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VISUAL INPUTS TO THE DORSOCAUDAL FASTIGIAL NUCLEUS OF THE CAT CEREBELLUM.

An experimental study using single unit recordings and horseradish peroxidase labelling

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INTRODUCTION

The caudal half of the fastigial nucleus (FN) receives afferents from the midvermis of the cerebellum and the brainstem (6, 10, 17, 37). The midvermis (lobules VI and VII) receives fibers from several structures in the brainstem, some of which are the recipients of inputs from the superior colliculus (SC), the pretectum and the ventral nucleus of the lateral geniculate body (1). Physiologically, the midvermal part of the cerebellum has been known to receive visual information (11, 12, 36, 39, 40). There are vermal Purkinje cells which change their firing rates at saccadic and smooth pursuit eye movements (23, 24, 29, 43). Lesion experiments have also demonstrated that dysfunction of the caudal FN elicits oculomotor symptoms similar to those seen after removal of the vermis (34, 44). The FN projects to the pretectal nuclei and the nucleus of Darkschewitch, as well as to the SC and the interstitial nucleus of Cajal (17, 28, 42), some of which are related to eye movements. These anatomical and physiological findings support the idea that the midvermis-FN link may play an important role in the control of visuo-oculomotor function.

The present study was carried out to analyse the neuronal activity in, and the pathways to, the caudal FN upon which visual information can be conveyed via the brainstem starting from the optic nerve, by using single unit recordings and horseradish labelling.

MATERIAL AND METHODS

Electrophysiological procedures.

Electrophysiological experiments were carried out on 11 cats (body weight 1.7-3.7 kg). The trachea and the femoral vein were cannulated under anesthesia with intramuscular injection of ketamine chloride (50 mg/kg). After the cannulation, surgical operations were performed under anesthesia administrated intravenously with alpha-chloralose (50 mg/kg)

and urethane (400 mg/kg). During the recording session, the cats were anesthetized and immobilized with continuous injection of urethane (17 mg/kg/h) and gallamine triethiodide (15 mg/kg/h) while artificially respirated.

Craniotomy was performed at two positions, through which electrodes were inserted into the optic chiasm (OCh) and the FN. The OCh was stimulated with bipolar electrodes (interpolar distance; 3 mm) introduced stereotaxically. The stimulating electrodes were made of stainless steels wires (shaft diameter; 125 μm), the tips of which were electrolytically sharpened. The electrodes had a diameter of about 50 μm at 75 μm from the tips and were insulated with Isonel 31, except for 70-100 μm from the tips. A mydriatic agent (tropicamide and phenylephrine hydrochloride) was instilled in both eyes. The electrodes were fixed at that point where the maximum field potential to a moving flash of light was recorded. The location of the electrode was identified histologically in the center of the OCh at the conclusion of the experiments. Experiments were performed in order to confirm that the responses of the FN cells resulted from electrical stimulation of the OCh, and not through current spread effects from surrounding structures. Threshold currents of the electrical stimulation of the OCh were measured for 51 cells. The threshold ranged from 110 μA to 520 μA, with an average value of 185 ± 124 μA (average ± standard deviation). Threshold curves were also obtained from 12 cells; the thresholds being determined at several depths along the electrode track in the OCh and the above-located structures. The threshold current curves varied among the cells. However, the threshold value was almost the same in each cell when the electrode tips were within the OCh, whereas the magnitude of currents required to evoke similar responses increased steeply when the tips were located outside the OCh. Response patterns of FN cells did not alter with changes of suprathreshold stimulus intensity, although the modulation of responses became weak with a decrease in the stimulus intensity. It was, therefore, considered that electrical stimulation of the OCh itself, but not of neighboring tissues, evoked significant responses in the FN cells. A constant current stimulus, whose strength and width were usually 3x threshold (less than 750 μA) and 100 μs, respectively, was applied to the OCh for every 1 or 3 s. In three experiments, a xenon flash lamp was used for photic stimulation to both eyes in order to supplement the electrical stimulation experiment.

Responses to moving pattern stimulation were tested by a reciprocating motion of a large visual field; a black and white stripe pattern (4 degrees interval). The pattern was projected on a tangent screen 76 cm in front of the animal's eyes. Soft contact lenses covered the eyes to prevent drying and to allow focusing on the screen. The tangent screen subtended 60° of visual field horizontally, and 45° vertically. The pattern was reflected by a mirror system driven by a galvanometer under control of an electric function generator. The pattern was moved horizontally as well as vertically at speeds ranging from 2 deg/s to 38 deg/s. To prevent the edges of the moving pattern from appearing on the screen, a black cardboard with a rectangular window was put between the mirror and the screen.

A stainless steel microelectrode (impedance; about 2MΩ at 330Hz) was introduced into the FN through vermal lobule VII, 0.8-2.0 mm from the midline at a 60° angle from the horizontal plane. Action potentials recorded extracellularly from the FN were identified as cell-body activity rather than axonal activity by the criteria described previously (23). Single cell discharges and stimulus signals were stored on magnetic tape. Peristimulus time (PST) histograms were constructed by an on-line system (FM 11 computer, Fujitsu, and a custom-made interface) and were stored on magnetic disk to be analyzed later.

In order to reconstruct the recording sites in histological sections, a DC current of 20 μA was applied through the recording electrode for 30 s to make an electrolytic lesion after completion of the recording. Two or three lesions were made in each animal. The animal was sacrificed at the end of experiments and perfused through the heart, with a solution of 10% formalin. The cerebellum was dissected free from the brainstem and cut parasagittally into a series of frozen sections (50 μm thick) which were stained with thionin. Recording sites were identified histologically and reconstructed as shown in Fig. 4.

Anatomical procedures.

A total of 18 adult cats (body weight 1.8-5.0 kg) were used for anatomical investigation. Animals were operated on under sodium pentobarbital (30 mg/kg) anesthesia. A small amount (see below) of a 50% solution of horseradish peroxidase (HRP, Toyobo Grade 1-C) or a 4% solution of wheat germ agglutinin (WGA) conjugated HRP (Sigma) was injected into the FN, the cerebellar vermal cortex, the vestibular nuclei (VN), or the medullary reticular formation (RF). In all animals, except 3 cats (*KEN 174, KEN 175*, and *KEN 206* where hydraulic pressure injections were made), HRP was injected iontophoretically (DC current; 2 μA, 500 ms ON and 500 ms OFF, for 4 min), using a glass micropipette (20-50 μm tip diameter).

Between 32 and 57 h after the injections, the animals were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.9% saline followed by a mixture of 0.4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3). The brains were dissected free and stored overnight in 0.1 M phosphate buffer containing 30% sucrose. The cerebellum was cut parasagittally and the brainstem was cut transversally on a freezing microtome. Every 5th section (50 μm thick) was treated according to the method of Mesulam *et al.* (32), and was counterstained with 1% neutral red. The positions of labelled cells observed under the microscope were entered on drawings of the sections made with the aid of a projection apparatus.

