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THE COURSE OF DIRECT PROJECTIONS FROM THE ABDUCENS NUCLEUS TO THE CONTRALATERAL MEDIAL RECTUS SUBDIVISION OF THE OCULOMOTOR NUCLEUS IN THE CAT

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SUMMARY

We have used autoradiography (tritiated leucine) to investigate the projections of a number of nuclear groups of the cat pons. Some cells of the abducens nucleus have axons that cross the midline, ascend in the opposite median longitudinal fasciculus (MLF) and synapse on the cells of the oculomotor complex which have been identified by others as those innervating the medial rectus muscle.

INTRODUCTION

It seems clear, both from pathological material in humans[10,15] and experiments in monkeys[8,34], that the median longitudinal fasciculus (MLF) carries a stimulus to the medial rectus subnucleus of the oculomotor complex to effect the adductive portion of lateral gaze. The importance of this pathway was emphasized by Shanzer et al.[34], who showed that section of the MLF of the monkey blocked adduction to stimuli from the ipsilateral frontal cortex, ipsilateral fields of Forel or contralateral paramedian pontine reticular formation (PPRF).

The essential nature of the ipsilateral paramedian pontine reticular formation in the generation of lateral gaze has had broad support for some time[4,27]. This concept has been developed by others, who have contributed important data using neuroanatomic[21] and physiologic[11,12,28] techniques showing that the region of the PPRF is indispensible[12] and temporally tightly linked[28] to lateral gaze.

The anatomic link between the motor nuclei (III and VI) and the supranuclear control center located in the ipsilateral PPRF has been more elusive. While there is

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evidence for a connection between the PPRF and the ipsilateral sixth cranial nerve nucleus[27], a direct projection from the PPRF to the contralateral medial rectus subnucleus via the contralateral MLF has not been seen in most cases. Goebel et al. found degenerating fibers in a bundle that ran lateral to the *ipsilateral* MLF following a lesion in the PPRF[21]. In a similar experiment, Nauta[31] found degenerating fibers in the *ipsilateral* tractus fasciculorum of Forel after reticular formation lesions. The lack of anatomic connection between the PPRF and the contralateral third cranial nerve nucleus has also been further supported by the work of Brodal[5], Buttner-Ennever and Henn[6], Eccles[16], Fuller[18], Lynch[29], Valverde[38] and Graybiel[25].

There has been some physiologic support for such a connection[26], but I know of only one supporting anatomic study[33]. This study has been often cited in support of the existence of a direct projection between the PPRF and the contralateral third cranial nerve nucleus, yet few details of this connection are available in the paper cited.

There are two other pontine nuclear groups that might send axons across the midline to the opposite third cranial nerve nucleus via the MLF. A heavy contribution of fibers to the MLF from the vestibular nuclei has been well documented[17,19,35]. However, the primacy of these nuclei, in the generation of non-vestibular eye movements, may well be questioned, since lesions in them do not cause significant gaze paralysis[30], and since a lesion in the PPRF blocks eye motion induced by the vestibular system.

Investigating another candidate, Carpenter[7,8] reported on degeneration studies following lesions in the abducens nucleus and in the MLF. A lesion in the abducens nucleus which included the nearby MLF resulted in degenerating fibers in the reticular formation and in the vestibular nuclei, in addition to causing paresis of lateral gaze. A lesion in the MLF caused retrograde cellular changes in the abducens nuclei and in the vestibular nuclei but not in the reticular formation. Retrograde degeneration in cells of the abducens nucleus was also reported by Christoff et al.[9], following section of the MLF.

Graybiel[25] was able to trace retrograde transport of horseradish peroxidase from the third cranial nerve nucleus to the contralateral sixth cranial nerve nucleus, but not to any of the nuclei of the PPRF. Two 'short communications' attempt to better define a possible connection between the sixth cranial nerve nucleus and the opposite third cranial nerve nucleus using autoradiography of tritiated amino acids. Graybiel[25] used tritiated proline to label a volume of the pons including cells of the perihypoglossal nucleus, cells of the reticular formation and cells of the sixth cranial nerve nucleus of the cat. She was able to demonstrate a collection of tritiated proline in the medial rectus subdivision of the contralateral third cranial nerve nucleus. The details of the pathways taken by the axons is not discussed. Buttner-Evener and Henn[6] mention a similar autoradiographically demonstrable connection between a broad area about and including the sixth cranial nerve nucleus and the contralateral medial rectus subnucleus in the monkey. From the line-drawing supplied, it seems that there was labeling of cells of the cerebellum, the nucleus prepositus hypoglossi and possibly the vestibular nuclei. Once again, details of the exact course of the axons connecting the injection site with the third cranial nerve nucleus are lacking.

If some of the cells of the sixth cranial nerve nucleus project via the MLF to the contralateral third cranial nerve nucleus, these are likely to be other than those whose axons create the sixth cranial nerve. There is no degeneration of fibers in the MLF following section of the abducens nerve[7]. Warwick[39], using retrograde degeneration, felt that there was only one population of cells in the sixth cranial nerve nucleus of the monkey. Since then, however, there has been ample anatomical and physiological evidence for the existence of more than one type of cell in the sixth cranial nerve nucleus of the cat[3,20,22,23,36].

In summary, then, there has been evidence presented by others that the population of cells in the sixth cranial nerve nucleus is not homogeneous. Using horseradish peroxidase, some cells of the sixth cranial nerve nucleus appear to project to the third cranial nerve nucleus. Using retrograde degeneration techniques, some cells of the sixth cranial nerve nucleus appear to project into the opposite MLF and finally, using a anterograde technique (autoradiography), some cells, in an area about and including the sixth cranial nerve nucleus, project to the contralateral medial rectus subnucleus using a pathway yet to be defined.

