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Connections of the Superior Paraolivary Nucleus of the Rat: Projections to the Inferior Colliculus

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Abstract

GABAergic neurotransmission contributes to shaping the response properties of inferior colliculus (IC) neurons. In rodents, the superior paraolivary nucleus (SPON) is a prominent and well-defined cell group of the superior olivary complex that sends significant but often neglected GABAergic projections to the IC. To investigate the trajectory, distribution and morphology of these projections, we injected the neuroanatomical tracer biotinylated dextran amine into the SPON of albino rats.

Our results demonstrate that: 1) The SPON innervates densely all three subdivisions of the ipsilateral IC: central nucleus (CNIC), dorsal cortex (DCIC) and external cortex (ECIC). The SPON also sends a sparse projection to the contralateral DCIC via the commissure of the IC. 2) SPON axons are relatively thick (diameter > 1.2 μ m), ascend to the midbrain tectum in the medial aspect of the lateral lemniscus, and, for the most part, do not innervate the nuclei of the lateral lemniscus. 3) SPON fibers ramify profusely within the IC and bear abundant *en passant* and terminal boutons. 4) The axons of neurons in discrete regions of the SPON form two laminar terminal plexuses in the ipsilateral IC: a medial plexus that spans the CNIC and DCIC parallel to the known fibrodendritic laminae of the CNIC, and a lateral plexus located in the ECIC and oriented more or less parallel to the surface of the IC. 5) The projection from SPON to the ipsilateral IC is topographic: medial SPON neurons innervate the ventromedial region of the CNIC and DCIC and the ventrolateral region of the ECIC, whereas more laterally situated SPON neurons innervate more dorsolateral regions of the CNIC and

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Author contribution

E.S. designed the experiments and supervised the project. M.A.A. did some experiments and prepared the illustrations. V.F.S. did most of the tract-tracing experiments. E.S., M.A.A., and A.S.B. wrote the manuscript.

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DCIC and more dorsomedial regions of the ECIC. Thus, SPON fibers follow a pattern of distribution within the IC similar to that previously reported for intracollicular and corticocollicular projections.

Keywords

Auditory brainstem; Superior olivary complex; Medial nucleus of the trapezoid body; Lateral lemniscus; Tract-tracing; GABAergic inhibition

For the last two decades, numerous electrophysiological investigations have revealed the importance of inhibition in central auditory processing, and the inferior colliculus (IC), the largest auditory center of the mammalian brainstem, has become a favorite model to study cellular mechanisms of synaptic inhibition. Inhibitory inputs play a critical role in shaping the response properties of IC neurons; in particular the inhibitory neurotransmitter GABA has been shown to sharpen tuning curves, modulate temporal firing patterns, alter sensitivity to interaural intensity and interaural temporal disparities and frequency modulated sweeps, and to contribute to the duration tuning characteristics of IC neurons (Yang et al., 1992; Park and Pollak, 1993; Casseday et al., 1994; LeBeau et al., 1996; Palombi and Caspary, 1996; Koch and Grothe, 1998; Caspary et al., 2002). Moreover, abnormal GABAergic neurotransmission in the IC has been associated with audiogenic seizures (Faingold, 2002).

Identified sources of GABAergic inhibition to the IC include ascending projections from lower auditory nuclei, and intrinsic and commissural fibers (González-Hernández et al., 1996; Zhang et al., 1998; Saldaña and Merchán, 2005; Hernández et al., 2006). Investigations of the sources of ascending GABAergic projections have typically focused on the dorsal and ventral nuclei of the lateral lemniscus (DNLL and VNLL, respectively), thereby largely neglecting the superior paraolivary nucleus (SPON), a well-defined GABAergic cell group of the rodent superior olivary complex that contains considerably more neurons than the DNLL (Kulesza and Berrebi, 2000; Saldaña and Berrebi, 2000; Kulesza et al., 2002).

Available knowledge of the projection from SPON to the IC comes exclusively from studies in which retrograde tracers were placed in the IC. According to such experiments, the vast majority of SPON neurons innervate the ipsilateral IC (Saldaña and Berrebi, 2000). While SPON neurons were labeled following tracer injections into the central nucleus (CNIC) or dorsal cortex of the IC (DCIC), this was not the case after injections into the external cortex (ECIC) (Coleman and Clerici, 1987; González-Hernández et al., 1996). The projection from SPON to the CNIC is topographically organized such that medial and lateral SPON neurons innervate ventromedial and dorsolateral regions of the CNIC, respectively (Willard and Ryugo, 1983; González-Hernández et al., 1996; Kelly et al., 1998; Saldaña and Berrebi, 2000). This arrangement corresponds to the tonotopic organization of the nucleus, with higher characteristic frequency SPON neurons located medially and lower characteristic frequency neurons situated laterally (Behrend et al., 2002; Dehmel et al., 2002; Kulesza et al., 2003).

Previous connectional studies provided an initial framework for future investigations of SPON-to-IC projections, but an informed understanding of the physiological relevance of SPON-mediated inhibition in the IC requires precise knowledge of the trajectory, morphology, distribution and density of SPON fibers and synaptic boutons that innervate this structure. Such information can only be obtained by labeling SPON axons with anterograde tracers. Thus, we have injected the sensitive bidirectional tracer biotinylated dextran amine (BDA) into the SPON. We utilized albino rats because in this species SPON neurons are morphologically, neurochemically and electrophysiologically homogeneous (Saldaña and Berrebi, 2000; Kulesza and Berrebi, 2000; Kulesza et al., 2003; Kadner et al., 2006), and virtually all of them innervate the IC (Saldaña and Berrebi, 2000).