Abbreviations

AM : anteromedian nucleus
Amb : nucleus ambiguus
AVC : anteroventral cochlear nucleus
Brc : brachium conjunctivum
Brp : brachium pontis
CG : central gray
Cr : restiform body
D : descending vestibular nucleus
D. : down
DAO : dorsal accessory olive
DC : dorsal cochlear nucleus
EW : Edinger-Westphal nucleus
FLM : fasciculus longitudinalis medialis
FN : fastigial nucleus
f.p.l. : posterolateral fissure
f.pr. : fissura prima
f.p.s. : posterior superior fissure
f.sec. : fissura secunda
IO : inferior olive
L : lateral vestibular nuclei
L. : left
LGB : lateral geniculate body
LM : medial Lemniscus
M : medial vestibular nucleus
MAO : medial accessory olive
MGB : medial geniculate body
mX : dorsal motor nucleus of vagus
Nce : external cuneate nucleus

NIA : anterior interpositus nucleus
Nint : interstitial nucleus of Cajal
NIP : posterior interpositus nucleus
Nll : nuclei of the lateral lemniscus
NOT : nucleus of the optic tract
NPA : anterior pretectal nucleus
NPC : nucleus of the posterior commissure
NPH : perihypoglossal nuclei
NPP : posterior pretectal nucleus
Nrl : lateral reticular nucleus (nucleus of lateral funiculus)
NRm : magnocellular red nucleus
Nrp : paramedian reticular nucleus
NRTP : nucleus reticularis tegmenti pontis
NtrspV : spinal tract nucleus of the trigeminal nerve
Ped : corticospinal and corticopontine fibers in the cerebral peduncle
Pt : pretectum
PVC : posteroventral cochlear nucleus
R. : right
Rgc : nucleus reticularis gigantocellularis
Rpo : nucleus reticularis pontis oralis
Rv : nucleus reticularis ventralis
S : superior vestibular nucleus
SC : superior colliculus
SN : substantia nigra
SO : superior olive
SOL : lateral superior olive
SOM : medial superior olive
T : trapezoid body
Trs : solitary tract
U. : upper
III : oculomotor nucleus
VI : abducens nucleus
VII : facial nucleus
VIIg : genu of the facial nerve
X : dorsal motor nucleus of vagus
XII : hypoglossal nucleus
VI, VIIA, VIIB, VIII, IX and X in cerebellum: Lobules of Larsell's nomenclature.

RESULTS

I. Electrophysiological study

1. *Response patterns of the dorsocaudal FN cells to electrical and photic stimulation.* — A total of 158 cells responded to electrical stimulation of the OCh. The responsive cells were classified into three groups according to typical response patterns. Seventy-six cells (48%) in the first group (type-1) showed prominent facilitation after a transient suppression. The second group (type-2) included 61 cells (38%) which exhibited suppression of firing following a transient facilitation. The third group (type-3) consisted of 21 cells (14%) which showed suppression

with no signs of facilitation. Spontaneous discharge rates for 5 s in these cells were 14.2±9.5 spikes/s (mean±SD) for type-1 cells (n=20), 18.5±11.1 spikes/s for type-2 cells (n=21), and 10.9±9.0 spikes/s for type-3 cells (n=8). There was no significant difference among these cells at the 5% probability level.

Response patterns of a representative type-1 cell are illustrated in Fig. 1A. The cell ceased to fire at 12 ms after stimulation. This early suppression continued for 35 ms, which was followed by facilitation (late facilitation) lasting for about 220 ms. Latencies of the early suppression and the late facilitation were 10.2±8.8 ms and 42.6±21.8 ms (n=20), respectively. In 16 of the 76 type-1 cells, the late facilitation was followed by the second long suppression, lasting for about 200 ms as illustrated in Fig. 1A.

Response patterns of type-2 cells were characterized by early facilitation followed by late suppression. The early facilitation of the cell in Fig. 1B started about 17 ms after stimulation. The maximum firing rate reached 140 spikes/s. Cellular discharges were suppressed for 230 ms, starting 67 ms after the onset of stimulus. In some type-2 cells, weak facilitation of their firing was also observed after a late suppression. In 21 type-2 cells, the latencies of early facilitation and late suppression were 13.7±4.8 ms and 47.8±27.7 ms, respectively. When the response patterns of type-1 and type-2 cells are compared, it appears that facilitation and

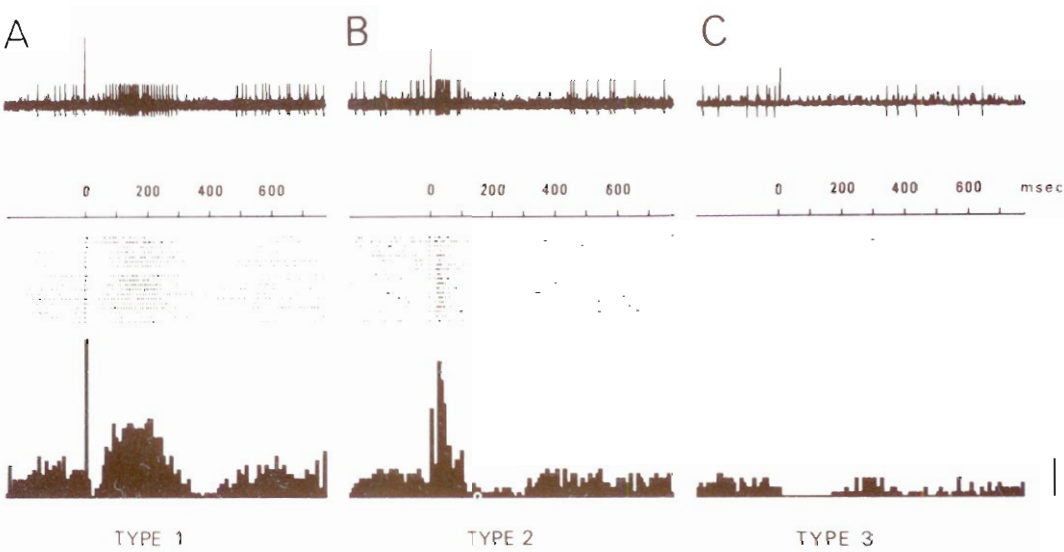

Fig. 1. – *Response pattern in type-1 (A), type-2 (B), and type-3 (C) cells to OCh stimulation.*

Upper, middle, and bottom rows show original spike recordings, rasters, and peristimulus time histograms (PST) constructed from 20 sweeps aligning to onset of stimulus (0 ms), respectively. Bin width in PST was 8 ms. Cellular discharges were recorded for 1024 ms in each sweep. A vertical bar indicates 5 spikes/bin. A: type-1 cell response showing long lasting facilitation after a transient suppression. B: type-2 cell response showing a long lasting suppression after a short facilitation. C: type-3 cell response whose discharges were strongly suppressed for more than 200 ms.

suppression in type-2 cells corresponded temporally with suppression and facilitation in type-1 cells.

