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Characterization of a directional selective inhibitory input from the medial terminal nucleus to the pretectal nuclear complex in the rat

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Abstract

The receptive field properties of neurons in the medial terminal nucleus of the accessory optic system (MTN) that project to the ipsilateral nucleus of the optic tract (NOT) and dorsal terminal nucleus (DTN), as identified by antidromic electrical activation, were analysed in the anaesthetized rat. The great majority (88%) of MTN neurons that were antidromically activated from NOT and DTN preferred downward directed movement of large visual stimuli while the remaining cells preferred upward directed stimulus movement. Distinct retrograde tracer injections into the NOT/DTN and the ipsilateral inferior olive (IO) revealed that no MTN neurons project to both targets. MTN neurons projecting to the ipsilateral NOT/DTN were predominantly found in the ventral part of the MTN, whereas those projecting to the IO were found in the dorsal part of the MTN. *In situ* hybridization for glutamic acid decarboxylase (GAD) mRNA was used as a marker for GABAergic neurons. Up to 98% of MTN neurons retrogradely labelled from the ipsilateral NOT/DTN also expressed GAD mRNA. Earlier studies have shown that MTN neurons that prefer upward directed stimulus movements are segregated from MTN neurons that prefer downward directed stimulus movements. It also has been demonstrated that directionally selective neurons in the NOT/DTN prefer horizontal stimulus movements and receive an inhibitory input from ipsilateral MTN. Our results indicate that this input is mediated by GABAergic cells in the ventral part of MTN, which to a large extent prefer downward directed stimulus movements, and that the great majority of MTN neurons that prefer upward directed stimulus movements project to other targets one of which possibly is the IO.

Introduction

In order to allow an elaborate analysis of visual information a stable retinal image is required. Stabilization of the retinal image is achieved by a number of oculomotor reflex mechanisms for which different subcortical visual nuclei serve as neuronal control centres. Among these oculomotor reflexes in mammals, the optokinetic nystagmus (OKN) is of particular interest. The adequate visual stimulus to elicit OKN is the occurrence of a low speed shift of the retinal image, i.e. the retinal image slip (Collewijn, 1969; Cohen *et al.*, 1977; Evinger & Fuchs, 1978), and the basic anatomical and physiological properties of the underlying neuronal control circuits are very similar across different mammalian species (Simpson, 1984; Simpson *et al.*, 1988b).

One of the most striking basic features of OKN control in all mammals is that neurons sensitive for different directions of retinal slip are found in topographically separate nuclei. However, all nuclei that take part in the control of slow compensatory eye movements are strongly coupled and most of their interconnections are reciprocal (reviewed by Simpson *et al.*, 1988b). Thus, neurons that specifically respond to low speed vertical displacements of whole field visual stimuli are found in the medial and lateral terminal nuclei (MTN and LTN, respectively) of the accessory optic system (AOS). According to their preferred directions, two neuronal populations can be distinguished, one that prefers upward and another one that prefers downward directed motion (rabbit: Walley, 1967; Soodak & Simpson, 1988; rat: Natal & Britto, 1987; cat: Grasse & Cynader, 1982). At least in MTN, the two populations seem to be anatomically separated because neurons in the dorsomedial portion predominantly prefer upward moving visual stimuli while most neurons in ventrolateral MTN prefer downward movements (Van der Togt *et al.*, 1993). In contrast to MTN and LTN, neurons that specifically respond to low speed horizontal stimulus displacements are found in the pretectal nucleus of the optic tract (NOT) and the adjacent dorsal terminal nucleus (DTN) of the AOS (rabbit: Collewijn, 1975; rat: Cazin *et al.*, 1980; cat: Hoffmann & Schoppmann, 1981; ferret: Klauer *et al.*, 1990; monkey: Hoffmann & Distler, 1989; Mustari & Fuchs, 1990). Despite their topographical segregation, however, all OKN-related pretectal and accessory optic nuclei share oculomotor-related efferent targets in the medulla, one of which is dorsal cap of Kooy of the inferior olive (IO).

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The anatomical and physiological organization of MTN, NOT/DTN, and the projection from MTN to NOT/DTN has been subject of several anatomical as well as physiological studies (Blanks *et al.*, 1982; Giolli *et al.*, 1984, 1985, 1988; Simpson *et al.*, 1988b; Clarke *et al.*, 1989; Van der Togt *et al.*, 1991; Van der Togt & van der Want, 1992). Anatomically, the MTN can be subdivided into a ventral (MTNv) and a dorsal (MTNd) portion (Hayhow *et al.*, 1960; Gregory & Giolli, 1985), that are strongly interconnected by intranuclear projections (Giolli *et al.*, 1968, 1992; Van der Togt *et al.*, 1993). Although MTN neurons that project to IO are predominantly found in MTNd (Van der Togt *et al.*, 1993), the functional significance of this segregation is not completely understood, apart from the presumed constitution of a reciprocal inhibitory connection between an upward directional selective neuronal population in MTNd and a downward directional selective population in MTNv.

Axon terminals of MTN to NOT/DTN neurons in rat were reported to contain GABA (Van der Togt *et al.*, 1991; Giolli *et al.*, 1992) and among their targets are those NOT/DTN neurons which project to IO (Van der Togt & van der Want, 1992). Both observations were also confirmed physiologically, by demonstrating an inhibitory influence of the MTN projection onto neurons in NOT/DTN that are sensitive to horizontal image movements (Van der Togt & Schmidt, 1994). This seems functionally appropriate if one assumes that input from MTN neurons selective for vertical image movements is used to shape the directional selectivity of NOT/DTN neurons that are selective for horizontal image movements. However, the intranuclear segregation of either upward or downward preferring neurons in MTN and the observation that more neurons from MTNv project to NOT/DTN than from MTNd (Blanks *et al.*, 1982), suggests a more complex organization of the MTN to NOT/DTN projection.

In order to elucidate further how the different pretectal and accessory optic nuclei are functionally connected we studied the projection from MTN to NOT/DTN in rat by combining single unit recording and electrical stimulation with retrograde tracing techniques. Also, to gain additional evidence for a GABAergic nature of the MTN projection to NOT/DTN we combined retrograde tracing with *in situ* hybridization for the GABA synthesizing enzyme GAD. It will be demonstrated that MTN neurons that constitute the NOT/DTN projection are physiologically and anatomically independent from those MTN cells that project to IO, and that they express GAD mRNA.

Materials and methods

Animals and anaesthesia

Recording experiments were conducted on five adult pigmented rats (Long Evans strain), and retrograde tracing experiments were done on another six animals, with body weights between 200 and 350 g. All experimental animals were taken from our own breeding colony. Care was taken to avoid any pain and discomfort for the animals due to experimental procedures which were all carried out according to governmental laws.

Anaesthesia and immobilization of the animals were induced with an intraperitoneal injection of urethane (1 g/kg body weight) and an intramuscular injection of ketamine dihydrochloride (Ketavet®; 30 mg/kg). Anaesthesia was maintained during the recording by subsequent urethane injections (250–500 mg/kg) as required. The body temperature was measured rectally and maintained at 38 °C by means of a heating pad.

