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RESEARCH ARTICLE

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Intrinsic connectivity of human superior colliculus

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Abstract The superior colliculus (SC) is believed to play an important role in sensorimotor integration and orienting behavior. It is classically divided into superficial layers predominantly containing visual neurons and deep layers containing multisensory and premotor neurons. Investigations of intrinsic connectivity within the SC in non-human species initially led to controversy regarding the existence of interlaminar connections between superficial and deep layers. It now seems more likely that such connections exist in a number of species, including non-human primates. In the latter, anatomical data concerning intrinsic SC connectivity are restricted to a limited number of intracellularly labeled neurons. No studies have been conducted to investigate the existence of intrinsic connections of human SC. In the present study, DiI (1,1′-dioctadecyl-3,3,3′,3′- tetramethylindocarbocyanine perchlorate) and BDA (biotinylated dextran amine) were two tracers used in post-mortem human brains to examine intrinsic SC connections. Injections into the superficial layers revealed tangential connections within superficial layers and radial superficial-layer to deep-layer connections. Within superficial layers, horizontal connections were found over the entire rostro-caudal axis and were mostly directed laterally, i.e. toward the brachium of the inferior colliculus. Superficial-layer to deep-layer connections were more prominent in sections containing the injection site or located close to it. In these sections, an axon bundle having roughly the same diameter as the injection site crossed all deep layers, and individual axons displayed en passant or terminal boutons. The present results suggest that intrinsic connections within superficial layers and radial superficial-layers to deep-layers exist in human SC. The putative roles of these connections are discussed with regard to visual receptive field organization, as well as visuomotor and multisensory integration.

Keywords Sensorimotor · Multisensory · BDA · DiI · Tracing

Abbreviations ACSF: Artificial cerebrospinal fluid · BDA: Biotinylated dextran amine · DAB: 3,3′-Diaminobenzidine tetrahydrochloride · DiI: 1,1′-Dioctadecyl-3,3,3′,3′- tetramethylindocarbocyanine perchlorate · PAG: Periaqueductal gray · SC: Superior colliculus · SGI: Stratum griseum intermediale · SGP: Stratum griseum profundum · SL: Stratum lemnisci · SO: Stratum opticum · SGS: Stratum griseum superficial · SZ: Stratum zonale · TB: Tris buffer · TBS: Tris phosphate buffer · TBST: Tris phosphate buffer tween

Introduction

The dorsal aspect of the human tectal plate is composed of the inferior and superior colliculi. While the former contains a central nucleus surrounded by several subdivisions (Olszewski and Baxter 1982; Tardif et al. 2003), the latter consists of six main layers visible on Nissl-stained and myelin-stained sections (Olszewski and Baxter 1982). Functionally, the SC is classically divided into two divisions: (i) superficial layers including stratum zonale (SZ), stratum griseum superficial (SGS) and stratum opticum (SO); (ii) deep layers including stratum griseum intermediale (SGI), stratum lemnisci (SL) and stratum griseum profundum (SGP).

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This subdivision into superficial and deep layers is based on hodological, physiological and behavioral evidence from animal studies. Superficial layers have been shown to be interconnected mainly with vision-related structures, whereas the connectivity of deep layers is much more complex and mostly motor-related (for review see, e.g. Huerta and Harting 1984). Although retinal or cortical input carrying visual information is mainly restricted to its superficial layers, several high-order visual areas also project to the deep collicular layers (Lynch et al. 1985; Huerta et al. 1986; Harting et al. 1991; Lock et al. 2003). This is in agreement with the finding that superficial layers contain a topographic representation of the contralateral visual space, whereas multimodal and motor maps are found in the deep layers (Cynader and Berman 1972; Sparks 1988; Wallace et al. 1996).

Several lines of evidence suggest close interaction between superficial and deep layers, while others speak in favor of relative independence. The development of the auditory space map found in deep collicular layers was shown to depend on input from superficial layers (King et al. 1998). On the other hand, selective superficial versus superficial-plus-deep collicular lesions were shown to produce different behavioral deficits (Casagrande et al. 1972). Furthermore, the visually related functions are carried out by the deep layers of the SC despite the lack of strong direct visual input to the deep layer neurons (Stein and Meredith 1991).

