

A Quantitative Comparison of the Hemispheric, Areal, and Laminar Origins of Sensory and Motor Cortical Projections to the Superior Colliculus of the Cat

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ABSTRACT

The superior colliculus (SC) is a midbrain structure central to orienting behaviors. The organization of descending projections from sensory cortices to the SC has garnered much attention; however, rarely have projections from multiple modalities been quantified and contrasted, allowing for meaningful conclusions within a single species. Here, we examine corticotectal projections from visual, auditory, somatosensory, motor, and limbic cortices via retrograde pathway tracers injected throughout the superficial and deep layers of the cat SC. As anticipated, the majority of cortical inputs to the SC originate in the visual cortex. In fact, each field implicated in visual orienting behavior makes a substantial projection. Conversely, only one area of the

auditory orienting system, the auditory field of the anterior ectosylvian sulcus (fAES), and no area involved in somatosensory orienting, shows significant corticotectal inputs. Although small relative to visual inputs, the projection from the fAES is of particular interest, as it represents the only bilateral cortical input to the SC. This detailed, quantitative study allows for comparison across modalities in an animal that serves as a useful model for both auditory and visual perception. Moreover, the differences in patterns of corticotectal projections between modalities inform the ways in which orienting systems are modulated by cortical feedback. *J. Comp. Neurol.* 524:2623–2642, 2016.

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Across modalities, the principle function of sensory systems is to form an accurate representation of the world around us through the sensation and perception of environmental stimuli. To ensure that potentially relevant stimuli are attended to, a system exists that reorients sensory organs and directs behavioral responses toward appetitive stimuli, such as potential prey, and away from aversive stimuli. Central to this orienting system is the superior colliculus (SC), a midbrain structure composed of multiple layers (Kanaseki and Sprague, 1974). The SC is capable of integrating information from diverse sensory systems (Stein, 1998; King, 2004) with maps of visual and auditory space, and somatosensation across the body surface that are in register across the structure (Stein, 1984; see May, 2006 for a review of SC structure and gross connectivity). More-

over, the intermediate and deep laminae of the SC in the cat have been shown to contain maps of pinna and eye movements that are in register with sensory topographies, suggesting an efficient mechanism for sensorimotor integration (Stein and Clamann, 1981). Indeed, a model of integration that involves the remapping of sensory inputs to motor coordinates, to direct overt

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behavior via a common pathway, appears to hold across species (Sparks, 1988; Stein et al., 1995). Such a pathway has been demonstrated electrophysiologically in monkeys, in which retinal and auditory signals, although initially encoded in different coordinate systems, share a common efferent pathway via the SC for the generation of saccadic eye movements (Jay and Sparks, 1987).

Signal processing in the SC can be modulated by corticotectal projections from the sensory cortex (Diamond et al., 1969; Wallace et al., 1993). Early studies suggested that top-down regulation of SC function was particularly critical for visual orienting relative to other sensory systems (Stein, 1978). However, subsequent studies have reinforced the critical influence of both somatosensory (Clemon and Stein, 1986) and auditory (Meredith and Clemon, 1989) cortices on SC-mediated orienting behaviors. In some species, a differential pattern of innervation has been observed between the medial and lateral poles of the SC. For example, the upper half of the rat's visual field is involved in predator recognition and avoidance and projects to the medial SC, whereas the lower half of the field is involved in prey recognition and projects laterally. These field representations are consequently involved in defensive and approach behaviors, respectively, and have been shown to have largely non-overlapping cortical projections (Comoli et al., 2012). Although this pattern of differential projection would not be expected to hold for larger species, in which the division between behavioral outputs of the visual field representations is much less clear, it nevertheless underscores the importance of the SC for sensorimotor integration and the control of relevant behavior.

A number of studies have attempted to identify or quantify the sources of top-down projections to the SC, typically within a particular sensory modality. Within the visual system, a number of cortical areas have been implicated in regulating orienting behavior. Early studies revealed that deactivation of lower-level striate (area 17) and extrastriate (areas 18 and 19) cortices results in drastic decreases in SC activity in the cat (Wickelgren and Sterling, 1969; Stein, 1978). Similar deficits are observed following deactivation of areas along the banks of the posterior middle suprasylvian sulcus, including the posteromedial and posterolateral lateral suprasylvian areas (PMLS and PLLS, respectively; Lomber et al., 1994b). Indeed, anatomical evidence of direct corticotectal projections between each of these areas and the SC has been demonstrated (Harting et al., 1992).

As in the visual system, deactivation of at least one of the core areas of the cat auditory cortex (the primary

auditory cortex; A1) results in a deficit in spatial orienting behavior (Lomber et al., 2007a; Malhotra et al., 2008). Moreover, similar deficits are observed following deactivation of the dorsal zone (DZ), posterior auditory field (PAF), and auditory field of the anterior ectosylvian sulcus (fAES; Lomber et al., 2007a; Malhotra et al., 2008). Anatomical examinations of corticotectal projections from auditory areas have been limited in scope, or have provided contradictory results. A robust projection from the fAES to the SC has been described (Meredith and Clemon, 1989; Chabot et al., 2013), and it was noted that stimulation of the fAES drove auditory neurons of the SC, whereas this was not the case for any other field of the auditory cortex (Meredith and Clemon, 1989). However, some studies note sizeable projections to the SC from other nonprimary auditory fields, including the second auditory cortex (A2; Winer et al., 1998).

The cortical areas implicated in somatosensory orienting behavior include both primary (Burton and Sinclair, 2000) and secondary areas (Burton et al., 1997). Early electrophysiological studies demonstrated that stimulation of somatosensory cortex elicits field potentials (Tamai, 1973) and unit responses (Kassel, 1982) in the ipsilateral SC (but see Stein, 1978). Previous anatomical examinations of somatosensory projections to the SC of the cat found only weak projections from primary (S1) and secondary (S2) somatosensory cortices (Stein et al., 1983). Instead, input has been shown to be dominated by the fourth somatosensory area (S4; Stein et al., 1983; McHaffie et al., 1988), and areas located along the rostral suprasylvian sulcus (rSS), including parts of the medial division of the second (S2m), and the fifth somatosensory (S5) areas (Clemon et al., 2007). Somatosensory representations within the S4 and along the rSS show a topographical relationship to somatosensory receptive fields in the SC, and these cortical areas have been shown to modulate the responses of SC neurons (Clemon and Stein, 1984, 1986), including descending output neurons of the SC that are directly involved in initiating orienting behaviors (Wallace et al., 1993). Indeed, electrical stimulation in and around area S4 in the unrestrained cat appears to elicit gaze shifts, reaching movements of the contralateral limb, and adjustments in body posture (Jiang and Guitton, 1995). Although a number of the studies described above have addressed the top-down connectivity between sensory cortices and the SC, it remains difficult to interpret these projections in a holistic manner, as these studies have typically been undertaken within a single modality, and across a number of species (Table 1). The few studies that have looked across visual, auditory, and somatosensory modalities were limited to descriptive discussions. Moreover, those

studies that have attempted to provide more quantitative analyses often fail to describe the source of corticotectal projections with respect to sensory system (Manger et al., 2010), or provide a comparison across hemispheres (Powell, 1976; Tortely et al., 1980; Galetti et al., 1981; Berman and Payne, 1982; Wallace et al., 1993). Thus, the current study aims to compare the bilateral patterns of corticotectal projections arising from auditory, visual, somatosensory, motor, and limbic cortices of the cat. The retrograde tracer wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) is employed to allow for ease of comparison with previous studies. Although it is anticipated that the visual cortex will dominate the corticotectal projections to the SC, the current approach will reveal the principal auditory and somatosensory inputs and, importantly, allow for within-animal comparison across modality and cortex of origin.

MATERIALS AND METHODS

Five adult (>6 months old) female domestic cats were examined. All the cats were obtained from a US Department of Agriculture (USDA)-licensed commercial animal breeding facility (Liberty Laboratories, Waverly, NY). All surgical and experimental procedures were conducted in accordance with the US National Research Council's *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* and the Canadian Council on Animal Care's *Guide to the Care and Use of Experimental Animals* and were approved by the Western University Animal Use Subcommittee of the University Council on Animal Care.

Surgical procedures

The afternoon prior to each surgical procedure, cats were fasted, lightly anesthetized with ketamine (4 mg/kg, i.m.) and Domitor (Zoetis, Florham Park, NJ; 0.05 mg/kg, i.m.), and an indwelling catheter was inserted into the cephalic vein to deliver antiinflammatory medication (dexamethasone, 0.05 mg/kg, i.v.). On the day of surgery, each cat was given atropine (0.02 mg/kg, s.c.) to minimize respiratory and alimentary secretions, acepromazine (0.02 mg/kg, s.c.), an additional dose of dexamethasone (0.5 mg/kg, i.v.), and buprenorphine (0.01 mg/kg, s.c.). Sodium pentobarbital (25 mg/kg to effect, i.v.) was administered through the catheter to induce general anesthesia. The cat was positioned in a stereotaxic apparatus and prepared for surgery using aseptic procedures. Body temperature, respiration rate, and heart rate were monitored throughout the procedure. Temperature was maintained at 37°C using a water-filled heating pad (Gaymar, Orchard Park, NY).

A midline incision was made and the temporalis muscle was reflected laterally. A large craniotomy was made over the marginal, posterolateral, and middle suprasylvian gyri of the left hemisphere. The bone piece was removed and stored in sterile saline for later replacement, and the dura was reflected laterally. Mannitol (25 mg/kg, i.v.) was infused to make the cerebrum more malleable and to permit lateral displacement. This approach has been repeatedly shown to permit direct visualization of the dorsal surface of the SC, just anterior to the bony tentorium (Lomber et al., 2001, 2002, 2007b; Chabot et al., 2013). To better visualize the dorsal surface of the SC, the posterior half of the splenium of the corpus callosum was ablated by aspiration.