Extracellular recording and electrical stimulation

The animals were fixed in a stereotaxic head holder and the skull overlying the cerebellum and the pretectum was trepanned. Double-barrelled glass micropipettes were used both for extracellular recordings in the MTN and electrical stimulation in the NOT/DTN. The pipettes were pulled on a standard vertical puller and the tips were broken to an outer diameter of 6–8 μm and filled with 2 M sodium acetate which resulted in an impedance of 1–2 MΩ.

The MTN and the NOT/DTN were approached as described earlier (Van der Togt & Schmidt, 1994). In brief, receptive field positions of neurons in the superior colliculus (SC) were used to guide consecutive penetrations towards the NOT/DTN until neurons that showed directional selectivity to horizontal movements of a whole-field stimulus were recorded. Such cells were found rostral and lateral to those SC neurons that had their receptive fields located frontal and inferior in the visual field. With the NOT/DTN electrode left in place, a second pipette tilted at 32° caudally was inserted at the rostral pole of the cerebellum 1.5 mm lateral to the mid-line. This electrode was lowered until MTN neurons were identified by their directional selectivity to low speed vertical stimulus movements at depths between 7 and 9 mm below the cerebellar surface. If necessary, additional penetrations spaced 0.2 mm were made until the MTN was identified.

Extracellular responses were conventionally amplified and filtered and processed on-line as peristimulus time histograms (PSTH), averaged over 16 stimulus repetitions, by a microcomputer. Spike times were digitally stored for off-line analysis.

Electrical stimuli applied to the NOT/DTN electrode were 60–100 μs bipolar, constant current pulses with amplitudes up to 0.5 mA. Electrically evoked spikes were regarded antidromic if, first, the latencies of 10 consecutive spikes differed by less than 0.1 ms, and, second and most critically, if collision appeared between spontaneous and electrically evoked spikes (Fig. 1). Antidromic spikes were usually elicited at stimulus amplitudes below 0.1 mA.

For visual stimulation, a pseudo-random square pattern (60 × 60° total size; 4 × 4° pixel size) was projected via a galvanometer mounted double mirror array on to an orthogonal screen in front of the animal. Stimulus movements in different directions and at various speeds were induced by galvanometer movements controlled by a function generator. In general, the pattern was either moved along a linear track, with one direction followed by the opposite direction, or along a circular path, which allowed all stimulus directions to be tested within a single stimulus cycle. In both cases, the orientation and the speed, usually in the range of 5–20°/s, of the stimulus remained constant.

Retrograde tracing

In animals used for retrograde tracing, one to three dye injections were made stereotaxically into the IO on one side according to the protocol described by Horn & Hoffmann (1987). For injections into the NOT/DTN, directionally selective neurons ipsilateral to the IO injection were first localized electrophysiologically as described above. Then, the recording electrode was replaced by a dye filled micropipette mounted to a microlitre syringe. Tracers injected into IO or NOT/DTN were 0.1–0.3 μL 2% fast blue (FB, Illing) or 2.5% fluorogold (FG, Fluorochrome Inc., Englewood, CO, USA) dissolved in a 2% dimethylsulphoxide aqueous solution. Following injections all surgical wounds were sutured and treated with antibiotics. Animals were allowed to completely recover from the anaesthesia before they were returned to their home cages where they survived for 4–6 days.

Histology

At the end of the experiments the animals were sacrificed with an overdose of sodium pentobarbital (Nembutal®), perfused transcardially with 0.9% NaCl followed by freshly prepared 4% phosphate-

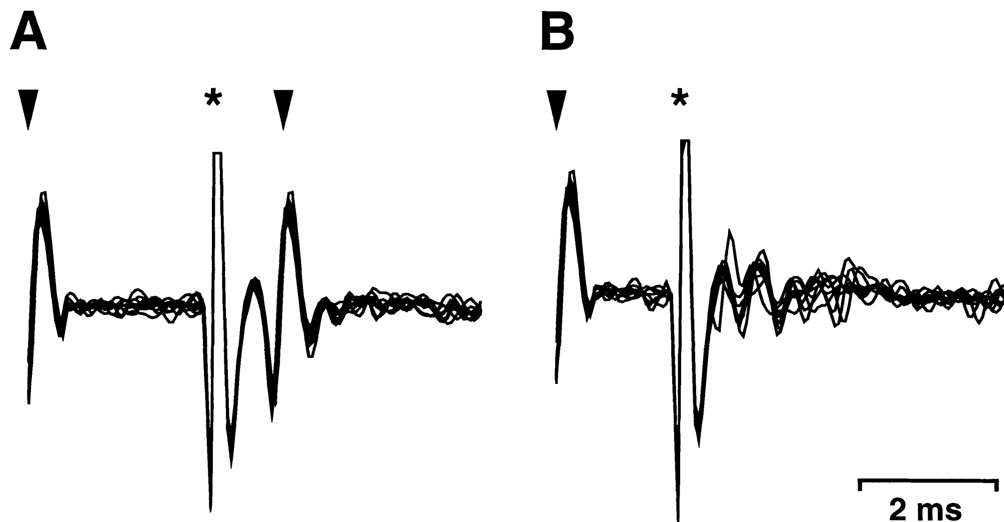

FIG. 1. Antidromic activation of a medial terminal nucleus neuron after ipsilateral nucleus of the optic tract/dorsal terminal nucleus of the accessory optic system stimulation demonstrated by collision of spontaneous and electrically evoked spikes. (A) The stimulus (star) was delayed 2.0 ms to the spontaneous spike (left arrowhead). The antidromic spike (right arrowhead) appeared with a latency of 1.0 ms. (B) The stimulus was delayed 1.0 ms to the spontaneous spike. Owing to collision no antidromic spike appeared. Each graph shows 10 consecutive stimulations. Scale bar = 1 ms.

buffered paraformaldehyde at pH 7.2. Two alternating series of 50 μm thick transverse vibratome sections that included the MTN and the NOT/DTN were collected in phosphate buffer. In the retrograde tracing experiments, one series was mounted on gelatinized slides, air dried and inspected for the tracer distribution. The second series was processed for immunocytochemistry. The distribution of retrograde label was plotted using a computer-aided microscope reconstruction system (NTS, Eutectics Inc., Raleigh, NC, USA). Brain sections obtained from animals used in physiology experiments were stained with cresyl violet and Luxol fast blue for the inspection of recording and stimulation electrode tracks.

In situ *hybridization*

Two animals receiving injections of latex beads into the NOT were processed for *in situ* hybridization to reveal the inhibitory phenotype of NOT-projecting MTN neurons. Briefly, animals were sacrificed and perfused as described above. The mid-brain was cryoprotected in sterile 25% sodium phosphate buffered sucrose and cut into coronal 25 μm thick sections with a cryostat. Hybridization was performed exactly as previously described (Wahle *et al.*, 1994; Reimann & Schmidt, 1996) using a Digoxigenin-UTP-labelled antisense riboprobe against GAD mRNA transcribed from a 2.3 kb GAD cDNA cloned downstream of a T7 promoter in pBS. Hybrid molecules were detected after stringent washes using alkaline phosphatase (AP)-conjugated sheep-anti-DIG F(ab) fragments (Boehringer Mannheim) diluted 1 : 2000 for 2 h, followed by an overnight development of the AP reaction product with nitroblue tetrazolium and X-phosphate. Double-labelled neurons contained the retrogradely transported latex beads and the blue amorphous AP reaction product in the somata.

