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Connections of the Auditory Brainstem in a Songbird, *Taeniopygia guttata*. III. Projections of the Superior Olive and Lateral Lemniscal Nuclei

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Abstract

Sequential to companion articles that report the projections of the cochlear nucleus angularis (NA) and the third-order nucleus laminaris (NL) to the central nucleus of the inferior colliculus (MLd) and to the superior olive (OS) and lateral lemniscal nuclei (LLV, LLI, and LLD) (Krützfeldt et al., *J Comp Neurol*, this issue), we here describe the projections of the latter group of nuclei using standard tract-tracing methods. OS projects on LLV and both have further ascending projections on LLI, LLD, and MLd. LLV also provides auditory input to the song system, via nucleus uvulaeformis, and to the thalamo-telencephalic auditory system, via nucleus ovoidalis (Ov), thus bypassing MLd. The two divisions of LLD (LLDa and LLDp) project across the midline via the commissure of Probst each to innervate the homologous contralateral nucleus and MLd. Both, particularly LLDp, also project on Ov. Injections in LLD and LLV resulted in anterograde labeling of caudal nucleus basorostralis (Bas) in the frontal telencephalon, but retrograde tracing so far suggests that only LLI is a real source of this projection (Wild and Farabaugh [1996] *J Comp Neurol* 365:306–328). OS and LLV also have descending projections on the ipsilateral NA, NM, and NL, and LLV also projects on OS. The ascending inputs to MLd and more rostral nuclei may contribute importantly to mechanisms of auditory pattern (song) recognition. Consistent with previous studies, some of the descending projections may be inhibitory.

INDEXING TERMS

dorsal; ventral, and intermediate nuclei of the lateral lemniscus; zebra finch; avian

In a systematic attempt to describe the connections of the auditory brainstem in a songbird, two companion articles charted the projections of the cochlear nucleus angularis (NA) and the third-order nucleus laminaris (NL) to the inferior colliculus (Krützfeldt et al., 2010a) and to the superior olive and lateral lemniscal nuclei (Krützfeldt et al., 2010b). In the present article we describe the heterogeneous projections of the latter group of nuclei, some of which both ascend and descend the neuraxis. Some of these projections have been described previously, briefly in the zebra finch and in some other avian species, but others have either never before been described or have not been described in songbirds (Wild et al., 1985, 2001, 2009; Hall et al., 1993; Wild and Farabaugh, 1996; Coleman et al., 2007). The justification for this series of studies in the zebra finch was given in the introduction to the first article (Krützfeldt et al., 2010a), and therefore need not be repeated in detail here. Suffice it to say that, because songbirds, like humans, rely on auditory feedback from

conspecifics and self to learn their species specific vocalizations (song) and to produce it accurately during adulthood (Krützfeldt et al., 2010a), a knowledge of the brainstem pathways that mediate auditory input to forebrain structures is a necessary preliminary to an understanding of the neural mechanisms underlying auditory-vocal learning.

MATERIALS AND METHODS

The methods used were essentially the same as those described in detail in the first article (Krützfeldt et al., 2010a). In brief, in a series of adult male zebra finches the superior olive (OS) and the ventral and dorsal nuclei of the lateral lemniscus (LLV and LLD) each received electrophysiologically guided injections of either biotinylated dextran amine (BDA, Invitrogen, Eugene, OR; 10K MW, 10% in phosphate-buffered saline [PBS], pH 7–4) or cholera toxin B-chain (CTB, List Biological Laboratories, Campbell, CA; 1% in PBS). The intermediate nucleus (LLI) was not specifically injected because the projections of this nucleus have been described previously (Wild and Farabaugh, 1996). In another series of cases, injections of these and other tracers (e.g., Fast Blue, Polysciences, Warrington, PA; 2% aqueous) were also made into the putative targets of these nuclei in order to validate nuclear origins by retrograde labeling. Most injections were made using iontophoresis (2–4 μ A positive current for 10–30 min, 7 sec on, 7 sec off) delivered via glass micropipettes pulled on a David Kopf (Tujunga, CA) vertical puller and broken back to yield tip sizes of 10–20 μ m internal diameter, but some were made using air pressure delivered via a picospritzer (General Valve, Fairfield, NJ). Electrophysiological recordings of auditory-evoked neural activity were made using standard methods (see Krützfeldt et al., 2010a), and either tungsten microelectrodes (Frederick Haer, Bowdoinham, ME) or the injection pipette, which also replaced the tungsten electrode following positive identification of an auditory nucleus, made in combination with stereotaxis (unpublished atlas of the zebra finch brain [Akutagawa and Konishi], kindly supplied by Dr. M. Konishi). Brain tissue sections were treated immunohistochemically for the visualization of BDA and CTB as described in Krützfeldt et al. (2010a).