Abbreviations

A1	Primary auditory cortex	PMLS	Posteromedial lateral suprasylvian area
A2	Second auditory cortex	PS	Posterior suprasylvian area
AAF	Anterior auditory field	Psb	Postsubiculum
AEV	Anterior ectosylvian area	Rsp	Retrosplenial area
ALLS	Anterolateral lateral suprasylvian area	S1	Primary somatosensory cortex
AMLS	Anteromedial lateral suprasylvian area	S2	Second somatosensory area
ca	Anterior commissure	S3	Third somatosensory area
CgA	Anterior cingulate area	S4	Fourth somatosensory area
CgP	Posterior cingulate area	S5	Fifth somatosensory area
CVa	Cingulate visual area	S*	Somatosensory representation on the lateral bank of the anterior ectosylvian sulcus
DLS	Dorsal lateral suprasylvian area	SC	Superior colliculus
dPE	Dorsal posterior ectosylvian gyrus	SGI	Stratum griseum intermediale
DZ	Dorsal zone of auditory cortex	SGP	Stratum griseum profundum
EPp	Posterior posterior ectosylvian field	SGS	Stratum griseum superficiale
fAES	Auditory field of the anterior ectosylvian sulcus	SVA	Splenial visual area
FRS	Multisensory-auditory field of the rostral suprasylvian sulcus	T	Temporal auditory cortex
IC	Inferior colliculus	V1	Primary visual cortex
Ipe	Intermediate posterior ectosylvian gyrus	VAf	Ventral auditory field
IN	Insular auditory cortex	VLS	Ventral lateral suprasylvian area
MGB	Medial geniculate body	VPAF	Ventral posterior auditory field
mSS	Middle suprasylvian sulcus	vPE	Ventral posterior ectosylvian gyrus
PAF	Posterior auditory field	WGA-HRP	Wheat-germ agglutinin conjugated to horseradish peroxidase
Pag	Periaqueductal gray	wm	White matter
PLLS	Posterolateral lateral suprasylvian area		

TABLE 1.
Summary of Corticotectal Connectional Studies

Species	Authors	Modality			Antero from cortex	Retro from SC	Quantified	Compared
		Visual	Auditory	Somatosensory				
Cat	Holländer, 1974	X				X	No	No
Cat	Magalhães-Castro et al., 1975	X				X	No	No
Cat	Updyke, 1977	X			X		No	No
Rat	Wise and Jones, 1977			X	X	X	No	No
Cat	Kawamura et al., 1978	X				X	No	No
NWM	Graham et al., 1979	X			X		No	No
Rat	Sefton et al., 1981	X				X	No	No
OWM	Leichnetz et al., 1981	X			X	X	No	No
NWM	Tigges and Tigges, 1981	X			X		No	No
Cat	Baleydier et al., 1983	X			X	X	No	No
Cat	Stein et al., 1983			X	X	X	No	No
Cat	Segal and Beckstead, 1984	X			X	X	No	No
NWM	Cusick, 1988	X			X		No	No
Rat	Harvey and Worthington, 1990	X			X		No	No
Cat	Norita et al., 1991	X			X	X	No	No
Cat	Harting et al., 1992	X			X		No	No
OWM	Lui et al., 1995	X			X		No	No
Opossum	Martinich et al., 2000	X				X	No	No
Cat	McHaffie et al., 2001	X			X	X	No	No
Cat	Baleydier, 1977	X	X			X	No	No
Cat	Kawamura and Konno, 1979	X	X	X		X	No	No
Cat	Tortely et al., 1980		X	X		X	No	No
Rat	Thong and Dreher, 1986	X	X	X		X	No	No
OWM	Lock et al., 2003	X	X	X		X	No	No
Tree shrew	Baldwin et al., 2013	X	X	X		X	No	No
Cat	Meredith and Clemo, 1989		X		X	X	Yes (Retro)	No
NWM	Collins et al., 2005	X				X	Yes	No
Rat	Hoffer et al., 2005			X	X		Yes	No
Ferret	Bajo et al., 2010		X		X	X	Yes (Antero)	No
Cat	Chabot et al., 2013		X			X	Yes	No
OWM	Fries, 1984	X	X	X		X	Yes	No
Tree shrew	Casseday et al., 1979	X	X	X		X	No	Yes
Hedgehog	Künzle, 1995	X	X	X		X	No	Yes
Tree shrew	Baldwin et al., 2013	X	X	X		X	No	Yes
NWM	Baldwin and Kaas, 2012	X	X	X		X	No	Yes
Ferret	Manger et al., 2010	X	X	X		X	Yes	Yes

Abbreviations: SC, superior colliculus; OWM, Old World monkey; NWM, New World monkey.

Severing the posterior end of the corpus callosum permitted the injection pipette to enter the SC orthogonal to its dorsal surface.

Tracer deposits

WGA-HRP was deposited into the left SC of five cats. WGA-HRP has previously been demonstrated to be a highly sensitive retrograde neuronal tracer (particularly when used in conjunction with a tetramethyl benzidine [TMB] protocol); it has been used previously to successfully quantify corticotectal inputs in the cat (Chabot et al., 2013), and allows for direct comparison with the majority of anatomical examinations of the cortical projections to the SC. Each animal received three penetrations, and two separate injections were made at each penetration between 1,000 and 2,000 μm below the

surface of the SC to optimally inject both the superficial and deeper layers (Fig. 1). Each of the six total injections was made with a microliter syringe (Hamilton, Reno, NV), and consisted of 0.025–0.050 μl of 5% WGA-HRP pressure injected through a 30–35 μm -diameter pipette tip. Any leakage of WGA-HRP tracer over the surface of the SC during the injections was removed with sterile saline flushes to prevent tracer contamination of surrounding brain tissue. Following each deposit, the pipette remained stationary for 5 minutes. As is true of any protocol involving penetrations into neural tissue, there was likely some uptake of tracer by damaged fibers of passage in the current study; however, our protocol aims to minimize this uptake through the combination of multiple, small-volume injections and by allowing sufficient time

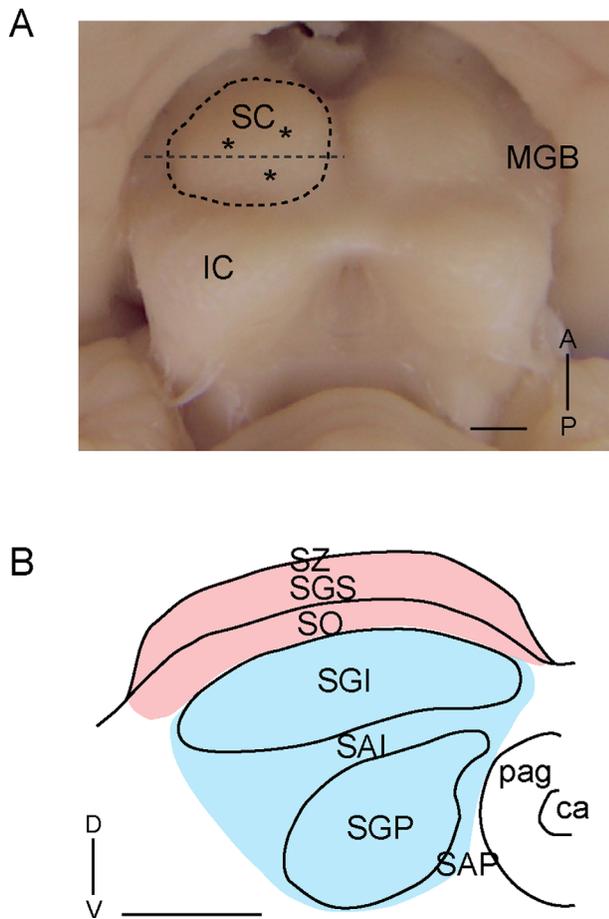


Figure 1. Injection locations and layers of the superior colliculus. **A:** Dorsal view of the mesencephalon: superior colliculus (SC) and inferior colliculus (IC). The anteroposterior axis is indicated at the bottom right. The dashed line bisecting the SC represents the level at which the SC is presented in B. The three asterisks indicate locations of the penetrations. **B:** The superior colliculus contains alternating gray and white layers. From dorsal to ventral, these layers are: the stratum zonale (SZ), superficial gray layer (SGS), stratum opticum (SO), intermediate gray layer (SGI), intermediate white layer (SAI), deep gray layer (SGP), and stratum album profundum (SAP). The superficial zone (pink shading; SZ, SG, and SO) receives the visual inputs, whereas the deep zone (blue shading; SGI, SAI, SGP, and SGP) receives multisensory inputs. For abbreviations, see list. Scale bar = 500 μ m in A,B.

between injections. When all three penetrations were complete, the bone piece was replaced and secured with dental acrylic and stainless steel skull screws. Dermal incisions were sutured with 3-0 silk.

Postsurgical procedures

The indwelling catheter was removed, and half-strength lactated Ringer's solution (20 ml/kg, s.c.) was administered as needed during the first 4 hours following surgery. Heart rate, respiratory rate, and tempera-

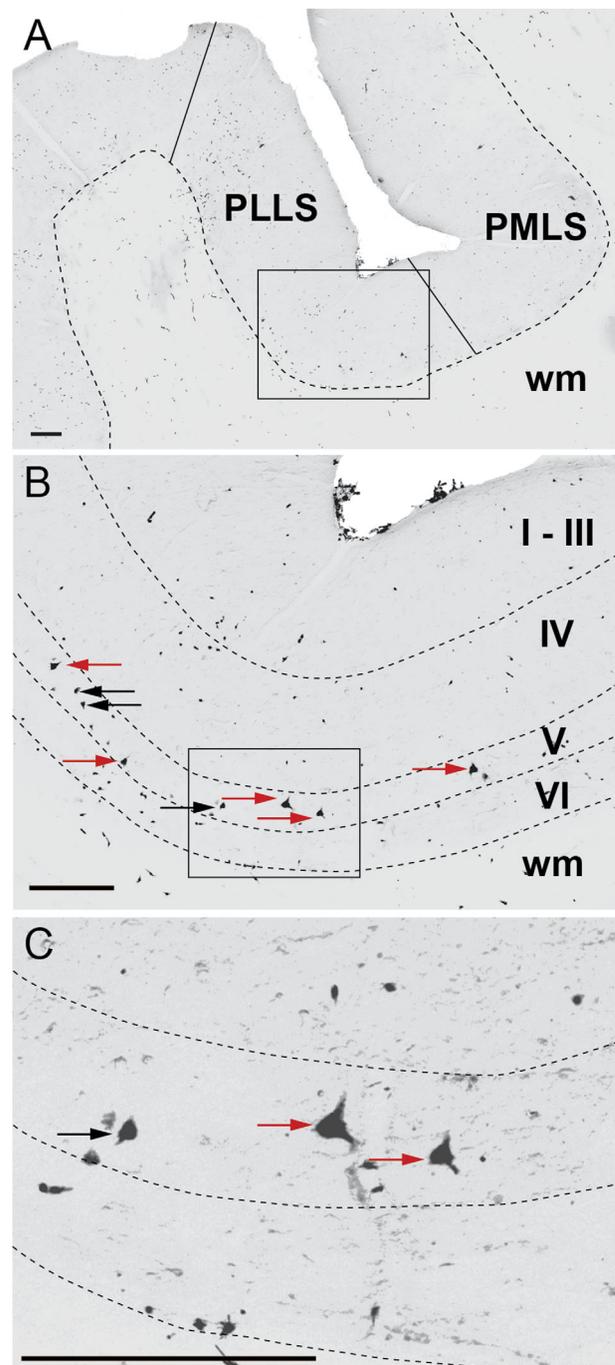


Figure 2. Labeled neurons in the posterolateral lateral suprasylvian (PLLS) area. The dashed lines indicate the boundaries between gray and white matter (**A**) or between cortical layers (**B,C**). The red arrows indicate labeled neurons. To be considered a labeled neuron, the nucleus and the entirety of the somatic membrane had to be present. The black arrows point to neurons that are too faintly labeled, and were not included in the count. For abbreviations, see list. Scale bar = 50 μ m in A-C.

