Cortical Input to the Nucleus of the Optic Tract and Dorsal Terminal Nucleus (NOT-DTN) in Macaques: a Retrograde Tracing Study

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Using retrograde tracing methods, we investigated the cortical projection to the nucleus of the optic tract and dorsal terminal nucleus of the accessory optic system (NOT-DTN) in macaque monkeys. Tracer injections at electrophysiologically identified recording sites in the NOT-DTN resulted in retrogradely labelled neurons in layer V of various cortical areas. The strongest projection always arose from the middle temporal area (MT) and the adjoining cortex anterior to MT in the superior temporal sulcus. A less dense projection came from the middle superior temporal area (MST). In addition, retrogradely labelled cells were consistently found in areas V1 and V2 at moderate to high density. Furthermore, sparse to moderate labelling occurred in prestriate area V3. These findings were compared with the label resulting from control injections into the superior colliculus in two additional cases. Our results indicate that the cortical input to the NOT-DTN as the sensorimotor interface for the pathway subserving stabilizing eye movements during the optokinetic reflex and smooth pursuit mainly arises from the motion-sensitive areas MT and MST in the superior temporal sulcus, as well as from areas V1 and V2. Clearly the projection to the NOT-DTN does not arise from a single cortical area.

Introduction

Visual cortical areas have to connect to subcortical structures to exert their influence on oculomotor behaviour such as smooth pursuit and optokinetic nystagmus. In normal frontal-eyed mammals such as the cat, monkey and man, horizontal optokinetic nystagmus (hOKN) is symmetrical, i.e. monocular visual stimulation in the temporonasal and nasotemporal directions elicits comparable optokinetic responses. Unilateral cortical lesions lead to characteristic directional deficits, i.e. monocular hOKN elicited via either eye becomes deficient during stimulation towards the lesioned side, especially at higher stimulus velocities (Zee *et al*., 1987; Dürsteler and Wurtz, 1988; Heide *et al*., 1996).

The subcortical neuronal substrate of the optokinetic reflex has been investigated in many mammals [for subprimate literature see Distler and Hoffmann (Distler and Hoffmann, 1993); for the monkey see Hoffmann *et al*. and Mustari and Fuchs (Hoffmann *et al*., 1988; Mustari and Fuchs, 1990)]. In all of these animals, the pretectal nucleus of the optic tract and the dorsal terminal nucleus of the accessory optic tract (NOT-DTN), with its strongly direction-selective neurons, links the visual information from the retina and visual cortex, via projections to the inferior olive, the nucleus praepositus hypoglossi, the nucleus reticularis tegmenti pontis and the dorsolateral pontine nucleus, with the cerebellum and the oculomotor structures [for a review of the anatomy see Simpson *et al*. (Simpson *et al*., 1988)]. In most mammals, the direct retinal input to the NOT-DTN comes almost exclusively from the contralateral eye, whereas in the monkey the retinal input is strongly bilateral (Kourouyan and Horton, 1997; Telkes *et al*., 2000).

Using orthodromic and antidromic electrical stimulation, we recently identified cortical neurons in the superior temporal sulcus (STS) mediating binocular information to the NOT-DTN of macaques (Hoffmann *et al*., 1991, 1992; Ilg and Hoffmann, 1993). However, with this approach it is not feasible to screen the entire cortical hemisphere for putative projection sites. Thus, in the present study we used tracer injections at electrophysiologically identified sites in the NOT-DTN and searched for retrogradely labelled neurons in the whole ipsilateral cortical hemisphere. To judge the specificity of the cortical label we also performed control injections into the superior colliculus (SC). Our data show that the motion-sensitive areas in the superior temporal sulcus (STS) provide the main input to the NOT-DTN. In addition, fewer labelled cells were found in the primary visual cortex (V1) and other extrastriate areas. These findings confirm and extend our earlier electrophysiological results.

Materials and Methods

Subjects

All experiments had been approved by the local ethics committee, and were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86 609 EEC) and NIH guidelines for care and use of animals for experimental procedures. In the present investigation five adult macaques of both sexes – three *Macaca mulatta* and two *M. fascicularis* – were used. The three *M. mulatta* had previously been used for chronic recording in the awake state in unrelated investigations. At the termination of these experiments they received unilateral tracer injections into the NOT-DTN and/or the SC. In one of the *M. fascicularis* monkeys tracer deposits were placed in both NOT-DTNs at the beginning of an acute electrophysiological experiment. The retinae of some of the animals were used for the analysis of the retinal input to the NOT-DTN in monkeys (Telkes *et al*., 2000).

Surgery and Injections

The animals were initially anaesthetized with ketamine hydrochloride (10 mg/kg i.m.). An i.v. catheter was put in place, the animals were intubated through the mouth and, after additional local anaesthesia with bupivacainhydrochloride 0.5% (Bupivacain^{R}) or prilocainhydrochloride 0.5% (Xylonest^{R}), placed into a stereotaxic apparatus. During surgery they received additional doses of pentobarbital as needed. During the whole session the animals were artificially ventilated with nitrous oxide:oxygen at a 3:1 ratio containing 0.3–1% halothane. Heart rate, SPO_2, blood pressure, body temperature and endtidal CO_2 were monitored and kept at physiological levels. A craniotomy was performed according to stereotaxic coordinates to allow access to the midbrain and pretectum.

The NOT-DTN was then localized electrophysiologically according to its position just anterior and lateral to the foveal representation in the SC, and by its characteristic preference for ipsiversive stimulus movement (Hoffmann *et al*., 1988). When a putative injection site in the NOT-DTN or the SC was localized, the recording electrode was replaced by a glass pipette containing a recording wire. The pipette was connected via a short tube to a Hamilton syringe. Before the injection, the previously recorded response properties were verified. Then, 2% Fluorogold (FG) in 1% NaCl, 15% rhodamine dextran (RD) mol. wt 3000 in 0.1 M citrate NaOH, pH 3.0, 2–2.5% wheatgerm agglutinin coupled to horseradish

peroxidase (WGA-HRP)/10% HRP in 0.1 M phosphate buffer (PB), pH 7.4, or 2% Granular Blue (GB) in distilled water was slowly injected over a 30 min period (Table 1). After 20 min the pipette was withdrawn after aspiration to avoid leakage into the overlying tissue. Only in case 1 was limited local tracer uptake in the cerebral cortex observed. Then the wound was closed in appropriate layers and the animals were allowed to recover. They were treated with analgesics and antibiotics for at least 5 days after surgery.