Evidence from non-human studies suggests that the SC plays an important role in orienting movements, including saccadic eye movements (see Sparks 1999 for review) and in multisensory orientation (Burnett et al. 2004). Because the motor map of the deep collicular layers is in register with the space map of the superficial layers (Schiller and Stryker 1972) and the retinotopic visual input is largely confined to the superficial layers, early on the idea of an extensive downward interlaminar communication emerged (Sprague 1975). Such superficial-layer to deep-layer connections may also play a role in multisensory interactions that occur in the deep collicular layers (Wallace et al. 1996; Bell et al. 2001). Early studies in cats suggested that such interconnectivity does not exist (Edwards 1980), but further investigations have demonstrated connectivity from superficial to deep collicular layers in a variety of non-human species (see Isa 2002 for a review) including rat (Hilbig et al. 2000; Özen et al. 2000), hamster (Mooney et al. 1988; Rhoades et al. 1989; Mooney et al. 1992), tree shrew (Hall and Lee 1997), ferret (Doubell et al. 2003), cat (Behan and Appell 1992) and squirrel monkey (Moschovakis et al. 1988a, b). To our knowledge, the latter study is the only one that demonstrated connectivity from superficial-to deep-layers in the SC of non-human primates. No studies have investigated such connectivity in humans.

In the study conducted by Moschovakis et al. (1988a, b), intracellular injections in non-human primates allowed a precise description of morphological features of individual neurons participating in intrinsic collicular connectivity. However, the limited number of individual neurons injected makes it difficult to estimate the extent of overall connectivity between the superficial and deep collicular layers in primates, as well as the extent of intrinsic connectivity occurring within superficial layers. Very little is known about SC connectivity in humans. Human studies have shown that commissural connections of SC mainly involve the deep collicular layers (Tardif and Clarke 2002) and a Golgi study suggested the existence of horizontal connections within superficial and deep layers (Laemle 1981, 1983).

We report here on the intrinsic connections of human SC using two tracing techniques, DiI and BDA, in post-mortem tissue.

Materials and methods

The tectum of eight adult human brains was used in this study. The brains were obtained from a donor program at the Faculty of Medicine in Lausanne (brain 8) or from authorized autopsies at the Institute of Pathology, University of Basel, Switzerland (brains 1–7; Table 1). The recommendations of the Declaration of Helsinki were followed. Permission for brain autopsy was obtained from patients' relatives. All subjects died from causes unrelated to the brain; three did not have signs of

Table 1 Description of cases

Case number	Sex	Age	PMD (h)	Cause of death	Neuropathology	Tracer; injection side
1	M	84	25	Pneumonia	Braak stage VI	BDA; L
2	F	89	24	Cardiac failure	Braak stages III and IV	BDA; L
3	F	76	8	Respiratory Insufficiency	AgD	BDA; L + R
4	F	89	15	Myocardial infarct	Braak stage III	DiI; R
5	F	76	10	Acute myeloid leukemia, pulmonary bleeding	–	DiI; R
6	M	71	7	bronchopneunonia	–	DiI; L
7	F	80	23	Cardiac failure	Braak stage III	DiI; R
8	M	83	10	Cardiac failure	–	DiI; L

M male, *F* female, *PMD* post-mortem delay, *AgD* Argyrophilic grains disease (presence of argyrophilic grains in the CA1 region of the hippocampus), *L* left, *R* right. Braak stages were determined by histological examination of the entorhinal cortex using the description of Braak and Braak (1991). No other neuropathological changes were present

brain disease (cases 5, 6 and 8) while the other five had macroscopically and/or microscopically diagnosed neurological conditions, which did not involve the SC.

Tracing with DiI

After removing the brain from the skull, the tectal plate was fixed by immersion in 4% paraformaldehyde in phosphate buffer for 12–24 h. A small DiI crystal (1,1′-dioctadecyl-3,3,3′,3′- tetramethylindocarbocyanine perchlorate; Molecular Probes, The Netherlands) was placed in the superficial layers of the SC using the tip of a glass micropipette. An operating microscope equipped with a calibrated grid was used to assess the crystal size (ca. 400 μm in diameter). The actual depth of the crystal was determined on histological sections. Injected material was stored at 37° for 12–32 months in 4% paraformaldehyde in phosphate buffer. Each SC was dissected from the tectal plate, embedded in 4% agar and cut with a vibratome (Leica, model VT 1000 S) into 40 μm thick serial sections. These were collected in wells containing 4% paraformaldehyde in phosphate buffer. Sections were mounted on glass slides in the same fixative, coverslipped and sealed with nail varnish. Charts of axonal labeling were compiled using a computer-assisted camera lucida and photomicrographs were taken with an argon-krypton confocal microscope (see Tardif and Clarke 2002 for details). Following microscopic analysis, the coverslip was removed and the section was counterstained with cresyl-violet in order to delineate collicular layers (Figs. 1 and 2).