The purpose of this paper is to report on a study of the course of some rostral projections of the sixth cranial nerve nucleus using an anterograde technique (autoradiography).

METHODS

Fifty-nine cats were used in a study of the projections of the PPRF and the sixth cranial nerve nucleus. Under barbiturate general anesthesia, an occipital craniotomy and spreading of the hemispheres allowed access to the bony tentorium. A micropipette, loaded with from 1–2 μCi of tritiated leucine dissolved in 0.1 μl of normal saline, was passed through a dental drill hole in the tentorium after stereotaxic localization. The tritiated leucine was injected over a 15-min period, and the skin was closed. In successful experiments, the animals were allowed to survive from 3 to 7 days. Shorter or much longer survival time proved unreliable. Standard histologic techniques were used to cut paraffin sections. It was important not to limit the sectioning to the same plane in all experiments. Individual axons were easy to follow autoradiographically when the plane of section is parallel to them but harder to detect in cross-section. Mounted sections were dipped in three-quarter strength Kodak NTB-2 emulsion, dried and exposed under cold, light-free conditions for 6–8 weeks and developed in Dektol. Anatomic landmarks were defined by lightly staining with toluidine blue.

The use of radioactive compounds for autoradiographic tracing of CNS connections is still evolving. The summary article by Cowan[14] and the cautions, posted by Graybiel in her chapter[24], point out the many problems. Our working definition of what we felt to be positive identification of a connection between soma, axon and synaptic field was as follows. One, the soma of the neuron must have had greater overlying photographic change than the background in the area. In other words, the soma appears to be concentrating radioactivity. Two, radioactive axons that did not seem to have their origins elsewhere must be seen leaving the region of the cell bodies.

Finally, in this set of experiments, axons were traceable to and stopped at a cloud-like burst of radioactivity — the 'terminal field'. We think that, when audioradiography is used to define the whole neuron, problems of misinterpretation are less likely.

RESULTS

This report is limited to the projections of the sixth cranial nerve nucleus.

Eight cats had injections that unilaterally labeled the sixth cranial nerve nucleus without spread to the vestibular nuclei or cerebellum (Fig. 1). Five of these 8 had two axon systems that projected in the following manner. First, the axons of the ipsilateral sixth cranial nerve were labeled. Second, there was a group of axons that emerged from the rostral half of the nucleus and immediately crossed the midline to ascend in the contralateral MLF to reach the third nerve nucleus where they ended in a terminal field in the dorsal and rostral portion of the contralateral third cranial nerve nucleus (Figs. 2–7). This portion of the third cranial nerve nucleus corresponds to that region identified by Gacek as belonging to the medial rectus[20].

Two of these 5 cats that had projections to the contralateral third cranial nerve nucleus had injection sites limited to the sixth cranial nerve nucleus without apparent spread to any other neighboring nuclear mass. In particular, neither of these had injections that spread to the nucleus propostus hypoglossi or any cells of the reticular formation using the following criteria. First, only the cells of the sixth cranial nerve

INJECTION OF TRITIATED LEUCINE INTO THE SIXTH CRANIAL NERVE NUCLEUS				
PONTINE CELL BODIES LABELED	NO. OF CATS	AXON PROJECTIONS		
1 SIXTH NUCLEUS	2	IPSILAT SIXTH CRANIAL NERVE	TO THE CONTRALAT THIRD NUCLEUS VIA THE MLF	
1 SIXTH NUCLEUS 2 PPRF	2	IPSILAT SIXTH CRANIAL NERVE	TO THE CONTRALAT THIRD NUCLEUS VIA THE MLF	TO THE CONTRALATERAL PREF IPSILATERAL ROSTRAL TO AREA OF PRETECTUM IPSILATERAL CAUDAL VIA THE MLF-DESTINATION UNKNOWN CONTRALATERAL CAUDAL VIA THE MLF-DESTINATION UNKNOWN
1 SIXTH NUCLEUS (CAUDAL POLE) 2 PPRF	3	IPSILAT. SIXTH CRANIAL NERVE		SAME AS ABOVE
1 SIXTH NUCLEUS 2 NUCLEUS PREPOSITUS HYPOGLOSSI	1	IPSILAT SIXTH CRANIAL NERVE	TO THE CONTRALAT THIRD NUCLEUS VIA THE MLF	

Fig. 1. Summary of injections limited to the sixth cranial nerve nucleus and its immediate neighbors.