EXPERIMENTAL PROCEDURES

Fifteen female Sprague-Dawley rats (body weight 190–210 g), obtained from the Animal Core Facility of the University of Salamanca, were cared for and used in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) regulations concerning the use of animals in biomedical research, and the experimental procedures were approved and supervised by the Animal Care and Use Committee of the University of Salamanca. For the surgical procedures, including the transcatheter perfusion of fixatives, the animals were deeply anesthetized with a mixture of ketamine HCl (80 mg/kg body weight) and xylazine (6 mg/kg body weight) administered intramuscularly.

Under stereotaxic guidance, glass micropipettes loaded with the neuroanatomical tracer BDA (10,000, Molecular Probes, Eugene, OR; 10% in 0.1M sodium phosphate buffer, pH 7.4) were placed into the SPON of deeply anaesthetized rats, and the tracer was delivered by iontophoresis using a pulsed 5 μ A DC positive current (7 seconds on / 7 seconds off) for 5–15 minutes. The current was then stopped and the pipette left in place for an additional 15–20 minutes prior to withdrawal, in order to minimize leakage of the tracer along the injection tract. To avoid damaging the prominent transverse sinus, the pipettes were lowered into the brain in a dorsocaudal to ventrorostral direction, so that their trajectory formed a 16° angle with the coronal plane.

Following 7–10 days survival, the rats were again anesthetized deeply and their brains fixed by transcatheter perfusion of buffered 4% formaldehyde (prepared from freshly depolymerized paraformaldehyde) and 0.1 % glutaraldehyde. After cryoprotection in 30 % sucrose in PB, the brains were cut coronally on a freezing microtome at a thickness of 40 μ m. To visualize the tracer, the sections were first processed by the avidin-biotin-peroxidase complex procedure (ABC, Vectastain, Vector Labs, Burlingame, CA) following the manufacturer's specifications, and then by standard histochemistry for peroxidase, with or without heavy-metal intensification (i. e., López et al., 1999). For cytoarchitectural reference, every fourth section was counterstained with cresyl violet.

Sections were photographed at high resolution with a Zeiss Axioskop 40 microscope using a Zeiss AxioCam MRc 5 digital camera and plan semi-apochromatic objective lenses 2.5 $\times$ (NA 0.075), 5 $\times$ (NA 0.15), 10 $\times$ (NA 0.30), 20 $\times$ (NA 0.50) and 40 $\times$ (NA 0.75). Camera lucida drawings were made with India ink using a Leica DMRB microscope fitted with a drawing tube, and subsequently scanned at high resolution. The brightness and contrast of images were adjusted with Adobe Photoshop software, and the illustrations were arranged into plates using Canvas software.

RESULTS

The information described herein comes from 15 selected experimental cases with single injections of BDA into the SPON of the albino rat. In 13 cases, the injection site was wholly contained within the limits of the nucleus (Fig. 1). In the remaining 2 cases, the injection site was located in the ventrolateral region of the SPON and encroached upon the neighboring medial superior olive (MSO). The locations of injection sites of various representative cases is illustrated schematically in figure 1C.

In all cases, abundant BDA-labeled fibers and neuronal somata were observed outside the injection site. The fibers could be followed to their destinations where they ramified and gave rise to terminal branches bearing *en passant* axonal varicosities and terminal specializations. Most retrogradely labeled neurons displayed a dense reaction product that filled the soma and usually spread to the primary, secondary or even more distal dendritic branches (Fig. 2).

As expected from the known anisotropic organization of the SPON, whose dendritic trees and plexuses of afferent fibers extend within nearly parasagittal planes (Banks and Smith, 1992; Schofield, 1995; Saldaña and Berrebi, 2000), the injection sites of BDA into the nucleus tended to be elongated in the rostrocaudal and dorsoventral dimensions and narrow mediolaterally. The mediolateral diameter of the center of the injection site ranged from 150 to 380 μm (Fig. 1).

Distribution of retrogradely labeled neurons

Injections of BDA confined to the SPON resulted in the labeling of abundant neurons in the ipsilateral medial nucleus of the trapezoid body (MNTB) (Fig. 1A, B, 2A) and in the contralateral ventral cochlear nucleus (VCoN) (Fig. 2D–F). Neurons were also labeled in the ipsilateral lateral nucleus of the trapezoid body (LNTB) (Fig. 2B), ipsilateral VCoN (Fig. 2C) and ipsilateral tectal longitudinal column (TLC; Fig. 2G). It was noted that the ipsilateral and contralateral IC were virtually devoid of labeled cell bodies (not shown).

Retrogradely labeled neurons in the ipsilateral MNTB displayed the morphologic features characteristic of the principal neurons of this nucleus (Fig. 2A; see also Kuwabara and Zook, 1991; Banks and Smith, 1992; Sommer et al., 1993). The mediolateral position of these labeled neurons varied as a function of the mediolateral position of the injection site, thus confirming the previously reported topography of the projection from the MNTB to the SPON (Banks and Smith, 1992; Sommer et al., 1993).