Tracing with BDA

For BDA injections, an adaptation of the procedure from Dai et al. (1998) was used. Briefly, the brain was removed from the skull and dampened with artificial cerebrospinal fluid (ACSF; NaCl, 120 mM; KCl, 3 mM; NaH_2PO_4, 1.4 mM; D-glucose, 10 mM, pH. 7.3) at 4°C. The tectal plate was then dissected and immersed into ACSF supplied by 95% O_2 + 5% CO_2 for 2 h. The tissue block was then glued on a metallic plate, immersed in ACSF, and kept at 4°C by surrounding the tissue with ice cubes. A glass micropipette (tip from 1 to 5 μm diameter) containing 5% BDA (biotinylated dextran amine, 10,000 MW) was held by a micromanipulator (WPI; Taurus, Berlin, Germany) and inserted perpendicularly into the superficial layers of the SC. Iontophoretic injections were made by using a 7-μA positive current for 7 s on, 7 s off over 5–10 min. This yielded focal injections whose core was approximately 300–800 μm with very little diffusion around the site. Tissue blocks were then put in ACSF supplied by 95% O_2 + 5% CO_2 at room temperature for 12 h, fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH. 7.4) for 7 days, and then placed in 20% sucrose in phosphate buffer until they sank. Each SC was dissected from the tectum and quickly frozen in isopentane surrounded by dry ice. It was kept at −20°C until it could be sectioned with a cryostat. Serial 40 μm sections were collected and stored in 0.05 M TBS (Tris phosphate buffer; 0.05 Tris, 0.9% NaCl, pH. 8.0).

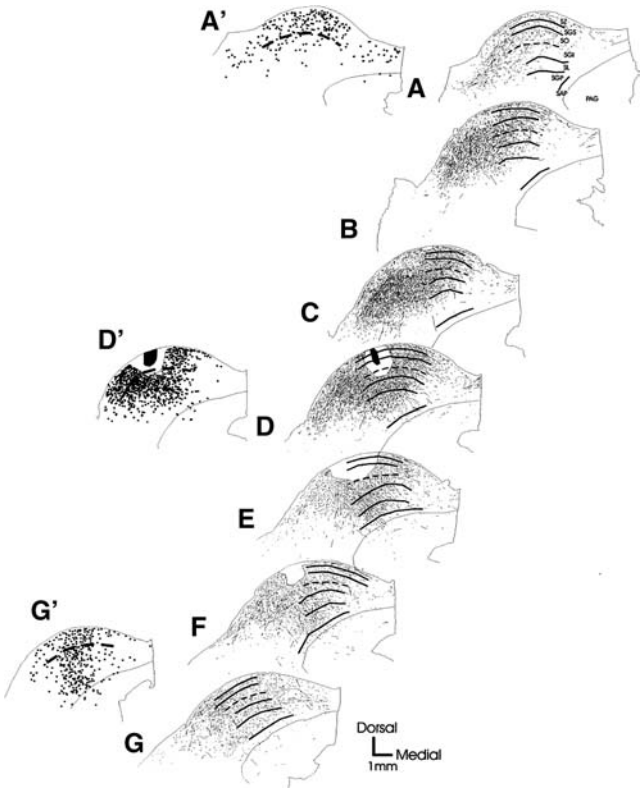

Fig. 1 Distribution of axonal labeling from a DiI injection in serial coronal sections of the left SC (*A–G* is an anterior to posterior series. Distance between sections is ~500 μm). *Thin lines* represent single DiI labeled axon segments; *thick lines* delineate collicular layers as assessed on the same Nissl-stained section; *dotted lines* indicate the limit between superficial and deep collicular layers. The *black area* in *D* indicates the location of the DiI crystal and the surrounding white area is the region of diffusion of the tracer in the extracellular space. Note the tangential spread of labeled axons in the superficial layers in all sections and a radial axon bundle crossing all collicular layers under the injection site in E. In addition, horizontally running axons are present in deep layers in sections E–G and obliquely running axons in sections A–D. A′, D′ and G′ are sections adjacent to A, D and G, respectively, and show the distribution of axonal segments with at least one en passant or terminal boutons