Results

In total, 250 directionally selective single units were recorded in the MTN and tested for antidromic activation from the ipsilateral NOT/DTN. All recorded MTN neurons showed strong sustained responses to vertically moving whole-field stimuli with only little signs of adaptation to the stimulus (Fig. 2). According to the stimulus direction that elicited the stronger response, 136 of these cells were classified as upward-preferring (Fig. 2A) and 114 were characterized as downward-preferring (Fig. 2B). Responses to a stimulus moving on a circular path were additionally recorded from 46 cells. This allowed a cell's directional selectivity to be characterized more precisely because all possible stimulus directions are presented in a single stimulus sweep (Fig. 2C,D). Usually, the strongest responses to this stimulus were elicited by movements that were not perfectly vertical but had a horizontal component always pointing temporally. However, this horizontal component was always considerably smaller than the vertical component. Thus, whenever stimulus movement along a circular path was also used, the classification of an individual cell as being either upward preferring or downward preferring, as achieved with pure vertical stimulus movements, was confirmed (Fig. 2). Therefore, using pure vertical stimulus movements was considered sufficient to classify a cell as preferring upward or downward stimulus directions. Although different stimulus velocities were not tested systematically, all neurons gave strongest responses to stimulus speeds below 10°/s. Therefore, the usual stimulus speed used for characterization of a cell's directional selectivity was 5°/s.

In single electrode penetrations, upward preferring cells were usually recorded more dorsally and downward preferring cells were recorded more ventrally. Although this topographical segregation of upward and downward preferring cells was not entirely perfect, i.e. few downward preferring cells were recorded dorsal to an upward preferring cell, it seemed a general feature of MTN organization (Fig. 3; see also Van der Togt *et al.*, 1993).

Antidromic activation

With single electric shocks delivered to the ipsilateral NOT/DTN, 32 units could be antidromically activated in the MTN, as derived from the positive collision test (Fig. 1). Antidromic response latencies of these neurons varied between 1.0 and 2.6 ms (mean 1.4 ms ± 0.4; Fig. 4). Neurons antidromically activated were preferentially recorded ventrally within single electrode penetrations. Consequently, when tested with a vertically moving visual stimulus for their directional

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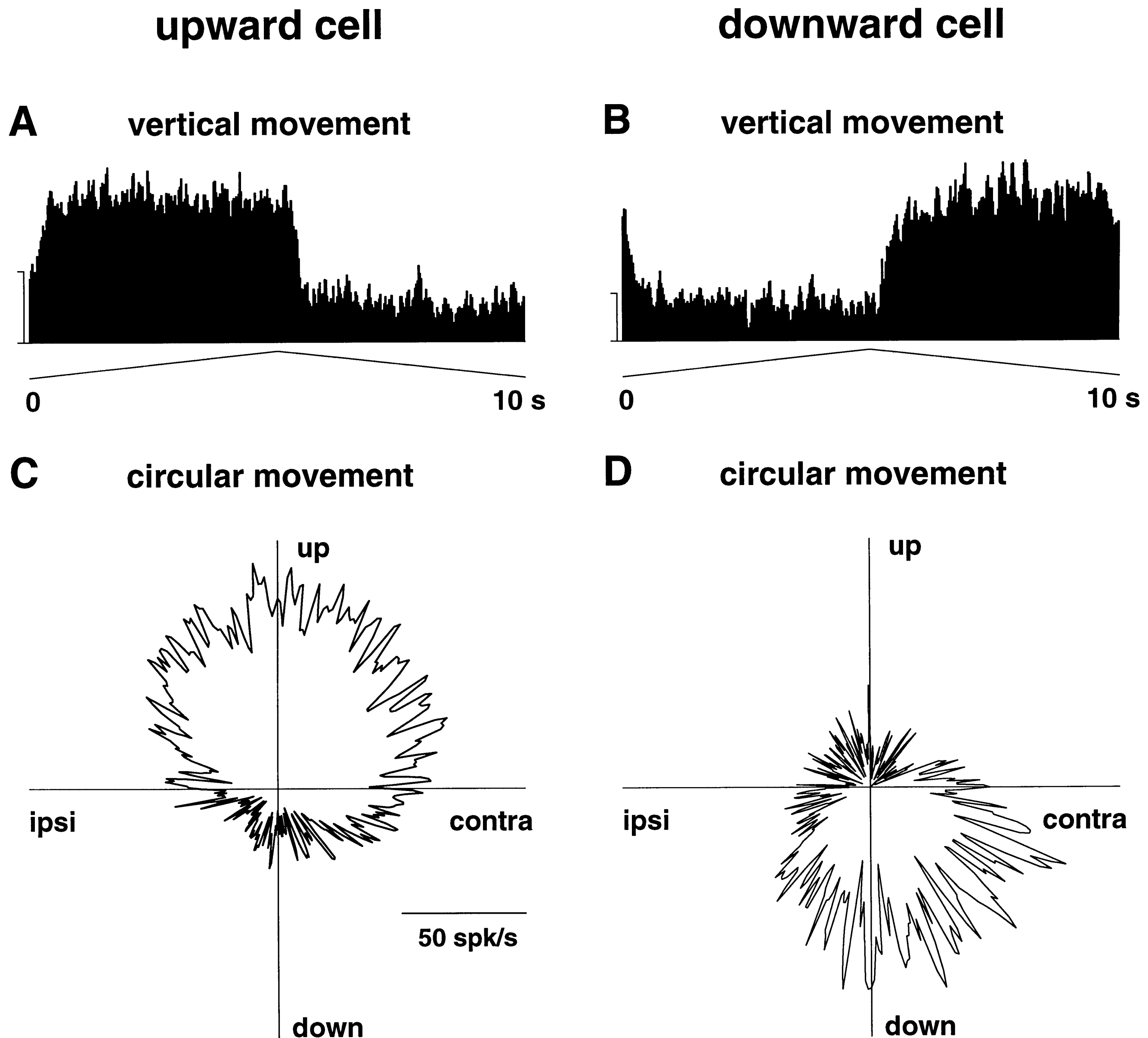


FIG. 2. Responses of two representative medial terminal nucleus of the accessory optic system neurons to a moving visual stimulus. (A,B) Peristimulus time histograms to vertical stimulus movements, at a constant speed of 5°/s. Stimulus position is indicated by the line below histograms, upward deflection represents upward movement. The scale bars to the left indicate 40 spikes/s, histogram binwidth, 25 ms, responses to six stimulus sweeps are averaged. In C and D the activity of the neurons when the stimulus was moved along a circular path is shown in a polar plot. Note that the polar plots are not corrected for the cells' response latencies. Stimulus movement started ipsiversively, e.g. towards the recorded side, and was continued in clockwise direction at a constant speed of 5°/s.

selectivity, 28 of the antidromic neurons (88%) were downward-preferring cells, and four (12%) were upward preferring. Neurons antidromically activated from NOT/DTN did not differ in their response characteristics from neurons not antidromically activated. Based on the strength of their responses, the modulation between preferred and non-preferred directions, and their velocity preference, the antidromic neurons did not form a functional subpopulation within the MTN neuronal population.