Type-3 cells showed suppression after electrical stimulation of the OCh. A typical example is shown in Fig. 1C, where the suppression persisted for about 220 msec. It was difficult, however, to determine accurately the onset of suppression in many cells because of rather low spontaneous discharge rates. In 8 cells in which analysis was possible, the latencies and durations of the suppression were 10.8 ± 2.5 ms and 97.1 ± 50.0 ms, respectively.

In thirty-two cells which responded to electrical stimulation of the OCh, we examined whether or not they also responded to photic stimulation. Eight of the cells (25%) clearly responded to photic stimulation as well as to electrical stimulation of the OCh. Three types of response pattern to photic stimulation were observed and they were identical to those found by electrical stimulation; 3 type-1 cells, 4 type-2 cells, and one type-3 cells.

2. *Responses to moving pattern stimulation.* — Twenty-nine FN cells, which responded to OCh stimulation, were tested for responses to moving pattern stimulation; 13 type-1 cells, 11 type-2 cells and 5 type-3 cells. Seven (4 type-2 cells and 3 type-3 cells) of the cells showed clear modulation in association with horizontal movement of the pattern (Figs. 2 and 3). None of the type-1 cells tested exhibited any modulation to moving pattern stimulation.

A representative type-2 cell which was identified by OCh stimulation is shown in Fig. 2A. This cell showed clear modulation to a sinusoidally moving pattern, with excitation on leftward movement and inhibition on rightward movement (Fig. 2B), while no significant modulation was observed to a vertical movement (Fig. 2C). In all seven cells, it was observed that these modulations were dependent upon the velocity of the moving pattern. In another type-2 cell, the modulation in response to the moving pattern was manifested at 0.6 Hz (Fig. 3A) and 0.4 Hz (Fig. 3B) as compared with at 0.3 Hz (Fig. 3C). Similar patterns of modulation were observed in other type-2 and type-3 cells. The relationship between the velocity of the moving pattern and the depth of modulation was, therefore, analysed in detail in the seven cells (Fig. 3D). The depth of modulation was defined as the difference between peak and peak points of a cycle of the sinusoidal curve inserted in the histogram of Figs. 3A-C, which was calculated by the first harmonic component of fast Fourier transformation from the histogram of activity. In all the cells analysed, the depth of modulation (spikes/s) increased with velocity of the moving pattern, ranging from 2.8 deg/s to 23.5 deg/s. However, the relationship between the two parameters varied among the cells as shown in Fig. 3D.

3. *Location of responsive cells.* — The recording sites of responsive cells were located in a limited area of the dorsocaudal part of the FN, although recording tracks were made all over the FN rostrocaudally and mediolaterally (Fig. 4). The responsive cells were localized to a region 1.6-1.9 mm from the midline; the extent of the area was about 1 mm rostrocaudally, 0.3 mm mediolaterally, and 0.4 mm

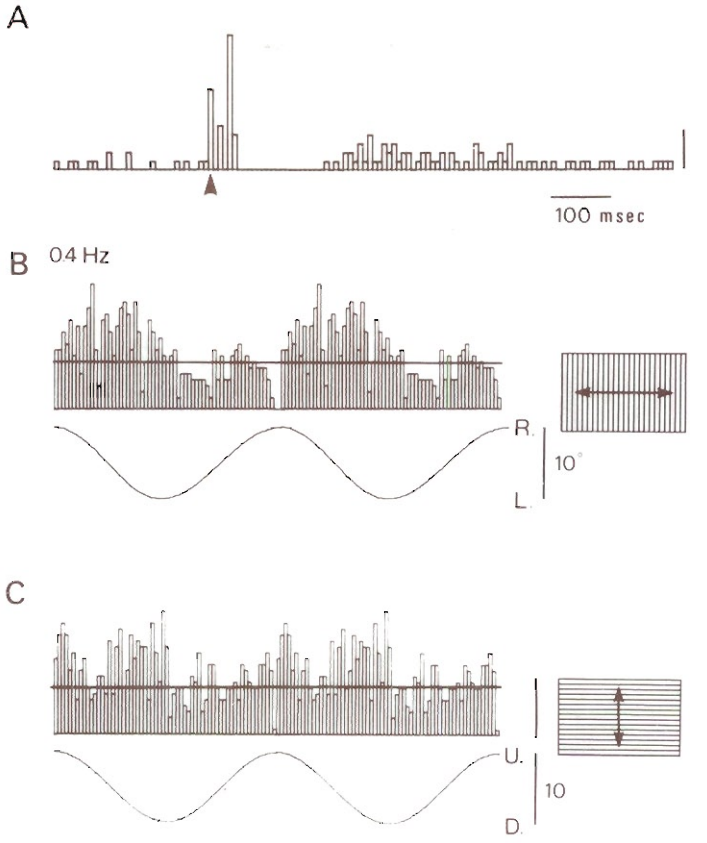

Fig. 2. – *Response patterns of a type-2 cell to electrical stimulation of the OCh (A), and to a sinusoidally moving pattern in the horizontal (B) and vertical (C) direction at 0.4 Hz; a maximum speed of 12.6 deg/s.*

The cell showed clear modulation of activity with excitation on leftward movement and inhibition on rightward movement (B). However, no significant changes of activity were observed when the pattern moved in vertical direction (C). The histogram of one cycle generated from 10 trials was repeated. Horizontal lines inserted in the histograms of B and C denote average spontaneous discharge rate in the dark. A vertical bar to the right of the histogram of C means 20 spikes/sec. An arrow in A indicates the onset of OCh stimulation. Sinusoidal curves below the histograms in B and C mean the movement of visual pattern of a black and white stripe, indicated to the right.

dorsoventrally. Positions of the 3 types of cells were apparently intermingled in the area. However, there was a tendency for type-1 cells to be located in the dorsocaudal part of the area, for type-2 cells to be more caudal, and for type-3 cells to be more rostral than the sites of type-1 cells. No responsive cells were found in the rostral or the ventrocaudal part of the FN.