Sections were rinsed in TBS pH 8.0 3×10 min and incubated for 10 min in methanol 70 + 0.5% H_2O_2 to reduce intrinsic peroxidase activity. They were then rinsed in TBST (tris phosphate buffer tween; 50 mM Tris buffer (TB) + NaCl 0.9% + Tween 0.5%, pH 8.0) 3×30 min before incubation in avidin-biotin-horseradish peroxidase complex (ABC kit; Vector, Peterborough, UK) in TBST for 130 min. Sections were rinsed in TB (50 mM, pH. 8.0) 4×20 min and put into TB with 0.4% nickel-ammonium sulfate (pH 8.0). Sections were preincubated in TB with 0.4% nickel-ammonium sulfate (pH 8.0) and 0.02% 3,3′-diaminobenzidine

tetrahydrochloride (DAB) for 10 min. The same volume of TB with 0.4% nickel-ammonium sulfate (pH 8.0), DAB 0.02 and 0.003% H_2O_2 was then added for 20–30 min. Sections were rinsed in TB (pH 8.0) 3×10 min, mounted on gelatine-coated glass slides, then dehydrated and coverslipped. Regularly spaced sections were counterstained with cresyl-violet.

The distribution of labeled axons was assessed in two ways. First, four to seven charts of axonal labeling (spaced by ~ 500 μm) were compiled using a computer-microscope system with a Lucivid micromonitor (MicroBrightField, Colchester, USA) connected to the microscope and to a PC computer. Neurolucida software (MicroBrightField) was used to chart the section contours, the limit between the SC and periaqueductal gray (PAG; visible on unstained sections) and the injection site using a 10 × objective. The distribution of labeled axons outside the injection site was assessed using a 25 × objective. The distribution of axonal segments with synaptic boutons has been charted using a 40 × objective. Each axon segment that contained at least one en passant or terminal bouton was entered on the chart. Boutons were identified as axonal enlargements of more than 4 times the local axonal diameter. Collicular layers (Olszewski and Baxter 1982) were determined in the same sections, which were Nissl-stained after charting. Second, photomicrographs of DiI labeling were taken with a confocal laser-scanning microscope (Leica TCS NT). DiI excitation was obtained with an argon-krypton laser set at 568 nm, and the emitted light was filtered through a LP 590 filter. Images of 1024×1024 pixels were taken with 10 ×, 25 × or 100 × objectives. For each photomicrograph, stacks of optical sections spaced at 1 μm were collected and projected on a single plane. In Fig. 3 and 4, labeled axons are represented in black. For some low-power photomicrographs, montages were made using the Photoshop 5.5 software (Adobe Systems, San Jose, Calif., USA). Photomicrographs of BDA labeled elements were imaged using a Zeiss microscope (AxioPlan 2) equipped with axiocam digital camera (resolution of 3900×3090 pixels) and axiovision software (Zeiss, Feldbach, Switzerland). Images were slightly adjusted for brightness and contrast, but no other transformations were made.

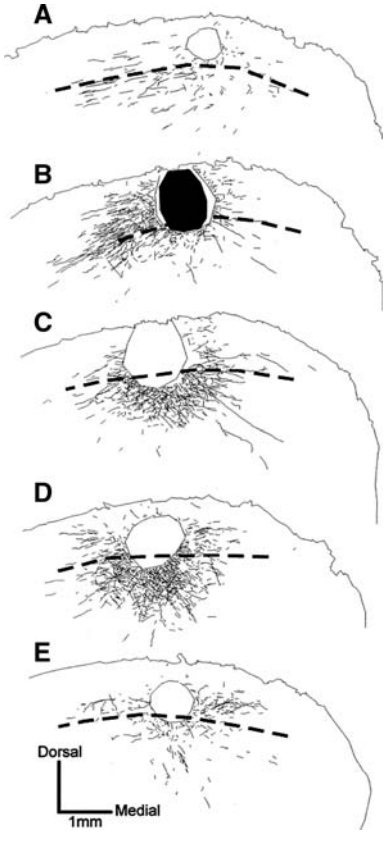

Fig. 2 Distribution of axonal labeling from a BDA injection in serial coronal sections of the left SC (*A–E* is an anterior to posterior series. Distance between sections is ~350 μm). *Dotted lines* indicate the limit between superficial and deep collicular layers. In the rostral section shown in *B*, most labeled axons are located in superficial layers and oriented toward the lateral part of the SC. Labeled axons in deep layers were found in more caudal sections (C–E)