Neurons labeled in the contralateral VCoN were identified as octopus cells (Fig. 2D), globular bushy cells (Fig. 2E), or multipolar/stellate neurons (Fig. 2F). These neuronal types were easily recognizable by their shape and position, and by the caliber and trajectories of their axons (Brownell, 1975; Spirou et al., 1990; Smith et al., 1991, 1993; Cant and Benson, 2003). Multipolar cell axons were relatively thin and usually identified in the ventral or middle part of the trapezoid body, whereas the axons of globular bushy cells, which traveled in the ventral half of the trapezoid body, were somewhat thicker than those of multipolar neurons. The axons of octopus cells were even thicker and coursed within the intermediate acoustic stria (not shown). Virtually all neurons labeled in the ipsilateral VCoN were identified as globular bushy cells (Fig. 2C).

The neurons labeled in the ipsilateral LNTB were morphologically homogeneous and had small, rounded or oval cell bodies (11–13 μm in maximum diameter). Finally, the neurons labeled in the ipsilateral TLC tended to be multipolar (Fig. 2G) and were distributed throughout the rostrocaudal extent of the nucleus (Saldaña et al., 2007).

Distribution of labeled axons and terminal fields

In all cases, BDA-labeled fiber plexuses were observed in the ipsilateral lateral superior olive (LSO), LNTB, VCoN, dorsal cochlear nucleus (DCoN), VNLL, DNLL, and IC. Some BDA-labeled fibers and terminal boutons were also found in the contralateral IC.

Within the ipsilateral LSO, labeled fibers formed a dense plexus oriented in parallel to the major axis of the neurons of the nucleus (i.e., Scheibel and Scheibel, 1974). The thickness of the plexus depended on the mediolateral diameter of the injection site (Fig. 1A, B), and the position of this plexus shifted along the tonotopic axis of the LSO in accord with the mediolateral position of the injection site. Moreover, this plexus of labeled fibers in the LSO extended ventrally to innervate the LNTB (Fig. 1B). As discussed below, our interpretation is that these labeled fibers do not originate from SPON neurons, but instead we presume that they belong to retrogradely labeled MNTB neurons.

The number of fibers labeled in the ipsilateral VCoN was usually low, and these fibers entered the nucleus via the trapezoid body and then coursed mediolaterally. For a number of reasons we also conclude that these fibers do not represent a genuine projection of SPON neurons (see Discussion).

Abundant fibers were labeled in the ipsilateral lateral lemniscus and these could be classified as belonging to three seemingly different types. The fibers of the first type were thick (1.2–2.5 μm in diameter) and ascended in the medial part of the lateral lemniscus, occupying a position medial to the VNLL and the DNLL (Figs. 3A, B). These fibers crossed the rubrospinal tract and then traversed the medial paralemniscal nucleus (Feliciano et al., 1995; Varga et al., 2008) without innervating it. Although some of these thick fibers gave rise to collateral branches that coursed laterally to innervate the dorsal half of VNLL or, less frequently, the DNLL (Fig. 3C), most ascended to the IC without branching (Fig. 3B). For reasons that will become evident in the Discussion, we have concluded that these thick fibers arise from SPON neurons.

The fibers of the second type were thinner (caliber $< 1 \mu\text{m}$). Many of them ascended outside the VNLL in the caudal, lateral and rostral aspects of the lateral lemniscus (Fig. 3B). These fibers gave rise to collateral branches that innervated densely the VNLL but apparently did not ascend past the dorsal border of this nucleus. These features led us to conclude that these fibers belong to neurons of the ipsilateral MNTB.

The fibers of the third type were also thin and traveled within the VNLL, contributing to the innervation of the nucleus. These latter fibers proceeded dorsally, crossing and innervating the DNLL before reaching the IC. Their most likely origin is the contralateral VCoN (see Discussion).

In each case the VNLL contained several discrete terminal fields of labeled fibers which were distributed throughout the dorsoventral extent of the nucleus (Fig. 3B). It appeared that these terminal fields were formed, for the most part, by the two types of thin fibers.

Within the DNLL, the labeled fibers formed a sparse terminal field arranged circumferentially, paralleling the contour of the nucleus. The position of this plexus depended once again on the mediolateral position of the SPON injection site: after medial injections, the plexus appeared in the periphery of the DNLL, whereas more lateral SPON deposits were associated with more centrally positioned plexuses in the DNLL. This topographic arrangement follows the reported concentric anisotropy and tonotopy of the DNLL (Merchán et al., 1994; Saint Marie et al., 1999). We conclude that the most likely origin of the terminal fibers found in the DNLL is the contralateral VCoN.

Fibers labeled in the IC—Most labeled fibers that entered the IC were of thick diameter (Fig. 3D) and their point of entrance into the nucleus was relatively medial and rostral. Within the IC, the thick fibers ramified profusely to create dense terminal fields. The few thinner fibers that reached the IC entered the nucleus more caudally and laterally than the thicker axons. Once in the IC, they ramified and their terminal branches intermingled with those of the thick fibers.