RESULTS

Ascending projections of the superior olive

Figure 1G shows an injection of BDA in the left superior olive (OS). It was concentrated in the ventral part of OS, but spread throughout the nucleus, to which it was very largely confined. The ascending projections from this and other OS injections ($n = 5$) were bilateral, but predominantly contralateral (Fig. 1A–F, H). Anterograde (and retrograde) labeling was present in the contralateral OS, which was reached by fibers that ascended through the tegmentum dorsomedially, crossed to the other side dorsal to the medial longitudinal fasciculus, and then descended ventrolaterally to their target. This trajectory is quite distinct from that of NA and NL fibers that project to the ipsilateral OS and then beyond by crossing the midline in the ventral tegmentum (Krützfeldt et al., 2010a). Anterograde label from OS injections was also present in LLV, LLI, LLDp, and MLd, but was largely absent from LLDa (Fig. 1A–F). The label in LLI extended from LLIC, at the ventro-lateral tip of the principal sensory trigeminal nucleus (PrV), all the way through LLIr (Fig. 1I–O). The label in the contralateral OS, LLV, and MLd was most dense in the ventral parts of these nuclei, possibly reflecting the concentration of the injection in the ventral part of OS. In another OS case, the injection was concentrated in the ventromedial part of OS and the anterograde label in MLd was most dense in the ventrolateral part of the nucleus.

Descending projections of OS

The injection shown in Fig. 1G, and others like it, also produced anterograde labeling predominantly in the ipsilateral cochlear nuclei (nucleus angularis [NA] and nucleus magnocellularis [NM]) and in nucleus laminaris (NL; Figs. 2A–D, 3A–C). In NL punctate labeling was apparent along both medial (internal) and lateral (external) dendrites of NL cell bodies, as well as on the NL cell bodies themselves (Fig. 3B). Retrograde labeling also accompanied the anterograde labeling in NA and the labeled processes of these cells contributed to the apparent density of labeling in the nucleus. Nevertheless, anterograde labeling could be seen to be considerably denser in the lateral, higher-frequency part of NA, than in the medial, lower-frequency part, consistent with the ventral predominance of label in the ventral, higher-frequency part of MLd (see above and Krützfeldt et al., 2010a). This could imply that low to high acoustic frequencies are represented dorsoventrally in OS, i.e., that there is a tonotopic representation in OS, as in barn owls (Moiseff and Konishi, 1983), but further experiments are required to confirm this in zebra finches.

Ascending projections of the LLV

A typical BDA injection ($n = 5$) in the right LLV is shown in Fig. 4A. Terminal labeling was present in the ipsilateral LLI (both LLlc and LLlr), LLDp, and MLd, but was largely absent from LLDa (Fig. 4B, C). The label in the more ventral part of LLDp was more dense than that in the more dorsal part of LLDp (Fig. 4C), and in MLd it was extremely dense, filling the entire nucleus (Fig. 4D, E). In the diencephalon, terminal labeling was present in the lateral part of nucleus uvulaeformis (Uva; Fig. 4F), in nucleus ovoidalis (Ov), predominantly ipsilaterally (Fig. 4G), and as a tiny, dense patch dorsomedial to the occipitomesencephalic tract (OM; Fig. 4H). In the telencephalon terminal labeling was present, again predominantly ipsilaterally, in the most caudal part of nucleus basorostralis (Bas), immediately ventral to the rostral pole of the entopallium (Fig. 4I).

Descending projections of LLV

The injection in LLV shown in Fig. 4A, and others like it, produced terminal labeling in OS bilaterally (Fig. 2E, J), and in NA, NM, and NL ipsilaterally (Figs. 2F–I, 3D–F). The labeling in NA, NM, and NL was considerably less dense than that produced by OS injections, but was present as fibers and beaded terminations throughout the entire nuclei. It should also be noted that the terminal labeling in NA, NM, and NL produced by LLV and OS injections was ipsilateral to the injection site in each case, but whereas the projections from NA and NL to OS are predominantly ipsilateral, those to LLV are predominantly contralateral (Krützfeldt et al., 2010a).