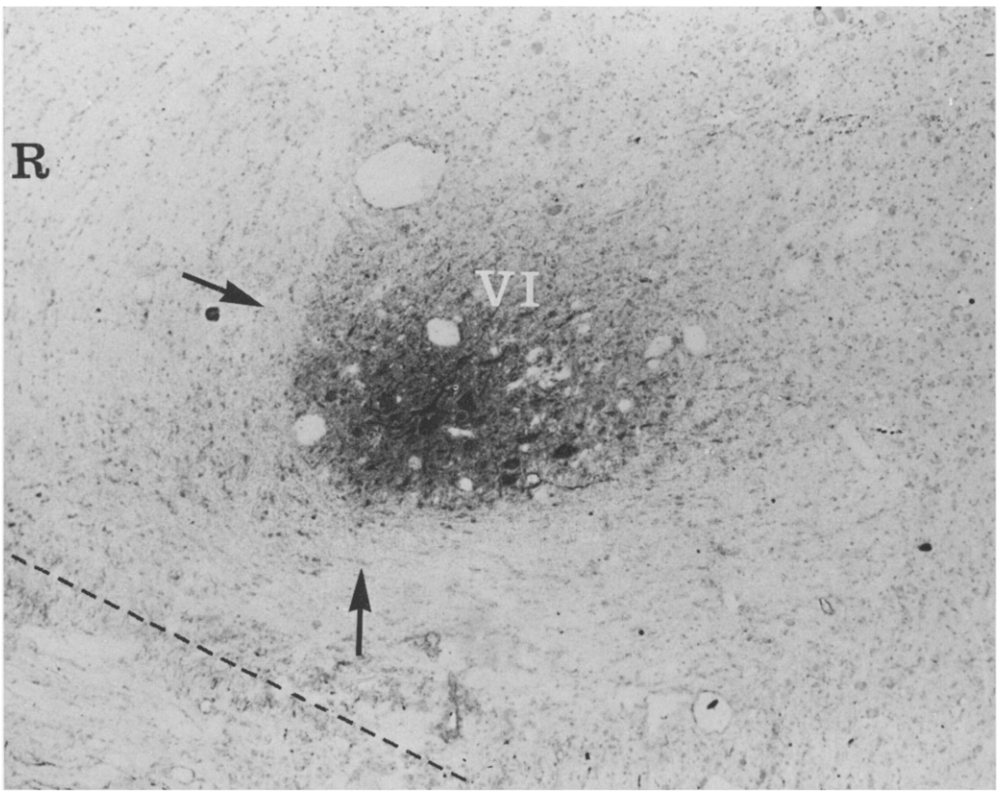


Fig. 2. Cat 101475; injection site in sixth cranial nerve nucleus (VI). Medial and rostral (R) borders of the nucleus outlined by genu of facial nerve (arrows are in nerve). Dashed line is midline. Horizontal section.

nucleus appeared to be labeled. Second, no known axon projections of the reticular formation (rostral ipsilateral, caudal or contralateral) could be seen[5,32,38].

Two of the three remaining cats, with projections to the contralateral third cranial nerve nucleus, were contaminated by spread to the underlying paramedian pontine reticular formation using the second criterion (V.S.). The other of the 3 remaining cats had spread of the injection site to the overlying nucleus prepositus hypoglossi using the first criteria (V.S.).

Since the injection sites were centered in the nucleus, the two cats with injections limited to the sixth cranial nerve nucleus did not help localize which part of the nucleus projected to the third cranial nerve nucleus. Indirect evidence on this matter was obtained from the 3 final cats of the 8, which had combined injections of the sixth cranial nerve nucleus and the reticular formation without projection to the contralateral third cranial nerve nucleus. In these 3, only the caudal half to one-third of the sixth cranial nerve nucleus was injected along with the underlying reticular formation. In none of these were any projections to the contralateral third cranial nucleus seen, in spite of intensive labeling of some fibers of the ipsilateral sixth cranial nerve. This negative result, along with the fact that the axons destined for the third cranial nerve