ture were continually monitored until the animal was sternally recumbent. The analgesic buprenorphine (0.01 m/kg, s.c.) was administered every 6 hours for

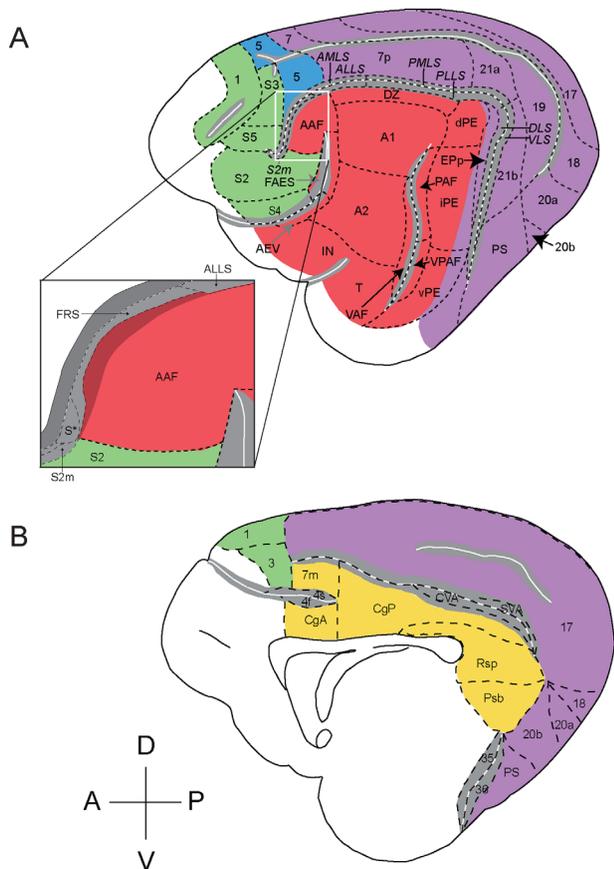


Figure 3. Schematic views of the cat cerebrum showing the visual, auditory, somatosensory, motor, and limbic cortical areas. **A:** Lateral view of the cat brain showing visual (purple), auditory (red), somatosensory (green), and motor areas (blue). Sulci (gray) are open to represent the sulcal cortex. Inset provides detailed structure of the anterior ectosylvian sulcus, and is adapted from Clemo et al. (2007). Dashed lines indicate cortical area borders. Dorsoventral and anteroposterior axes are indicated at bottom left. **B:** Medial view of the cat brain with the same general organization as in A. Limbic areas are presented in yellow. For abbreviations, see list.

the first 24 hours, and every 12 hours for the subsequent 24 hours. Animals also received the systemic antibiotic Convenia (Zoetis; 8 mg/kg, s.c.) to guard against possible infection. Cats received dexamethasone (0.05 mg/kg, s.c.) every 24 hours for 2 days after surgery. In all cases, recovery was uneventful.

Perfusion and tissue processing

Forty-eight hours following tracer injections, the cephalic vein was cannulated, and the cat was deeply anesthetized (sodium pentobarbital, 25 mg/kg i.v). The anticoagulant heparin (10,000 U; 1 ml), and the vasodilator 1% sodium nitrite (1 ml) were administered. The cat was then perfused through the ascending aorta with physiological saline for 5 minutes. Next, aldehyde

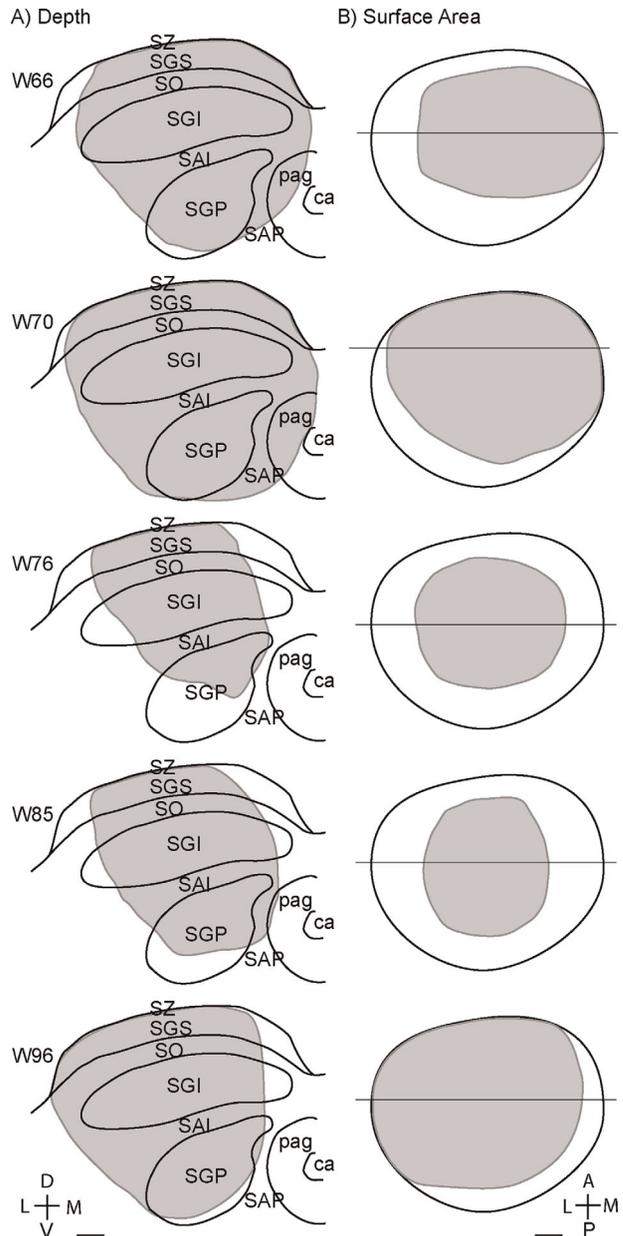


Figure 4. Schematic representations of the five cases in the present study, presented on a standardized superior colliculus. **A:** The tracer spread across the coronal plane of the SC for each animal. **B:** The tracer spread along a dorsal view in the same animals. Note that the tracer spread throughout the superficial and deep layers of the superior colliculus. Tracer spread ventromedially into the periaqueductal gray (pag) in two cases, but there is no evidence of spread into the contralateral SC or ipsilateral pretectum in any case. For abbreviations, see list. Scale bar = 500 μ m in A,B.

fixatives (1% paraformaldehyde/1.5% glutaraldehyde) were perfused for 20 minutes, followed by 10% sucrose for 5 minutes. All solutions were buffered to a pH of 7.4 with 0.1 M Sorenson's buffer and infused at a rate of 100 ml/min. Immediately following the perfusion, the head was mounted in a stereotaxic frame. The brain

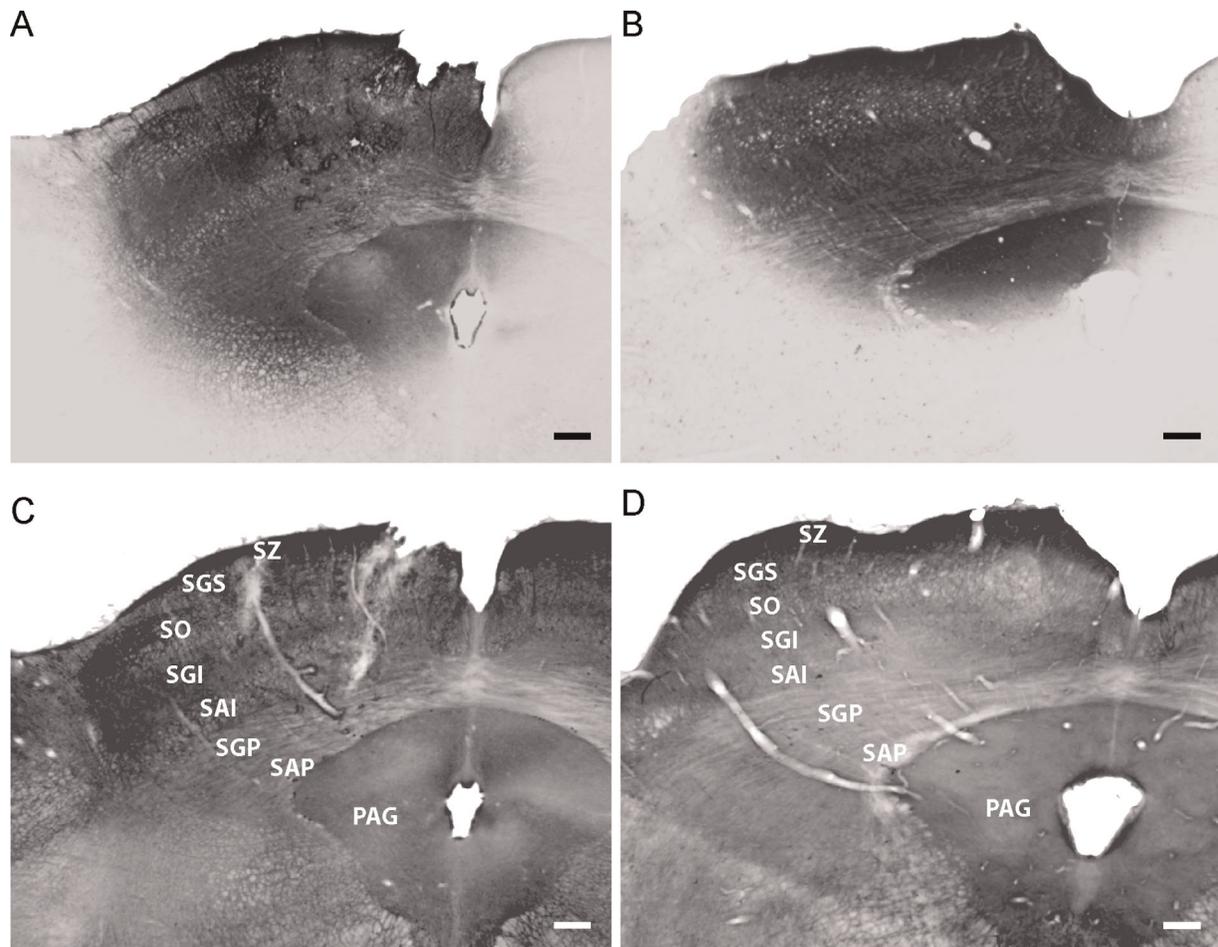


Figure 5. Photomicrographs of coronal sections passing through the superior colliculus of cases W70 (A,C) and W66 (B,D). A and B depict the injection sites, and C and D present photomicrographs from the cytochrome oxidase series with layers of the superior colliculus indicated. A track left by the Hamilton syringe is visible in C. Note that the WGA-HRP tracer spread into all the layers of the superior colliculus, with some spread into the periaqueductal gray in these two cases only. For abbreviations, see list. Scale bar is 500 μ m in A–D.

was then exposed, blocked in the coronal plane at Horsley–Clarke level A25 (for review, see Schurr and Merrington, 1978), and removed from the cranium. Each brain was photographed to provide a permanent record, and immersed in 30% sucrose in 0.1 M Sorenson's buffer until it sank, to cryoprotect it for histological processing.