Results

Both DiI and BDA injections produced a dense core surrounded by a zone where the tracer was present in the extracellular space. Both tracers were likely to be picked up by neural elements and transported further along or within the axonal membrane. The uptake region (the core and surrounding zone) was approximately 500–1000 μm in diameter. In all cases the injection core was limited to the SZ, SGS and SO with very limited diffusion into the SGI (Fig. 1D).

As shown in the DiI case illustrated in Fig. 3, injections in the superficial layers yielded dense axonal labeling in the ipsilateral SC. In superficial layers most labeled axon segments were parallel to the pial surface and extended laterally up to 6 mm from the border of the injection site. In the anterior part of the SC, dense horizontally oriented bundles were predominantly present in SO and ran more laterally than medially, i.e. they were oriented toward the lateral brachium of the SC (Fig. 3a). This suggests that fibers either entering or leaving the SC were labeled. Structures resembling synaptic boutons were also observed in SZ, SGS and SO. In the anterior part of the SC, SGP and SAP were almost devoid of labeling (Fig. 1A–C). The lateral spreading of labeled axons was also present in SGI and SL, particularly in sections containing the injection site and in anterior sections (Fig. 1A–D). In sections located posteriorly, a majority of distant axons (i.e. more than 2 mm from the border of the injection site) were present in superficial layers. In addition, more axons were found in the SGP and SAP with a few axon segments extending into the PAG (Fig. 1D–G).