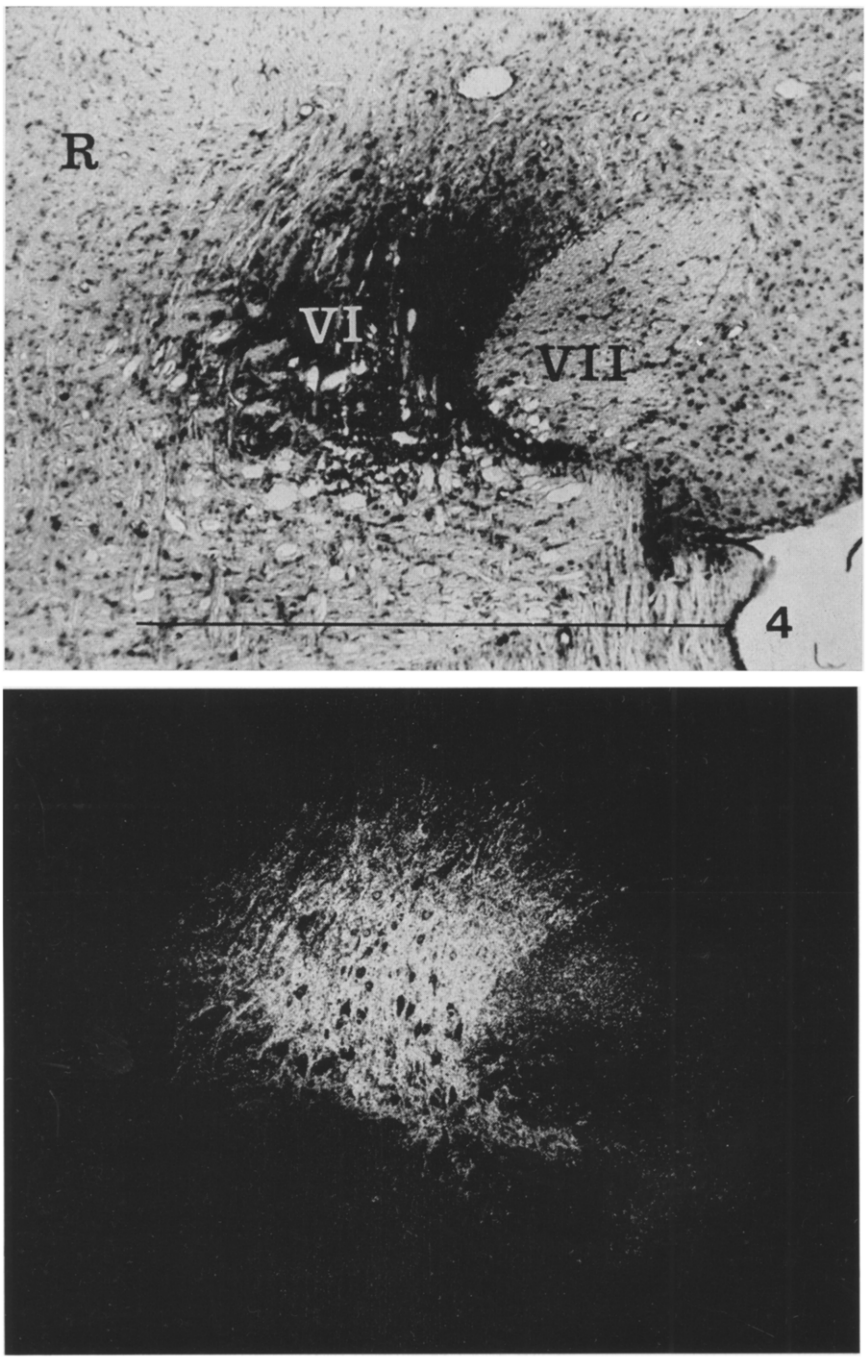
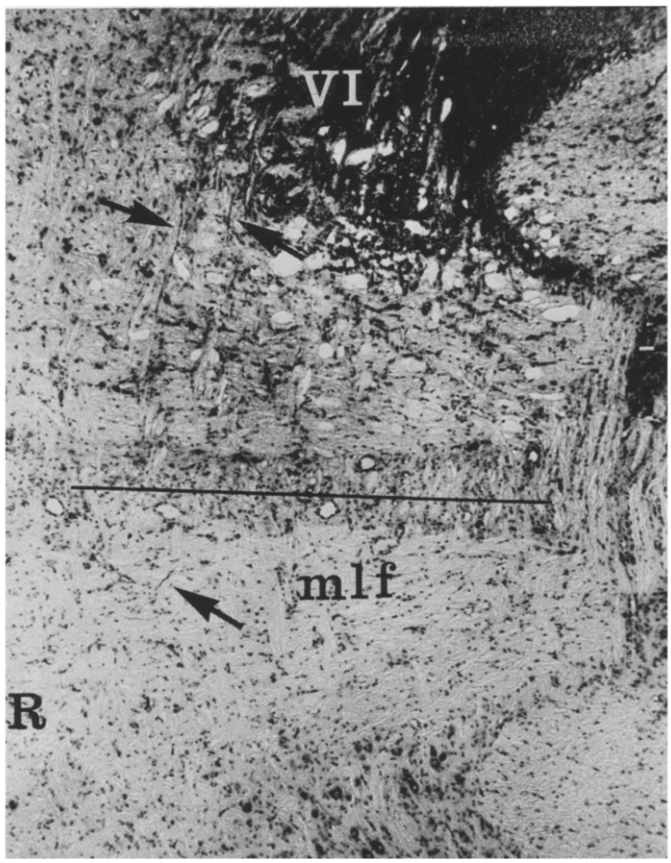
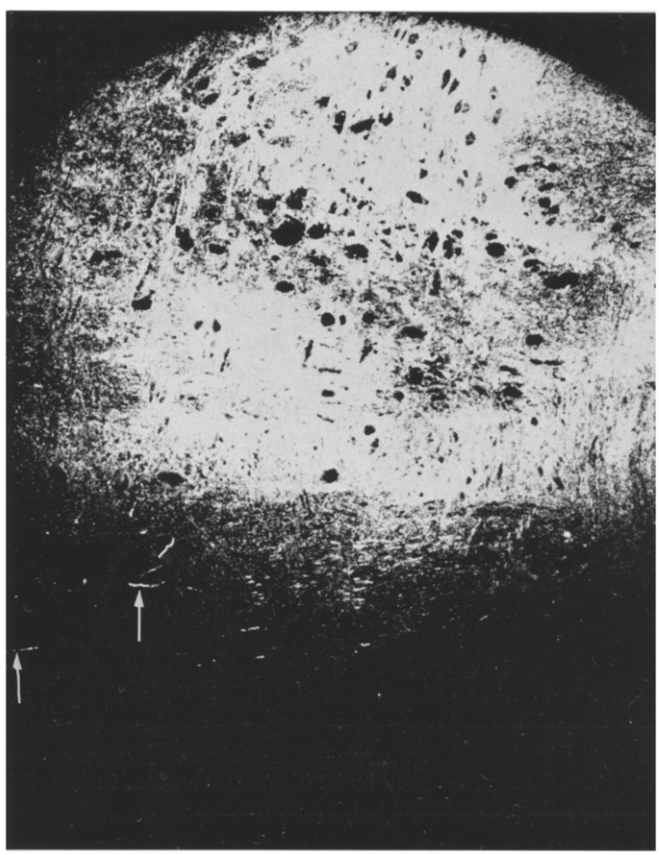


Fig. 3. a: cat 91675-1; bright-field photomicrograph of injection site in sixth cranial nerve nucleus (VI). Caudal part of the sixth cranial nerve nucleus limited by seventh nerve (VII). Midline shown by solid line up to fourth ventricle (4). Rostral (R) to left. Horizontal section. b: dark-field of a.