Histology

After an appropriate survival time the animals were again anaesthetized with ketamine hydrochloride and sacrificed with an overdose of pentobarbital. They were then perfused through the heart with 0.9% NaCl followed by paraformaldehyde–lysine–periodate containing 4% paraformaldehyde. After postfixation overnight, the tissue was cryoprotected in 10% and 20% glycerol in 0.1 M PB, pH 7.4, shock-frozen in isopentane and stored at –70°C. For localization of the injection sites 50-µm-thick frozen sections of the midbrains were cut either coronally (three animals) or perpendicularly to the layers of the SC (two animals). At least two alternating series were cut, one for verification of the tracer deposit and one for Nissl stain.

The cortical hemispheres were cut at 50 µm thickness on a freezing microtome in the parasagittal (five cases) or the frontal plane (one case). Five alternate series were cut and used for visualization of retrogradely labelled cells, Nissl stain, Klüver–Barrera stain, for myeloarchitecture (Gallyas, 1979) [as modified by Hess and Merker (Hess and Merker, 1983)], and for SMI-32 immunohistochemistry (Hof and Morrison, 1995) and *Wisteria floribunda* agglutinin histochemistry (Brückner *et al*., 1994). Sections containing fluorescent tracers were mounted immediately after cutting from 0.45% saline, dried on a hot plate, defatted in xylene (2 × 1 min) and coverslipped with DEPEX. Horseradish peroxidase was visualized using tetramethylbenzidine as a chromogen [modified after van der Want *et al*. (van der Want *et al*., 1997)].

Data Analysis

The locations of retrogradely labelled neurons were charted onto enlarged drawings of Nissl-stained sections of the entire hemisphere spaced 1 mm apart. Two-dimensional reconstructions of the cortex were made by bending wires along layer IV of such sections at 2 mm intervals for each hemisphere. After marking landmarks as lip and fundus of sulcus on these wires, they were soldered together to form appropriate three-dimensional models. These models were then unfolded to form two-dimensional maps of the cortical hemisphere (Van Essen and Maunsell, 1980), with the anatomical data and myeloarchitectonic borders transferred onto them. The area-specific myeloarchitecture was used to distinguish extrastriate areas V2, V3, V4, V4t, middle temporal area (MT), the densely myelinated zone of the middle superior temporal area (MST), the visual area at the floor of the superior temporal sulcus (FST) and LIPv [literature summarized elsewhere (Distler *et al*., 1993)]. Note that even though the approximate location of area FST is given on the maps and the sections of Figures 1–10 in most cases no borders are indicated because in sagittal sections area FST cannot be distinguished based on myeloarchitecture. The areal borders were verified with the material stained for SMI-32 and Wisteria floribunda agglutinin (Brückner *et al*., 1994; Cusick *et al*., 1995; Hof and Morrison, 1995).

In addition, in four of the cases retrogradely labelled cells were counted in certain areas of the brain, i.e. V1 in the calcarine sulcus, and on the operculum, areas V2, V3, V4, MT and MST, and their density was expressed as no. of cells/mm in layer V. The cells were counted in sections spaced 1 mm apart. The densities were then averaged over all sections containing a particular area. These data were used to quantify the relative labelling density of these areas in an individual case.

Results

The location of the injections, the tracers used, and the survival times are summarized in Table 1. After NOT-DTN and SC injections retrogradely labelled projection neurons in most areas were exclusively found in cortical layer V.

Injections into the NOT-DTN

Cases 1 and 2l

Case 1 received an RD injection into the left NOT-DTN extending ~1.2 mm anterior–posteriorly. The centre of the injection site, together with the two-dimensional map of the ipsilateral STS, is shown in Figure 1. Representative sections through the left cortical hemisphere of this case are shown in Figure 2. Case 2 l received an injection of FG into the left NOT-DTN. The injection site and the two-dimensional map of

Table 1
Summary of injection sites

Animal	Species	Injection site	Tracer	Volume (µl)	Survival time (days)
Case 1	*M. mulatta*	left NOT-DTN	15% RD	0.7	10
Case 2	*M. fascicularis*	left + right NOT-DTN	2% FG	0.5	4
Case 3	*M. mulatta*	right NOT-DTN	2.5% WGA-HRP/ 10% HRP	0.25	3
Case 4	*M. mulatta*	right SC	2% GB	0.25	8
Case 5	*M. fascicularis*	right SC	15% RD	1	8