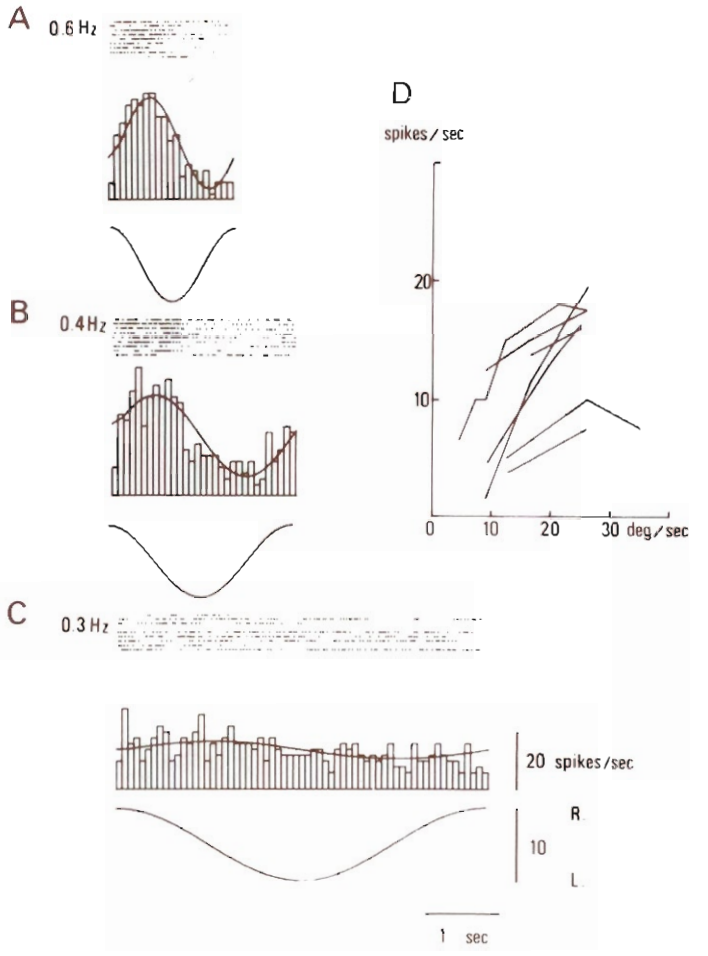

Fig. 3. – *Response pattern of a type-2 cell to a sinusoidally moving pattern in horizontal direction at three different speeds in A, B and C.*

A: 0.6 Hz (maximum speed = 18.8 deg/s). B: 0.4 Hz (maximum speed = 12.6 deg/s). C: 0.3 Hz (maximum speed = 9.4 deg/s). Sinusoidal curves inserted in the histograms mean the first harmonic component of fast Fourier transformation obtained from the histogram of activity. Sinusoidal curves below the histograms indicate the movements of visual pattern. D indicates the depth of modulation as a function of the velocity of a moving pattern stimulus in 4 type-2 cells and 3 type-3 cells. The depth of modulation increased with the velocity applied.

II. Anatomical Study

Findings of representative 7 cases of HRP experiments where the enzyme was confined to the aimed areas accompanied by the least diffusion, if any, to neighboring regions are presented below.

1. *Projections to the caudal FN.* — After iontophoretic injection of HRP, the

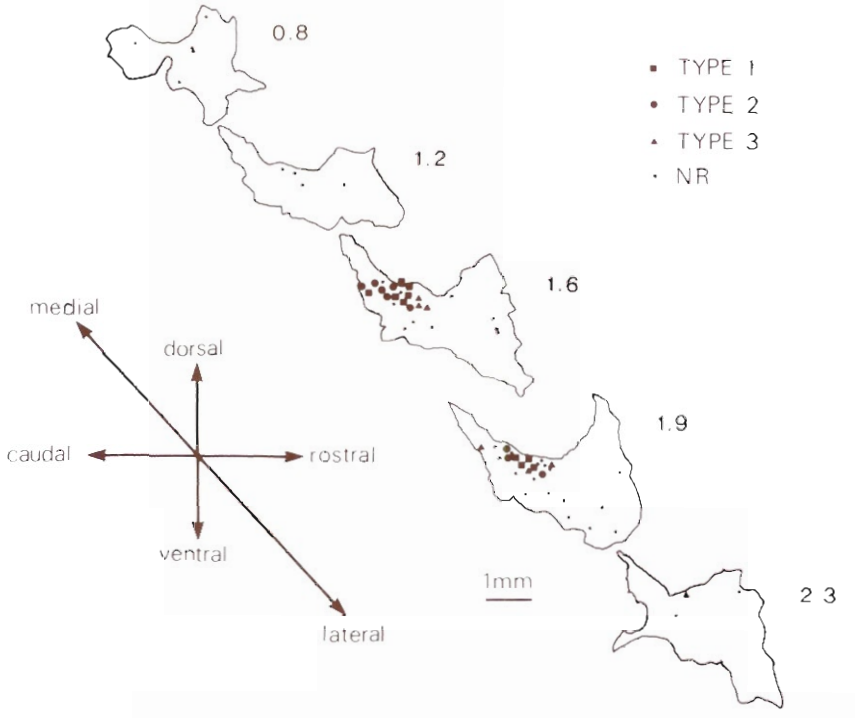

Fig. 4. - *Recording sites of visually responsive FN cells.*

Profiles of FN are presented on sagittal planes at 0.8, 1.2, 1.6, 1.9 and 2.2 mm from the midline. Locations of type-1, type-2, and type-3 cells are represented by squares, circles, and triangles, respectively. Small dots represent unresponsive cells (NR). Visually induced cell responses are recorded only in the dorsocaudal part of the FN.

central and caudal part of the left FN were affected in *cat KEN 182* (Figs. 5a and 6). The glass pipette passed through sublobules Vc, Vd, and Vld of the cerebellar cortex in which slight diffusion of the enzyme was present.

In the cortex, a large number of Purkinje cells were retrogradely labelled in extensive areas of lobules VI-VIII. In the inferior olive, many labelled cells were found in the caudal half of the contralateral (right) medial accessory olive (MAO), predominantly in the central part. In the VN, labelled cells occurred in the four main subnuclei (superior (S), lateral (L), medial (M), descending (D)), predominantly in the caudal parts of the M and the D (Fig. 5b) of the ipsilateral side. The gigantocellular reticular nucleus (Rgc) on both sides also contained some labelled cells of small size. A few cells were labelled in the Edinger-Westphal nucleus (EW), the dorsal raphe nucleus, the nucleus locus coeruleus, the nucleus reticularis tegmenti pontis (NRTP), the perihypoglossal nuclei (NPH) and the group x of the VN. In all sections examined, only 8 labelled cells were found in the dorsolateral pontine nucleus.