Fig. 4. a: cat 91675-1; slightly more ventral section to that of Fig. 3a. Axons (arrows) are emerging from the heavily labeled sixth cranial nerve nucleus (VI), some have already crossed (arrow) the midline (solid line) and are proceeding rostrally (R) in the contralateral MLF (mlf). Bright-field, horizontal section. b: dark-field of a. Axons (arrows) have emerged from the sixth cranial nerve nucleus, have crossed the midline and are proceeding rostrally. Microscope condensor closed to enhance axon visibility.


VI
mlf
R



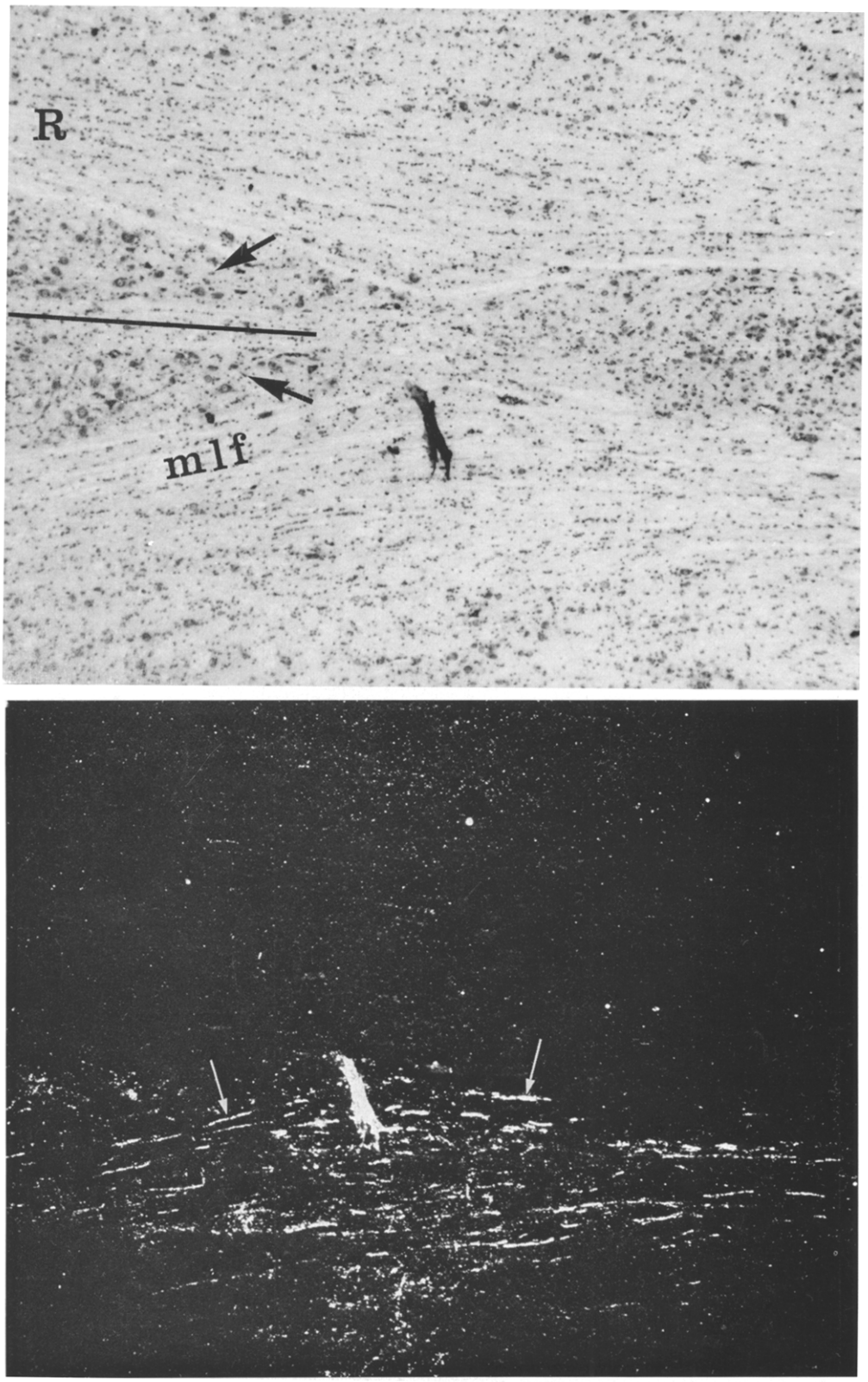


Fig. 5. a: cat 91675-1; bright-field photomicrograph of caudal poles of the third cranial nerve nucleus (arrows). Midline indicated by solid line. Rostral indicated by R. Horizontal section. b: dark-field of a. Axons (arrows) can be seen in the MLF contralateral to the injection site.