Distribution of retrogradely labelled neurons

In order to study the topographical distribution of neurons in MTN that project to the ipsilateral NOT/DTN, the retrograde tracer fluorogold was injected into the NOT/DTN, and labelled neurons were plotted from serial sections of the MTN. The NOT/DTN injection sites, that were selected for analysis, were centred in the caudal third of the pretectal nuclear complex and involved the NOT, the DTN, the dorsal posterior pretectal nucleus (PPN) and the most dorsal part of the anterior pretectal nucleus (APN). Some spread of the tracer into the most rostral and lateral part of the superior colliculus was also observed.

Within the anatomical borders of the MTN, numerous retrogradely labelled neurons were observed that formed a densely packed cell group close to the ventral border of the mesencephalon (Fig. 5). Labelled neurons were much more frequent in the ventral part of MTN and only few retrogradely labelled cells were found in the dorsal part. The most strongly labelled neurons had round to oval

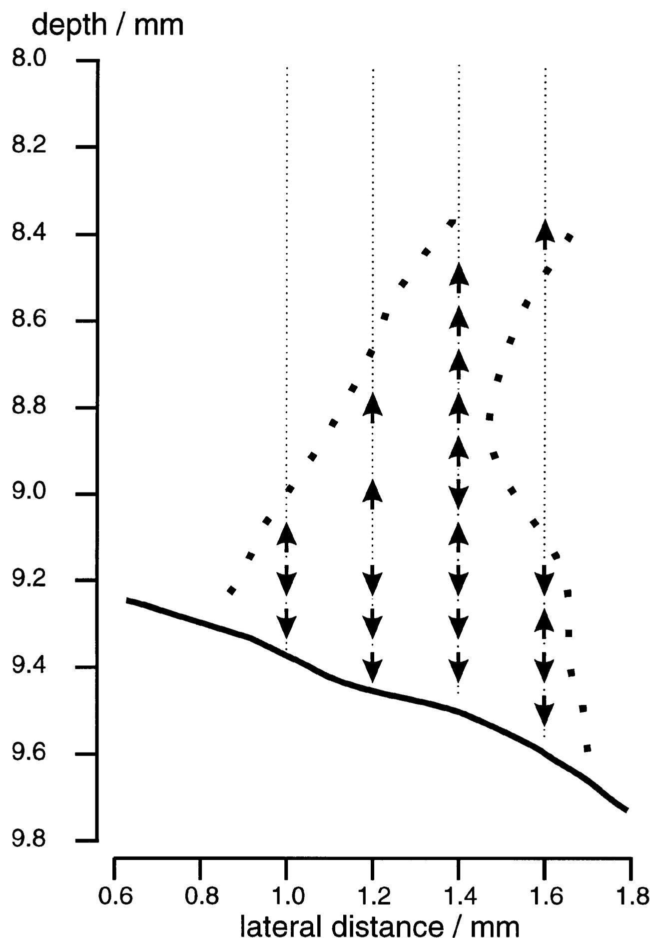


FIG. 3. Distribution of preferred directions of medial terminal nucleus (MTN) neurons reconstructed from four consecutive electrode penetrations (indicated by thin dotted lines) in a frontal view. Preferred directions of recorded neurons obtained with a vertically moving whole-field stimulus are indicated by upward or downward pointing arrows along each electrode track. The solid line indicates the ventral surface, thick dotted lines indicate the MTN nuclear borders. Abscissa, distance from the mid-line in millimetres; ordinate, depth below cerebellar surface in millimetres.

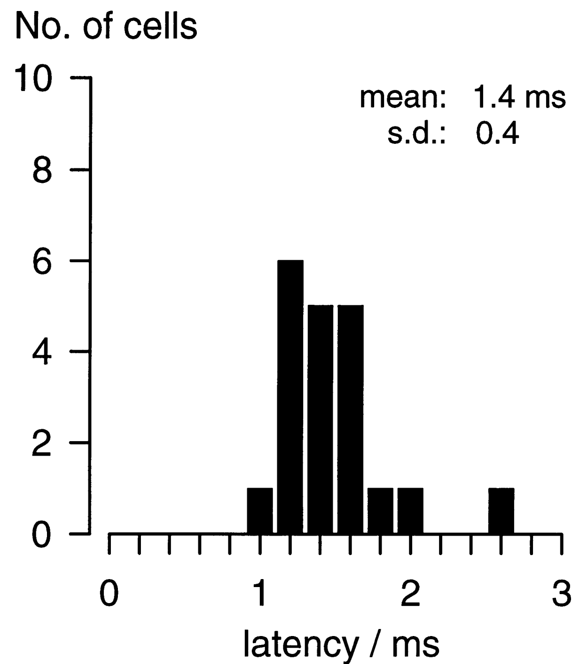


FIG. 4. Distribution of antidromic response latencies after ipsilateral nucleus of the optic tract/dorsal terminal nucleus stimulation. Abscissa, latency in milliseconds; ordinate, number of cells; mean, mean response latency; s.d., standard deviation.