Although the position of the terminal fibers within the IC changed depending on the position of the injection site in the SPON, their distribution followed a similar pattern in all cases. Thus, in all cases with an injection site confined to the SPON, there were two plexuses of terminal fibers in the ipsilateral IC. These plexuses crossed the IC rostrocaudally and their thickness varied depending on the mediolateral diameter of the injection site (Figs. 4–8). One of these plexuses (the *medial plexus*) was continuous throughout the CNIC and DCIC, and the other (the *lateral plexus*) was positioned in the ECIC.

In individual coronal sections, the medial plexus appeared as a band of fibers that crossed the CNIC and entered the DCIC (Fig. 4) and whose ventrolateral-to-dorsomedial orientation coincided with that of the fibrodendritic laminae of the CNIC (Faye-Lund and Osen, 1985; Malmierca et al., 1993). This band appeared slightly more vertical in rostral sections than in caudal sections and it was composed of terminal fibers bearing abundant *en passant* and terminal boutons (Fig. 5A). Most of these fibers showed a conspicuous orientation, traveling in various ventrodorsal or rostrocaudal directions within the plane of the plexus (Fig. 5A); labeled fibers with mediolateral orientation were less frequently observed within the plexus.

Similarly, in individual coronal sections, the lateral plexus appeared as a band of fibers situated in the deep region of the ECIC, more or less parallel to the lateral surface of the IC, with its ventral end somewhat deeper than its dorsal end (Fig. 4B). Like the fibers of the medial plexus, those of the lateral plexus possessed abundant *en passant* and terminal swellings. Their orientation was, in general, more heterogeneous than that of the fibers of the medial plexus (Fig. 5B).

In cases where the tracer was injected into the medial SPON, the medial plexus occupied a ventromedial position within the CNIC and DCIC and the lateral plexus occupied a ventrolateral position in the ECIC (Fig. 6), whereas when the tracer was injected into more lateral regions of the SPON, the medial IC plexus shifted dorsolaterally and the lateral plexus shifted dorsomedially (Fig. 7). These observations provided compelling evidence that the projection from the SPON to the ipsilateral IC is topographically organized, as depicted schematically in Figure 9.

In two of our cases the injection site was situated in the ventrolateral region of the SPON and encroached upon the neighboring MSO (Fig. 1C). These placements resulted in an additional plexus of labeled fibers located dorsally within the IC, at the border between the CNIC and the ECIC (Fig. 8D). Since it only appeared in these two experimental cases, this additional plexus is assumed to represent the projection from the MSO.

In all cases, we observed some BDA-labeled fibers extending beyond the dorsomedial end of the medial plexus to cross the commissure of the IC. These fibers reached the contralateral DCIC, where they gave rise to sparse collateral branches that terminated locally (Figs. 4, 6–8). Due to the scarcity of this crossed projection, a topographic arrangement similar to that of the ipsilateral projection was not apparent.

Within the commissure of the IC, labeled fibers formed two terminal fields, one on each side of the midline (Figs. 4, 6–8) which innervated the recently identified TLC (Saldaña et al., 2007; Marshall et al., 2008). We also noted the presence of labeled axons in the ipsilateral medial geniculate body. Detailed reports on these novel projections from the SPON will be the subject of separate accounts.

DISCUSSION

Technical considerations

The tracer used in this study, BDA, labels the axons of neurons at the injection site, but it is also known to label axons that innervate or cross the injection site, as well as their parent cell bodies. Consequently, BDA gives rise to so-called collateral transport, whereby the tracer taken up by a given axonal branch is transported retrogradely to a bifurcation in the axon, and then anterogradely into another branch (e.g., De Olmos and Heimer, 1977; Merchán et al. 1994; Warr et al. 1997; Doucet and Ryugo, 2003). Because it is not possible to distinguish axons labeled by collateral transport from axons labeled anterogradely, the labeling observed following our injections into the SPON could arise from SPON neurons or belong to any of

the neuron types labeled retrogradely. Thus, as in all studies that employ this tracer, caution must be exercised when interpreting the origin of BDA labeled fibers and boutons.

Origin of fibers labeled in the LSO and LNTB—For several reasons, we interpret the terminal fibers labeled in the LSO as originating from the ipsilateral MNTB: 1) In our experiments, numerous MNTB neurons displayed a dense, diffuse labeling of their cell bodies and dendrites, and their axons could be followed to the injection site; 2) The MNTB is known to innervate the ipsilateral LSO and the axons forming this projection cross the SPON (Banks and Smith, 1992; Sommer et al, 1993); 3) The morphology and orientation of the fibers labeled in the LSO in our cases are similar to those obtained with injections confined to the MNTB (Banks and Smith, 1992; Sommer et al, 1993; our own unpublished observations); 4) The systematic displacement of the terminal fibers in the LSO as the injection site was shifted along the mediolateral axis of the SPON is consistent with the known tonotopy of MNTB-to-LSO projections (Banks and Smith, 1992; Sommer et al, 1993); and 5) No projections from the SPON to the ipsilateral LSO have been previously reported. This latter point is strengthened by the fact that our own injections of BDA into the LSO of the rat have not caused the retrograde labeling of SPON neurons (unpublished observations).