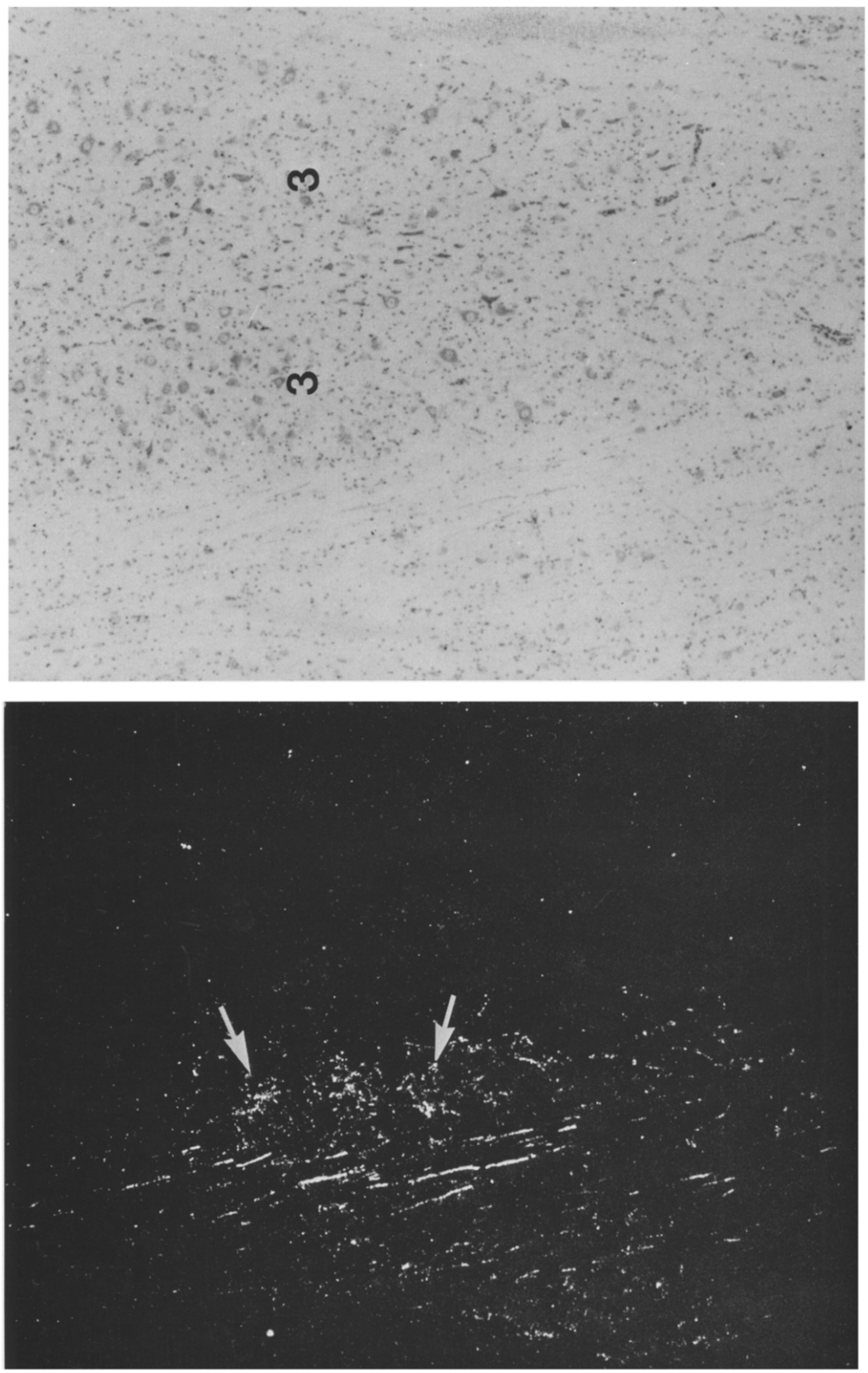


Fig. 6. a: cat 91675-1; bright-field photomicrograph of the paired third cranial nerve nuclei (3). Horizontal section. b: dark-field of a. A terminal field (arrows) is beginning to appear in the third cranial nerve nucleus.