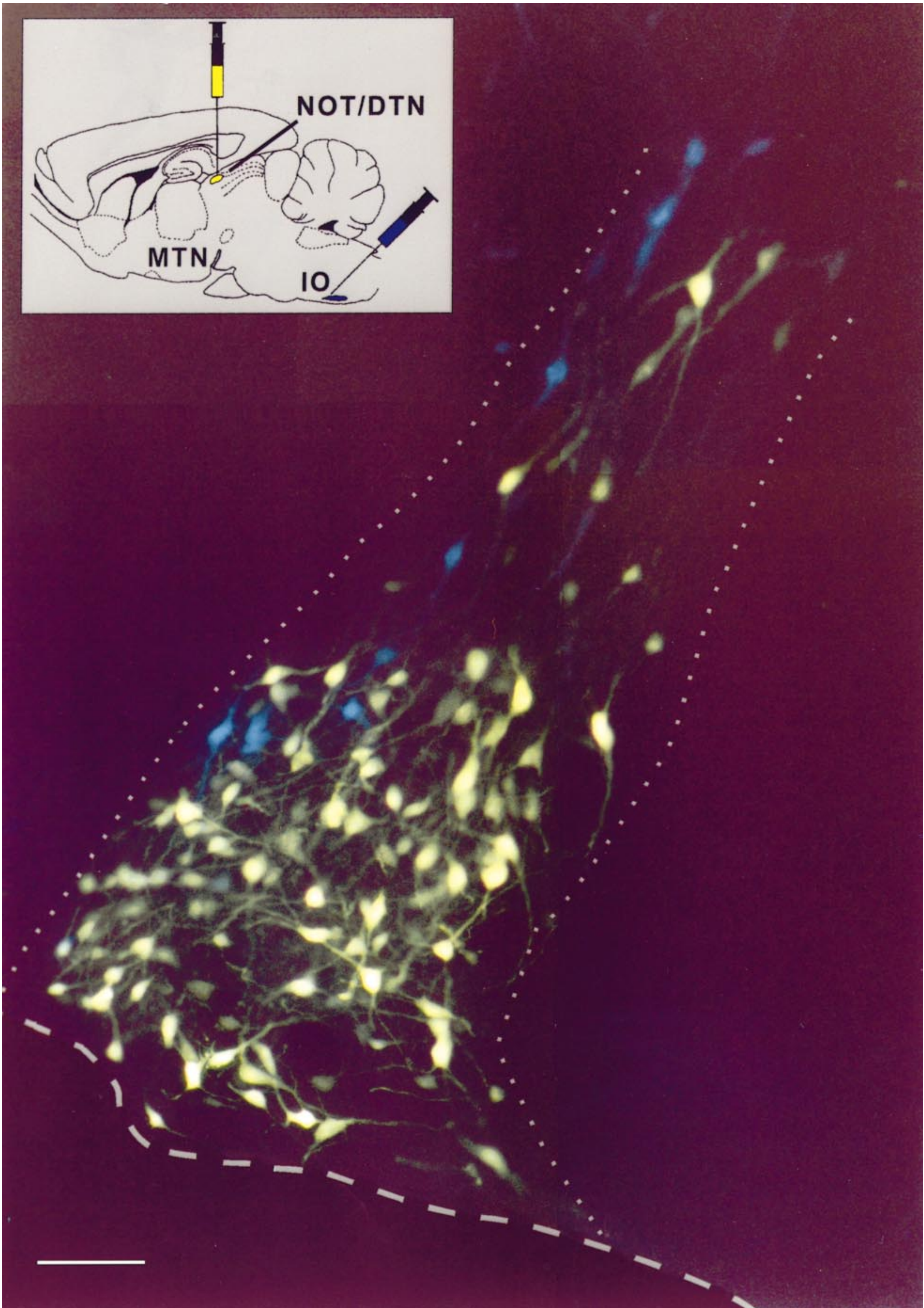
cell bodies. When the retrograde label, which rarely extended beyond the primary dendrites, allowed inspection of their dendritic tree, neurons located ventrally had multipolar to fusiform appearance and thus resembled the 'small-multipolar' cell type described by Gregory & Giolli (1985). The few labelled neurons in the dorsal MTN had an elongated dendritic tree that followed the long axis of the MTN outline, e.g. their dendritic trees were orientated from dorsolateral to ventromedial. Such cells have been termed 'linear-bipolar' or 'linear-multipolar' cells (Gregory & Giolli, 1985).

In order to confirm the results from the physiology, namely that the segregation of MTN cells that prefer either upward or downward stimulus movements is paralleled by a segregation of their efferent targets, double retrograde tracing was performed. For this, one retrograde tracer (usually FG) was injected into the ipsilateral NOT/DTN and a second tracer (usually FB) was injected into the IO which is a common efferent target for the NOT/DTN and the MTN. In the case of the NOT/DTN, injections were centred into the NOT proper with considerable involvement of the NPP beneath. In addition, some spread of the tracer was found into the most rostral and lateral aspect of the superior colliculus. Injections into IO were centred in the mediolateral extent of its dorsal part with considerable spread of the tracer into more dorsal, adjacent medullary structures. However, involvement of the contralateral IO was not observed. Because in both cases no other known efferent target of MTN neurons apart from NOT/DTN and IO was co-injected the label was considered specific.

In all cases neurons labelled from the NOT/DTN were almost completely segregated topographically from those labelled from the IO. Moreover, it was not possible to find any neurons labelled with both retrograde tracers (Fig. 5). NOT/DTN-projecting FG-labelled neurons were found ventral in the MTN, as described above after single retrograde tracing. On the other hand, IO-projecting FB-labelled neurons were predominantly found dorsal in the MTN and were mainly distributed close to the medial border of the nucleus (Fig. 6). Their dendritic architecture mostly resembled that of 'linear-bipolar' or 'linear-multipolar' cells and only the few ventrally located cells had a 'multipolar' appearance (Gregory & Giolli, 1985). Neurons labelled from NOT/DTN outnumbered those labelled from IO at least by a factor of five (e.g. 419 vs. 56 for the case shown in Fig. 6), although the tracer injections always entirely covered the target structures.

In situ *hybridization*

In the two animals that were used for *in situ* hybridization numerous MTN neurons were found that clearly had accumulated the GAD mRNA marker. The majority of these neurons was located ventral in the MTN and only a few scattered neurons were found more dorsally (Fig. 7A). Although the AP reaction product not always filled the


NOT/DTN
MTN
IO

entire somata but was concentrated close to the nucleus, most cells labelled for GAD mRNA had large somata. Because the *in situ* hybridization does not label primary dendrites no description of the dendritic morphology of the putative GABAergic neurons can be given.

The number of GAD mRNA expressing neurons clearly outnumbered those retrogradely labelled from NOT/DTN in both cases. Although the retrograde label by fluorescent beads was much less intense than the *in situ* hybridization reaction product it was possible to evaluate quantitatively the number of neurons that contained both the beads and the *in situ* label (see examples in Fig. 7B–E). Results from these experiments are given in Table 1. In both cases bead injections were strictly confined to the NOT/DTN. As shown in the table, the vast majority of NOT/DTN-projecting MTN cells (case 1, 85%, case 2, 98%) also expressed the mRNA for GAD.

Discussion

The results demonstrate that neurons in the rat MTN that project to the ipsilateral NOT and DTN form a physiologically distinct neuronal population. The great majority of these neurons is characterized by their directional selectivity to visual stimuli moving downwards in the visual field, and they are located predominantly in the ventral part of the MTN. Only about 10% of the neurons projecting to the NOT/DTN prefer upward directed stimulus movements; however, also these neurons are found ventrally. Within the MTN, the population of neurons that project to NOT/DTN is separated from neurons that project to the IO and that are located predominantly in the dorsal part of the MTN.

The interconnections between accessory optic nuclei and the pretectal nuclear complex have been the subject of a number of anatomical studies (reviewed by Simpson *et al.*, 1988b). In particular, the substantial projection from the MTN to NOT/DTN confirms earlier studies in the rat (Blanks *et al.*, 1982; Giolli *et al.*, 1984, 1992; Clarke *et al.*, 1989; van der Togt *et al.*, 1991; van der Togt & van der Want, 1992), and a similar projection is present in other mammals (rabbit, Giolli *et al.*, 1984; monkey, Mustari *et al.*, 1994).

A prominent anatomical feature of the MTN to NOT/DTN projection neurons is their topographical distribution within the MTN. As could be also confirmed in the present study, NOT/DTN projecting neurons in the MTNv have been reported to outnumber those in the MTNd (Blanks *et al.*, 1982; Giolli *et al.*, 1992). In addition to the different location, NOT/DTN projecting MTN neurons seem to differ from IO projecting MTN neurons in their dendritic morphology. NOT/DTN projecting MTN neurons can be mostly characterized by their round to fusiform soma shapes and multipolar dendritic trees while IO projecting MTN cells show spindle to fusiform shaped somata and elongated dendritic trees. It has to be emphasized, however, that the dendritic architecture of an individual neuron depended primarily on its location within the MTN, e.g. dorsal or ventral, than on its efferent target. Because the morphological features of neurons from the two cell populations show considerable overlap this difference is only qualitative and does not quantitatively delineate two distinct populations.