By the same token, the labeled fibers observed in the LNTB probably originate for the most part in the MNTB, as this projection has been reported previously (Banks and Smith, 1992; Sommer et al., 1993). Moreover, the terminal fields in the LNTB seemed to be a ventral extension of the terminal fields in the LSO, which, as discussed above, presumably originate in the MNTB. We cannot rule out with certainty, however, that some of the labeled fibers in LNTB represent the local axonal collaterals of LNTB neurons retrogradely labeled by SPON injections.

It is noteworthy that we did not observe labeled terminal fibers in the MSO in our experiments. This result was unexpected given that projections from the MNTB to the MSO have been repeatedly documented (Spangler et al., 1985; Adams and Mugnaini, 1990; Banks and Smith, 1992; Kuwabara and Zook, 1992; Sommer et al., 1993; Henkel and Gabriele, 1999; Werthat et al., 2008). However, close inspection of the MNTB neurons intracellularly labeled by Banks and Smith (1992) suggests that within the MNTB of the rat, neurons that innervate the MSO are different from those innervating the SPON; this would explain the absence of axonal labeling in the MSO following injections into the SPON.

We also never observed retrogradely labeled neurons in the MSO. Thus, this particular aspect of auditory brainstem circuitry in the rat appears to be differently organized than in the gerbil, in which a projection from the MSO to the ipsilateral SPON has been reported (Kuwabara and Zook, 1999).

Origin of fibers labeled in the ventral cochlear nucleus—In the rat, there are no direct projections from the SPON to the VCoN, as SPON neurons are not labeled following injections of retrograde tracers into the rat VCoN (Faye-Lund, 1986, our own unpublished observations). Therefore, it is very unlikely that the axons labeled in the VCoN in our cases belong to SPON neurons. A more likely possibility is that they originate from MNTB neurons, as direct projections from MNTB to VCoN have been demonstrated in rats and guinea pigs (Faye-Lund, 1986; Schofield, 1994).

Origin of fibers labeled in the nuclei of the lateral lemniscus and the inferior colliculus—When our injections of BDA were confined to the SPON, we found three types of fibers ascending in the lateral lemniscus: thick fibers in the medial part of the lateral lemniscus that reached the IC, without entering the nuclei of the lateral lemniscus; thin fibers that ascended outside the nuclei of the lateral lemniscus without reaching the level of the DNLL;

and thin fibers that ran through the VNLL and the DNLL to reach the IC. To assist in determining the source of these various fiber types, we compared the results of a typical SPON injection (case 97097) with those of additional representative experimental cases, obtained for other purposes. In one of these additional cases, BDA was injected into the MNTB, and in another the same tracer was placed into the anterior VCoN. Each of these injection sites was wholly contained within the corresponding nucleus and the tracer injection and visualization procedures were identical to those used in the present study. Figure 10 shows micrographs of coronal sections through the lateral lemniscus in each of these cases.

The thick, medially located axons were labeled following injection into the ipsilateral SPON (Fig. 10A), but not after injections placed in either the ipsilateral MNTB (Fig. 10B) or the contralateral VCoN (Fig. 10C). Therefore, we conclude that the thick, medially situated labeled axons belong to SPON neurons. Further support of this conclusion is the fact that injections of the retrograde tracer cholera toxin B subunit in the medial paralemniscal nucleus, which in our material is crossed by the thick labeled axons, result in abundant neurons labeled in the ipsilateral SPON, but not in the ipsilateral MNTB or the contralateral VCoN (Varga et al., 2008).

Our results show that only a small percentage of SPON axons give off collaterals to the nuclei of the lateral lemniscus before innervating the ipsilateral IC. This observation is consistent with the relatively modest number of neurons labeled in the SPON following small injections of retrograde tracers confined to either one of the nuclei of the lateral lemniscus (Varga et al., 2008; Kelly et al, 2009).

In cases with injections into the MNTB (Fig. 10B), numerous thin axons were labeled in the lateral lemniscus, outside the VNLL. Such axons contributed to the innervation of the VNLL, but the labeling in the DNLL and IC was very sparse. Projections from the MNTB to the ipsilateral VNLL that do not reach the level of the IC have been reported in various species, including rats (Banks and Smith, 1992; Sommer et al., 1993; Kelly et al., 2009), cats (Glendenning et al., 1981; Spangler et al., 1985) and bats (Huffman and Covey, 1995). We therefore conclude that the thin axons that ran outside the VNLL belong to MNTB neurons.

Finally, in cases with injections into the VCoN, thin axons were seen running through the VNLL and DNLL before entering the IC (Fig. 10C). This observation suggests that the thin labeled axons observed coursing through the VNLL following injections into the SPON originate from the contralateral VCoN. Indeed, projections from the VCoN to the contralateral nuclei of the lateral lemniscus have been repeatedly documented (Glendenning et al., 1981; Friauf and Ostwald, 1988; Huffman and Covey, 1995; Schofield and Cant, 1997; Kelly et al., 2009).

The labeling of terminal fibers in the DNLL was denser following VCoN injections than SPON injections, indicating that the contribution of VCoN axons to the labeling observed in the DNLL following injections into the SPON was relatively modest. This, in turn, indicates that the contribution of VCoN axons to the labeling observed in the IC following injections into the SPON was also very modest. Consequently, we conclude that the labeling observed in the IC following SPON injections represents, for the most part, the projections of SPON neurons.