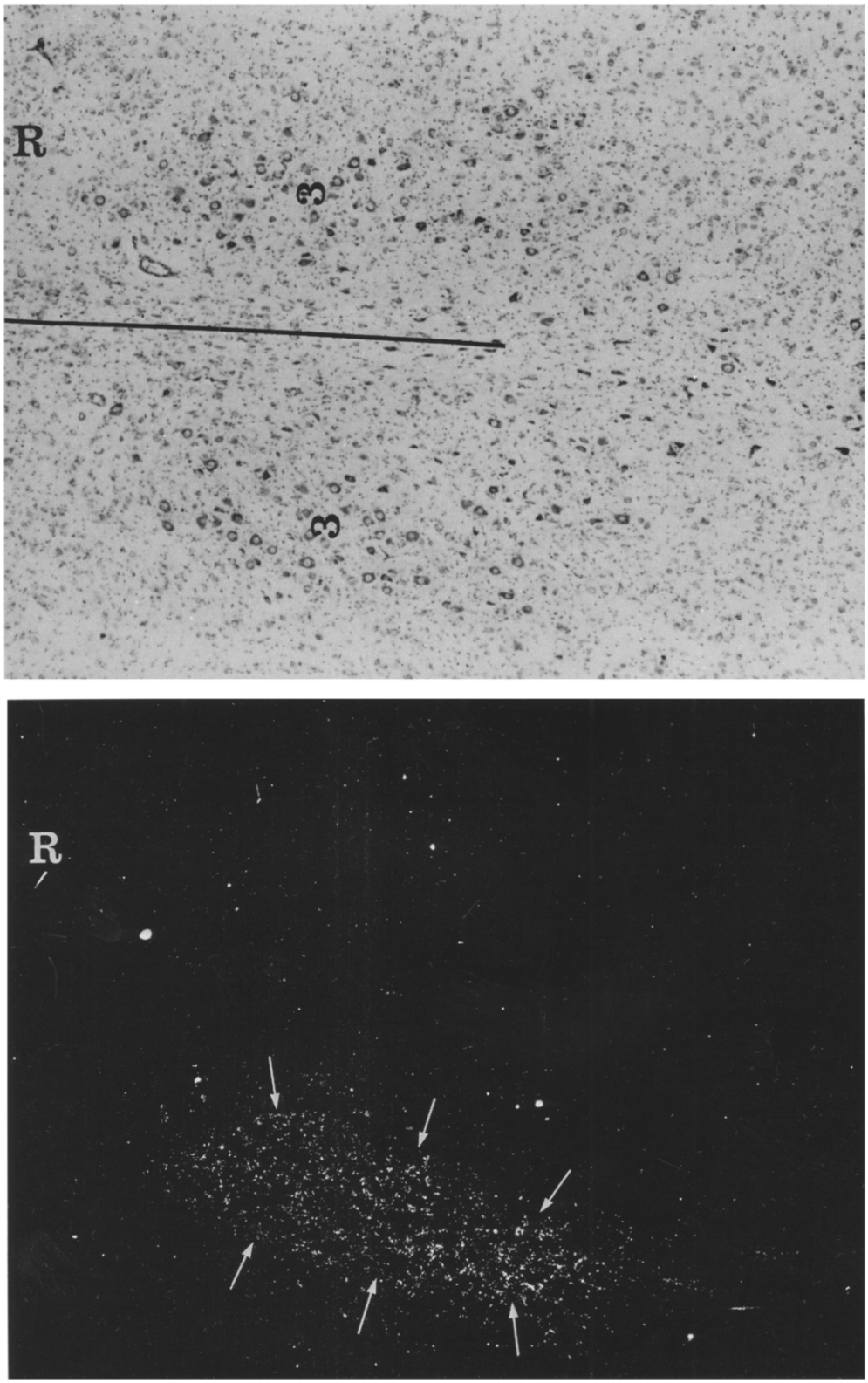


Fig. 7. a: cat 91675-1; bright-field photomicrograph of horizontal section through the dorsum of the paired third cranial nerve nuclei (3). Midline identified by solid line. R indicates rostral. b: dark-field of a. Terminal field (outlined by tips of arrows) correlates exactly with motor nucleus contralateral to injection site. R indicates rostral.

nucleus seemed to exit from the rostral half of the nucleus, suggests that some cells in the rostral half of the sixth cranial nerve nucleus are the source of these fibers.

DISCUSSION

The results of these experiments follow logically from the results of others[1,2,6–13,21,25,34,37]. Lateral gaze is a physiologic, not an anatomic entity. Thus, the assumption that we have traced the course taken by the direct supranuclear command for the adductive component of lateral gaze must depend on our fitting known physiologic principles. We feel that the pathway traced from the sixth cranial nerve nucleus to the contralateral third cranial nerve nucleus conforms to all the functional information that has been gathered about this system. Proceeding from the nuclear to the supranuclear level, the physiology asks for a synapse on the medial rectus subnucleus, a passage in the MLF ipsilateral to the medial rectus subnucleus and an origin in either the contralateral sixth cranial nerve nucleus or the cells of the contralateral PPRF. Since the cells of the PPRF apparently do not fulfill the first two criteria, the sixth cranial nerve nucleus was the most logical source for this connection. We were pleased to see this confirmed by those experiments in which the sixth cranial nerve nucleus was labeled.

We are not aware of any morphological differences among the cells of the sixth cranial nerve nucleus. Largely on the basis of negative evidence, we concluded that these somata were located in the rostral half of the nucleus.

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REFERENCES

1 Baker, R. and Berthoz, A., Is the prepositus hypoglossi nucleus the source of another vestibulo-ocular pathway?, *Brain Research*, 86 (1975) 121–127.
2 Baker, R., Gresty, M. and Berthoz, A., Neuronal activity in the prepositus hypoglossi nucleus correlated with vertical and horizontal eye movement in the cat, *Brain Research*, 101 (1975) 366–371.
3 Baker, R. and Highstein, S. M., Physiological identification of interneurons and motoneurons in the abducens nucleus, *Brain Research*, 91 (1975) 292–298.
4 Bender, M. and Shanzer, S., Oculomotor pathways defined by electrical stimulation and lesions in the brainstem of the monkey. In M. Bender (Ed.), *The Oculomotor System*, Harper and Row, New York, 1964.
5 Brodal, A., *The Reticular Formation of the Brain Stem: Anatomical Aspects and Functional Correlations*, Oliver and Boyd, London, 1957.
6 Buttner-Ennever, J. A. and Henn, V., An autoradiographic study of the pathways from the pontine reticular formation involved in horizontal eye movements, *Brain Research*, 108 (1976) 155–164.
7 Carpenter, M. and McMasters, R., Disturbances of conjugate horizontal eye movements in the monkey: II. Physiological effects and anatomical degeneration resulting from lesions in the medial longitudinal fasciculus, *Arch. Neurol.*, 8 (1963) 347–368.
8 Carpenter, M., McMasters, R. and Hanna, G., Disturbances of conjugate horizontal eye movements