MTN neurons that project to NOT/DTN express at least two different markers for GABAergic neurons, namely immunoreactivity for GABA (Giolli *et al.*, 1992), and GAD mRNA in our *in situ* hybridization experiments. Furthermore, fibre terminals in NOT/DTN that stem from MTN cells all show strong GABA immunostaining (Van der Togt *et al.*, 1991). In addition to the anatomical evidences, an inhibitory influence of the MTN to NOT/DTN projection has also been demonstrated electrophysiologically, because electrical stimulation of the MTN results in a complete inhibition of directionally selective NOT/DTN cells (Van der Togt & Schmidt, 1994). Although after bead injections the total number of labelled cells was smaller than after the fluorogold injections (see Table 1), it seems very unlikely that the beads were selectively taken up or transported only by a subpopulation of MTN neurons that project to NOT/DTN. Furthermore, the fluorescent label was less intense after the *in situ* hybridization procedure so that the number of neurons double labelled with beads and the GAD mRNA marker may be underestimated. However, the great majority of retrogradely labelled cells in one case and nearly all labelled cells in the second case also expressed GAD mRNA. Taking into account the physiological and the anatomical evidences, we regard the projection from MTNv to NOT/DTN as being homogeneously GABAergic. Still, a considerable number of GAD mRNA expressing cells was not retrogradely labelled. These could, at least partly, be cells that project to NOT/DTN but that were not labelled by the bead injections or they could represent another GABAergic cell population in the MTN. Inhibitory connections between MTNv and MTNd have been described (Van der Togt *et al.*, 1993) and it remains to be determined whether this projection is formed by a neuronal population separate from cells that project to NOT/DTN.

The anatomical segregation in the rat MTN of neurons that project to the NOT/DTN from those that terminate in IO is paralleled by a clear physiological difference between the two populations. The response properties of MTN neurons are characterized by a strong directional selectivity for large vertically moving visual stimuli and individual neurons prefer either upward or downward directed stimulus movements (Walley, 1967; Grasse & Cynader, 1982; Natal & Britto, 1987; Soodak & Simpson, 1988). Directional selectivity, however, is spatially organized within rat MTN as to upward preferring cells being located dorsally and downward preferring cells being located ventrally (Van der Togt *et al.*, 1993). The spatial overlap of the distribution of downward selective neurons achieved in recording experiments (Van der Togt *et al.*, 1993) with the distribution of NOT/DTN projecting neurons in the tracing experiments is as complete as is the overlap of upward selective neurons with IO-projecting cells.

Functional considerations

Because the distribution of upward and downward preferring cells perfectly matches the distribution of IO and NOT/DTN projecting MTN neurons, respectively, our finding that NOT/DTN projecting MTN neurons identified by antidromic activation are almost exclusively downward preferring cells is not surprising. Instead, it corroborates the view that the MTN may be regarded functionally as a composite of two distinct nuclei, referred to in part of the literature

FIG. 5. Photomicrograph montage of retrogradely labelled neurons in medial terminal nucleus of the accessory optic system (outlined by dotted lines) after a fluorogold injection into nucleus of the optic tract/dorsal terminal nucleus (NOT/DTN) and a fast blue injection into the inferior olive (IO) (as indicated schematically in the top left inset). Note that, first, no neuron is double labelled with both tracers, and, second, that the two populations are topographically segregated into a ventrolateral, fluorogold-labelled, NOT/DTN-projecting, and a dorsomedial, fast blue-labelled, IO-projecting population. Medial, left and dorsal, up; stippled line, ventral border of section; scale bar = 100 µm.