Our injections into the SPON also labeled neurons in the ipsilateral LNTB. Because little is known about the projections of LNTB neurons, it would be speculative to estimate their contribution to the plexuses labeled in the IC. However, it seems unlikely that the few labeled LNTB neurons could account for a significant percentage of the fibers labeled in the IC.

In summary, we conclude that the vast majority of labeled fibers and terminals in the IC represent the direct projections from the SPON. Therefore, the remainder of this section will

focus on a detailed discussion of the morphofunctional organization of the SPON-to-IC projection.

The projections from SPON to the IC are predominantly ipsilateral

Our experiments confirm numerous previous reports in rats (Beyerl, 1978; Druga and Syka, 1984; Faye-Lund, 1986; Coleman and Clerici, 1987; Aschoff and Ostwald, 1988; Merchán et al., 1994; Okoyama et al., 1995; González Hernández et al., 1996; Kelly et al., 1998, 2009; Saldaña and Berrebi, 2000; Ito et al., 2008) and other species (mouse [Willard and Ryugo, 1983], gerbil [Nordeen et al., 1983], chinchilla [Saint Marie and Baker, 1990], guinea pig [Aschoff and Ostwald, 1988; Saint-Marie and Baker, 1990; Schofield and Cant 1991], cat [Adams, 1983; Brunso-Bechtold et al., 1981], ferret [Moore, 1988], opossum [Willard and Martin 1983, 1984], mole [Kudo et al., 1990] and several bat species [Schweizer, 1981; Zook and Caseday, 1982; Ross et al., 1988; Frisina et al., 1989; Grothe et al., 1994]) which describe the projection from SPON (or the dorsomedial periolivary region of the SOC) to the IC as almost exclusively ipsilateral. According to Coleman and Clerici (1987), of all SPON neurons retrogradely labeled from the rat CNIC, only 2% were found contralateral to the injection site. Similarly, following injections of the retrograde tracer FluoroGold into the rat IC, only 1.2 % of all labeled SPON neurons were observed on the side opposite to the injection site (Ito et al., 2008).

The weak projection we observe from the SPON to the contralateral IC is in line with a previous observation in the guinea pig. Using a double fluorescent tracer paradigm, Schofield (1991) showed a small number of SPON neurons with contralateral projections to the IC, most of which were also labeled from the ipsilateral IC. According to our data, SPON axons in the rat reach the contralateral IC via the commissure of the IC. Therefore, the SPON joins a number of nuclei whose axons cross the midline in this commissure, including the IC, nucleus sagulum, nuclei of the lateral lemniscus and auditory cortex (reviewed by Saldaña and Merchán, 2005).

SPON innervates all three subdivisions of the IC

Our experiments clearly revealed the organization of the SPON projection to the ipsilateral CNIC. The ventrolateral-to-dorsomedial orientation of SPON axons coincides with that of the main axis of most CNIC dendritic arbors (Faye-Lund and Osen, 1985; Malmierca et al., 1993), with which they intermingle. Therefore, SPON fibers must be considered an integral component of the fibrocellular laminae of the rat IC.

Besides the expected projection to the CNIC, our experiments also revealed dense projections to the ipsilateral ECIC and weaker projections to the DCIC of both sides, mainly ipsilaterally. While the projection to the ipsilateral DCIC had already been shown with retrograde tracers (Coleman and Clerici, 1987; González-Hernández et al., 1996), the projection to the ECIC came as a surprise, as no SPON neurons were labeled following horseradish peroxidase injections restricted to the rat ECIC (Coleman and Clerici, 1987; González-Hernández et al., 1996). The reasons for this marked discrepancy remain unclear, but may be related to the differences in sensitivity of the tracers used.

In our experiments, the position of the plexuses labeled in the CNIC-DCIC and in the ECIC changed systematically with the mediolateral position of the injection site, as expected from the known tonotopic organization of both SPON (Kulesza et al., 2003) and IC (Clopton and Winfield, 1973; Malmierca et al., 2008). Thus, the topographic projections from SPON to all three subdivisions of the IC support the notion that the IC is made of loosely concentric fibrocellular laminae that span the three subdivisions, as already proposed on the basis of the

distribution of the intrinsic and commissural projections of the CNIC and DCIC, and of corticocollicular projections (Saldaña and Merchán, 1992, 2005; Saldaña et al., 1996).

Functional considerations

Despite the fact that the SPON has only recently received the attention of the auditory neuroscience community, several electrophysiological reports provide clues as to the potential functional role of this nucleus. Single unit recordings in the rat have shown that SPON neurons respond transiently to the offset of pure tones and noise bursts (Kulesza et al., 2003, 2007; Kadner and Berrebi, 2008). Moreover, when two sequential tones or noise bursts are separated by a silent gap, the SPON offset response to the first tone demonstrates sensitivity to gaps of less than 1 ms duration. In addition, SPON neurons respond with precise synchronization and relatively high spike counts to sinusoidally amplitude modulated (SAM) tones with relatively low modulation rates of up to ~200 Hz. Thus, as observed in response to gaps, SPON responses to SAM stimuli are triggered by episodes of low stimulus energy, represented in the latter case by the troughs of the amplitude modulation. The apparent dependence of SPON responses on episodes of low stimulus energy, and the preference for low modulation rates, has led to the proposal that the SPON represents a *discontinuity detector* that is particularly sensitive to very short, but relatively widely spaced interruptions in acoustic signals (Kadner and Berrebi, 2008). This discontinuity information is relayed to the ipsilateral IC *via* the dense, topographic GABAergic projections described in this paper, which likely serve to segment ongoing responses in collicular target neurons. Such temporal segmentation is observed in responses of primary-like units in the mouse IC to pure tones with gaps (Walton et al., 1997), where ongoing responses are interrupted by a transient reduction in firing rate indicating the presence of the gap in the stimulus.

