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AFFERENT CONNECTIONS TO THE ABDUCENT NUCLEUS IN THE CAT

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INTRODUCTION

By means of anterograde degeneration techniques and under the supervision of the late Professor Alf Brodal one of us (G.H.H.) more than ten years ago studied the projection from the reticular formation to the cranial nerve motor nuclei. In the abducent nucleus a scanty bilateral terminal degeneration was revealed, but it was not possible from that material to decide the exact origin of these afferents. No degeneration was found in the oculomotor or trochlear nuclei (Hoddevik, unpublished results). Recently, in the cat the abducent afferents have been studied by means of retrograde transport of free horseradish peroxidase (HRP) (18), or the wheat germ agglutinin-horseradish peroxidase conjugate (WGA-HRP) (15). However, in both these studies the injected tracer had spread outside the borders of the abducent nucleus. Furthermore, when free HRP is used, an additional pitfall is that this tracer appears to be taken up and transported retrogradely even by presumably unlesioned fibres passing through the periphery of an injection site (30). Ozaki and Okamura (23) studied the distribution of premotor neurons by retrograde-retrograde transneuronal transport of WGA-HRP after injections centred in the lateral rectus muscle, but this study also included afferents to the accessory abducent nucleus.

A more reliable method for the study of afferents to small nuclei is now available through a modification of the implantation technique described by Mori *et al.* (22). WGA-HRP is used as tracer (10), since this conjugate appears not to be taken up and transported by unlesioned fibres passing through the stained area at the implantation site (4; see also refs. 9, 24, 26). In the present investigation seven cats with WGA-HRP implants, of which four were almost entirely restricted to the abducent nucleus, were used to study the origin of its non-cortical afferents. The results obtained with this refined technique will be described below.

MATERIAL AND METHODS

Seven cats (weight 1.9 to 3.5 kg) with WGA-HRP implants in the abducent nucleus were selected for this study. A glass micropipette was filled with paraffine, and the paraffine near the tip was then dissolved in ethyl ether and the tip filled with crystalline WGA-HRP. Under deep pentobarbital anesthesia and from a dorsal approach the pipette was then

stereotactically inserted into the abducent nucleus, fixed to the skull with dental cement and left in situ. (For further details concerning this implantation technique, see refs. 6 and 22). The following day the cats were killed under deep anesthesia by intracardiac perfusion with physiological saline followed by a solution of 1% paraformaldehyde and 1.25% glutaraldehyde in 1M phosphate buffer at pH 7.4, and finally with a buffered 10% sucrose solution. The brain stem and two upper segments of the cervical spinal cord were dissected out and cut in serial transverse sections at 50μm on a freezing microtome. The sections were collected in groups of five, and two sections from each group were selected, processed with tetramethyl benzidine as described by Mesulam (21) and mounted as two parallel series. One series was weakly stained with neutral red, the other left unstained. In six animals (all cases except cat B.St.L. 1229), all sections through the brain stem were activated and mounted. All mounted sections were examined in bright field microscopy, and the extent of the stained area at the implantation site and the distribution of retrogradely labelled cells (in the cervical spinal cord and all parts of the brain stem including thalamus) were plotted in individual drawings.

RESULTS

The abducent nucleus is situated dorsally in the rostral part of the medulla oblongata, just ventral to the root fibres of the facial nerve. The nucleus consists of motoneurons and so called internuclear neurons (*i.e.*, neurons projecting to other nuclei in the central nervous system). The readers are referred to Evinger *et al.* (8) for the morphology and distribution of abducent nuclear neurons. One of our cases (cat *B.St.L. 1244*; Figs. 1 D and 2 A) had an implant entirely confined to the nucleus, another cat (*B.St.L. 1251*; Fig. 1 E) had a restricted implant with a slight additional involvement of the genu of the facial nerve. In these animals the majority of the retrogradely labelled neurons were found in the medial and descending vestibular nuclei, the reticular formation proper and the oculomotor nucleus [1]. Similar findings were made in two animals in which there was a very slight staining of the adjacent part of the reticular formation (cats *B.St.L. 1225* and *1226*; Fig. 1 A, B). Three other cats, *B.St.L. 1229* (Fig. 1 C), *1237* and *1247*, had a somewhat greater spread of tracer to the immediately surrounding reticular formation, and these cases had a more widespread distribution of retrogradely labelled cells. In the description given below, the findings from one case with a restricted, and one case with a larger implantation site are presented in more detail. Examples of retrogradely labelled neurons are shown Fig. 2 B-E.