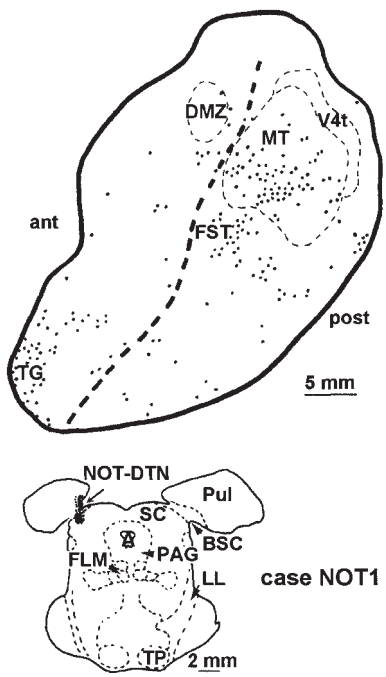

Figure 1. The lower part shows a line drawing of a frontal midbrain section through the centre of the NOT-DTN injection site in case 1. The solid black label indicates the RD injection site proper, the dotted area around it indicates the area of tracer spread. The upper part shows a two-dimensional map of the ipsilateral (left) superior temporal sulcus derived from parasagittal sections. Posterior is to the right, anterior to the left. The thick outline represents the upper (left) and lower (right) lip of the sulcus, the thick dashed line represents the fundus of the sulcus. The thin dashed lines indicate the borders of areas V4t, MT and the densely myelinated zone of MST based on myeloarchitecture. Retrogradely labelled neurons are indicated by dots. The density of the dots semiquantitatively represents the density of labelled neurons. Note that only layer V neurons are indicated on the map. The scale bars represent 2 mm for the midbrain section and 5 mm for the cortical map. A, aqueduct; BSC, brachium of the superior colliculus; DMZ, densely myelinated zone of MST; FLM, fasciculus longitudinalis medialis; LL, lemniscus lateralis; PAG, periaqueductal grey; Pul, pulvinar; TP, tractus pyramidalis.

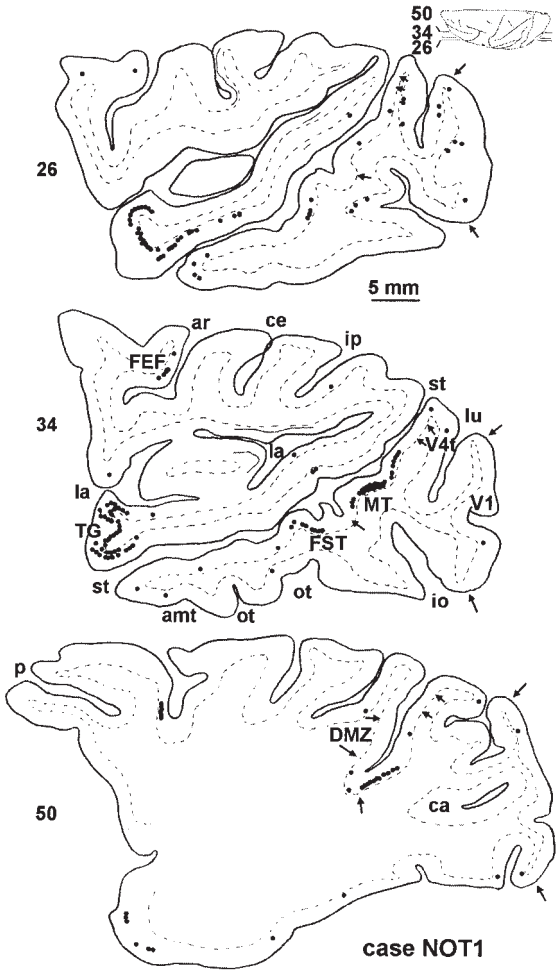

Figure 2. Line drawings of three representative parasagittal sections through the left hemisphere of case 1. The position of these sections in the brain is indicated on the dorsal view of the brain on the upper right. The broken line indicates the border of the white matter. Each dot represents a retrogradely labelled neuron. The areal borders of V1, MT, V4t and DMZ based on myeloarchitecture are indicated by arrows. The scale bar indicates 5 mm. amt, anterior mediotemporal sulcus; ar, arcuate sulcus; ca, calcarine sulcus; ce, central sulcus; io, inferotemporal sulcus; ip, intraparietal sulcus; la, lateral sulcus; lu, lunate sulcus; ot, occipitotemporal sulcus; p, principal sulcus; st, superior temporal sulcus.

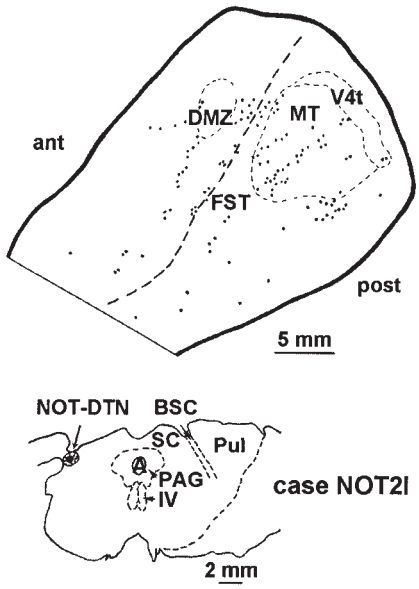

Figure 3. Lower part: line drawing of a midbrain section through the centre of the FG injection site into the left NOT-DTN of case 2l. In case 2, the midbrain was cut perpendicularly to the layers of the SC. Upper part: two-dimensional map of the left STS of case 2l derived from parasagittal sections. For conventions see Figure 1. IV, nucleus trochlearis.

the ipsilateral STS are shown in Figure 3. This injection was rather small (anterior–posterior extent ~750 μm). In both cases there was only marginal spread of the tracer to the neighbouring SC and the overlying medial pulvinar. In the midbrain, these injections resulted in retrogradely labelled neurons in the contralateral NOT-DTN as well as in the contralateral and ipsilateral nucleus parabigeminalis. No label was observed in the contralateral pulvinar.

The densest label in the entire ipsilateral hemispheres was located in the posterior bank of the STS, mainly in area MT but not restricted to it. The labelled area continued anteriorly of area MT. Area MST in the upper bank of the sulcus was largely devoid of label. A second patch of labelled cells occurred in the most anterior part of the upper bank of the sulcus and the neighbouring gyrus (area TG) of case 1. Area TG was the only location in the whole hemisphere where, in addition to layer V, retrogradely labelled cells were also found in layer VI. Comparison with other cases as well as the literature, however, indicates that this label could be due to some involvement of the medial pulvinar into the injection site (Trojanowski and Jacobson, 1976; Baleydier and Mauguiere, 1985; Levitt *et al.*, 1995) or, more likely, because there was no label in the contralateral pulvinar, it could be due to local tracer uptake along the penetration track in the latero-posterior part of the cingulate sulcus (Vogt and Pandya, 1987) observed in this case. Layer VI label is not included on the STS map in Figures 1 and 3. Outside the STS few scattered cells were found in the operculum of V1 and in the most lateral part of the lunate and the infraoccipital sulci, thus representing the central visual field in areas V1, V2 and V3 (Gattass *et al.*, 1981, 1988). Additionally, few cells were labelled in the lateral intraparietal area (LIP) in the posterior bank of the intraparietal sulcus, and a moderately dense patch of labelled cells was present in the frontal eye field in the anterior bank of the arcuate sulcus (Fig. 2, Table 2).