It is noteworthy that a structure bearing morphological similarities to the SPON has recently been identified in postmortem human brainstems (Kulesza, 2008). Assuming that the human and rat SPON have similar functions, the inhibitory projection from SPON to IC may serve an important function in the perception of speech, which is segmented by gaps at the boundaries between words, syllables and occasionally between, or even within, phonemes. Psychophysical studies demonstrate that the acuity with which gaps are detected between closely spaced sounds or components of sounds plays an important role in speech perception (Tyler et al., 1982; Irwin and McAuley, 1987; Glasberg and Moore, 1989; Snell and Frisina, 2000; Snell et al., 2002). Interestingly, the gaps occurring in speech have durations of several tens of milliseconds and are usually spaced hundreds of milliseconds apart, suggesting that they should evoke strong responses from the SPON. Therefore, it is quite plausible that the segmentation of IC responses by SPON-derived inhibition plays an important role in the processing of speech.

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Abbreviations

Aq, Cerebral aqueduct
 AVCoN, Anteroventral cochlear nucleus
 BDA, Biotinylated dextran amine
 CNIC, Central nucleus of the inferior colliculus
 DCIC, Dorsal cortex of the inferior colliculus
 DCoN, Dorsal cochlear nucleus

DNLL, Dorsal nucleus of the lateral lemniscus
 ECIC, External cortex of the inferior colliculus
 ECIC-r, Rostral part of the external cortex of the inferior colliculus
 I.A., Interaural
 IC, Inferior colliculus
 LNTB, Lateral nucleus of the trapezoid body
 LSO, Lateral superior olive
 MNTB, Medial nucleus of the trapezoid body
 MPL, Medial paralemniscal nucleus
 MSO, Medial superior olive
 rs, Rubrospinal tract
 SC, Superior colliculus
 SPON, Superior paraolivary nucleus
 TLC, Tectal longitudinal column
 VCoN, Ventral cochlear nucleus
 VNLL, Ventral nucleus of the lateral lemniscus
 VNTB, Ventral nucleus of the trapezoid body

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Fig. 1.

Injection sites of BDA into the SPON of the rat. (A) Photomicrograph of a coronal section through the central rostrocaudal third of the superior olivary complex from case 97084, whose injection site is located in the dorsomedial region of the SPON. Numerous retrogradely labeled cell bodies are observed in the medial half of the MNTB and LNTB, and a dense plexus of labeled axons occupies the medial limb of LSO. The distribution of anterogradely labeled fibers in the IC is shown in Fig. 4A, B. Note in the upper right corner of the micrograph the labeled thick axons of octopus cells. (B) Similar photomicrograph from case 99044, with a smaller injection site located in the dorsomedial region of the SPON. Compared to the case depicted in A, fewer neurons were retrogradely labeled in the MNTB and LNTB and they show a more

restricted distribution; likewise, the fiber plexus in the medial limb of LSO is narrower. (C) Schematic representation of the size and location of the injection site of various representative cases. Other abbreviations: MSO, medial superior olive; VNTB, ventral nucleus of the trapezoid body. Scale bar = 500 μm for A and B, 450 μm for C.

Fig. 2. Photomicrographs of neurons retrogradely labeled following injections of BDA into the SPON of the rat. (A) Principal neurons of the ipsilateral MNTB. (B) Small round neurons of the ipsilateral LNTB. (C) Globular bushy cells of the ipsilateral VCoN. (D) Octopus cells of the contralateral posterior VCoN. (E) Globular bushy cell of the contralateral VCoN. (F) Multipolar cell of the contralateral VCoN. (G) Neuron of the ipsilateral TLC. In all photomicrographs, dorsal is to the top. Scale bar in A (50 µm) applies to all seven micrographs.

Fig. 3.

Photomicrographs of axons labeled in the lateral lemniscus following an injection of BDA into the ipsilateral SPON. (A) SPON axons are thick and, upon leaving the dorsal aspect of the superior olivary complex, course dorsally, medially and rostrally toward the lateral lemniscus. The thick SPON axons intermingle with thinner axons from other sources (see text). (B) The thick SPON axons ascend in the medial fascicles of the lateral lemniscus without entering the VNLL or the DNLL. Some of these axons give off horizontal collaterals that course laterally toward the dorsal half of the VNLL, whereas other, thinner fibers are seen traversing the VNLL or in fascicles lateral to it. (C) A labeled SPON axon in the medial part of the lateral lemniscus gives off a thinner collateral that courses laterally toward the DNLL. (D) Upon entering the IC,

labeled SPON fibers ramify profusely to create dense terminal fields. The orientation arrows in B apply to all four micrographs. Other abbreviations: MPL, medial paralemniscal nucleus; rs, rubrospinal tract.