Brains were frozen, and sections were cut in the coronal plane at 60 μ m using a freezing microtome, and collected serially through the entire brain. Six series of sections at 360 μ m intervals were created. Individual series were processed for: 1) HRP–diaminobenzidine (HRP–DAB) intensified with cobalt–nickel (Adams, 1981); 2) HRP–tetramethyl benzidine (HRP–TMB) histochemistry (Mesulam, 1978; as modified by Olucha et al., 1985); 3) cytochrome oxidase (Payne and Lomber, 1976); and 4) cresyl violet (Nissl stain). The remaining series were extras and were discarded after all tissue was success-

fully mounted onto gelatin-coated slides, air-dried, cleared, and coverslipped.

Data analysis

Tissue was analyzed using standard light/darkfield microscopy with a Nikon E600 microscope mounted with a DXM 1200 digital camera. The contours of sections and the injection sites were traced, and labeled cells were examined, quantified, and plotted using Neurolucida software (MicroBrightfield, Williston, VT; RRID:nif-0000-10294). The quantification was performed using the tissue series processed for HRP–TMB due to the sensitivity of this approach, and used an exhaustive search paradigm that ensured all tissue was examined and labeled cells were identified. An additional series from each animal was reacted using the HRP–DAB procedure as a backup (the DAB reaction is more stable over time than TMB); however, in all cases the series processed using

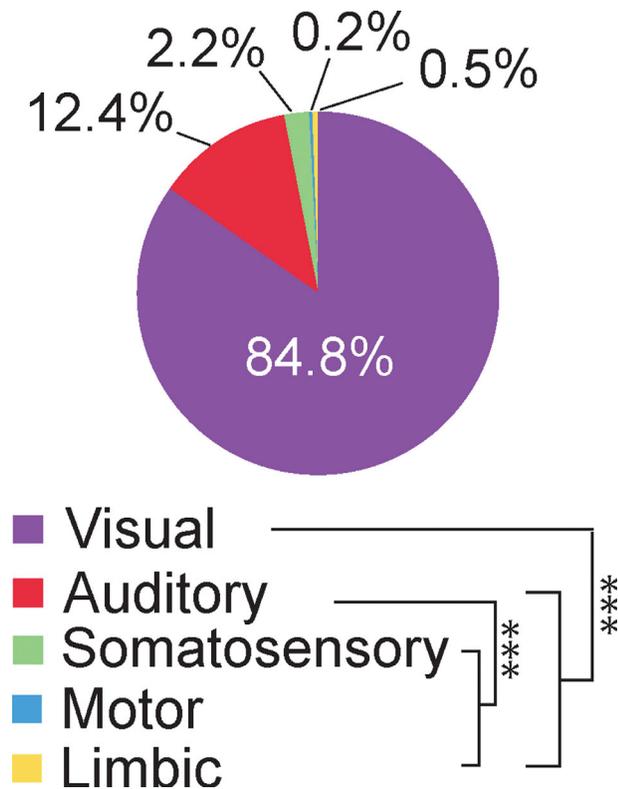


Figure 6. Pie graph illustrating the distribution of corticotectal projections by modality in the cortex. The percentages of labeled cells arising from each modality are presented on the graph. The magnitude of corticotectal projection differed significantly by modality ($F(4,16) = 344.53$, $P < 0.001$). The results of corrected pairwise comparisons are indicated (***) denotes $P < 0.001$.

the HRP-TMB protocol were reacted and quantified successfully, and thus the HRP-DAB series were ultimately redundant. Photomicrographs presented in this manuscript were adjusted for brightness and contrast using Adobe (San Jose, CA) Photoshop 12.1 (RRID:SciRes_000161), but were not otherwise altered.

For a neuron to be considered HRP-positively labeled and not merely an artifact of the reaction process, the nucleus had to be visible and the entirety of the somatic membrane had to be present (Fig. 2). Portions of a cell or remnants of membrane were not counted. When labeled cells were determined to lie on the border of two cortical areas, or within a transitional zone between areas, the cells were equally distributed between the two areas. The focus of the microscope lens was adjusted throughout the z-plane to ensure that the full thickness of each section was examined.

Labeled neurons were assigned to cortical areas on an individual-animal basis based on cytoarchitecture and sulcal and gyral landmarks defining areal borders. The cortical area and layer in which each labeled cell

was located were determined from superimposed images of adjacent sections stained for the presence of Nissl or cytochrome oxidase. Borders between the posterior lateral suprasylvian areas (PLLS and PMLS), and the dorsal and ventral lateral suprasylvian areas (DLS and VLS) of the visual cortex were placed on the lateral bank of the middle suprasylvian sulcus and the dorsal bank of the posterior limb of the suprasylvian sulcus, respectively (as per Palmer et al., 1978, Updyke, 1986, Rauschecker et al., 1987). This convention is supported by cytoarchitectonic methods in the visual system (van der Gucht et al., 2001). Overall, previously published cytoarchitectonic descriptions were utilized to determine boundaries between auditory, visual, somatosensory, motor, and limbic areas (Sanides, 1969; Meredith and Clemo, 1989; Clascá et al., 1997, 2000; Winer and Prieto, 2001). Figure 3 presents lateral and medial views of the cat cerebrum showing these delimitations with abbreviations based on the wide body of literature examining different sensory and motor fields of the cat cortex. A univariate analysis of variance (ANOVA) was performed with each cortical field projecting to the SC included, and a Tukey's HSD test was performed to compute all pairwise comparisons between fields. The same tests were also applied to compute the relative strength of projections at a modality level.

RESULTS

Tracer injections site and tracer spread

Five cats received deposits of WGA-HRP that spread throughout the superficial and deep layers of the left SC. The injection sizes were very similar to those of Meredith and Clemo (1989) and Chabot and colleagues (2013). The extents of injections in all five animals are illustrated on the coronal plane (Fig. 4A) and on a dorsal view of the SC (Fig. 4B). In all cases, the tracer was exposed to axon terminals in the stratum griseum superficiale (SGS), stratum griseum intermediale (SGI), and stratum griseum profundum (SGP; Fig. 5) and covered the majority of the SC (Fig. 4B). In two cases, the tracer spread into the periaqueductal gray (Fig. 4A, W66 and W70), whereas there was no evidence of spread into any portion of the pretectal nuclei or the inferior colliculus in any case. There was also no evidence of tracer spread to the contralateral SC.

Profile of labeling by modality, hemisphere, and layer

All neurons in the cerebral cortex showing positive labeling were counted. On an individual-animal basis, the number of labeled neurons in each cortical area was expressed as a percentage of the total number of

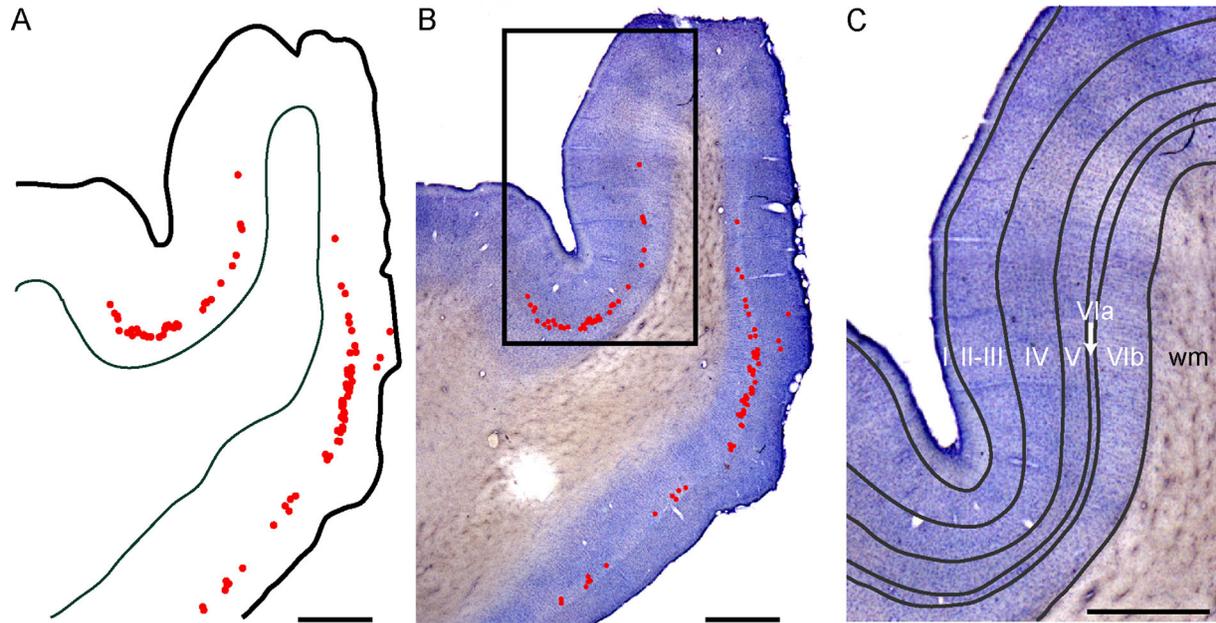


Figure 7. Determining layer-specific origins of corticotectal projections. **A:** Location of all labeled neurons in a partial cortical section. The outline of the section is shown as a thick black line, and the gray/white matter border is shown as a thin gray line. Labeled neurons are plotted as red dots. **B:** An adjacent Nissl-stained section. The labeled cells from A have been superimposed to identify their laminar location. **C:** A detailed view of the region outlined by the black box; laminar borders are denoted with gray lines. wm = white matter, scale bar = 1 mm.

labeled cells in the entire cortex to allow for meaningful conclusions despite variability in tracer uptake and spread, as well as in the tissue processing used to visualize labeled neurons.