Projections of LLD

Because of the proximity of LLDa and LLDp, and because of the way LLDp axons partly traverse LLDa, confining injections to either one of them proved virtually impossible; regardless, no descending projections from either nucleus were observed. An injection of BDA centered on LLDa, with some involvement of LLDp, is depicted in Fig. 5H. That the projections labeled by this injection arise predominantly from LLDa is indicated by the fact that, although retrogradely labeled neurons were present in both NL and NA contralaterally, the ones in NA were lightly labeled and relatively sparse, while those in NL were heavily labeled and numerous (Krützfeldt et al., 2010b). Krützfeldt et al. (2010a) showed that the ascending input to LLDa arises specifically from NL and not from NA. Labeled fibers left the LLD injection site dorsomedially to cross the midline in the commissure of Probst to innervate the contralateral LLDa (Fig. 5G–J) which, because of the reciprocity of the projection, also contained retrogradely labeled cells (Fig. 5J). Fibers also passed through and slightly dorsal to the contralateral LLDa to innervate the contralateral MLd throughout its

dorsomedial half to two-thirds (Fig. 5E, F). In addition, there was a less dense, but a virtual mirror image of this terminal field in the ipsilateral MLd (Fig. 5D). This is assumed to have arisen via collaterals of contralateral LLDa (and possibly LLDp) neurons, because injections in MLd did not produce retrograde labeling in the ipsilateral LLDa (and very few retrogradely labeled neurons in the ipsilateral LLDp; see below). In the diencephalon, sparse anterograde labeling was present within the ipsilateral Ov, but was confined to its periphery (Fig. 5B), and heavily labeled fibers and terminations were present in the most caudal part of the ipsilateral Bas (Fig. 5A, C).

Another injection in LLD, this time of CTB, was also centered on LLDa, but had more involvement of LLDp (Fig. 5K). This produced a similar scenario of crossed projections as that described above, except that, in addition to anterograde and retrograde labeling in the contralateral LLDa, there was anterograde and retrograde labeling in part of LLDp also (Fig. 5L). The location of the terminal fields in the contralateral and ipsilateral MLd were also mirror images of each other, but the location was different from that described for the injection shown in Fig. 5H. Label was present throughout most of MLd, but was concentrated in the ventrolateral third of the nucleus, possibly suggesting a greater input from LLDp.

Retrograde assessment of putative orthograde projections

Injections relevant to this assessment (of BDA, CTB, or Fast Blue) were made in NA, NM, NL, OS, LLV, LLI, LLD, MLd, Uva, Ov, and Bas. Data from some of these were used in the companion articles (Krütfeldt et al., 2010a, b) or a previous article (Wild and Farabaugh, 1996), but others were made specifically for the present purpose. Injections in NA and NL produced retrograde labeling in OS and LLV (Fig. 6A–D); that in OS was predominantly ipsilateral from both NA and NL (Fig. 6A, B) but that in LLV was predominantly ipsilateral from NA (Fig. 6C) and sparse and ipsilateral only from NL (Fig. 6D). Injections in OS produced retrograde labeling in the contralateral LLV (Fig. 6E) and injections in LLV produced retrograde labeling in OS, predominantly contralaterally (Fig. 6F). Injections aimed at LLr retrogradely labeled cells in NA and OS, predominantly contralaterally, NL contralaterally, and LLV ipsilaterally (data taken from cases used in Wild and Farabaugh, 1996). An injection of BDA centered on LLDa, but with a small involvement of LLDp (Fig. 5H), retrogradely labeled cells in the contralateral LLDa (Fig. 5J), and a few in the contralateral LLDp. No LLV cells were labeled and only an occasional cell was labeled in OS. A few cells were labeled on the medial edge of the ipsilateral MLd, but whether these target LLD, more caudal nuclei, or the contralateral MLd—their crossing axons being labeled by uptake from damage caused by the injection pipette—is not known. In contrast, a similar injection of CTB, also centered on LLDa, but with more involvement of LLDp (Fig. 5K), retrogradely labeled cells in the contralateral LLDa and LLDp (Fig. 5L) and numerous cells in the ipsilateral LLV and in OS, predominantly contralaterally, confirming the anterograde results from injections in LLV and OS (see above). Injections in MLd (e.g., Fig. 7) produced retrograde labeling in NA and NL contralaterally (see Krütfeldt et al., 2010a), in OS predominantly contralaterally (Fig. 7E, F), densely in LLV, predominantly ipsilaterally (Fig. 7C, D), in LLDa contralaterally only (Fig. 7B), and in LLDp, largely contralaterally (Fig. 7A, B). Injections in MLd also resulted in dense anterograde, terminal labeling in the ipsilateral LLDa and less dense anterograde labeling (and a few retrogradely labeled cells) in LLDp (Fig. 7A). But because injections in LLD produced only minimal retrograde labeling in the ipsilateral MLd (see above), the anterograde labeling in LLD following MLd injections is assumed to have arisen, at least in large part, from contralateral LLD neurons that project to LLD and MLd via branched axons. This is also indicated by the direction of labeled fibers adjacent to LLDa, which appear to have entered the nucleus via the commissure of Probst from the opposite side (Fig.