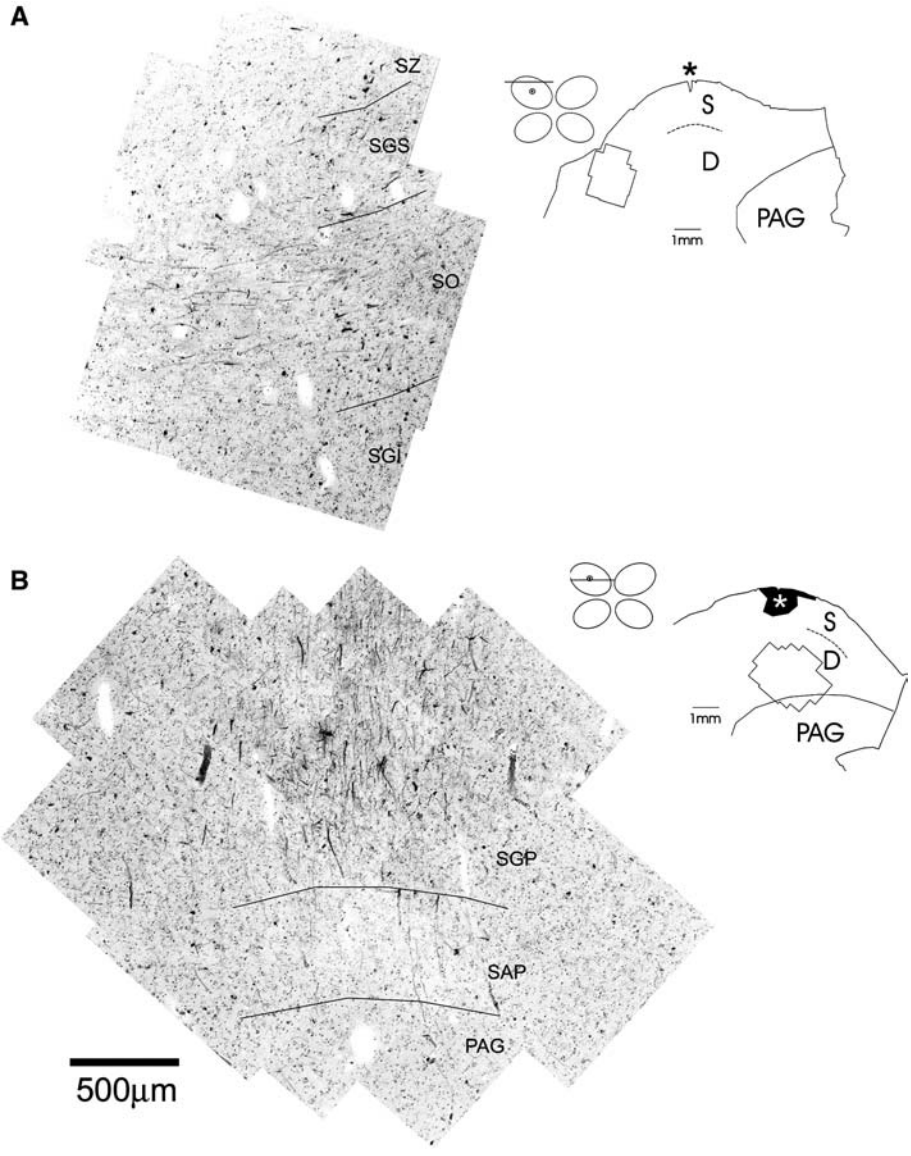

Fig. 3 Confocal photomicrographs showing DiI labeled axons (represented in *black*) within the SC. **A** *Left*: axon bundle running parallel to the pial surface within SO and directed toward the lateral part of the SC. The section was adjacent to that shown in Fig. 1A. *Right*: camera lucida drawing of the section in which the confocal photomicrographs were taken. *Inset* shows the actual location of the photomicrographs shown at left. Ovals are schematic drawing of the tectal plate illustrating the section's level and the injection site. **B** *Left*: axon bundle running radially and crossing superficial and deep layers. The section was adjacent to that shown in Fig. 1e. *Right*: same conventions as in **A**. *S* superficial layers, *D* deep layers, *PAG* periaqueductal gray. *Asterisk* indicates the level of the injection site

In a limited number of sections in the proximity of the injection site or containing it, the most striking feature was a radially oriented dense bundle of labeled axons. These axons left the injection site and crossed the SGI, SL and SGP to reach the SAP as shown in the DiI experiments (Fig. 1E, 3B). This bundle had a diameter corresponding roughly to that of the injection site and contained structures resembling en passant or terminal boutons (Fig. 4). The distribution of axons containing synaptic-like boutons was charted in one DiI case (Fig. 1, left). Boutons were more present in superficial layers on rostral sections (Fig. 1A′). More caudally, they were present in all collicular layers; most of them being located below the injection site level (Fig. 1D′, G′). Although the lateral spreading of axons was still present in superficial layers (Fig. 1D–F), fewer axons extended laterally within the deep layers (Fig. 3B). The BDA injections led to fewer labeled axons and a shorter range of tracing than DiI. Reconstruction of a BDA injection is shown in Fig. 2. As for DiI injections, labeled axons in rostral sections (i.e. Fig. 2B) were predominantly located in superficial layers and directed toward the lateral part of the SC. In more caudal sections, connections were found between superficial and deep layers (Fig. 2C–E). Individual BDA labeled axons were found running radially from the injection site through the deep collicular layers. Their morphological features were similar to those of the DiI labeled axons, including the presence of presumed synaptic boutons (Fig. 5).

Discussion

This anatomical investigation is the first tracing study on intrinsic connectivity in the human SC. The lipophilic tracer DiI travels anterogradely and retrogradely (Honig and Hume 1989) within axons while BDA (10 k) is believed to be preferentially transported anterogradely

(Reiner et al. 2000). Although the same pattern of labeling was found with both tracers, BDA injections led to less diffusion around the injection site and fewer labeled axons. It cannot be excluded that some DiI labeled axons found in the deep layers were retrogradely labeled and therefore had their origins in the deep layers, sending their axons to the superficial layers. Such deep-layer to superficial-layer connections were shown to exist in the cat but are probably restricted to SGI cells projecting to superficial layers (Behan and Kime 1996). The anterograde properties of BDA may therefore explain in part the fact that fewer labeled axons were found with this tracer.