Case 2r

The injection site of FG and the corresponding ipsilateral STS in the right hemisphere of case 2r are demonstrated in Figure 4. This somewhat larger injection (anterior–posterior extent ~2 mm) was again centred in the NOT-DTN and involved the brachium of the superior colliculus (BSC) and possibly part of the medial pulvinar. Spread of the tracer to the SC was not evident.

In the STS, the highest concentration of labelled neurons was again found in area MT and in the adjoining cortex anterior to MT. Even though part of the STS (the shaded area in the two-dimensional map in Fig. 4) could not be fully analysed because of tissue damage, relatively few cells were found in the anterior bank of the STS, including area MST. In this case, V1 was also analysed. Numerous labelled cells were located in the central and lower part of the lateral operculum, corresponding to the central visual field representation, as well as in the lower bank of the calcarine sulcus, representing the peripheral visual field.

Table 2
Summary of labelled cortical areas after NOT-DTN or SC injections

Case	V1	V2	V3	V4	V4t	MT	MST	FST	Ant. STS	STPa	TG	Ia	LIP	FEF
NOT 1	+	+	+	+	–	+++	+	+	+	–	+	–	+	+
NOT 2l	o	o	o	o	–	+++	+	+	+	–	o	o	o	o
NOT 2r	+++	o	o	o	–	+++	+	+	–	–	o	o	o	o
NOT 3	+	+	+	–	–	+++	+	+	+	+	–	–	+	–
SC 4	+++	+	+	+++	–	+++	–	+	–	–	–	–	–	+
SC 5	+	+	+	+	+	+	+	+	+	+++	+++	+	+	+

The density of labelled cells was judged relative to the highest density found in a particular case. +++, strong label; ++, moderate label; +, a few or scattered labelled cells; –, no labelled cells; o, not analysed. STPa, anterior part of the superior temporal polysensory area.

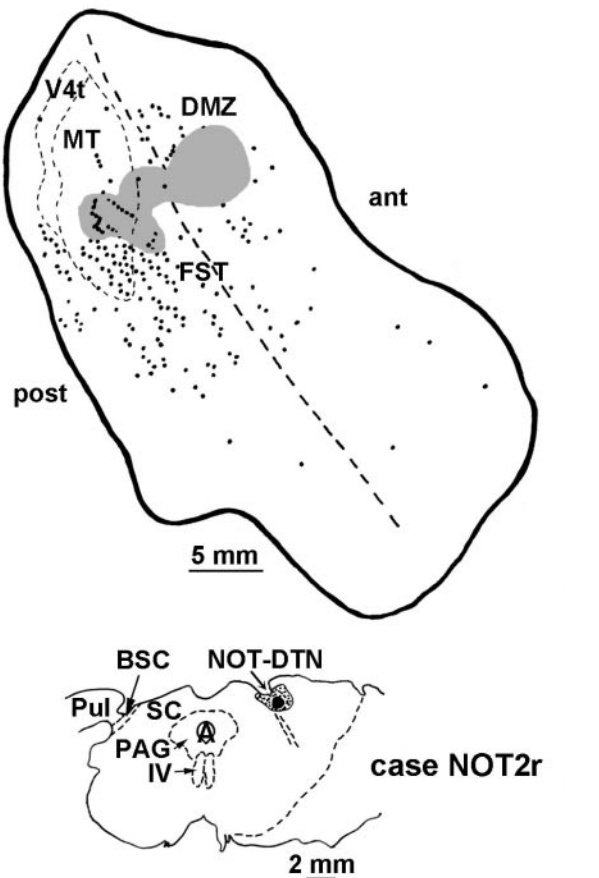

Figure 4. Lower part: line drawing of a midbrain section through the centre of the FG injection site into the right NOT-DTN of case 2r. Upper part: two-dimensional map of the right STS of case 2r derived from parasagittal sections. Posterior is to the left, anterior to the right. The shaded area indicates the cortical region that could not be fully analysed due to tissue damage. For other conventions see Figure 1.

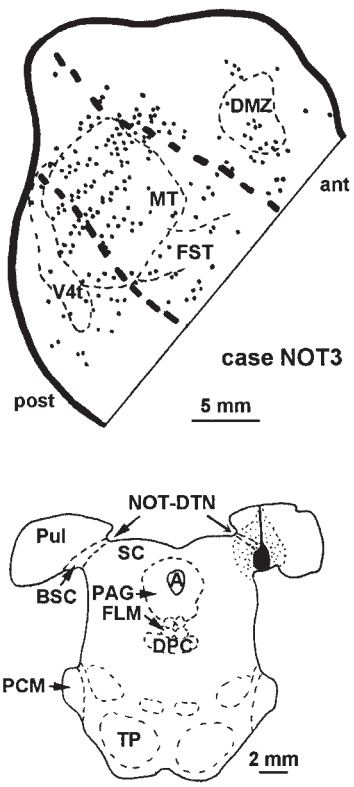

Figure 5. Lower part: line drawing of a midbrain section through the centre of the WGA-HRP/HRP injection site into the right NOT-DTN of case 3. Upper part: two-dimensional map of the posterior part of the right STS of case 3 derived from frontal sections. For conventions see Figures 1 and 3. A, aqueduct; DPC, decussatio pedunculorum cerebellaris superiorum; PCM, pedunculus cerebellaris medialis.