As expected, the greatest direct projection to the SC emerged from the visual cortex (84.8%), with much smaller inputs from the auditory (12.4%), somatosensory (2.2%), motor (0.2%), and limbic cortices (0.5%; Fig. 6). The limbic projections arose from the anterior and posterior cingulate areas and accounted for 0.5% of all labeled cells (areal data not plotted). Labeled cells originated overwhelmingly from the infragranular layers (99.6%) of the ipsilateral cortex (93.4%). Infragranular projections arose almost entirely from layer V with few labeled cells present in layer VI. Although no further subdivision of cortical layers was explicitly made in the current study, labeled cells in layer VI appeared to be predominantly located in the VIa, suggesting an association with corticocortical projections. An example of this pattern of labeling and laminar delineation is provided in Figure 7. The sparse supragranular labeling observed was confined to layer II, and presented no discernible areal pattern of distribution. Thus a specific layer-wise comparison was not undertaken due to the lack of statistical power resulting from layers with few (layers II and VI) or no (layers I, III, and IV) labeled cells. Instead, cells were considered to be either supragranular or infragranular in nature to allow for a

gross comparison of the laminar origin of cortical projections.

Profiles of labeling across cortical fields

A representative plot of labeled cells in one animal (W76) is presented in Figure 8. Labeling in the visual cortex covered the entirety of the visual field, from the representation of the fovea at the occipital pole to the most peripheral point of the visual field at the anterior portion of the visual cortex. Interestingly, the areas involved in visual orienting behavior, namely, areas 17, 18, 19, and the PMLS and PLLS, showed the strongest projections to the SC. The primary visual cortex (area 17) was the predominant source of projections to the SC, making up 36% of labeled cells across animals. Labeling was heaviest at the posterior aspect (upper visual field), with fewer labeled cells toward the anterior end (lower visual field). The next largest projections arose from the PLLS (13%), area 18 (9%), area 19 (7%), and the PMLS (7%; Fig. 9). Labeling in areas 18 and 19 was heaviest anteriorly, whereas the PMLS, PLLS, and the anterolateral and anteromedial lateral suprasylvian areas (ALLS and AMLS, respectively) were labeled uniformly along the anteroposterior axis. The remaining areas of the visual cortex are not involved in orienting behavior, and each made up less than 3% of the total labeled cells.

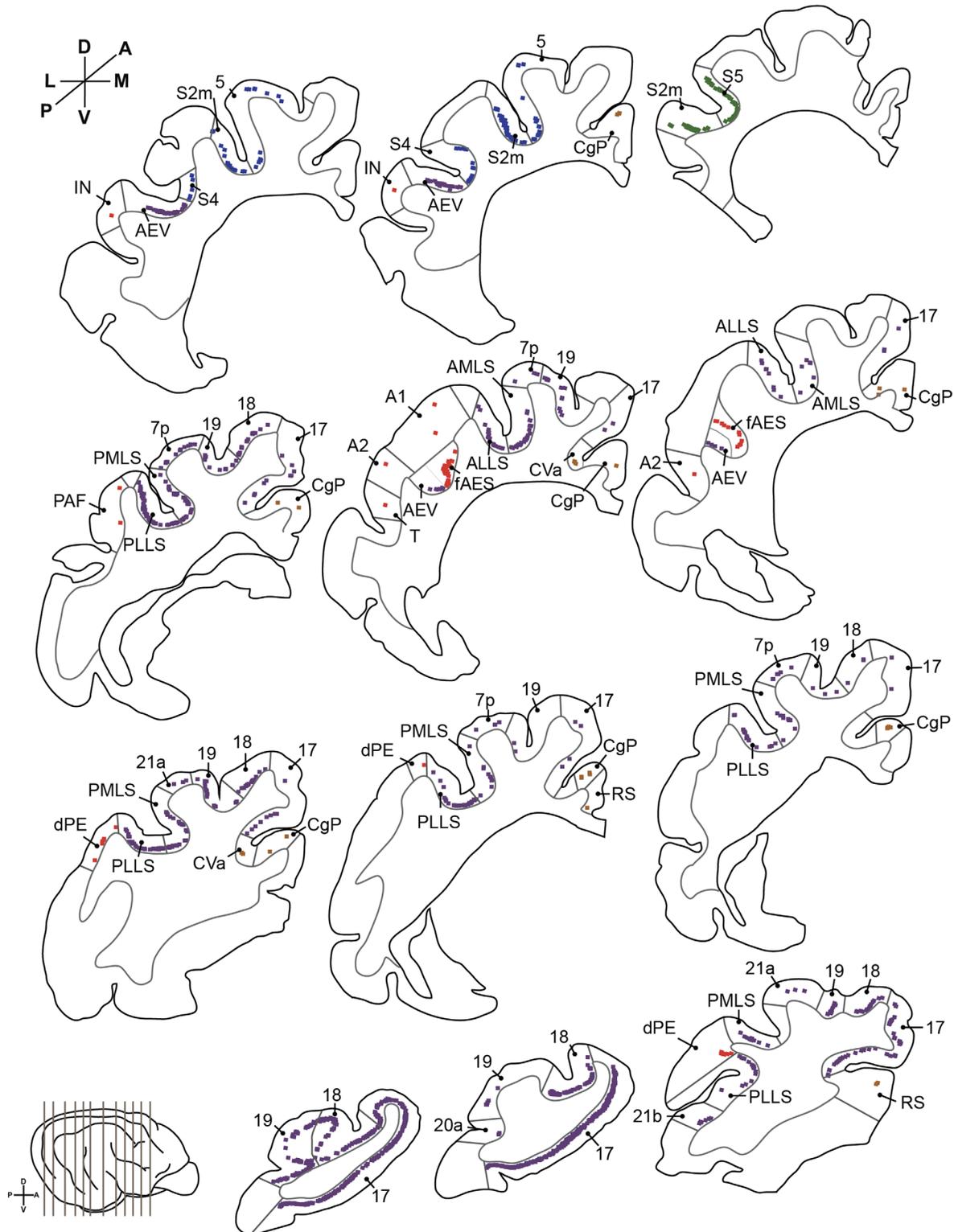


Figure 8. Representative distribution of visual (purple), auditory (red), somatosensory (green), motor (blue), and limbic (brown) corticotectal projections. The lateral schematic of the brain (lower left) shows the levels from which these mapped coronal sections through the cortex were taken. Note that visual areas 17, 18, 19, 20, 21, PLLS, PMLS, ALLS, AMLS, and area 7 are all labeled, with the most substantial labeling in the primary visual cortex (area 17). Labeled cells cover the entirety of area 17 from posterior to anterior. Contrary to the primary visual cortex, the primary auditory cortex is poorly labeled, as are the PAF and DZ. Labeled cells in the AI and PAF are found primarily in their posterior extent, adjacent to the dPE and iPE. Conversely, the fAES is heavily labeled following a superior colliculus injection. Similar to the auditory system, the S1 (areas 1 and 3) is devoid of labeling, with somatosensory cells projecting to SC confined to S2. For abbreviations, see list.

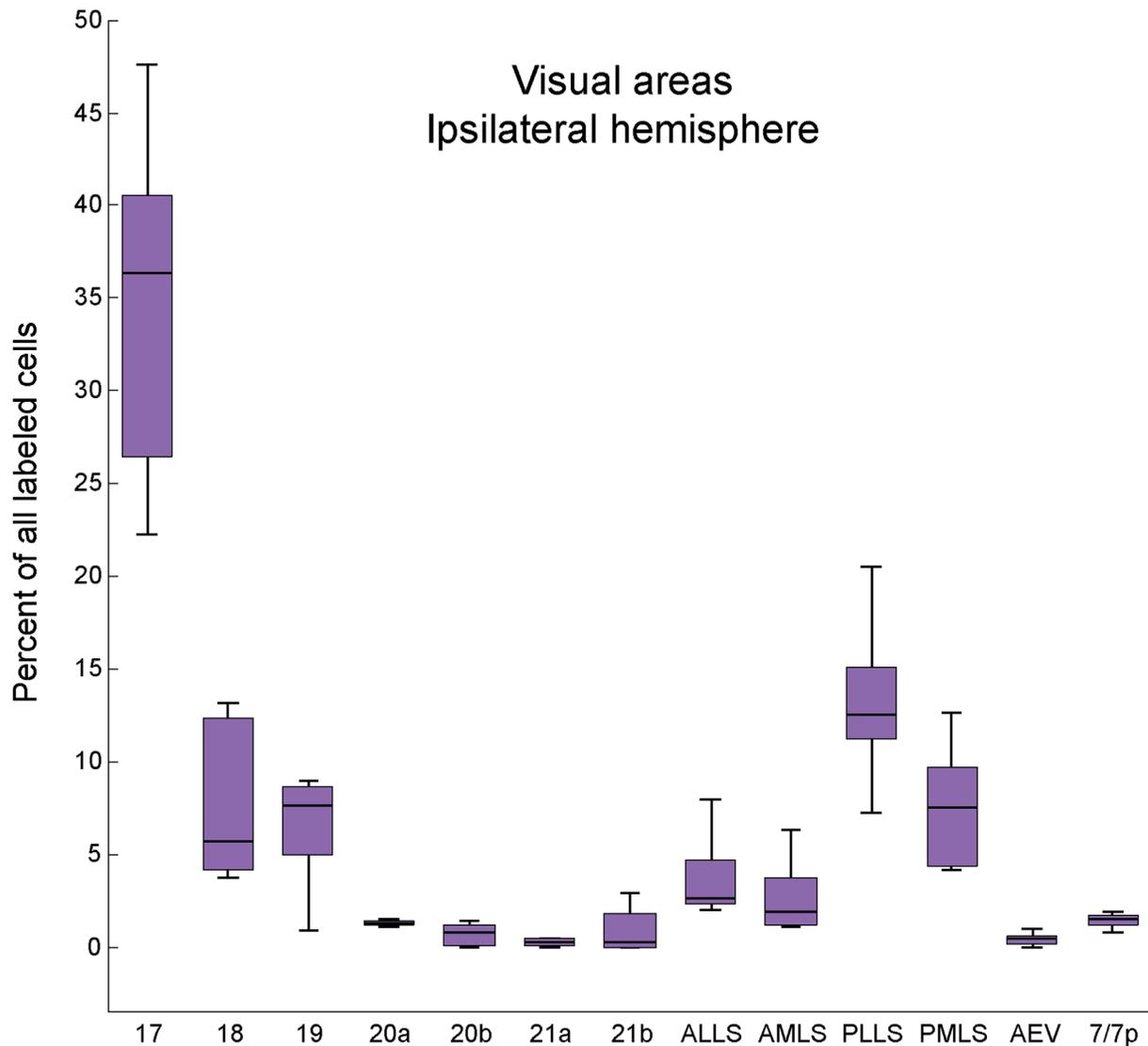


Figure 9. Box-and-whisker plot illustrating the distribution of visual corticotectal projections by cortical area. The y-axis indicates the percentage of labeled neurons, and whiskers extend a maximum of 1.5 times the interquartile range. The two major connections from the visual cortex originate from the primary area 17 (median = 36.4%) and PLLS (median = 12.6%). The other visual areas involved in visual orienting behavior (18, 19, PMLS) show weaker connections with the superior colliculus (median of 5.7%, 7.6%, and 7.5%, respectively). For abbreviations, see list.