The staining at the implantation site in cat B.St.L. 1244 was confined to the abducent nucleus (Fig. 1 D and 2 A). Retrogradely labelled neurons were found bilaterally in the medial and descending vestibular nuclei, mainly in their ventral and medial parts. In the oculomotor nucleus labelled cells were present bilaterally, but with contralateral preponderance. They were located along its entire rostrocaudal extent except for its rostralmost pole. The cells lay in all subdivisions of the oculomotor nucleus, with the majority in the dorsolateral division (see ref.

[1] Cat B.St.L. 1251 had one labelled cell in the facial nucleus.

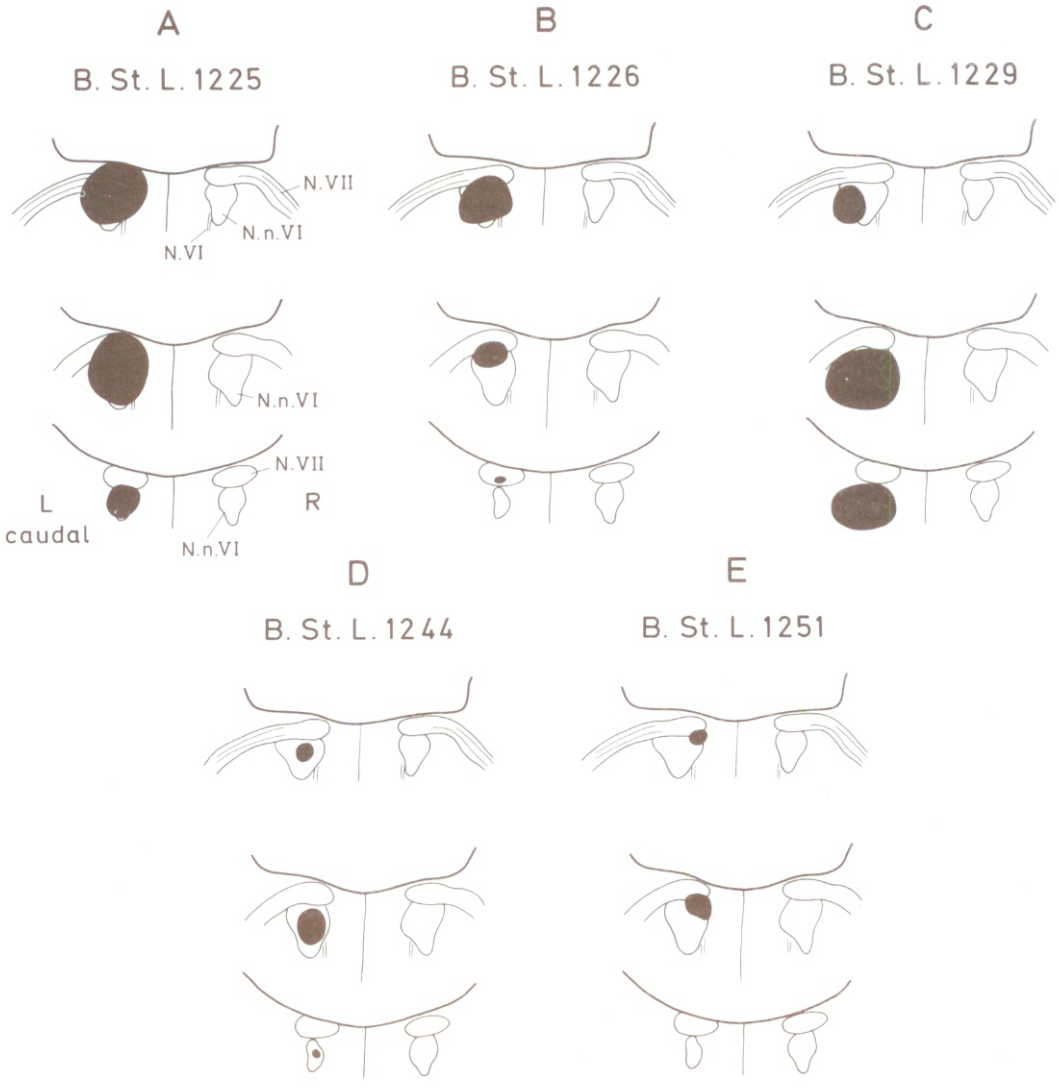

Fig. 1 A-E. – *Diagrams of transverse sections from caudal to rostral through the abducent nucleus showing the implantation sites of WGA-HRP in five of the cases described in the text.*

Abbreviations: Nn VI, abducent nucleus; N VII, facial nerve; N VI, abducent nerve; L, left; R, right.