7A). Of all the auditory nuclei, only LLV was retrogradely labeled by injections in Uva (not shown here; but see Coleman et al., 2007). Injections in Ov, however, produced retrograde labeling in LLDp, a very few in LLDa, and in LLV, all predominantly ipsilaterally (Fig. 8A, B). Injections aimed at the most caudal part of Bas produced retrograde labeling only in LLI, except in one case in which a couple of labeled cells were present in the more ventral part of LLDp.

In summary, the retrograde data were mostly consistent with and supported the anterograde data, only the retrograde data from Bas injections being largely inconsistent with the anterograde data (see Discussion).

DISCUSSION

Technical considerations

Fibers of passage—The nuclei of the lateral lemniscus, being embedded within the lateral lemniscus, represent a classic case of injection hazard because of fibers of passage: a great many fibers having more caudal origins in lower auditory nuclei, such as OS, NA, and NL, project through and adjacent to LLV, LLI, and LLD, en route to MLd. In addition, many fibers destined to terminate in LLD pass through and adjacent to LLV and LLI; similarly, those destined for LLI pass through and adjacent to LLV. Therefore, injections in any of the three nuclei cannot avoid a certain amount of uptake by fibers of passage, a fact that must be taken into account when considering and interpreting the data.

Use of different tracers—During the course of many tracing studies conducted over more than 15 years, in which we have been using combinations of BDA and CTB, it has become abundantly clear that BDA is the tracer of choice for anterograde labeling (Veenman et al., 1992), whereas CTB is more sensitive for retrograde labeling, in the sense of labeling more neurons than BDA from similar-sized injections. These differences should be borne in mind, especially when considering the retrograde data resulting from BDA versus CTB injections.

Branched axons—A possibility that we have made no explicit attempt to assess here is the extent to which the axons of brainstem auditory neurons branch to innervate more than one nucleus (cf. Wild et al., 2009). Although this can be indicated by somatopetal and somatofugal transport to and from retrogradely labeled neurons—a phenomenon we suspect is common in the auditory brainstem—double fluorescent retrograde labeling would be required to confirm this, as it has been for the descending projections of OS (see below). Figures 9 and 10 summarize the findings made in this series of three studies, based on single injections into relevant nuclei, i.e., without recourse to double retrograde labeling. This limitation does not affect, however, our claims for target specificity based on anterograde and retrograde labeling.

Ascending projections of the OS

In the zebra finch the projections of OS were found to the contralateral OS and, as ascending fibers, to LLV, LLI, LLDp, and MLd, predominantly contralaterally. A projection to LLDa was conspicuous by its absence. Bilateral OS projections to MLd have been shown in the mallard (Arends, 1981) and chicken (Conlee and Parks, 1986; Westerberg and Schwarz, 1995) and the latter authors also noted a small projection to LLI (their LLIp). But the projection to LLI in the zebra finch is particularly noteworthy in that it defines without interruption the complete rostrocaudal extent of the nucleus, from its position ventrolaterally adjacent to the principal sensory trigeminal nucleus, through its rostral extent medial to the ventrolateral parabrachial nucleus. These data are nicely consistent with the conception of

LLI as defined by Arends and Zeigler (1986) in the pigeon, even to the extent of showing a slight reduction in the size of the terminal field about halfway through the rostrocaudal extent of LLI, before expanding again to its full size at its rostral pole. Since LLI relays a short latency, bilateral, and presumably excitatory projection on Bas in the frontal telencephalon that is thought to originate in NA and NL (Delius et al., 1979; Arends and Zeigler, 1986; Wild and Farabaugh, 1996), OS in the zebra finch could contribute to this projection in a substantial way, albeit with the addition of an extra synapse. However, since the descending projections of OS on the cochlear nuclei and NL are known to be inhibitory in other species (see below), it is conceivable that the OS projection on LLI is likewise inhibitory.