Although labeling in the auditory cortex was sparse relative to visual areas, projections arose from 9 of the 13 auditory fields. Neuronal labeling was particularly abundant in the auditory field of the anterior ectosylvian sulcus (fAES; 4.7%) relative to other auditory fields (all < 1%; Fig. 10). The remaining labeled cells were located in dorso-posterior areas, with more ventral fields showing little to no labeling. Interestingly, the primary auditory cortex (A1), the posterior auditory field (PAF), and the dorsal zone (DZ) showed very weak projections to the SC, with labeled cells confined to their posterior limits, adjacent to the dorsal and intermediate portions of the posterior ectosylvian sulcus (fields dPE and iPE, respectively).

In somatosensory and motor cortices, labeled neurons were observed in a small number of fields. These fields included the second (0.6%), fourth (0.5%), and fifth (0.5%) somatosensory areas (S2, S4, and S5, respectively), area 4 (0.2%), and area 5 (0.6%; Fig. 11). As in the auditory cortex, the primary somatosensory cortex has been implicated in orienting behavior, but does not have a substantial direct projection to the SC. The remaining fields involved with orienting, S2 and area 5, showed weak projections to the SC. It should be noted that blocking the brain at Horsley–Clarke level A25 prior to sectioning, as described above, precluded the quantification of labeled cells in the most anterior

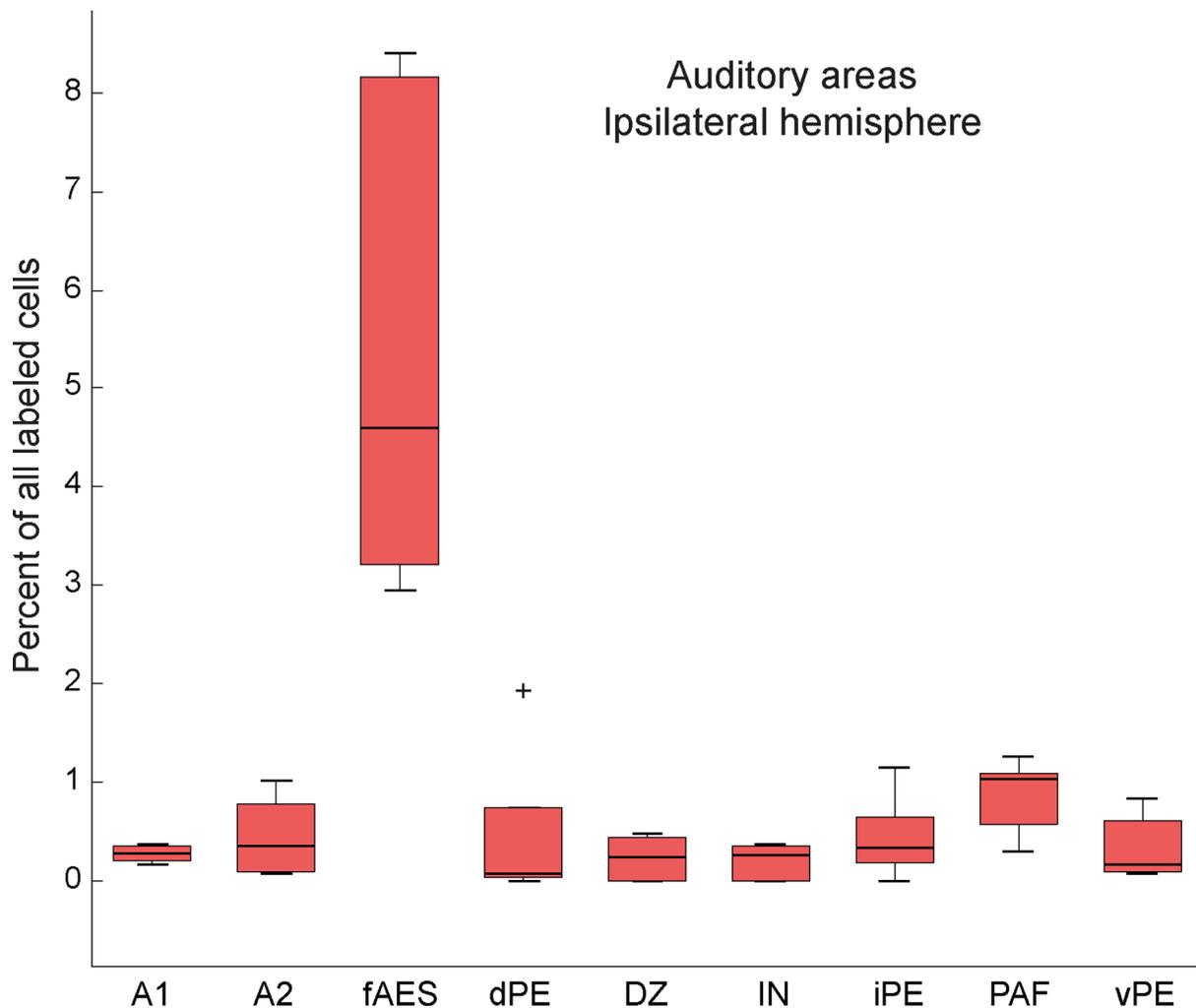


Figure 10. Box-and-whisker plot illustrating the distribution of auditory corticotectal projections by cortical area. The y-axis indicates the percentage of labeled neurons, whiskers extend a maximum of 1.5 times the interquartile range, and outliers are denoted by a black cross. The majority of the auditory cortical areas involved in the acoustic orienting behavior (A1, DZ, and PAF) do not show substantial direct axonal projections to the superior colliculus. However, the fAES is a significant source of projections to the superior colliculus (median = 4.6%). For abbreviations, see list.

fields of cat cortex. This includes areas that would be expected to have substantial inputs to the SC, including the medial portion of area 6, which has been shown to contribute to visually guided head orienting behavior in cats (Lomber and Payne, 2004) and which projects to the ipsilateral SC in the cat (Harting et al., 1992) and bilaterally to the SC in the macaque (Distel and Fries, 1982).

Considerably fewer labeled cells were identified in the contralateral than in the ipsilateral hemisphere (6.6% vs. 93.4%), and a somewhat different labeling profile was observed. Unlike in the ipsilateral hemisphere, labeling was very sparse in the visual cortex, and almost absent in the somatosensory cortical areas following an injection in the SC (Figs. 12 and 13). Within the auditory modality, the fAES remained the primary

source of projections to the SC (4.24% of labeled cells compared with less than 1% in all other auditory areas; Fig. 13).

DISCUSSION

The current study presents the first quantitative analysis of corticotectal projections originating from the visual, auditory, somatosensory, motor, and limbic cortices of the cat (see Fig. 14 for a summary). This within-animal study provides the unique opportunity to compare the strength of projections across modalities and hemispheres, and is of particular importance given that stimuli that elicit an orienting response from the SC are often multisensory in nature.

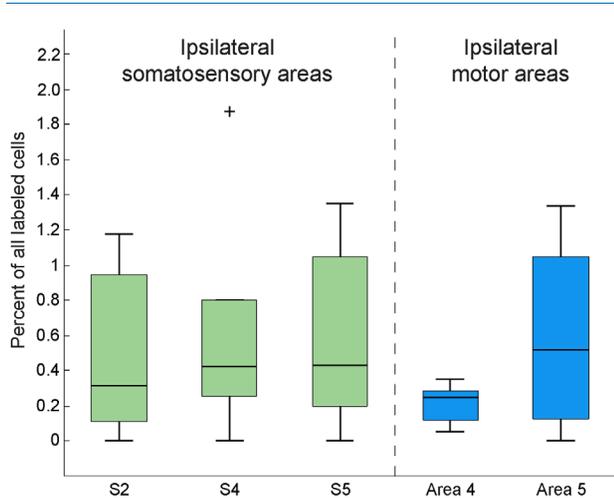


Figure 11. Box-and-whisker plot illustrating the distribution of somatosensory and motor corticotectal projections by cortical area. The y-axis indicates the percentage of labeled neurons, whiskers extend a maximum of 1.5 times the interquartile range, and outliers are denoted by a black cross. Note that the primary somatosensory area does not have a direct projection to the superior colliculus. For abbreviations, see list.

Ipsilateral projections by modality and cortical area

Retrograde pathway tracing with WGA–HRP confirms that corticotectal projections arise almost entirely from infragranular layers of cortex. This conforms to the laminar pattern observed across a number of other mammalian species (Kawamura et al., 1978; Tortelly et al., 1980; Baleyrier et al., 1983; Fries, 1984; Segal and Beckstead, 1984; Kunzle, 1995; Martinich et al., 2000; Lock et al., 2003; Bajo et al. 2010; Manger et al., 2010). At the modality level, the principal source of projections is the visual cortex, with smaller projections originating from the auditory, somatosensory, motor, and limbic cortices. Although the absolute strengths differ, this relative pattern echoes that observed in the ferret (Manger et al., 2010).

The tracer injections made in the current study ensured that all layers of the SC were exposed to WGA–HRP. This is critical to quantifying visual corticotectal projections, as two systems have been identified in the cat: one that projects from the primary and secondary visual cortices to the superficial layers of the SC, and one that projects from fields along the middle suprasylvian sulcus (mSS) to both superficial (Segal and Beckstead, 1984) and deeper layers of the SC (Segal and Beckstead, 1984; Ogasawara et al., 1984). In ensuring that all layers of the SC were exposed to WGA–HRP, some tracer spread occurred into the periaqueductal gray (PAG) in two of five animals. Patterns of cortical projections to the PAG in the cat have been

reported previously, and are dominated by fields of the frontal cortex including areas 6, 4, and 32 (Bandler et al., 19895). Although these oculomotor and motor areas are likely involved in defense-based reactions in response to sensory stimuli, they were not included in the analyses presented here due to the technical constraints outlined above. Smaller projections to the PAG have also been noted from sensory areas, including the primary and secondary somatosensory cortices (Bragin et al., 1984), auditory insular cortex (Winer et al., 1998), and fields surrounding the ventral extent of the anterior ectosylvian sulcus (Bandler et al., 1985; although that study is, itself, confounded by tracer spread from the PAG into adjacent tegmental and superior collicular divisions). The absence of projections from these fields in the current study suggests that the data presented here are not unduly confounded by tracer spread into the PAG. Moreover, animals with tracer spread into PAG (W66 and W70) showed no significant differences in the patterns of labeling across cortical fields when compared with those in which the injection was entirely confined within the SC (W76, W85, and W96).