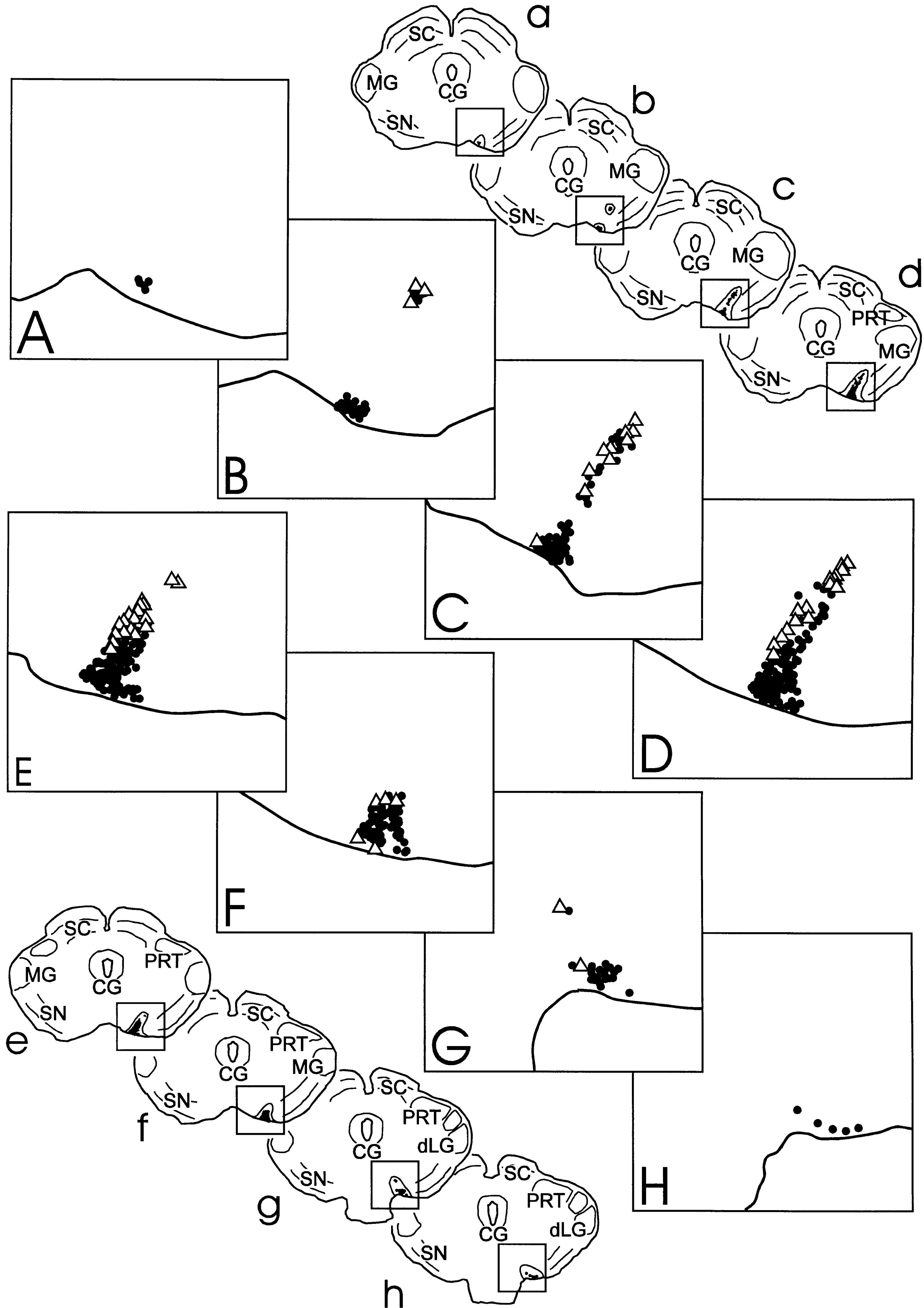


FIG. 6. Topographical distribution of neurons in medial terminal nucleus of the accessory optic system (MTN) retrogradely labelled after a fluorogold injection into ipsilateral nucleus of the optic tract/dorsal terminal nucleus (NOT/DTN) and a fast blue injection into ipsilateral inferior olive (IO). Line drawings were made from a series of frontal sections (a–h) through MTN arranged from most caudal (a) to most rostral (h). Insets show location of large-scale reconstructions (A–H) in which neurons retrogradely labelled from NOT/DTN are represented by black circles and neurons retrogradely labelled from IO are represented by open triangles. Box size = 2 × 2 mm.

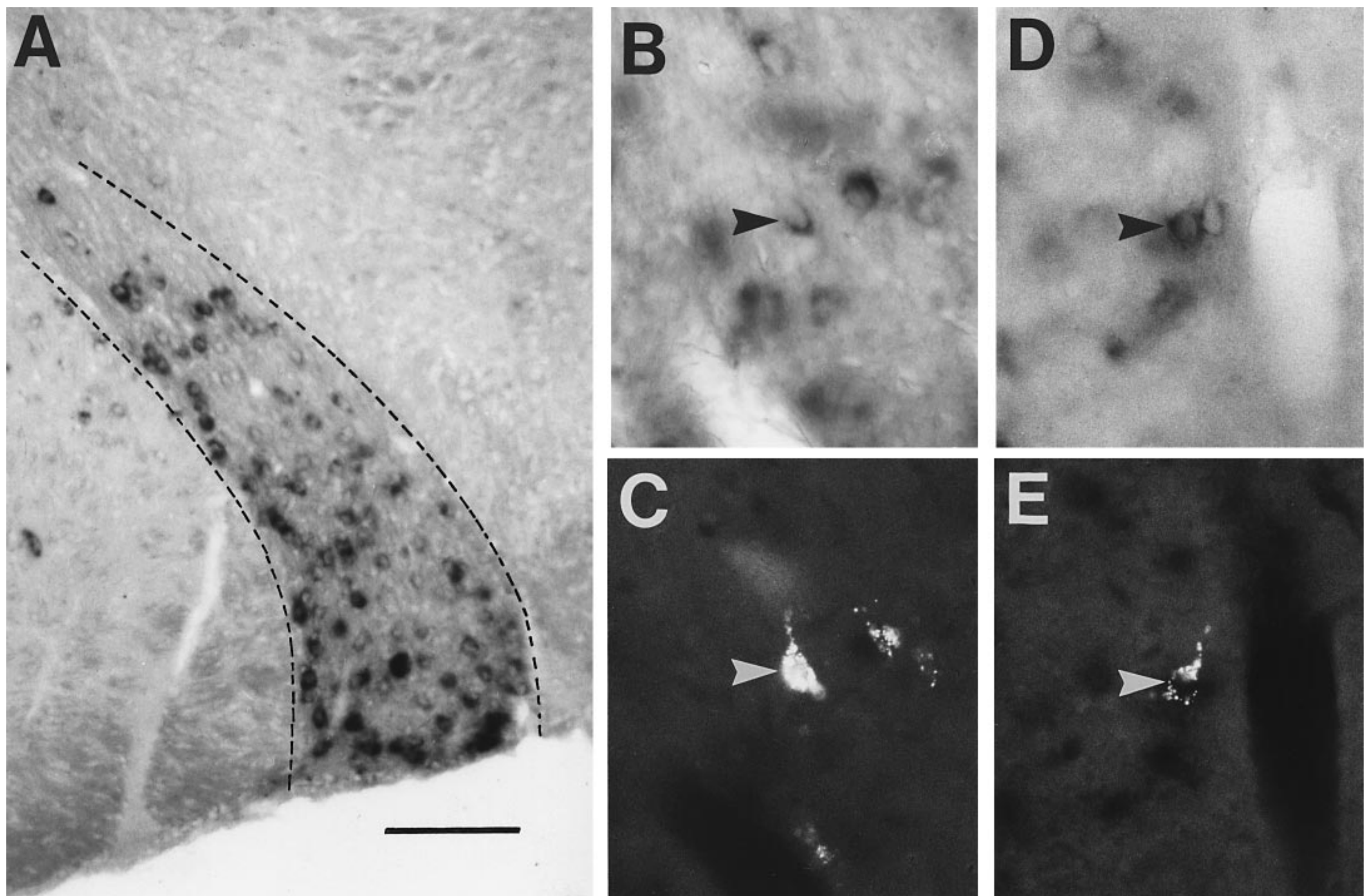


FIG. 7. Results from the *in situ* hybridization for glutamic acid decarboxylase (GAD) mRNA. (A) The distribution of GAD mRNA expressing neurons in medial terminal nucleus of the accessory optic system (MTN) is shown in a frontal section. (B–E) Two examples of MTN neurons that expressed GAD mRNA (B,C) and also were retrogradely labelled from nucleus of the optic tract/dorsal terminal nucleus with fluorescent beads (D,E). Medial, right; dorsal, up; scale bar represents 100 μm in A and 25 μm in B–E.

TABLE 1. Summary of the result from the double labelling experiments in which *in situ* hybridization for glutamic acid decarboxylase (GAD) mRNA in medial terminal nucleus of the accessory optic system (MTN) was combined with retrograde tracing from the nucleus of the optic tract/dorsal terminal nucleus. Numbers are given for cells labelled by the *in situ* hybridization (left column), by fluorescent beads (centre column), and for double-labelled cells (right column). Note that up to 98% of the MTN neurons retrogradely labelled from nucleus of the optic tract/dorsal terminal nucleus also expressed GAD mRNA

Case no.	GAD mRNA	Beads	GAD mRNA and beads
1	654	156	132
2	857	165	162

as MTNd and MTNv, that differ both in the response properties of their neurons and in their main projection targets. In functional terms, the activity of downward preferring MTNv cells seems to be used primarily, if not exclusively, to inhibit cells in NOT and DTN. In fact, directionally selective NOT/DTN neurons frequently show an upward component in their preferred direction up to 30° off the horizontal axis (Schmidt *et al.*, 1995). Although some of the antidromically activated neurons preferred upward movement their contribution to the MTN input to NOT/DTN is small as compared with the contribution of downward preferring neurons. Whether there are functional differences between neurons projecting to NOT/DTN that prefer downward movement and those that prefer upward movement is still an open question. One possible explanation is that they may contact different neuronal populations in NOT/DTN.