A similar distribution pattern of labelled cells was found in *cat KEN 170* (not

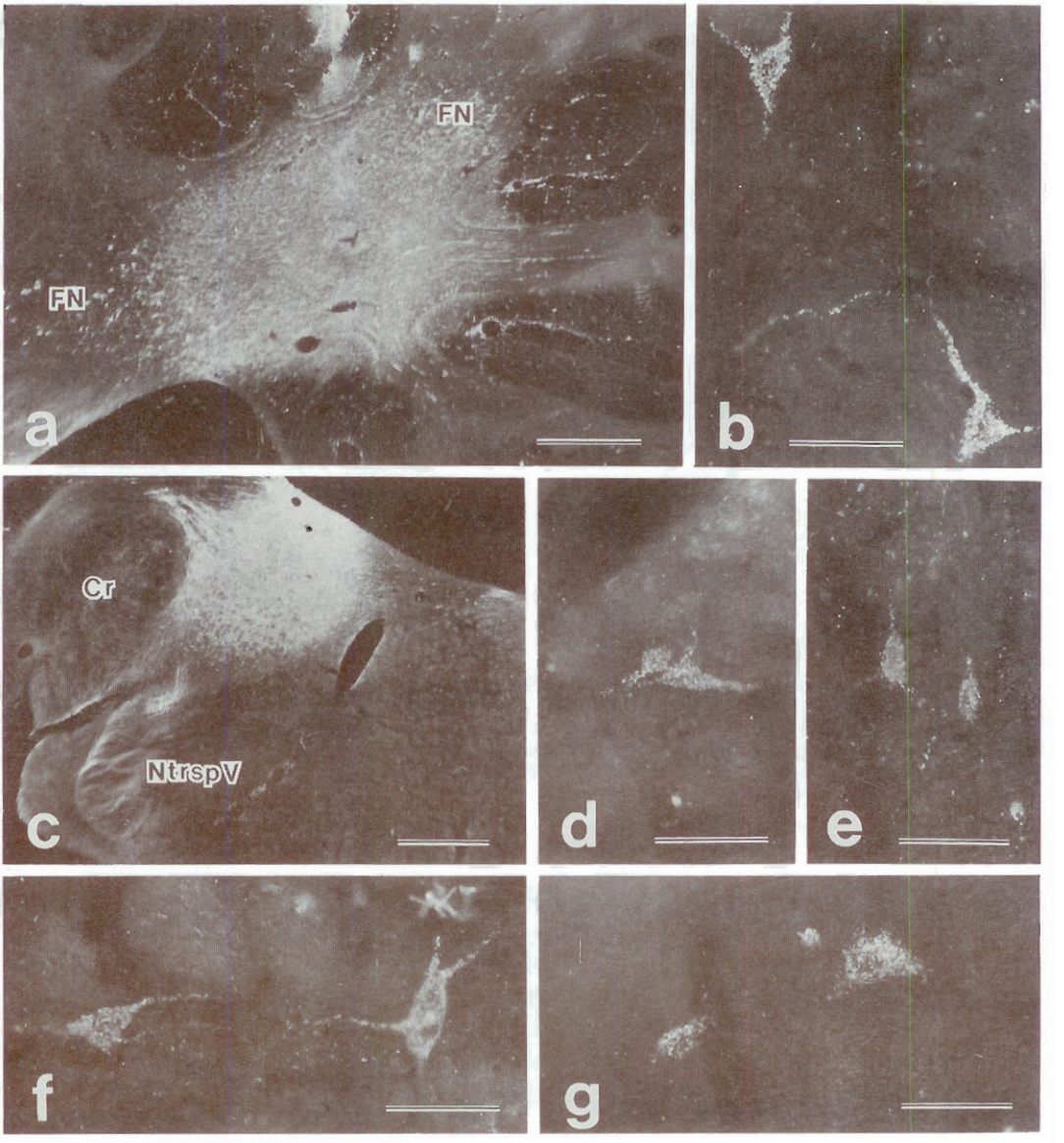

Fig. 5. – *Injection sites and labelled neurons.*

a and c: Polarizing photomicrographs to show the maximum extent of injected HRP in the left FN of *cat KEN 182* (a) and in the left VN of *cat KEN 193* (c). Scale bars indicate 1 mm.

b, d, e, f and g: Retrogradely labelled cells observed in the right D of *cat KEN 182* (b), in the left Nint (d), EW (e) and the dorsal part of the left Rgc (f) of *cat KEN 193*, and in the left NOT (g) of *cat KEN 194*. Polarizing microscope. Scale bars indicate 50 μm.

illustrated) where the central part of the left FN was injected, although in this case the number of labelled cells was much fewer than above (*cat KEN 182*).

In order to eliminate the possibility that cells projecting to the cerebellar cortex of lobules VI and VII were labelled by leakage along the needle track in these experiments (*cats KEN 182* and *KEN 170*), the following control experiments were made.

In *cats KEN 174* (not illustrated, 0.05 μl) and *KEN 175* (not illustrated, 0.02 μl), glass pipettes were inserted from the cerebellar surface of sublobule Vc to reach the floor of the primary fissure where sublobules Vld and Vlf were stained with HRP. The cerebellar nuclei were not involved. Some labelled cells were found in the pontine nuclei (PN), the NRTP and the MAO as well as a few in the Rgc and the VN.

2. *Projections to the VN and the RF.* — Since the main sources of input to the caudal FN were found to be the M and the D in the previous experiments, HRP was injected into the VN (the M and the D) of *cat KEN 193* in order to examine the cells of origin of the afferent projections (Figs. 5c-f and 7).

In this animal, the cannula passed through lobule V and the anterior interpositus nucleus in which there was some diffusion of the enzyme. Injected areas of HRP in the left VN (Fig. 5c) corresponded well to those regions where many vestibular cells were labelled in *cat KEN 182* (Fig. 6). There was no HRP staining in the RF. Many labelled cells were found bilaterally in the dorsal part of the Rgc (Figs. 5f and 7), at levels caudal to the abducens nucleus. Many cells were labelled bilaterally in the VN (the M, the D and the S) and some in the NPH (drawings 74 and 84 of Fig. 7). In the midbrain, some labelled cells were also found, with ipsilateral preponderance, in the Nint (Fig. 5d), the EW (Fig. 5e), the anteromedial nucleus (AM) of the EW and the rostromedial part of the mesencephalic RF. Small-sized cells were also found in and around the oculomotor nucleus.

In *cat KEN 194* (Figs. 5g and 8), the dorsal part of the left Rgc was stained with HRP. A small rostral part of the M was also slightly stained. In addition to the labelling of cells in the VN and the RF, many labelled cells were observed ipsilaterally in the Nint and the nucleus of the optic tract (NOT) (Fig. 5g). Some cells were also labelled in the dorsal raphe nucleus, the EW and the rostromedial part of the mesencephalic RF.

3. *Projections to the mid-vermal cerebellar cortex (lobules VI and VII).* — No data have been available so far from an experiment in which almost the entire part of lobules VI and VII was stained with HRP. In cat *KEN 206* (not illustrated, 0.1 μl×4, 45 h), most parts of lobules VI and VII were stained with HRP. There was minimum involvement of the caudal tip of FN.

Many labelled cells were found in several parts of the PN, the NRTP, and the caudal MAO. Some cells in the VN (mainly in the M and the D) and the NPH were labelled. Very few labelled cells occurred in the EW, the dorsal raphe nucleus and the RF.