25 for a description of the oculomotor nucleus and its morphological subdivision). In addition, faint anterograde terminal labelling was observed in the contralateral oculomotor nucleus, in the dorsolateral, dorsomedial and ventral division. Retrogradely labelled cells were also located contralaterally in the caudal pontine reticular nucleus, mainly in its medial part, and ipsilaterally in the gigantocellular reticular nucleus, mainly rostrally. Most of these neurons were medium-sized. No labelled giant cells were found. In addition, scattered retrogradely labelled cells were dis-

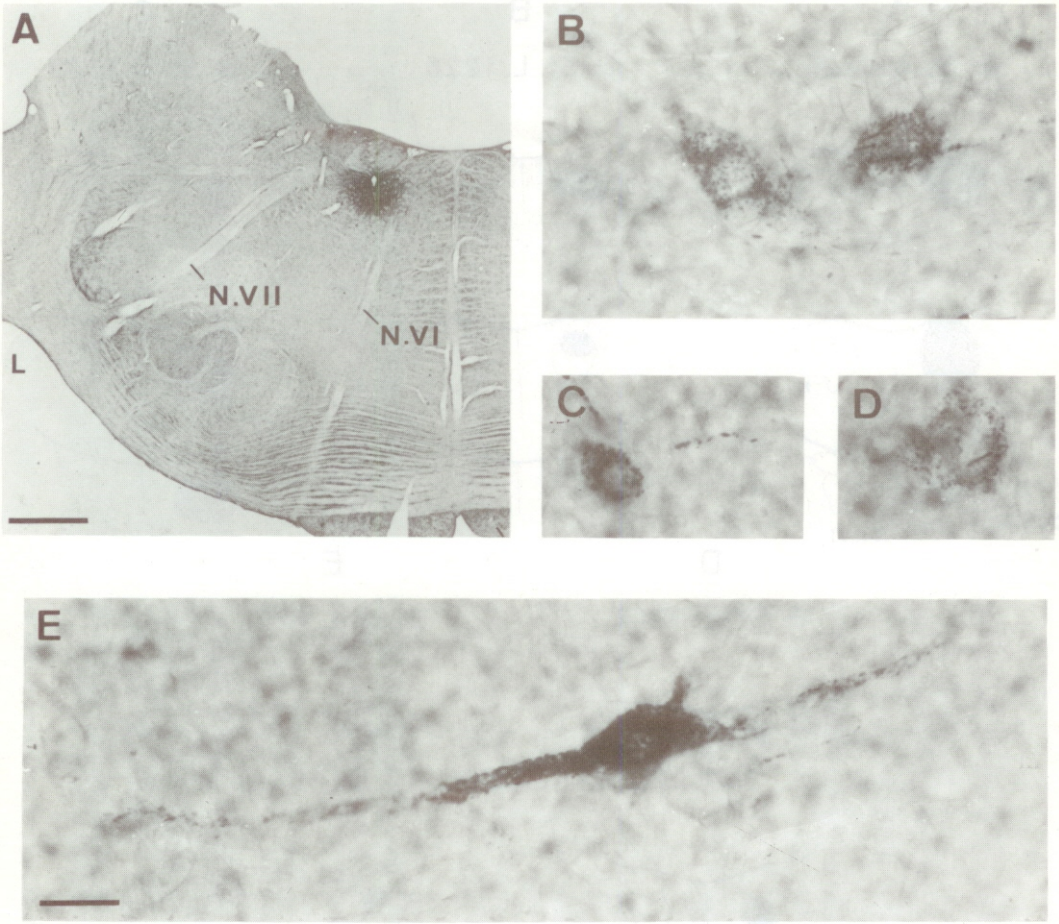

Fig. 2 A. – *Photomicrograph of the WGA-HRP implant in the left abducent nucleus in cat B.St.L. 1244.* Scale line 1 mm.