Case 3

This animal received a WGA-HRP/HRP injection centred in the right NOT-DTN. This injection had an anterior–posterior extent of ~1.5 mm. While there was little if any involvement of the SC, some of the tracer spread to the pulvinar (Fig. 5). In this case, the contralateral midbrain and thalamus were not subjected to TMB histochemistry because of a cortical tracer injection made in the course of an unrelated investigation. Thus, we have no information about contralateral projections resulting from this injection.

As in the other cases, the heaviest label in the ipsilateral STS occurred in area MT, whereas only few labelled cells were found in areas MST and FST (see the map in Fig. 5 and representative sections in Fig. 6). Note that while the whole cortical hemisphere was analysed, only the posterior part of the STS is shown in Figure 5. In the upper bank of the anterior part of the STS, mainly comprising areas TPO and IPa of Seltzer and Pandya (Seltzer and Pandya, 1989), an additional scarcely to moderately dense patch of retrogradely labelled cells was found. This retrograde label was accompanied by anterograde terminal labelling, thus suggesting that the label was due to involvement of the pulvinar into the injection site. Local tracer uptake along the penetration track in the cortex was not evident. Outside the STS, consistent labelling was found in the representation of the dorsal visual field of area V1 in the lower bank of the calcarine sulcus, and of areas V2 and V3v on the ventral surface of the brain. Scarce labelled cells were found in area V3A, and in area

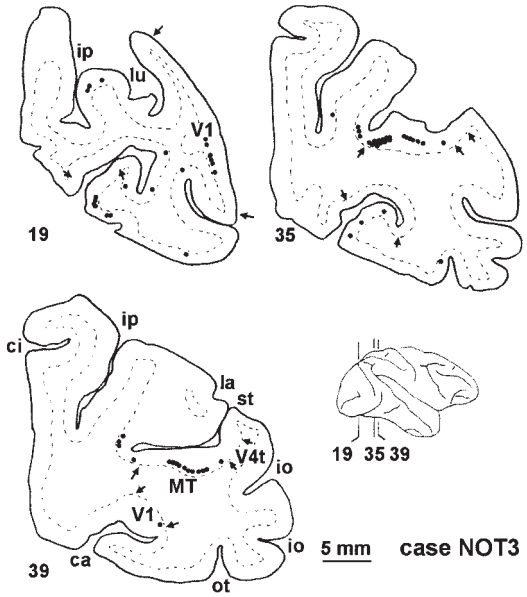

Figure 6. Line drawings of three representative frontal sections through the right hemisphere of case 3 the position of which in the brain is shown on the lateral view of the brain on the lower right. For conventions see Figure 2.

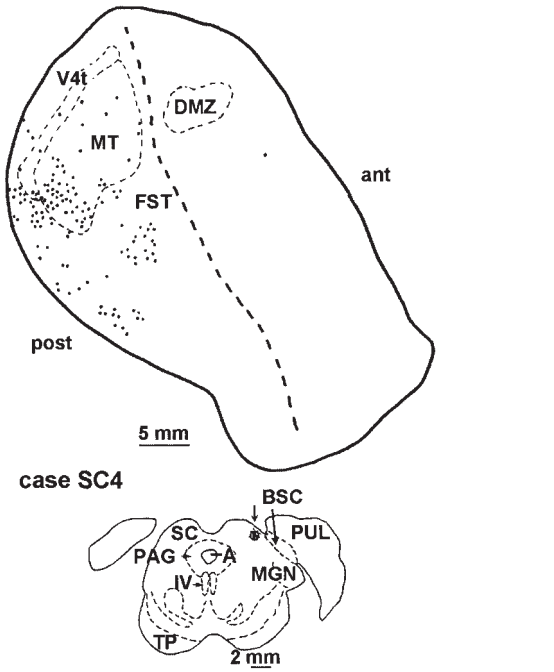

Figure 7. Lower part: line drawing of a midbrain section through the centre of the GB injection site into the foveal representation of the right SC close to the NOT-DTN of case 4. In case 4, the midbrain was cut perpendicularly to the layers of the SC. Upper part: two-dimensional map of the right STS of case 4 derived from parasagittal sections. Note that the patch of labelled cells in MT is restricted to the foveal representation. For conventions see Figures 1 and 3.

LIP in the lower bank of the intraparietal sulcus. The arcuate sulcus was devoid of label.

Thus, from these four cases a common labelling pattern after tracer injection into the NOT-DTN emerges. Consistently, the heaviest labelled zone in the ipsilateral cortical hemisphere occurred throughout area MT and the adjoining cortex anterior to it. The anterior bank of the STS including area MST was only sparsely labelled. Outside the STS, consistent labelling occurred in area V1. In one case this label was mainly located in the representation of the central visual field (case 1), in two other cases (case 2r and case 3) this label was concentrated in the dorsal field representation. While a clear topography has never been described within the NOT-DTN, the degree of involvement of neighbouring structures, i.e. SC and pulvinar, into the injection site could be the reason for the variability of labelling outside the STS. Thus, in two more cases we made tracer injections into the SC near to the NOT-DTN.

Control Injections into the Superior Colliculus

Case 4 and 5

Case 4 received a small Granular Blue injection (anterior–posterior extent ~1 mm) into the superficial layers of the foveal representation in the SC located very close to the NOT-DTN (Fig. 7). Within the ipsilateral STS the labelling was limited to a rather small but dense patch in the foveal representation in latero-posterior MT (Gattass and Gross, 1981; Van Essen *et al*., 1981), and a somewhat more prominent group of labelled neurons in V4 at the posterior and lateral border of the STS. Few labelled neurons were additionally found anterior to MT in the posterior bank of the sulcus.Outside the STS, dense labelling occurred in the central visual field representation in V1 on the operculum (Fig. 8). Less dense but still considerable labelling was seen in the central field representation in areas V2, V3 and V4 in the lunate and infero-occipital sulci, and on the prelunate gyrus. Few labelled cells were found in the representation of the ventral peripheral visual field in the calcarine sulcus. In addition, a small patch of neurons was labelled in the anterior bank of the arcuate sulcus in the frontal eye field.

