proportional to the size of the subnucleus itself. Thus, it may well be that each subnucleus has cells at all anteroposterior levels of the main somatic nucleus (also *2*, *24*) but with different preponderance. No indication was found to suggest some preferential organization of the subnuclei in either the dorsoventral or lateral direction.

The present results support those of Bach (*2*), Van Biervliet (*5*) and Schwabe (*18*), but are not in agreement with those of Abd-El-Malek (*1*), Szentagothai (*21*) or Danis (*10*) on the organization of the oculomotor nucleus in the cat. The reasons for the difference between Abd-El-Malek's results and mine are not immediately apparent. In the case of the latter investigators (*10*, *21*), however, the different technical approach could hardly be ignored. Specifically their observations during local stimulation were limited to the whole globe motion rather than to individual muscles. Furthermore, with their technique it is not possible to separate fiber tracts from cell bodies nor to rule out the spread of current to contiguous structures even across the midline.

Szentagothai (*21*) proposed a chieflly controlateral position for the SR and levator palpebrae subnuclei on the basis of a previous fiber degeneration study.

In the analysis of the unitary response to the various stimulations in the present study, it was hoped that the applied criteria were rigorous enough to exclude both fiber activity and synaptically driven units. Actually, and in spite of the fact that a number of units were "rejected" because they failed to meet enough of the criteria (Table 1),[3] very few spikes were encountered that could clearly be labelled as being characteristic of fiber or synaptically driven cells. The impression is therefore gained that when one stimulates the oculomotor nerve or one of its branches peripherally in the anesthetized cat, one is likely to record mainly antidromically activated spikes from cell bodies within the corresponding nucleus.

Although the technique used in this study might not always differentiate between motor and sensory units, it is unlikely that our data reflect activity in the latter since the cell bodies for afferent connection from the eye

[3] The 12 units rejected (in the case of the oculomotor nerve stimulation) with their spikes showing "A-B" break suggest that the latency requirements for the oculomotor nerve may have been too stringent.

muscles are generally (7, 8, 22–24) though not unanimously (9), thought to be in the mesencephalic nucleus of the trigeminal nerve. Synaptic excitatory influences on the motor cells by stimulation of proprioceptive afferents or even by stimulation of motor afferents are also possible. The details of their organization, however, have not been worked out for the eye muscles and, as mentioned above, the criteria for selection of units should minimize this possibility.

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