B-E. *Photomicrographs showing retrogradely labelled cells in cat B.St.L. 1225*, B in ventral part of ipsilateral medial vestibular nucleus, C in ventral division of ipsilateral oculomotor nucleus, D in ventral division of contralateral oculomotor nucleus, and E in ipsilateral nucleus of trapezoid body. Scale line in E 20 μm, same magnification in B-D.
Abbreviations, see Fig. 1.

persed bilaterally in the nucleus of the trapezoid body, the periaqueductal grey and the mesencephalic reticular formation just outside the oculomotor nucleus. Some labelled neurons were also found bilaterally in the tegmental reticular formation within and just outside the medial longitudinal fascicle (in the rostral part of the area sometimes referred to as the nucleus of the medial longitudinal fascicle). No retrograde cell labelling was found in the diencephalon or the cervical spinal cord.

The staining in cat B.St.L. 1229 (Fig. 1 C) comprised the abducent nucleus and the adjacent reticular formation on both sides, especially laterally. Retrogradely labelled neurons were distributed to the same areas as in the former case,

but were in addition present bilaterally in the lateral and superior vestibular nuclei, and in the paramedian reticular and tegmental pontine reticular nuclei. Furthermore, in the reticular formation proper, retrogradely labelled neurons were found bilaterally in the parvicellular and ventral reticular nuclei. Also, a few labelled cells were observed contralaterally in the principal and spinal trigeminal nuclei, and in the intercalatus and prepositus hypoglossal nuclei. Some large neurons were labelled ventrally in the contralateral superior colliculus, and finally a few faintly labelled neurons were found in the intralaminar nuclei of the thalamus.

DISCUSSION

Methodological remarks.

A major source of error in studies of afferent connections to nuclei surrounded by white matter and/or reticular formation, is uptake of tracer in fibres passing just outside the nuclear boundaries. However, since WGA-HRP appears not to be taken up by unlesioned passing fibres (see Introduction), it is likely that retrograde cell labelling in cases with no staining of the white matter along the needle track will only be found in neurons with axons/axonal branches terminating inside the stained area at the implantation site. Two of our cases (cats B.St.L. 1244 and 1251) had implants with staining restricted to the abducent nucleus with no contamination of the surrounding reticular formation. The retrograde cell labelling in these animals must therefore represent true afferents to the abducent nucleus.

None of our animals had visible damage to stained tissue in the reticular formation outside the abducent nucleus [1]. However, since the staining at the implantation site in five of the cases to a varying extent had spread to adjacent parts of the reticular formation, it can not be excluded that some of the retrogradely labelled cells found in these cases are afferents to the reticular formation (see below).

Another problem when WGA-HRP is used as a tracer, is the possibility of transneuronal transport. Labelling of somata may occur as a result of anterograde-retrograde, anterograde-anterograde or retrograde-retrograde transfer of WGA-HRP (see ref. 27 for review). However, neither of these types of transneuronal transport are likely to have occured in our material. The former two can be excluded since there was only very faint anterograde labelling of efferents from internuclear cells in the abducent nucleus, the latter since retrograde-retrograde transfer usually does not occur after only one day (13; see also ref. 5 for further discussion).

Afferents to the abducent nucleus.

The present study has revealed a more restricted and slightly different origin of afferents to the abducent nucleus than previously reported. Thus, we find that

[1] Some animals had faint staining of damaged white matter in the genu of the facial nerve.

non-cortical afferents to the abducent nucleus originate in the vestibular nuclei, the oculomotor nucleus with the adjacent part of the periaqueductal grey, certain parts of the reticular formation proper and the nucleus of the trapezoid body.

Earlier retrograde tracer studies in the cat (see especially ref. 18) described abducent afferents from several other brain stem nuclei, including the superior colliculus and nucleus prepositus hypoglossi. These findings can probably be ascribed to spread of tracer from the abducent nucleus to the reticular formation. An even more widespread origin of abducent afferents have been reported in the monkey (2, 18), but for the same reason it is impossible to reach conclusions as regards possible species differences in the projection here considered.

Our observations indicate that the afferents from the vestibular nuclei are the quantitatively most important. These fibers are well known (see ref. 3 for review). However, whereas Harvey *et al.* (14, rabbit) described bilateral connections only from the medial and lateral vestibular nuclei, Langer *et al.*(18, cat) reported an origin also from other parts of the vestibular complex. In our animals with implants restricted to the abducent nucleus retrogradely labelled cells were found only in the ventral and medial parts of the medial and descending vestibular nuclei. However, since three cases with staining of the reticular formation adjacent to the abducent nucleus (cats B.St.L. 1229, 1237 and 1247) had labelled cells also in the lateral and superior vestibular nuclei, it is highly probable that the latter nuclei do not project to the abducent nucleus, but to the adjacent reticular formation. Uptake of tracer in commissural vestibular fibres, which pass just outside the abducent nucleus (17), is not likely to have occurred since none of these three cases had staining of damaged tissue ventral to the abducent nucleus.