In case 5 a rather large RD injection (anterior–posterior extent ~2.5 mm) was placed into the representation of the ventral visual field in the intermediate layers of the SC (Fig. 9). Again, this injection was quite close to the NOT-DTN and involved a small part of the inferior colliculus.Within the ipsilateral STS, labelled neurons were found throughout area MT (Figs 9 and 10). In contrast to all other cases, the highest density of labelled cells was found throughout the anterior bank of the STS, including the densely myelinated zone of MST and the polysensory area STP.

The superior temporal gyrus and the posterior bank of the lateral sulcus were almost as densely labelled, while the anterior bank of the lateral sulcus contained less dense label. Moderate label was also found in the anterior bank of the lunate sulcus, including areas V3 and V3A, and in the posterior bank of the intraparietal sulcus, including area LIP. Less dense labelling was found in the posterior bank of the lunate in area V2 and in the dorsal bank of the calcarine sulcus. Both regions contain the representation of the ventral visual field. Furthermore, moderate densities of labelled cells were found throughout the arcuate sulcus, a few scattered labelled cells also being found in the principal sulcus.

Quantitative Comparison between NOT-DTN and SC Injections

Table 2 compares qualitatively the density of labelled cells within cortical areas of individual cases after both NOT-DTN and SC injections.The regions with the highest cell densities are labelled with three crosses. In Table 3 the quantitative data of cases NOT1 and NOT3 as well as of SC4 and SC5 are presented as average number of labelled cells per mm in striate cortex, prestriate

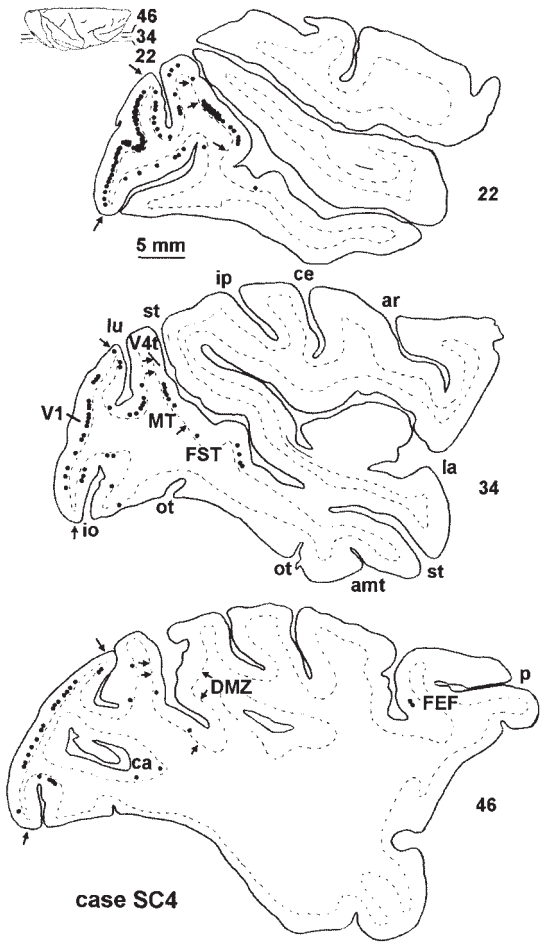

Figure 8. Line drawings of three representative parasagittal sections through the right hemisphere of case 4. For conventions see Figure 2. p, principal sulcus.

areas, MT and MST. Cases NOT2l and NOT2r were not included in this analysis because of the strong autofluorescence in this animal. To look for differences in labelling densities that related retinotopically to injection sites, we separated the data from V1 in the ventral and dorsal foveal field representation on the operculum, as well as the dorsal peripheral field representation in the calcarine sulcus. Likewise, we distinguished between the foveal and peripheral field representation in area MT. It is clear that there is considerable overlap in the cortical areas retrogradely labelled after injecting NOT-DTN and SC, but there are also clear quantitative differences. To make the comparison easier, we summed up the label outside MT after normalizing the average densities to the label in MT. This procedure shows that the heaviest label always occurred in area MT and the adjoining cortex after NOT-DTN injection, whereas after SC injections the cumulative label outside MT was at least three times higher than the label in MT. Furthermore, the cortical areas labelled after SC injections were in close retinotopic correspondence to the actual injection site. A similar covariance of label in cortical areas with the exact position of the injection within the NOT-DTN was never evident.

Discussion

The most consistent and strongest projection from cortex to NOT-DTN was found to originate from the motion sensitive area

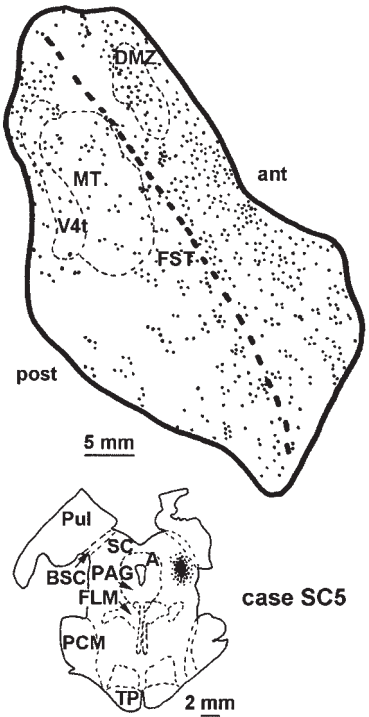

Figure 9. Lower part: line drawing of a midbrain section through the centre of the RD injection site into the representation of the ventral visual field in the right SC of case 5. Upper part: two-dimensional map of the right STS of case 5 derived from parasagittal sections. For conventions see Figures 1 and 3.