in the monkey: 1) Physiological effects and anatomical degeneration resulting from lesions of the abducens nucleus, and nerve, *Arch. Neurol.*, 8 (1963) 231–247.
9 Christoff, N., Anderson, P., Nathanson, M. and Bender, M., Problems in anatomic analysis of lesions of the median longitudinal fascicules, *Arch. Neurol.*, 2 (1960) 293–304.
10 Cogan, D., Kubik, C. and Smith, J., Unilateral internuclear ophthalmoplegia: report on 8 clinical cases with 1 postmortem study, *Arch. Ophthal.*, 44 (1950) 783–796.
11 Cohen, B. and Feldman, M., Relationship of electrical activity in pontine reticular formation and lateral geniculate body to rapid eye movements, *J. Neurophysiol.*, 31 (1958) 806–817.
12 Cohen, B. and Komatsuzaki, A., Eye movements induced by stimulation of the pontine reticular formation, *Exp. Neurol.*, 36 (1972) 101–117.
13 Cohen, B., Komatsuzaki, A. and Bender, M., Electro-oculographic syndrome in monkeys after pontine reticular formation lesions, *Arch. Neurol.*, 18 (1968) 78–92.
14 Cowan, W. M., Gottlieb, D. I., Hendrickson, A. E., Price, J. L. and Woolsey, J. A., The autoradiographic demonstration of axonal connections in the central nervous system, *Brain Research*, 37 (1972) 21–51.
15 Crosby, E., Relations of brain centers to normal and abnormal eye movements in the horizontal plane, *J. comp. Neurol.*, 99 (1953) 437–479.
16 Eccles, J. C., Nicoll, R. A., Taborikova, H. and Willey, T. J., Medial reticular neurons projecting rostrally, *J. Neurophysiol.*, 38 (1975) 531–538.
17 Fluur, E., Influences of semicircular ducts on extraocular muscles, *Acta Oto-Laryng. (Stockh.)*, Suppl. 149 (1959) 1–46.
18 Fuller, J. H., Brain stem reticular units: some properties of the course and origin of the ascending trajectory, *Brain Research*, 83 (1975) 349–367.
19 Gacek, R., Vestibulo-oculomotor projections in the cat, *Acta. Oto-Laryng. (Stockh.)*, Suppl. 293.
20 Gacek, R. R., Localization of neurons supplying the extraocular muscles in the kitten using horseradish peroxidase, *Exp. Neurol.*, 44 (1974) 381–403.
21 Goebel, H., Komatsuzaki, A., Bender, M. and Cohen, B., Lesions of the pontine tegmentum and conjugate gaze paralysis, *Arch. Neurol.*, 24 (1971) 431–440.
22 Gogan, P., Gueritaud, J. P., Horcholle-Bossavit, G. and Tyc-Dumont, S., Inhibitory nystagmic interneurons. Physiological and anatomical identification within the abducens nucleus, *Brain Research*, 49 (1973) 410–416.
23 Goldberg, S. J., Hull, C. D. and Buchwald, N. A., Afferent projections in the abducens nerve: an intracellular study, *Brain Research*, 68 (1974) 205–214.
24 Graybiel, A. M., *Wallerian Degeneration and Anterograde Tracer Methods in the Use of Axonal Transport for Studies of Neuronal Connectivity*, W. Maxwell Cowan and Michel Cuenod (Eds.), Elsevier Scientific Publishing, 1975.
25 Graybiel, A. M. and Hartwieg, E. A., Some afferent connections of the oculomotor complex in the cat: an experimental study with tracer techniques, *Brain Research*, 81 (1974) 543–551.
26 Highstein, S. M., Cohen, B. and Matsunami, K., Monosynaptic projections from the pontine reticular formation to the third nucleus in the cat, *Brain Research*, 75 (1974) 340–344.
27 Lorente de Nó, R., Vestibulo-ocular reflex arc, *Arch. Neurol. Psychiat.*, 30, 245–291.
28 Luschei, E. and Fuchs, A., Activity of brain stem neurons during eye movements of alert monkeys, *J. Neurophysiol.*, 35 4 (1972) 445–461.
29 Lynch, G., Smith, R. L. and Robertson, R., Direct projections from brain stem to telencephalon, *Exp. Brain Res.*, 17 (1973) 221–228.
30 McMaster, R. E., Weiss, A. H. and Carpenter, M. B., Degeneration resulting from discrete partial lesions of the vestibular nuclei in the monkey, *Amer. J. Anat.*, 118 (1966) 163–194.
31 Nauta, W. and Kuypers, G., Some ascending pathways in the brain stem reticular formation. In H. H. Jasper et al. (Eds.), *Reticular Formation of the Brain*, Little, Brown and Co., Boston, 1958, pp. 3–30.
32 Papez, J. W., Reticulo-spinal tracts in the cat. Marchi method, *J. comp. Neurol.*, 41 (1926) 365–399.
33 Scheibel, M. and Scheibel, A., Structural substrates for integrative patterns in the brain stem reticular core. In H. H. Jasper et al. (Eds.), *Reticular Formation of the Brain*, Little Brown and Co., Boston, 1958, pp. 31–55.
34 Shanzer, S., Wagman, I. and Bender, M., Further observations on the median longitudinal fasciculus, *Trans. Amer. neurol. Assoc.*, (1959) 14–17.
35 Szentagothai, J., The elementary vestibulo-ocular reflex arc, *J. Neurophysiol.*, 13 (1950) 395–407.

36 Taber, E., Cytoarchitecture of the brain stem of the cat, *J. comp. Neurol.*, 116 (1961) 27–69.
37 Uemera, T. and Cohen, B., Effects of vestibular nuclei lesions on vestibulo-ocular reflexes and posture in monkeys, *Acta. Oto-Laryng. (Stockh.)*, Suppl. 315 (1973) 1–71.
38 Valverde, F., Reticular formation of the pons and medulla oblongata. A Golgi study, *J. comp. Neurol.*, 116 (1961) 71–99.
39 Warwick, R., Representation of the extra-ocular muscles in the oculomotor nuclei of the monkey, *J. comp. Neurol.*, 98 (1953) 449–504.