Each of the areas previously demonstrated to be involved in visual orienting behavior in the cat—17, 18, 19, PLLS, and PMLS—show direct projections to the SC. This supports previous studies undertaken within the visual modality that involved either ablation techniques (Altman and Carpenter, 1961; Sprague et al., 1963, Kawamura et al., 1974; Baleyrier, 1977; Kawamura and Konno, 1979; Berson and McIlwain, 1983) or retrograde axonal transport of HRP (Kelly and Gilbert, 1975; Magalhães-Castro et al., 1975). Contrary to previous studies that have noted labeling of visual cortical areas that was limited to caudal fields (Baleyrier, 1977; Baleyrier et al., 1983), the current study reveals projections arising from the caudal to rostral extents of the visual cortex. The wider distribution in our results is likely the result of injections that exposed the entirety of the SC, rather than being confined to its central portion. The pattern of visual corticotectal projections observed here is in accordance with qualitative patterns observed in *Didelphis aurita* (South American opossum; Martinich et al., 2000) and in monkeys (Tigges and Tigges, 1981; Lock et al., 2003; Baldwin and Kaas, 2012). It fits well with the few quantitative analyses that have been undertaken (Fries, 1984; Collins et al., 2005). However, despite sharing similar organizational principles (Innocenti et al., 2002; Manger et al., 2005), the current results are discordant with those of the ferret, in which the principal visual corticotectal projections arise from areas 18 and 21 (Manger et al., 2010). Moreover, Berson and McIlwain

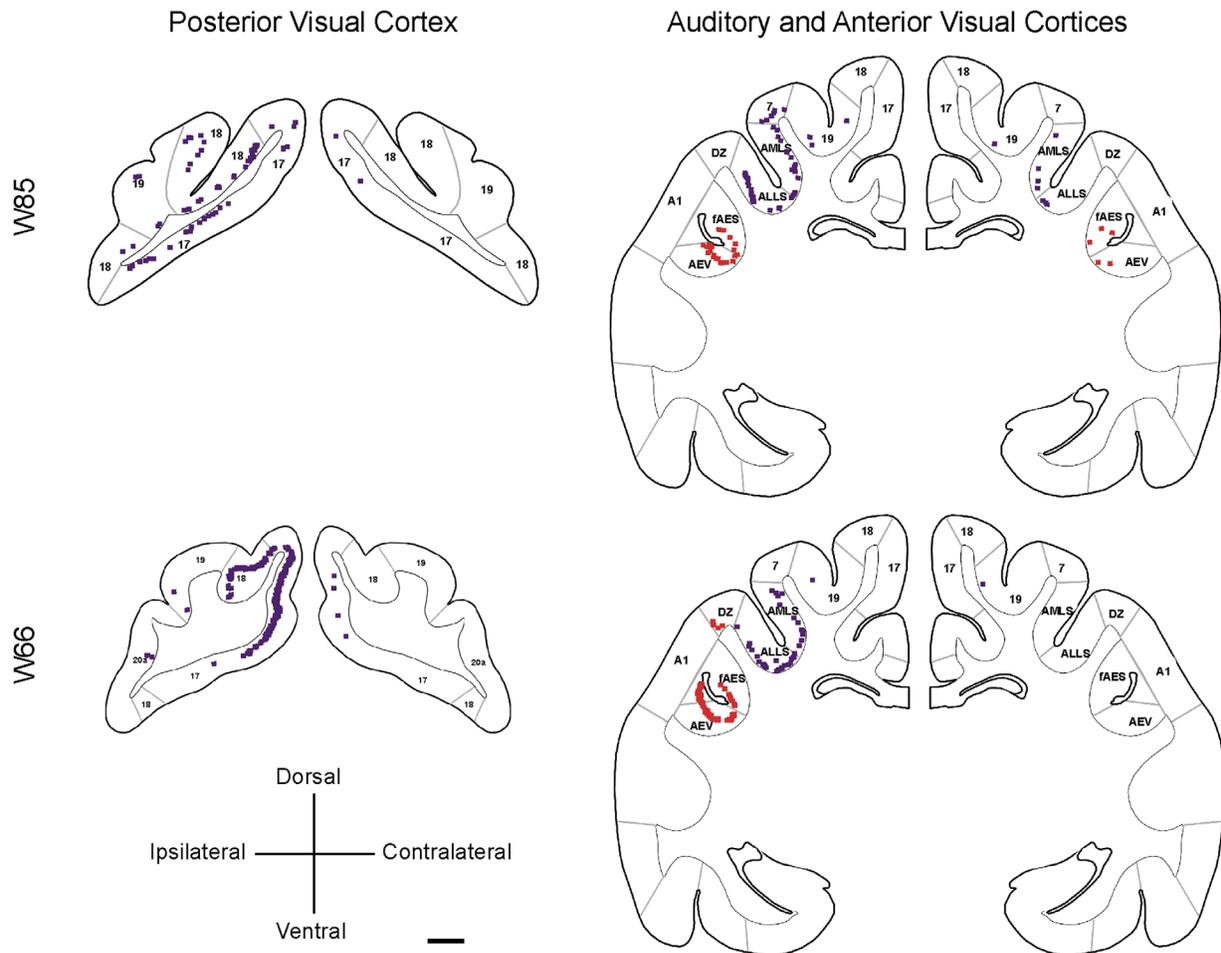


Figure 12. Profiles of HRP labeling in ipsilateral and contralateral hemispheres for two representative animals. In each case, individual animal data have been plotted on standardized coronal sections for ease of comparison. The left column shows plots of sections passing through the posterior visual cortices of the two animals. Plots of sections passing through the auditory and anterior visual cortices are presented on the right. Note that across modalities (visual in purple, auditory in red), the HRP-positive labeling in the contralateral hemisphere is quite sparse in comparison with that in the ipsilateral hemisphere. For abbreviations, see list. Scale bar = 2 mm.

(1983) observed that similar numbers of visual cells in the SC were driven by electrical stimulation of areas 17, 18, and 19, and the banks of the mSS, whereas the current study reveals that the projection from area 17 to the SC is 3 to 4 times larger than the projections from each of these other visual cortical areas. This may reflect the fact that, whereas the effects of cortical inputs are undoubtedly dependent on the number of neurons projecting from a given cortical area, there are other factors (including the number of terminals expressed on these projections, the cell types targeted, and the arrangement of synaptic terminals on target cell dendrites) that also contribute to synaptic strength. These additional factors are not measured using the current methodology. Contrary to what is observed in the visual system, only one of the areas considered to be involved in auditory orienting behavior—the fAES—shows a significant projection to the SC,

confirming the results of Meredith and Clemo (1989). Although the ferret does not have an fAES, the region surrounding the pseudosylvian sulcus is considered to be the functional analogue (Ramsay and Meredith, 2004; Bajo et al., 2010) and contains a robust projection to the SC. The absence of a substantive direct projection from the A1 to the SC is in accordance with previous anatomical data. For example, auditory cortical lesions do not result in the degeneration of fibers in the SC when these lesions are confined within the borders of the A1 (Diamond et al., 1969). Moreover, Lomber and colleagues (2007a) demonstrated that reversible deactivation of the superficial layers of the A1, DZ, and PAF was sufficient to impair auditory localization, suggesting a mechanism for these areas that does not necessitate a direct projection to the SC. However, these findings are at odds with neuroanatomical studies in the Mongolian gerbil (Budinger et al.,

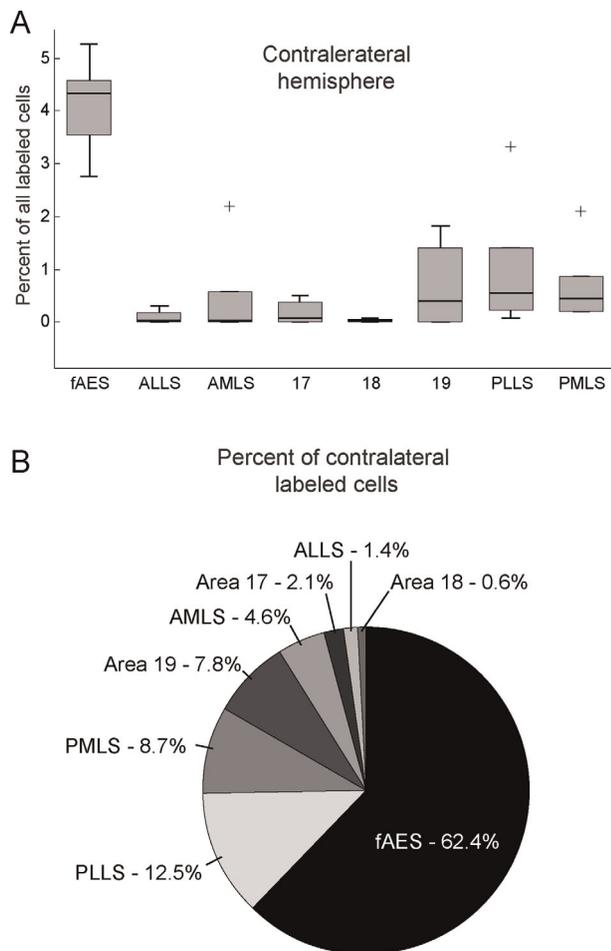


Figure 13. A: Box-and-whisker plot illustrating the distribution of contralateral corticotectal projections by cortical area. The y-axis indicates the percentage of labeled neurons, whiskers extend a maximum of 1.5 times the interquartile range, and outliers are denoted by a black cross. The majority of the contralateral cortical areas have a projection accounting for less than 1% of total labeled neurons. However, the fAES is a significant source of contralateral projections to the superior colliculus (median = 4.3%). **B:** Contralateral projections by cortical area expressed as the mean proportion of total contralateral projections. For abbreviations, see list.

2006, 2008; Budinger and Scheich, 2009) and the ferret (Manger et al., 2010), which describe projections from primary auditory fields to the SC. Also somewhat discordant with the current study, Winer and colleagues (1998) demonstrated corticotectal projections originating in the second auditory cortex (A2) and multisensory areas that are designated as auditory cortical fields in cats. Moreover, using an anterograde degenerative technique, Paula-Barbosa and Sousa-Pinto (1973) described significant projections from the A2 and DZ, in addition to the fAES. However, the thermocoagulation method is more invasive and less precise than the

retrograde labeling technique employed here, and this may account for the differences.