The auditory projection on LLIC described by Arends and Zeigler (1986) in pigeons was made on the basis of injections of wheat-germ agglutinin conjugated to horse-radish peroxidase (WGA-HRP) into NA and NL, but this projection was not confirmed retrogradely. The authors noted, however, that injections into the cochlear nuclei and NL produced retrograde labeling in OS, confirmed by Burger et al. (2005) in chickens and by us in the zebra finch (present study). But because neither Leibler (1975) nor Wild (1995) found a projection from NA or NL to LLIC in pigeons, and because we did not find a substantial projection from NA or NL to LLIC in zebra finches, but did find a projection from OS to LLIC, a possible explanation of the findings of Arends and Zeigler (1986) is that some OS neurons project to LLIC via collaterals of axons that project to NA. Such a possibility will require testing for double labeling of OS neurons by dual injections in LLIC and NA/NL. Alternatively, the findings of Arends and Zeigler (1986) could be explained by a real species difference in the source of innervation of LLIC. Takahashi and Konishi (1988b) in the barn owl also found a light projection from NA to the lateral part of the principal sensory trigeminal nucleus, but the similarity of this projection to that described by Arends and Zeigler (1986) in the pigeon is unclear.

In the present study there was a hint of a topographic, and possibly tonotopic, projection from OS to MLd, in that a ventral concentration of an injection in OS resulted in a ventral concentration of terminal labeling in MLd, where higher frequencies are known to be represented (Woolley and Casseday, 2004), but this will require further investigation using smaller injections in different parts of OS. The general pattern of ascending OS projections in the zebra finch distinctly resembles that of NA, which also projects to OS bilaterally, at least in the mallard (Arends, 1981), chicken (Conlee and Parks, 1986), owl (Takahashi and Konishi, 1988b), pigeon (Correia et al., 1982), and zebra finch (Krützfeldt et al., 2010a).

Descending projections of the OS

Descending projections of OS on the cochlear nuclei and nucleus laminaris have been reported in mallards (Arends, 1981), pigeons (Arends and Zeigler, 1986), barn owls (Carr et al., 1989), and chickens (Lachica et al., 1994; Westerberg and Schwartz, 1995). On the basis of double-labeling experiments in chickens, single OS neurons probably innervate all three of the ipsilateral NA, NM, and NL, but in a nontopographic manner (Burger et al., 2005). Similarly, in the zebra finch all three nuclei were anterogradely labeled following injections of BDA into the ipsilateral OS. The OS projection to NA, NM, and NL is known to be GABAergic (inhibitory) in owls and chickens (Müller, 1987; Carr et al., 1989; Code et al., 1989; von Bartheld et al., 1989; Lachica et al., 1994; Brückner and Hyson, 1998; Yang et al., 1999) and its function has been proposed to adjust the gain of the NM–NL timing circuit in a dynamic way, i.e., despite changes in overall intensity (Hyson, 2005). It can be noted that, since OS projects to the contralateral OS, albeit via neurons separate from those that project to the ipsilateral NA, NL, and NM (Burger et al., 2005), there is a possibility that each OS influences NA, NM, and NL bilaterally. As pointed out by Lachica et al. (1994) for the chicken, this connectivity, plus projections from NA to OS, also suggest that NA can

influence the activity in NM and NL, thereby enhancing the importance of intensity cues for binaural perception.

Ascending projections of LLV

The ascending projections of this nucleus are as varied as they are substantial. It projects, predominantly ipsilaterally, on LLI, LLDp, MLd, Uva, Ov, another tiny field in the mediodorsal thalamus, and possibly on Bas. Injections of BDA in LLV produced the greatest density of anterograde label in, and the most complete coverage of, MLd of any injection made in this series of studies. However, because of the fibers of passage problem noted above (Technical considerations), it may be that not all this label results from LLV. In order to assess the true density and extent of LLV projections to MLd, uncontaminated by NA, NL, and OS projections, it would be necessary first to surgically interrupt ascending fibers caudal to LLV, and then wait for degeneration of NA, NL, and OS axons before injecting LLV. Although we have not done this here, the large terminal fields produced by relatively small injections made in LLV nevertheless suggest that LLV makes a major contribution to the innervation of MLd in its own right. Retrograde tracing from MLd injections also suggest a substantial LLV input (confirmed in pigeons by Leibler, 1975). Furthermore, since LLV receives inputs from NA and NL (Krützfeldt et al., 2010b), LLV's extensive projection to MLd is consistent with the substantial overlap of direct NA and NL projections to MLd (Krützfeldt et al., 2010a).