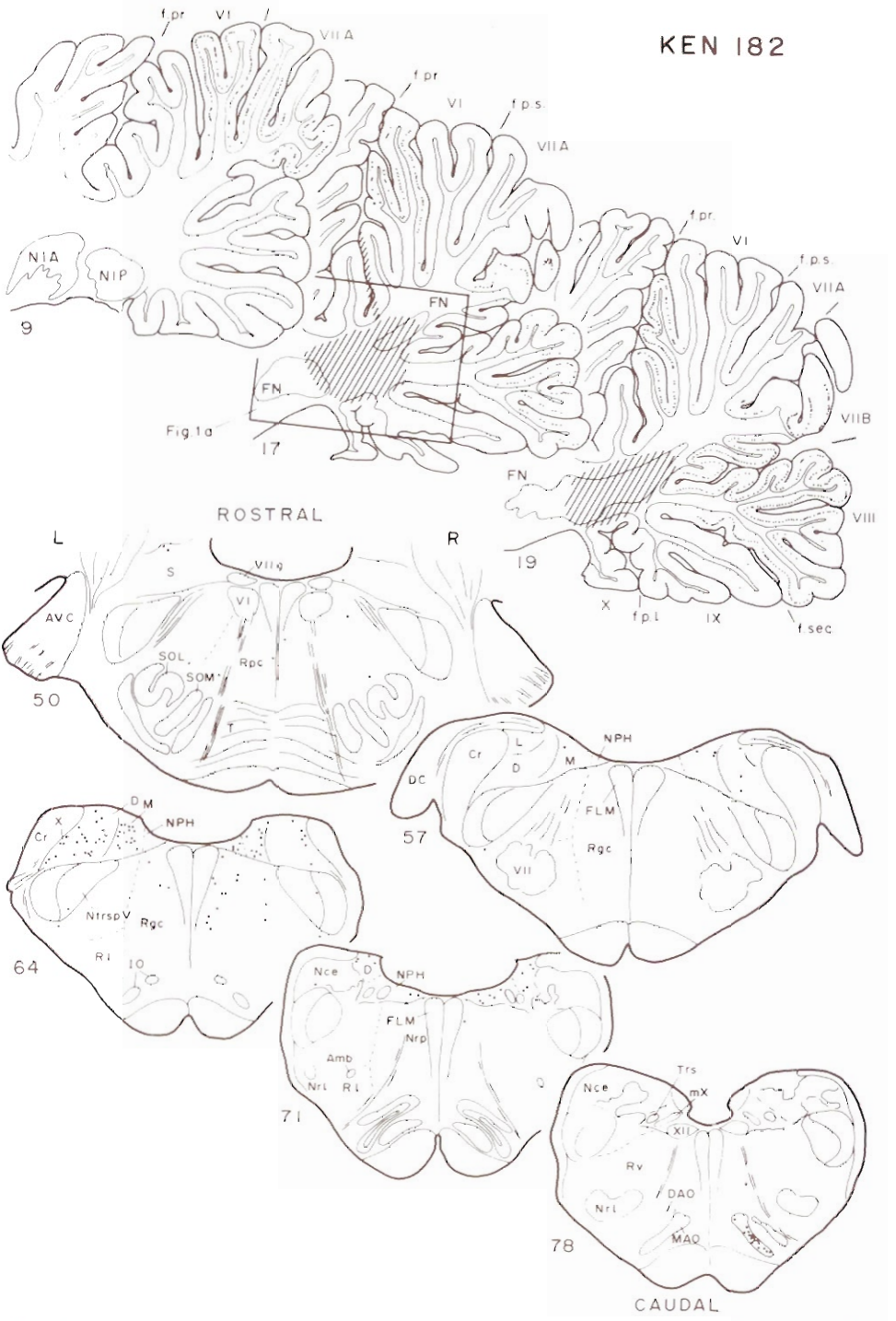

Fig. 6. – *Diagram to show positions of retrogradely labelled cells in sections of the brainstem and cerebellar cortex after injection of HRP in the caudal half of the FN in* cat KEN 182.

Areas stained with HRP are hatched. A single dot represents one labelled cell. In the brainstem, positions of labelled cells appearing in three neighboring sections were superimposed in this and following diagrams of Figs. 7 and 8.

DISCUSSION

The present study has revealed that cells responding to electrical stimulation of the OCh are located in the FN and can be classified into three groups. Type-1 cells (48%) showed early suppression and late facilitation, and type-2 cells (38%) exhibited early facilitation and late suppression, while type-3 cells (14%) showed long suppression (Fig. 1). Each cell type showed the same response patterns to photic stimulation as to electrical stimulation of the OCh. The responsive cells were located in a limited area of the dorsocaudal FN and the three types of the cells were intermingled, whereas no responsive cells were found in the rostral and ventral parts of the FN (Fig. 4). These data suggest that a restricted area of the dorsocaudal FN receives inputs from the visual system.

In confirming our previous degeneration study (17) the present experiment with injection of HRP into the caudal FN showed that lobules VI and VII project massively to the dorsocaudal FN (Fig. 6) where the cells responding to visual stimuli were recorded (Fig. 4). Lobules VI and VII of the cat vermis have been shown to be "the visual area of the cerebellar cortex" (11, 12, 40). Freeman (12) reported that Purkinje cells in these lobules showed a single pattern of response to photic stimulation. In the present study, three types of cells were observed in the dorsocaudal FN, and it is suggested that the midvermis-dorsocaudal FN link may play an important role in the integration of visual signals.

Ron and Robinson (35) reported that electrical stimulation of vermal lobules V-VII and the caudal FN induced eye movements, whereas similar stimulation of the rostral FN did not evoke any kinds of eye movement. There is a good correspondence in the sphere of the FN — in the dorsocaudal part — between the eye movement-induced area of Ron and Robinson (35, their Fig. 12) and the responsive area demonstrated in the present study (Fig. 4). A recent anatomical study of Yamada and Noda (45) also showed that the caudal part of the FN receives inputs from the oculomotor vermis of the monkey. Together, these data indicate that the dorsocaudal FN may participate in the control of visuomotor functions, receiving visual inputs via lobules VI and VII of the cerebellar vermis or directly via the brainstem (Fig. 6).

In analysing the possible pathways to the dorsocaudal FN in which the three types of visually evoked responses were obtained (Figs. 1-4), the findings of the present HRP experiments should now be considered. After injection of the enzyme in the caudal part of the unilateral FN, many labelled Purkinje cells occurred in lobules VI and VII of the cerebellar vermis, and a substantial number of labelled cells were also located bilaterally in the M and the D vestibular nuclei, the NPH, the inferior olive and the dorsal part of the medullary RF (Fig. 6).

Afferents to vermal lobules VI and VII mostly come from the PN and the NRTP (1, 14, 19) (see also experiment *KEN 206*, not illustrated). Other sources of afferents to lobules VI and VII are the caudal part of the MAO. Minor components also come from the VN (mainly from the M and the D), the EW, the dorsal raphe nucleus an the dorsolateral pontine tegmental area. It is possible,

therefore, that these structures in the brainstem may have some influence upon cells in the caudal part of the FN.