In addition to the vestibular, the projection from the pontine reticular formation to the abducent nucleus is well known, but its exact origin has remained undetermined (see esp. 12, 18, 28). While Langer *et al.* (18) described an ipsilateral projection, Graybiel (12), using anterograde autoradiographic tracing, like us found a crossed pathway. The present material has in addition revealed that the reticulo-abducent connection originates from the caudal pontine reticular and gigantocellular reticular nuclei.

Our findings concur with the results of previous studies (e.g., 18, 19, 23) that there is a conspicuous projection from the oculomotor nucleus. This connection originates mainly from the dorsolateral division of the nucleus, which harbors the motoneurons innervating the medial rectus muscle (see refs. 1 and 25). In addition to the afferent projection, also some abducent efferent fibers to the dorsolateral, dorsomedial and ventral divisions of the contralateral oculomotor nucleus were found in the present study. This observation is in accordance with the findings of Kairada (15), who described anterograde staining laterally in the oculomotor nucleus after abducent injections, and with Evinger *et al.* (8), who described a projection from the abducent nucleus to the dorsolateral division of the oculomotor nucleus. According to the illustrations of Ozaki and Okamura (23) also these authors made corresponding observations, but they describe labelled neurons only in the caudal half of the oculomotor nucleus.

A more widespread distribution of retrogradely labelled cells was found in cases where the staining at the implantation site also included adjacent parts of the reticular formation (see the description of cat B.St.L. 1247). Although in these animals it was impossible to decide if the widespread retrogradely labelled cells project to the abducent nucleus, to the reticular formation or to both regions, it can not be excluded that their axons reach the reticular formation only. Of special interest in this context are the projections from nucleus prepositus hypoglossi and the superior colliculus. These have previously been described as destined for the abducent nucleus (see esp. refs. 7, 18, 20). Although retrogradely labelled cells were found in these nuclei in several of our cases with a spread of tracer to the reticular formation, we failed to observe such cells in the cats with implants restricted to the abducent nucleus. Interestingly, a previous degeneration study (16) has given evidence that the axons descending from the superior colliculus pass in and adjacent to the medial longitudinal fascicle. Some of these fibres may terminate in the reticular formation medial to the abducent nucleus. In accordance with this, Ozaki and Okamura (23) found retrograde cell labelling in the superior colliculus and nucleus prepositus hypoglossi after pressure injection of WGA-HRP in (and, presumably, around) [1] the abducent nucleus. In their transneuronal transport experiments after injections centred in the lateral rectus muscle, no labelled cells were found in the superior colliculus, and only few and lightly labelled cells in the nucleus prepositus hypoglossi.

Another interesting observation made in our cases with a spread of tracer to the reticular formation was the presence of retrogradely labelled cells in the contralateral reticular formation, in the area corresponding to that involved at the implantation site. This finding appears to confirm Walberg's (29) description of a reciprocal crossed reticulo-reticular connection.

Functional considerations.

Various brain stem pathways participate in gaze control (see, e.g., ref. 11). The vestibular nuclei have a key position, and they are the major source of afferents to the abducent nucleus. Another important connection originates in the caudal pontine reticular and gigantocellular reticular nuclei. These in turn have been shown to receive projections from the cerebral cortex, the superior colliculus, the vestibular nuclei and the fastigial nucleus (16). The reticular formation therefore apparently serves as a centre for integration of information necessary for control of eye movements (see also ref. 31). However, it is obvious that smaller direct projections to the abducent nucleus, like those described here, also may be of importance for gaze control. In addition, the here described projection from the nucleus of

[1] The size of the injection sites compared to the nuclear boundaries is not evident from their figures.

the trapezoid body to the abducent nucleus may represent a link between auditory and oculomotor pathways.

SUMMARY

The afferent connections to the abducent nucleus in the cat were studied by means of retrograde transport of WGA-HRP after implantations of the tracer in crystalline form. Retrogradely labelled cells were found bilaterally in the medial and descending vestibular nuclei, mainly in their ventral and medial portions, in the rostral part of the ipsilateral gigantocellular reticular nucleus, in the medial part of the contralateral caudal pontine reticular nucleus and bilaterally in the oculomotor nucleus, mainly in its dorsolateral division. Some labelled cells were also found bilaterally in the mesencephalic reticular formation, the periaqueductal grey and the nucleus of the trapezoid body.

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