Fig. 4. Projection from the SPON to the IC. (A) Photomicrograph of a coronal section through the center of the IC from a case that received a large injection of BDA into the medial half of the ipsilateral SPON (Case # 97084; illustrated in Fig. 1A). In this case labeled fibers form a thick, medial plexus in the CNIC and DCIC, and a lateral plexus in the ECIC. (B) Camera lucida drawing of the fibers labeled in the section shown in A. Note the fibers crossing the commissure of the IC to end in the contralateral DCIC. In the drawing, the stippling represents terminal fields, and the lines are fibers of passage. The number below the drawing indicates the level of the section with respect to the interaural (I.A.) coronal plane. (C) Photomicrograph of a coronal section through the center of the IC from a case that received a small injection of BDA into the medial part of the ipsilateral SPON. The plexuses of terminal fibers are much thinner

than those of the case illustrated in A. Camera lucida drawings of other sections from this case are shown in Fig. 6. Scale bar and orientation arrows in A apply also to C.

Fig. 5.

High magnification micrographs showing the morphology of labeled SPON fibers in coronal sections of the ipsilateral CNIC (A) and ECIC (B). In both locations, terminal SPON fibers exhibit complex branching and abundant en passant and terminal varicosities. Note that most fibers forming the medial plexus (A) are oriented parallel to each other and run along the main axis of the plexus, which is approximately parallel to the lower border of the micrograph. In the ECIC (B) the plexus is less dense and the orientation of the fibers is more heterogeneous. The main axis of the lateral plexus is parallel to the lower border of panel B. Scale bar in B applies also to A.

Fig. 6.

Camera lucida drawings of coronal sections illustrating the pattern of labeling observed in the IC after injections of BDA into three different regions of the left SPON. The order of the serial sections is designated with capital letters, with A indicating the most rostral section. The numbers underneath each section indicate the distance from the interaural (I.A.) coronal plane. Within the IC, the lines represent fibers of passage, and the stippling represents terminal fields. For each case, the position of the injection site is illustrated in Fig. 1C.

Case with a small BDA injection into the medial region of the left SPON. Note that the medial and lateral plexuses of labeled SPON fibers span most of the ipsilateral IC rostrocaudally, entering the rostral ECIC (ECIC-r in A and B). The medial plexus occupies the ventromedial regions of the CNIC and DCIC, whereas the lateral plexus is located ventrally and laterally within the ECIC. A few labeled fibers cross the commissure of the IC to end in the contralateral IC. Other abbreviations: Aq, cerebral aqueduct; SC, superior colliculus.

Fig. 7.

Camera lucida drawings of coronal sections illustrating the pattern of labeling observed in the IC after injections of BDA into three different regions of the left SPON. The order of the serial sections is designated with capital letters, with A indicating the most rostral section. The numbers underneath each section indicate the distance from the interaural (I.A.) coronal plane. Within the IC, the lines represent fibers of passage, and the stippling represents terminal fields. For each case, the position of the injection site is illustrated in Fig. 1C.

Case with a small BDA injection into the ventral part of the central mediolateral third of the left SPON. The medial plexus is situated more dorsolaterally and the lateral plexus more dorsomedially than in the case shown in Fig. 6. Other abbreviations: Aq, cerebral aqueduct; ECIC-r, rostral part of the ECIC; SC, superior colliculus.

Fig. 8.

Camera lucida drawings of coronal sections illustrating the pattern of labeling observed in the IC after injections of BDA into three different regions of the left SPON. The order of the serial sections is designated with capital letters, with A indicating the most rostral section. The numbers underneath each section indicate the distance from the interaural (I.A.) coronal plane. Within the IC, the lines represent fibers of passage, and the stippling represents terminal fields. For each case, the position of the injection site is illustrated in Fig. 1C.

Case with a small BDA injection into the ventral part of the lateral third of the left SPON; this injection site encroached upon the neighboring MSO (Fig. 1C). The medial plexus is situated more dorsolaterally and the lateral plexus more dorsomedially than in the cases depicted in Figs. 6 and 7. The plexus at the dorsolateral border of the CNIC (surrounded by a dotted line in D) represents the projection from the MSO. Other abbreviations: Aq, cerebral aqueduct; ECIC-r, rostral part of the ECIC; SC, superior colliculus. Scale bar = 1 mm.

Fig. 9.

Schematic representation of the topographic organization of projections from the SPON to the ipsilateral IC in the rat. Three isofrequency bands or regions have been depicted in the SPON: a medial, higher frequency region (black); a central, medium frequency region (dark gray); and a lateral, lower frequency region (light gray). Regions of the ipsilateral IC innervated by each region of the SPON are represented with the corresponding shadowing.

Fig. 10.

Micrographs of coronal sections show labeling in the left nuclei of the lateral lemniscus and IC following single injections of BDA into the left SPON (**A**; case 97097, whose injection site is depicted in Fig. 1C), left MNTB (**B**) or right anteroventral cochlear nucleus (AVCoN) (**C**). Note that the thick labeled axons ascending in the medial part of the lateral lemniscus in panel A are not observed in panels B and C. Note also in panel B the near absence of labeled axons in the DNLL and IC. Other abbreviations: MPL, medial paralemniscal nucleus; rs, rubrospinal tract.