Horizontally oriented axons within superficial layers may be part of input or output pathways of the SC. Although the input and output terminal patterns of the SC are well known in non-human primates, the exact pathways taken by axons arriving and leaving the SC have not received much attention. Moreover, in the studies of Moschovakis et al (1988a, b), this lateral extension of collaterals in the superficial collicular layers is not described. An early study in cats (Sprague et al. 1975) suggested that cortical input from visual cortical areas enters the SC through its medial brachium, whereas the retinal input enters via its lateral brachium. Because we found a high number of labeled axons forming a bundle oriented toward the lateral brachium of the SC, some of these may be retrogradely labeled retinotectal axons. On the other hand, a portion of these labeled axons may also be part of an intrinsic network within the superficial layers of the SC. This interpretation is supported by the fact that these axons had relatively numerous boutons. Anatomical studies in the cat have shown similar

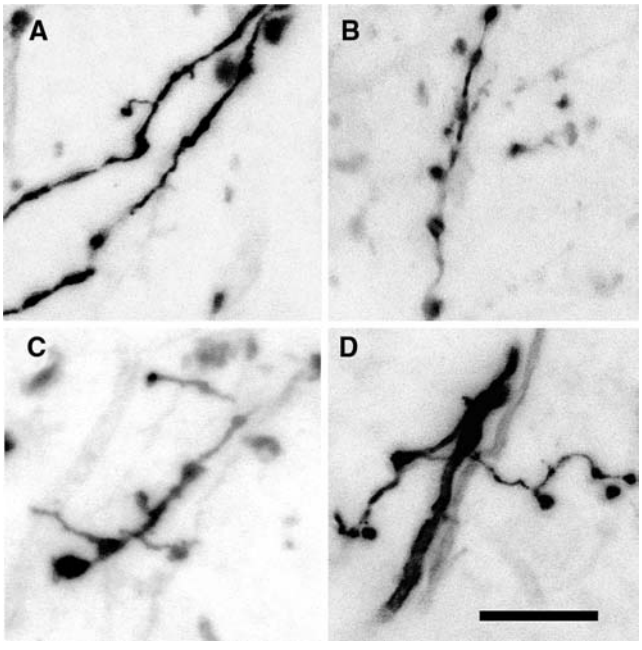

Fig. 4 High magnification confocal photomicrographs of labeled axon segments (represented in *black*) found in the deep collicular layer following a DiI injection in the superficial layers. Photomicrographs were taken in sections adjacent to that of Fig. 1E. Note the presence of axonal branching and en passant and terminal boutons. *Scale bar* 10 μm

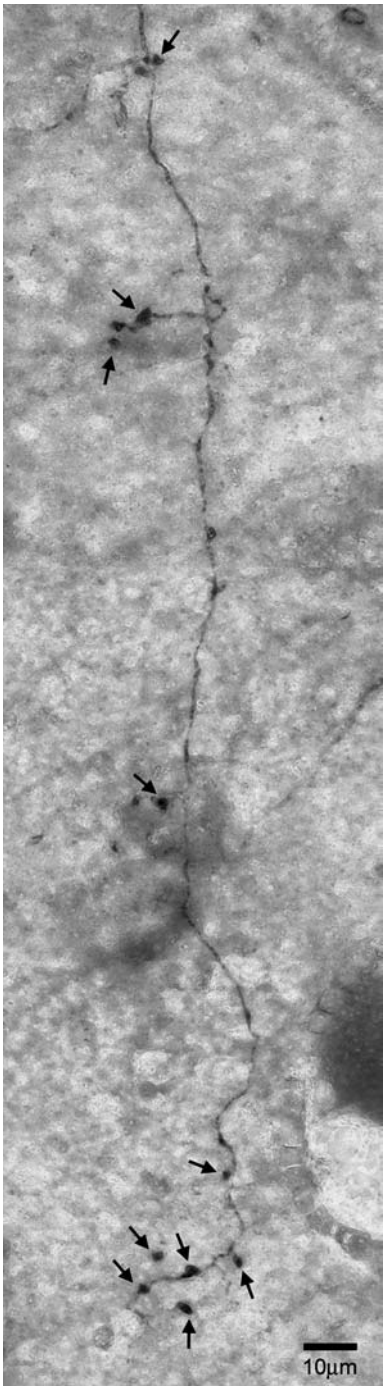

Fig. 5 Photomicrograph of a BDA labeled axon in deep collicular layers following an iontophoretic injection restrained to superficial layers of case 2. The axon is oriented perpendicularly to the pial surface. Note the presence of axonal branching and en passant as well as terminal boutons (*arrows*)

connections within superficial collicular layers (Behan and Appell 1992). Such lateral interactions within superficial layers may enable tectal neurons to integrate visual information from a large part of the visual space, and play a role in shaping the visual receptive fields. They may also underlie a phenomenon described by Rizzolatti et al (1974) where the response of a collicular neuron to visual stimulus is suppressed by the simultaneous presentation of a remote stimulus. The finding that ablation of all cortical areas only partially abolished this inhibitory phenomenon reinforced the hypothesis that is principally supported by intrinsic connectivity within the SC (Buchtel et al. 1979).