MT and the adjoining cortex anterior to it, whereas area MST was labelled less consistently. In addition, a significant projection was found to arise from the striate cortex, V2 and V3.

Comparison with Anterograde Tracing Studies

Using tritiated amino acids and HRP as anterograde tracers, Maunsell and Van Essen, Ungerleider *et al.* and Maioli *et al.* (Maunsell and Van Essen, 1983; Ungerleider *et al.*, 1984; Maioli *et al.*, 1989) were all unable to identify a projection from MT to the NOT-DTN or other pretectal nuclei in the macaque. In contrast, Hoffmann *et al.* (Hoffmann *et al.*, 1991) described labelled fibers and terminals in the NOT-DTN after HRP injections into MT, a finding later verified by Lui *et al.* (Lui *et al.*, 1995). A direct projection from MT to the NOT-DTN was also described in the marmoset (Spatz and Tigges, 1973) and in the owl monkey (Graham *et al.*, 1979), but not in the galago (Wall *et al.*, 1982). Both the additional motion-sensitive areas in the STS – MST and FST – have been described as projecting to the NOT-DTN in the macaque (Boussaoud *et al.*, 1992; Lui *et al.*, 1995).

Whether there is a direct projection of the primary visual cortex V1 to the NOT-DTN is also a matter of controversy. While studies in the macaque, in the owl monkey and in the squirrel monkey have described direct projections from V1 to the pretectum, including the NOT-DTN (Spatz *et al.*, 1970; Campos-Ortega and Hayhow, 1972; Graham *et al.*, 1979; Hoffmann *et al.* 1991), such a projection was not found by Lui *et al.* (Lui *et al.*, 1995) after injections of tritiated amino acids into V1 on the operculum close to the ectocalcarine sulcus. It was thus argued that possibly only parts of V1 representing the visual field around the vertical meridian at the V1/V2 border would project to the NOT-DTN. However, in all of our retrograde tracing experiments (cases 1–3 of this study, additional unpublished

observations) labelled cells in the operculum were not restricted to the neighbourhood of the V2 border. Additional but more variable labelling was found in the peripheral field representation in the calcarine sulcus. In addition, orthodromic electrical stimulation in V1 yielded responses in the NOT-DTN (Hoffmann *et al.*, 1991), and in another case electrical stimulation in the NOT-DTN yielded an antidromic response in opercular V1 (our own unpublished observation).

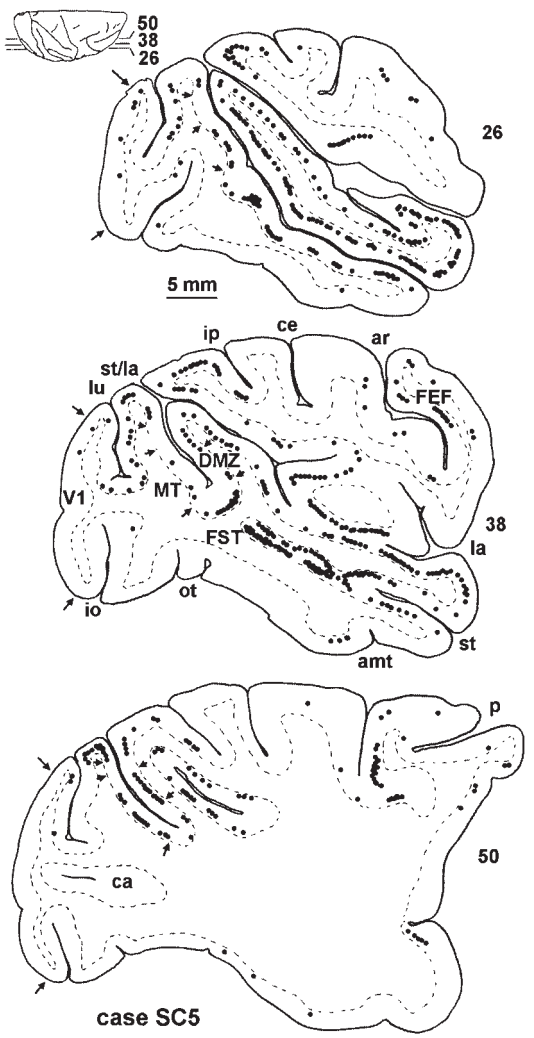

Figure 10. Line drawings of three representative parasagittal sections through the right hemisphere of case 5. For conventions see Figure 2.

NOT-DTN Injection Sites: Involvement of Neighbouring Structures?

All injections were made after electrophysiological identification of retinal slip cells characterized by their directional preference for horizontal ipsiversive stimulus movement and their position anterior and lateral to the foveal representation in the SC (Hoffmann *et al.*, 1988). The retinal slip cells lie between and below the fibers of the BSC running not only to the NOT-DTN but also to the SC and to other pretectal nuclei. Thus, involvement of fibers of passage running to the SC or pretectum or to the pons cannot be completely ruled out with any of the tracers used in the present study. In addition, neighbouring structures as the SC and the pulvinar could have been included in the area of tracer spread.

To judge whether our data are confounded by cortical projections to the SC we performed two control injections, close to the NOT-DTN. In both cases, the overall labelling in area MT was not stronger than labelling in the prestriate cortex (V2, V3, V4). In addition, there was a strict topographical relationship of the SC injection site and labelled cortical regions which was not seen after NOT-DTN injections, corresponding to the fact that there is little retinotopic order in this structure.Thus, we judge the influence of SC involvement in our NOT-DTN injections, especially concerning our data on the STS, as minimal. Comparison of our data with the studies of Asanuma *et al.* and Lynch *et al.* (Asanuma *et al.*, 1985; Lynch *et al.*, 1985) suggests that the labelling in LIP seen in our cases 1 and 3 (and our control case 5) could be due to involvement of fibers of passage to the intermediate and deep layers of the SC (the SC proper in case 5). We also believe that label in the frontal eye field (FEF) in case NOT 1 is due to fibers of passage to the intermediate layers of SC (Leichnetz *et al.*, 1981; Sommer and Wurtz, 2000).