Contrary to previous anatomical evidence (Stein et al., 1983; McHaffie et al., 1988), the current study does not characterize projections from somatosensory areas to the SC as substantial. Similar to these previous studies, we demonstrate no projection from the primary (0%) and small projections from the secondary (0.5%) somatosensory cortex. However, although the projection from the S4 has been described as dominating somatosensory input to the SC, the current study demonstrates balanced, small projections from the S2, S4, and S5 (all contributing $\sim 0.5\%$). The cause of this discrepancy is not immediately apparent. Clemo and Stein (1984) have noted that the subset of somatosensory neurons of the SC influenced by corticotectal inputs from the S4 are not distributed in a laminar pattern that distinguishes them from the total population of somatosensory cells; thus, the injections in the current study, which ensured that tracer spread throughout the SC, would be expected to successfully label these projections. It is worth noting again that, although the absence of a substantial projection from the S4 in the current study appears discordant with electrophysiological evidence showing that this cortical field is capable of modulating response properties of SC neurons (Clemo and Stein, 1984, 1986), the strength of electrophysiological responses is determined by more than the number of neurons projecting between areas of the brain.

Contralateral projections to the SC

Previous evidence for contralateral visual projections to the SC is mixed; some studies have suggested that areas involved in orienting behavior send sizable projections to the contralateral SC (Powell, 1976; Galletti et al., 1981; Berman and Payne, 1982). However, the current study supports the opposite finding that the contralateral visual cortex contains a small number of labeled cells (Baleyrier, 1977; Baleyrier et al., 1983). Instead, the only substantial contralateral projection arises from the fAES, a field of the auditory cortex that has been previously shown to project bilaterally to the SC (Fuentes-Santamaria et al. 2008, 2009; Chabot et al., 2013). Although they did not quantify labeled cells, Tortelli and colleagues (1980) also demonstrated bilateral corticotectal projections from the fAES, as well as from the banks of the suprasylvian sulcus. In the current study, the number of labeled cells in the areas of the cortex surrounding the contralateral suprasylvian sulcus (the ALLS, AMLS, PLLS, PMLS) are negligible when considered as a proportion of total labeled cells across hemispheres (Fig. 13A). However, if these

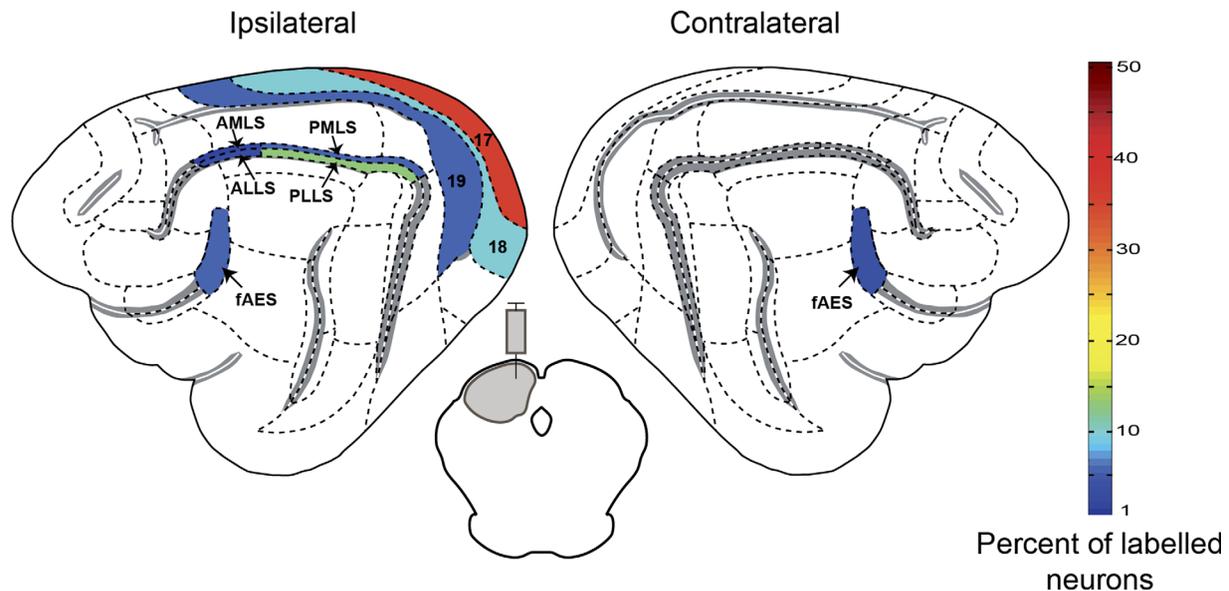


Figure 14. Summary of the visual, auditory, and somatosensory cortical projections to the SC. Areas containing greater than 1% of the total labeled cells are shown, with projecting strength color coded according to the color bar on the right. Each of the visual areas involved in orienting (17, 18, 19, PLLS, and PMLS) has a direct connection with the SC. Conversely, that majority of the auditory areas implicated in orienting behavior (A1, DZ, and PAF) shows no substantial projection to the SC. Only the fAES has a direct connection, which is of approximately equal strength bilaterally. Finally, only somatosensory area S2 and motor area 5 have direct connections to the SC. For abbreviations, see list.

projections are considered as a proportion of total contralateral labeling (Fig. 13B), the size of each is similar to its corresponding ipsilateral field. This alternate representation also serves to underscore the magnitude of the projection from the contralateral fAES. Indeed, a link between the fAES and acoustic orienting behavior is in accordance with findings that inactivation of the fAES by local anesthetic injection (Wilkinson et al., 1996) or by reversible cooling (Malhotra et al., 2004) results in profound deficits in acoustic localization. Interestingly, however, these deficits were shown to be robust for stimuli in the contralateral field, but negligible for ipsilaterally presented stimuli.

It is important to note that resection of the caudal splenium of the corpus callosum is not expected to have a substantial effect on the labeling of contralateral cortical neurons following injection into the SC. Previous research suggests that crossed corticotectal projections originating in visual (Powell, 1976; Galletti et al., 1981; Baleyrier et al. 1983; Harting et al., 1992) and auditory (Paula-Barbosa and Sousa-Pinto, 1973) cortical areas travel via the intercollicular commissure, rather than passing through the corpus callosum. Additionally, callosal resection does not selectively target interhemispheric connections of the posterior visual cortex as, unlike in some species (Pandya et al., 1971; Gould and Ebner, 1978; Dursteler et al., 1979;

de Lacoste et al., 1985), visual fibers in the cat are not confined to the caudal splenium. Instead, they pass through the entirety of the splenium and much of the body of the corpus callosum (Payne and Siwek, 1991; Lomber et al., 1994a). Thus, the patterns of contralateral labeling in the current study are unlikely to be biased by the surgical approach.

Unilateral visual, but bilateral auditory inputs

The current study demonstrates that whereas overall corticotectal input to the SC is dominated by visual cortical projections, the auditory field fAES makes the largest bilateral projection. Contralateral visual cortical fields located on the banks of the suprasylvian sulcus show projections to the SC that are comparable to ipsilateral fields when only contralaterally originating cells are considered. However, these projections appear much smaller when placed in the context of whole-brain projections, whereas the projection originating in the fAES remains compelling in either context. There are a number of ways that this differential pattern of projections might be interpreted. It is possible that the disparity between sensory cortices reflects differences in their intrinsic architecture. For example, thalamic afferents selectively target spiny stellate cells in layer

IV of the primary visual cortex (V1), whereas layer IV pyramidal cells are the preferential target in the A1 (Smith and Populin, 2001). Moreover, layer III cells in V1 make corticocortical connections that are almost exclusively ipsilateral (Fisken et al., 1975), whereas layer III cells in the A1 are commissural (Code and Winer, 1985). Such modality-specific architectures are established as early as the primary sensory cortex, persist throughout higher level sensory cortices (such as the fAES), and may underlie differences in corticotectal projections.

It may also be the case that modality-level differences in contralateral connectivity to the SC may reflect a contrast in the way that stimulus features, specifically those related to spatial representations and orienting behavior, are represented in these two modalities. In stark contrast to the retinotopic representations present in the primary visual cortex, there appears to be no topographic representation of acoustic space in the A1 (Middlebrooks and Pettigrew, 1981; Imig et al., 1990; Rajan et al., 1990a,b; Recanzone and Cohen, 2010). Moreover, although it is accepted that binaural cues to localization are established subcortically at the level of the superior olivary complex, the exact role of the auditory cortex in sound localization remains somewhat ambiguous. However, both electrophysiological (Stecker et al., 2005) and behavioral studies (Malhotra et al., 2004) suggest that interhemispheric connectivity plays a critical role. Based on these findings, Lee and Winer (2011) have suggested that commissural projections might serve to unify lateralized representations of stimulus features from the auditory cortices to improve the fidelity of the perceived auditory object. Thus, contralateral projections to the SC may arise from the auditory modality due to the functional necessity of bilateral cues for the perception of, and orientation toward, auditory stimuli. In addition, this projection may function cooperatively with smaller bilateral visual projections to establish a multisensory representation of stimulus location in the SC. Finally, it should be noted that injections in the current study sought to avoid tracer spread into the pretectal area, and as a consequence may have also limited spread into the anterior pole of the colliculus, where activity related to the ipsilateral visual field is represented (Feldon et al., 1970).

CONCLUSIONS

The present study quantifies and compares the patterns of corticotectal projections from visual, auditory, somatosensory, motor, and limbic cortices in the cat. Importantly, although all of the areas thought to contribute to visual localization have substantial inputs to

the SC, only one area involved with auditory orienting (the fAES), and no areas thought to contribute to somatosensory orienting, showed inputs of similar size. Additionally, we demonstrate that whereas ipsilateral visual cortical fields dominate the input to the SC, the only extensive contralateral input arises from a high-level field of the auditory cortex. We propose that this differential pattern of projections from visual and auditory cortices to the SC is due to the specific physiology and connectivity of these cortical structures. Further studies into the connectivity between these structures and electrophysiological examinations of function will be critical to an improved understanding of how multisensory environmental stimuli elicit orienting responses.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

ROLE OF AUTHORS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: BEB, NC, and SGL. Acquisition of data: BEB and NC. Analysis and interpretation of data: BEB and NC. Drafting of the manuscript: BEB. Critical revision of the manuscript for important intellectual content: BEB, NC, and SGL. Statistical analysis: BEB and NC. Obtained funding: SGL.

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