Unlike the widespread connections within superficial layers, superficial-to deep-layer connections were only observed in a limited number of sections: those at the level of the injection site or close to it. This suggests that a topographical arrangement of connectivity between superficial and deep collicular layers, as previously demonstrated in a variety of non-human species, may also exists in humans. In non-human primates, the contralateral hemifield is retinotopically represented in the superficial layers of the SC (Cynader and Berman, 1972). Electrical stimulation in the deep collicular layers results in a saccadic eye movement that redirects the fovea toward the part of the visual space represented in the overlying region of the stimulation site (Robinson 1972; Schiller and Stryker 1972). Our anatomical data support the hypothesis that intrinsic interlaminar connectivity may provide a route by which the visual activity in the superficial collicular layers can access the premotor neurons of the deep layer. In addition to this superficial-layer to deep-layer pathway, an intrinsic horizontally oriented collicular circuit exists within SGI; electric stimulation in the SGI may lead to excitatory or inhibitory effects on neighboring SGI neurons (McIlwain 1982; Meredith and Ramoa 1998).

In our study, all injections included the SZ, SGS and SO, which together form the superficial layers. Anatomical investigations in tree shrew suggested that SO is a distinct collicular layer that may act as an intermediate between SGS and the deep layers. The main interlaminar pathway of SGS neurons terminated in SO with only a few terminals in deeper layers (Hall and Lee 1993, 1997; Lee and Hall 1995). Thus, information from superficial layers may reach deep layers either through SO neurons projecting to deep layers or from SGI neurons extending their dendrites into the SO. Although partial invasion of apical dendrites of SGI neurons into SO has been described in cat (Moschovakis and Karabelas 1985) and in monkey (Moschovakis et al. 1988a, b), it seems to be much more limited than in nocturnal rodents (Mooney et al. 1984). In humans, previous Golgi studies in the SC are not informative about such extension of SGI apical dendrites because only neurons of the SGP and SAP were described (Laemle 1983).

The connectivity across collicular layers may also play a role in multisensory orientation. It has been shown in several non-human species that the visual space map present in superficial collicular layers is in register with the auditory receptive fields in deep layers, i.e. when the eyes are directed forward, the visual receptive field location of a visual neuron in superficial layers is related to the best azimuthal response of an auditory neuron immediately located in the underlying deep collicular layers (Wallace et al. 1996; King et al. 2000). Such map alignment may partially underlie the response enhancement or suppression obtained in multisensory neurons of deep collicular layers when spatially coincident visual and auditory stimuli are presented simultaneously (King and Palmer 1985; Frens and van Opstal 1998; Wallace et al. 1998; Bell et al. 2001; Populin and Yin 2002). However, superficial-to deep-layer connections are not the only pathway through, which visual input can reach the deep layers neurons, as suggested by tracing studies in non-human species. Although the retinotopic visual input from occipital cortical areas to the SC is mainly restricted to the superficial collicular layers, some high-order visual areas project directly to the deep collicular layers (Lynch et al. 1985; Huerta et al. 1986; Harting et al. 1991; Lock et al. 2003). In the cat, it has been shown that neurons in high-order multisensory cortical areas do project to multisensory neurons in the SC. However, those corticotectal neurons are modality-specific; suggesting that the multisensory integration observed in the SC is not pre-processed in the cortex, but instead occurs within the SC itself (Wallace et al. 1993).

In conclusion, multisensory neurons in the deep collicular layers receive visual input from two distinct channels, one from high-order cortical areas with imprecise or no retinotopic organization and the other from primary and early visual areas. It is likely that the latter input as well as the retinal input to multisensory neurons requires a superficial-to deep-layer pathway, and that such topographical organization of connections exists in human SC.

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