There are many reports of connections of striate and extrastriate visual areas and other cortical regions with the pulvinar. Involvement of the medial pulvinar overlying at least in part the NOT-DTN could contribute to the labelling in the anterior part of the upper bank of the STS and the adjoining area TG (see our cases 1 and 3), as well as to the labelling in FEF (Trojanowski and Jacobson, 1976). Area MT projects mainly to the inferior and the lateral pulvinar, and to a lateral portion of the medial pulvinar (Standage and Benevento, 1983; Ungerleider *et al.*, 1984). None of our injection sites involved these regions. In addition, areas MST and FST project mainly to middle to lateral portions of the medial pulvinar, as well as to the lateral pulvinar (Boussaoud *et al.*, 1992; Lui *et al.*, 1995; Adams *et al.*, 2000; Gutierrez *et al.*, 2000). The respective parts of the medial pulvinar could be included into the area of tracer spread in our case 3 so that part of the label seen here in areas MST and FST could be due to involvement of the pulvinar. To solve this problem we analysed the distribution of labelled cells in the cortical layers. As described above, NOT-DTN projection neurons were always found in layer V. A literature search revealed that projection

Table 3
Quantitative assessment of label density in striate and posterior prestriate areas

Case	V1 op d	V1 op v	V1 ca v	V2	V3d	V4	MT fov	MT per	MST
NOT 1	0.19 ± 0.16	0.04 ± 0.07	0.04 ± 0.1	0.12 ± 0.14	0.24 ± 0.27	0.06 ± 0.11	1.4 ± 0.97	0.5 ± 0.49	0.1 ± 0.12
NOT 3	0.06 ± 0.08	0.21 ± 0.2	0.42 ± 0.26	0.33 ± 0.22	0.07 ± 0.12	–	0.66 ± 0.21	1.27 ± 0.9	0.17 ± 0.23
SC 4	2.1 ± 2.3	0.6 ± 0.55	0.06 ± 0.06	1.1 ± 0.56	0.64 ± 0.14	1.5 ± 1.4	2.3 ± 1.3	0.2 ± 0.2	–
SC 5	0.18 ± 0.15	0.02 ± 0.05	0.05 ± 0.07	0.32 ± 0.26	0.63 ± 0.6	1.2 ± 0.8	1.4 ± 2.1	0.79 ± 0.36	1.2 ± 0.54

Values are the mean ± SD densities (no. of cells per mm in layer V) of retrogradely labelled neurons in striate cortex and several prestriate areas after NOT-DTN (cases 1 and 3) and SC injection (cases 4 and 5). V1 op d, V1 in the dorsal part of the operculum; V1op v, V1 in the ventral part of the operculum; V1 ca v, V1 in the ventral part of the calcarine sulcus; MT fov, foveal MT; MT per, peripheral MT.

neurons to the medial pulvinar lie in cortical layers V and VI of limbic areas (Baleydier and Maugiere, 1985). In area V2, neurons projecting to the pulvinar are predominantly located in layer VI and to a lesser degree in layer V (Levitt *et al.*, 1995). Our own, as yet unpublished, observations indicate that after tracer injections into various subregions of the pulvinar, retrogradely labelled cells within the STS are located in both layers V and VI. Thus, we can largely rule out involvement of the pulvinar as possible source for our label in areas MST and FST because in these regions all labelled cells in our NOT cases were restricted to layer V. The bilaminar label seen in area TG in NOT case 1 is possibly due to local tracer uptake along the penetration track in the depth of the posterior cingulate sulcus (Vogt and Pandya, 1987). To decide upon the origin of labelling in V1 is more difficult because it has been shown that V1 neurons projecting to the pulvinar are located in layer V similar to cells projecting to the SC and the pretectum (Lund *et al.*, 1975). However, V1 mainly projects to the inferior and the lateral pulvinar (Campos-Ortega and Hayhow, 1972; Graham, 1982; Ungerleider *et al.*, 1983; Lui *et al.*, 1995; Adams *et al.*, 2000). None of our injections included the target zones of striate projection neurons to the pulvinar. Thus, the V1 labelling after NOT-DTN injection cannot be explained by involvement of the pulvinar into the injection site. In conclusion, based on the control injections into the SC and on the laminar distribution of labelled cortical neurons, we feel confident that involvement of neighbouring structures in retrograde cortical label after our NOT-DTN injections was minimal.

Functional Interpretation

The present findings confirm our earlier results that the motion-sensitive areas in the STS, mainly area MT, provide the main cortical input to the NOT-DTN as the sensorimotor interface in the subcortical pathway for the optokinetic reflex. This projection arises from large parts of area MT and the neighbouring cortex, thus indicating that there is no specific cortical area responsible for coding of slow eye movements during OKN (and smooth pursuit) but that this function is distributed over the known motion-sensitive areas. Electrophysiological experiments using orthodromic and antidromic electrical stimulation have demonstrated a direct functional projection of MT upon retinal slip cells in the NOT-DTN (Hoffmann *et al.*, 1991, 1992). In summary, area MT is the prime source of cortical input to the NOT-DTN. In particular, cells in layer V of MT share the same properties as NOT-DTN neurons: strong direction-specific responses to large field random dot patterns and large receptive fields extending into the ipsilateral hemifield (Hoffmann *et al.*, 1992; Raiguel *et al.*, 1995). In addition, preliminary data indicate that only a specific subpopulation of MT cells coding for ipsiversive stimulus movement project to the NOT-DTN (Hoffmann *et al.*, 1992; Ilg and Hoffmann, 1993). This, then, would explain why, after specific MT and MST lesions (Dürsteler and Wurtz, 1988), direction-selective deficits in the slow phase of optokinetic eye movements occur towards the lesioned side even though these cortical areas are not biased for a certain direction of stimulus movement.

Notes

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