From our double retrograde labelling experiments it seems reasonable to conclude that the activity of upward preferring MTNd cells, in contrast to that of downward preferring MTNv cells, is transmitted to the dorsal cap of Kooy of the IO. However, most of the IO input that originates in MTN is relayed via the contralateral ventral tegmental relay zone (VTRZ) in rat and rabbit (Giolli *et al.*, 1985) so that MTN neurons retrogradely labelled from IO may not represent the principal MTN to IO input. To our knowledge, response properties of IO neurons in the dorsal cap have not been studied in the rat, so that data from other mammalian species have to be considered. Is has been shown in rabbits that dorsal cap neurons can be grouped into three classes according to their responses to large-field stimuli rotating about one of three particular axes (Leonard *et al.*, 1988). One cell group responds best to rotations about the vertical axis in the direction that causes ipsiversive horizontal stimulus movement. This response property represents the excitatory input from directional selective NOT/DTN neurons. The other two cell groups in IO prefer rotations about axes orientated horizontally at either 45° ipsilateral

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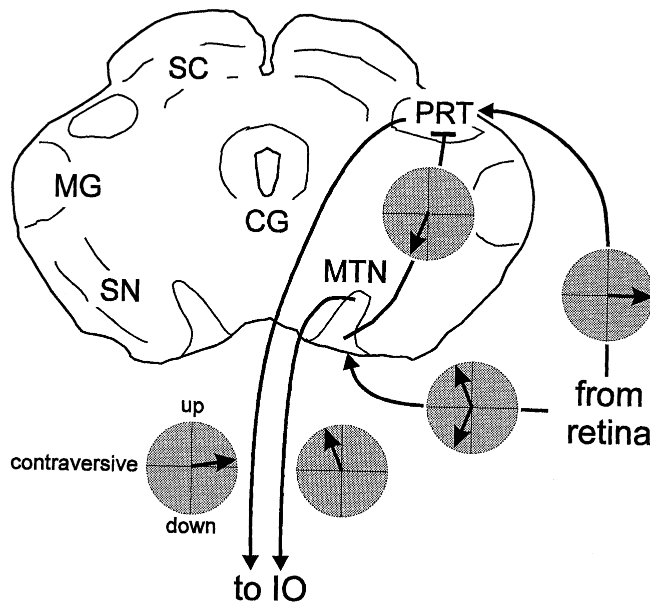

FIG. 8. Summary of directional selectivity (indicated by the arrows in the insets) within the connections studied. Directionally selective retinal ganglion cells that project to medial terminal nucleus of the accessory optic system (MTN) prefer stimulus directions either upward and contraversive or downward and contraversive, those that project to nucleus of the optic tract/dorsal terminal nucleus (NOT/DTN) prefer stimuli moving in ipsiversive direction. While directionally selective NOT/DTN neurons project to the inferior olive (IO), only upward directionally selective MTN neurons contribute to the IO projection. Downward preferring MTN neurons form an inhibitory projection to the NOT/DTN.

or 135° contralateral azimuth, termed anterior and posterior axis class cells, respectively. Their preference of rotational stimuli follows from opposite preferred directions in frontal and temporal subportions of their receptive fields. Because cells of the anterior axis class are dominated by the eye ipsilateral to the IO and cells of the posterior axis class are dominated by the contralateral eye, the preferred rotational directions for both cell groups cause upward stimulus movement in the frontal part of the visual field and downward stimulus movement in the posterior part of the visual field (Leonard *et al.*, 1988). Similar 'bipartite' receptive fields have been described in rabbit for neurons in the VTRZ and, although less frequent, in MTN (Simpson *et al.*, 1988a; Soodak & Simpson, 1988). Because we only stimulated the frontal visual fields of our rats we cannot comment on the presence of such 'bipartite' receptive fields in rat MTN. However, the IO cell responses described for the rabbit would represent input from AOS cells that prefer upward movement in our stimulation condition. In contrast to the dorsal cap, however, in the rabbit MTN cells can be found with preferred rotational directions that cause downward movement in the anterior part of their receptive fields and upward movement in the posterior part (Simpson *et al.*, 1988a). This group thus corresponds to downward preferring cells under our stimulus conditions.

Even though MTN input to IO is predominantly relayed via the contralateral VTRZ in rat and rabbit (Giolli *et al.*, 1985) is seems that information from AOS neurons that prefer downward movements is not transmitted to IO (Simpson *et al.*, 1988a; Leonard *et al.*, 1988). As IO inputs are used for OKN modulation one should expect that downward movements of a visual stimulus presented in the frontal visual field lead to weaker optokinetic responses than upward movements. In fact, monocular OKN gain in rabbit is highest to clockwise rotation around the 45° axis and to counterclockwise stimulus rotations around the 135° axis, which both cause upward stimulus movements in the frontal visual field (Tan *et al.*, 1993). In frontal eyed mammals, OKN performance shows an asymmetry in vertical directions resulting to higher gains for upward as compared with downward directed OKN (cat, Grasse & Cynader, 1988; monkey, Matsuo & Cohen, 1984; humans, Murasugi & Howard, 1989). Assuming that MTN to NOT/DTN projections are similar in all mammals this observation could well be explained by the fact that activity from downward preferring MTN neurons is not directly available for OKN generation and modulation. In fact, the information about downward retinal image slip not only remains disregarded for OKN generation but is even utilized to reduce OKN driving neuronal activity.

While the response properties of the neuronal elements that control OKN generation can explain the asymmetry in OKN performance, its functional significance remains unclear. Possibly, a strong optokinetic response to downward retinal slip is unwanted because during forward movement on a structured surface the optic flow of the retinal image is directed nasotemporal and downward. This would induce a downward directed OKN in a situation when the eyes should remain stationary. However, the downward component dominates optic flow during forward movements only in frontal eyed mammals. In lateral eyed mammals, the optic flow is dominated by a horizontal component that points temporally and that does not induce OKN because NOT/DTN neurons have opposite preferred directions. Still, vertical components are present in the optic flow field and because the flow is best described by a counterclockwise rotation about the optic axis of the eye it points downward in the anterior part of the visual field and upward in its posterior part. This matches the stimulus movement described above to which cells respond in rabbit MTN but not IO (Simpson *et al.*, 1988a; Leonard *et al.*, 1988).

The results of the present study can be summarized as follows (Fig. 8). Retinal information about downward directed retinal slip is transferred via directionally selective neurons located mainly in the ventral part of MTN to NOT/DTN where it is used to inactivate directionally selective neurons and, possibly, other NOT/DTN cell populations. Information about upward directed retinal slip, carried by directionally selective neurons in the dorsal part of MTN, is transferred to the IO.

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Abbreviations

AOS	accessory optic system
CG	central grey
dLG	dorsal lateral geniculate nucleus
DTN	dorsal terminal nucleus of the accessory optic system
FB	fast blue
FG	fluorogold
GAD	glutamic acid decarboxylase
IO	inferior olive
LTN	lateral terminal nucleus of the accessory optic system
MG	medial geniculate body
MTN	medial terminal nucleus of the accessory optic system
NOT	nucleus of the optic tract
NPH	nucleus prepositus hypoglossi

OKN optokinetic nystagmus
PRT pretectal nuclear complex
SC superior colliculus
SN substantia nigra

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