In order to find out possible routes to the VN, injection of HRP was made in *cat KEN 193* (Fig. 7). In agreement with the degeneration study of Hoddevik *et al.* (18), many cells in the dorsal part of the Rgc were found to send direct fibers to the VN. Furthermore, the dorsal part of the Rgc, in which eye-movement related cells were shown physiologically (5, 16), received substantial projections from the NOT, the Nint, and the AM of the EW nucleus (Fig. 8), which are very likely to be important visual sources. Among these three mesencephalic nuclei, however, the NOT is the only structure which receives direct retinal inputs (2, 3, 33). The direct projection from the NOT to the dorsal part of the Rgc, just caudal to the abducens nucleus, has been shown autoradiographically in the rabbit (21), but the present study is the first to demonstrate the presence of such a projection in the cat. A direct projection from the NOT to the VN has not so far been identified (see also our experiment *KEN 193*, Fig. 7).

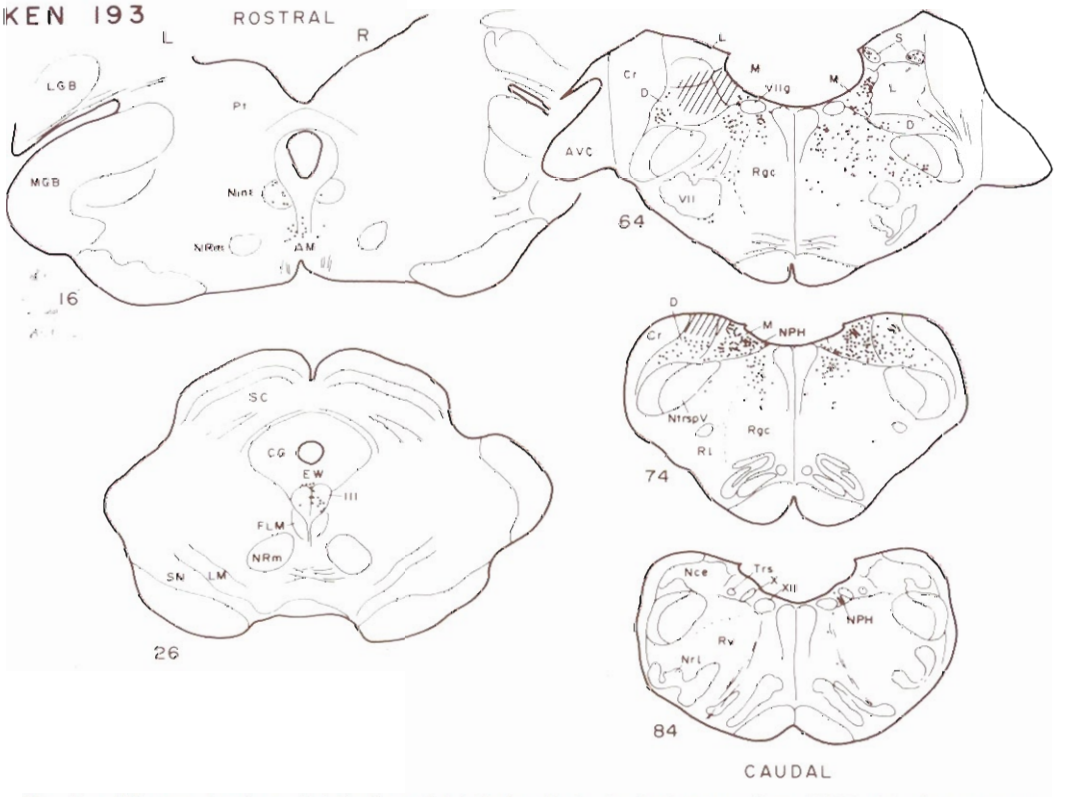

Fig. 7. – Diagram to show distribution of labelled cells in the brainstem of cat KEN 193 after injection of HRP in the left VN.

Principles of presentation as in Fig. 6.

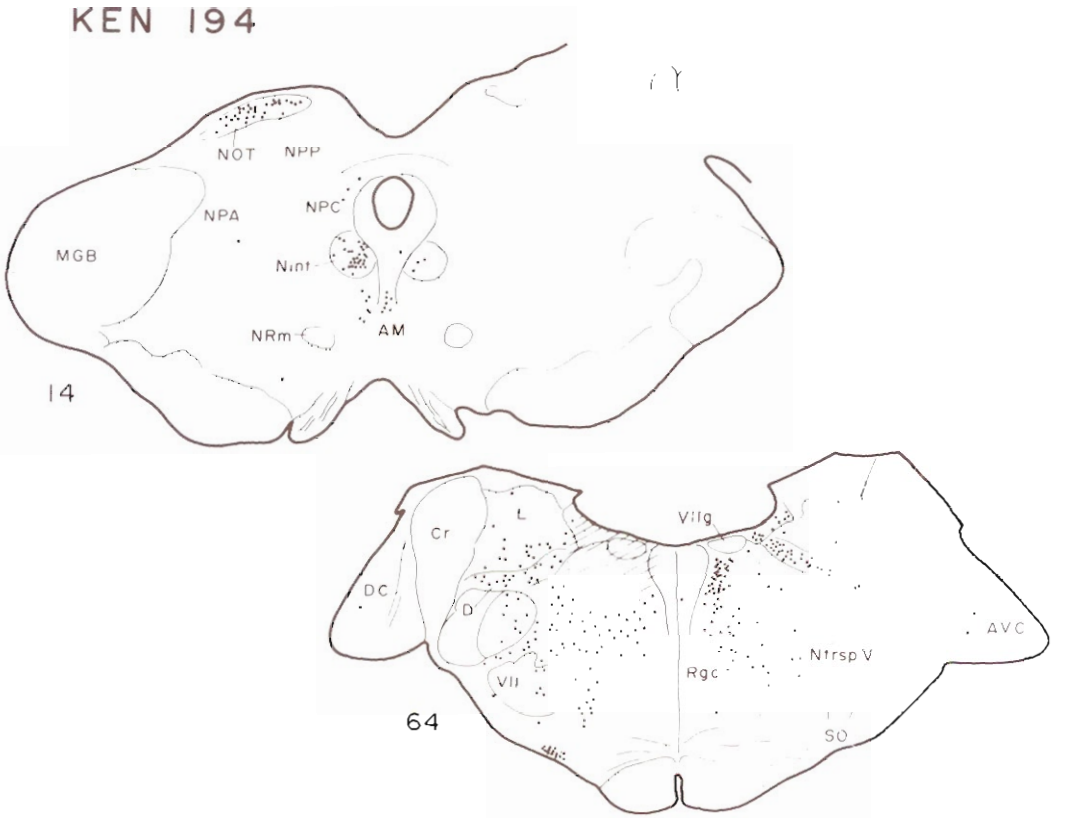

Fig. 8. – *Diagram to show distribution of labelled cells in a mesencephalic (drawing 14) and a pontine (drawing 64) levels of cat KEN 194 where the dorsal part of the Rgc was stained with injected HRP.*

Principles of presentation as in Fig. 6.

It can be suggested from the present morphological data that projections to the caudal part of the FN which propagate visual information may consist of two main routes. One is from the midvermal part of the cerebellar cortex, and the other is from the VN. The main pathways that reach the midvermal cortex were found to be from the SC via the PN and the inferior olive (see above). Concerning the projection to the VN, Cazin *et al.* (7) have indicated physiologically possible visual pathways involving the pretectum, the NRTP, and the contralateral VN. Based upon the present data as well as other studies, we may propose another possible route to the caudal FN: the NOT-RF-VN-FN. Tectoreticular fibers which terminate in widespread areas in the pontine and medullary RF (25, 26) may also contribute to fastigial projections involving the VN. Minor components of the visual or visuomotor inputs may also come indirectly from the EW by way of the VN, as well as directly to the FN (38, 41).

The afferent systems mentioned above provide some indication of the visual

sources leading to three types of the cells in the dorsocaudal FN. In the type-1 cells described in the present study, a sequence of suppression and facilitation to photic and electrical stimulation of the OCh was found to be the inverse of that of vermal Purkinje cell responses in terms of latency and duration (compare our Fig. 1A with Fig. 3C of ref. 12). Since FN cells are known to receive corticonuclear fibers of a monosynaptic inhibitory nature (13, 22) originating from the vermis (10), early suppression and late facilitation of type-1 cells may be caused by visually evoked discharges of Purkinje cells which receive visual signals mainly through the tecto-ponto-cerebellar cortex pathways (27, 30, 31).

The characteristic response pattern of type-2 cells, on the other hand, was identical to the temporal sequence of simple spike responses of vermal Purkinje cells (Fig. 3C of ref. 12), leading us to suggest that type-2 cells in the dorsocaudal FN and the vermal Purkinje cells receive visual inputs from common sources in the brainstem. As shown from the present HRP experiments (*KEN 182* and *KEN 206*), the M and the D vestibular nuclei are good candidates to convey visual information to both the FN and the vermis. Our unpublished observations revealed that the M vestibular cells show a response pattern similar to type-2 cells in the FN after electrical stimulation of the OCh, and that they are also activated antidromically by microstimulation (12-20 μA) of the ipsilateral caudal FN. These data highly suggest that type-2 cells in the dorsocaudal FN are influenced by visual inputs from the VN through the NOT-Rgc-VN-FN route. Other pathways providing the VN with visual signals may also be present (cf. Fig. 7).

Possible sources to induce the response of type-3 cells were largely unknown. It seems, however, that their activity is strongly inhibited by vermal Purkinje cell activity because they showed vigorous and long lasting suppression to photic and electrical stimulation of the OCh.

Our study has also presented evidence that dorsocaudal FN cells exhibit clear modulation of activity which reflects the velocity component of a moving pattern (Fig. 3). This type of modulation was found to be selective to the direction of movement (Figs. 2. and 3). Combined together with the stimulation experiment of the OCh (Fig. 2), it was revealed that all cells responding in this manner belonged to either type-2 or type-3 cells, and that none of them showed the features of type-1 cells, although the total number of cells recorded was small. It has been reported that cells in structures that mediate the passage of visual signals to the FN show modulation of their activity in association with the velocity component of a moving visual field during optokinetic nystagmus, or of a visual target during smooth pursuit eye movement. Thus, the NOT cells which are in the first station of the optokinetic system changed in their activity with the phase of velocity component of a sinusoidally moving visual field in a preferred direction (8, 9, 20). Furthemore, there is evidence that some cells in the VN, receiving inputs from the NOT as shown in the present study, exhibit a clear modulation in relation to the velocity component of a moving field rather than eye movement velocity during optokinetic nystagmus (4). These data suggest that the NOT-RF-VN route conveys the velocity signal of a moving visual field stimulus necessary for the

generation of optokinetic nystagmus. Since it can be assumed from the present study that type-2 cells, but not type-1 cells, are likely to be activated by visual impulses via this route, the modulation that we observed in type-2 cells seems to be transmitted through this route and to be optokinetic in nature.

On the other hand, it is obvious that Purkinje cells in lobules VI and VII of the alert monkey, which project to the dorsocaudal FN, receive signals of target velocity from the brainstem during smooth pursuit eye movement rather than signals of visual field motion (24, 43). Type-3 cells, which are influenced strongly via vermal Purkinje cells, showed a clear modulation with the phase of velocity component of a sinusoidally moving pattern (Fig. 3). It is likely that their modulation may be controlled by Purkinje cell activity reflecting target velocity during smooth pursuit eye movement. Type-1 cells, which did not respond to a moving visual pattern, are also suggested from the present stimulation experiment to receive inputs from vermal Purkinje cells. Therefore, type-1 cells are likely to be related to visually guided movements other than slow eye movements, such as visually guided saccades, because the majority of Purkinje cells of lobules VI and VII show modulation of their activity in temporal relation to saccades (23), and also because activity in some FN cells likewise changes in association with saccades (15). The present results suggest that several signals concerning visually-driven movements converge on the dorsocaudal FN, where there are cells functionally differentiated in the control of eye movements.

SUMMARY

Visual afferents to the cat fastigial nucleus (FN) have been studied with single unit recordings and horseradish peroxidase techniques. A total of 158 cells responding to electrical stimulation of the optic chiasm (OCh) were extracellularly recorded from the dorsocaudal part of the FN. They were classified into three groups: type-1 cells (48%) which showed early suppression and late facilitation; type-2 cells (38%) which showed early facilitation and late suppression; type-3 cells (14%) which exhibited long lasting suppression with no signs of facilitation. Eight of 32 cells tested showed the same response patterns to photic stimulation as to electrical stimulation of the OCh. None of the cells responding to electrical and photic stimulation, however, were found in the rostral and ventral parts of the FN. Furthermore, 29 cells which responded to electrical stimulation of the OCh were tested for responses to moving pattern stimulation. Seven (4 type-2 cells and 3 type-3 cells) of the 29 cells showed clear modulation, reflecting the velocity component of a horizontally moving pattern. However, none of 13 type-1 cells tested exhibited apparent modulation in relation to movement of the pattern. In order to trace the possible pathways mediating visual signals to this part of the FN, the horseradish peroxidase (HRP) method was used. Injection of HRP into the caudal FN resulted in the labelling of many cells, predominantly in the medial (M) and the descending (D) vestibular nuclei and in lobules VI and VII of the

cerebellar vermis. A series of experiments further indicated the presence of possible pathways propagating visual signals to the caudal FN. The main routes are: 1) via the nucleus of the optic tract (NOT) — the dorsal part of the medullary reticular formation — the M and the D vestibular nuclei — to the FN, and 2) via the superior colliculus — the pontine nuclei — vermal lobules VI and VII — to the FN. The different physiological response patterns of FN cells may indicate that several types of visual signals involved with visually guided movements impinge upon the dorsocaudal FN.

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