As noted in a separate report (Coleman et al., 2007), the projections of LLV to Uva permit an auditory input to the telencephalic song circuitry that is not routed through the classical relay of MLd and nucleus ovoidalis (Karten, 1967, 1968). The specific nature of this input to Uva has not been determined, but it appears to enable Uva to play a role in state-dependent gating of auditory activity in the song control nucleus HVC, to which Uva projects via direct and indirect pathways (Nottebohm et al., 1982; Wild, 1994).

A direct LLV (and LLD) projection to Ov was first noted in the pigeon (Wild, 1987) and is confirmed here for the zebra finch. In the pigeon, however, the projection was predominantly to the nucleus parovoidalis, which has a preferential input to the low frequency or "broad band" region of dorsolateral Field L in the telencephalon (Wild et al., 1993). It is unclear whether a similar organization exists in the zebra finch, because a separate parovoidal part of Ov is not apparent. Nevertheless, the LLV projection to Ov appears to have a concentration ventrally in the nucleus, in which lower frequencies are represented ventrally, at least in starlings (Bigalke-Kunz et al., 1987). This ventral part of Ov would then project on the dorsolateral part of Field L, where lower frequencies are represented, as in pigeons (Wild, unpubl. obs.).

In each case of LLV injections a tiny ($\approx 100 \mu\text{m}$ wide), dense terminal field was found dorsomedial to the occipitomesencephalic tract in the dorsal thalamus (Fig. 4H). Nothing is known about this curious, specific projection.

The most rostral terminal field resulting from LLV injections was in the most caudal part of Bas, the enigmatic multisensory nucleus of the frontal telencephalon (Wild and Farabaugh, 1996). When injections were aimed at this part of Bas, guided by auditory stimulus-evoked responses, retrogradely labeled cells were not found in LLV as a result. This is consistent with previous findings in zebra finches and other species, in which only LLI of the three lateral lemniscal nuclei has been found to project on Bas (Arends and Zeigler, 1986; Hall et al., 1993; Wild and Farabaugh, 1996; Wild et al., 1997, 2001). Whether the present findings mean that our LLV injections somehow resulted in uptake of tracer by LLI axons (which for this to happen would have to travel ventrally before ascending the neuraxis), or whether we have not accurately targeted the small, most caudal part of Bas that might receive a

projection from LLV, is presently unclear and awaits further investigation. The terminal field in Bas resulting from LLV injections is shown in Fig. 4I—despite the lack of retrograde confirmation—1) because we consider the density of anterograde labeling observed in Bas to be inconsistent with uptake by fibers of passage, and 2) no retrogradely labeled cells were found in LLI as a result of LLV injections. In other words, we suspect that there might be a projection from LLV to Bas. These arguments also apply to the putative LLD projection to Bas (see below).

Descending projections of LLV

Descending projections of LLV on NA were noted by Arends and Zeigler (1986) on the basis of retrograde labeling, but the present study also defines these projections anterogradely and shows their terminations in both cochlear nuclei (NA and NM) and in NL. It is perhaps significant that these projections are largely ipsilateral, whereas those from NA and NL to LLV are predominantly contralateral. If the LLV projections to NA, NL, and NM are inhibitory, like those of OS (see above), this could suggest a function of relatively enhancing the auditory input to LLV from the contralateral side. It is also possible that, since OS projects to both LLV and NA, NL and NM, some terminations in the cochlear nuclei and NL following LLV injections could, in principle, arise as a result of somatopetal and somatofugal transport to and from single OS neurons. This, however, appears not to be the case (Wild et al., 2009). Furthermore, this would not totally account for the LLV terminations in NA, NL, and NM, since retrogradely labeled neurons were present in LLV following injections in these nuclei.

Projections of LLI

Because the projections of this nucleus were shown in a previous study (Wild and Farabaugh, 1996), they were not examined in the present one. For the sake of completion, we can note that LLI has been shown in several species to be the source of a short latency auditory projection on caudal regions of nucleus basorostralis, a forebrain nucleus that was originally regarded as devoted to receiving sensory input from the beak and oral cavity, predominantly via trigeminal and, in ducks and parrots, also via glossopharyngeal and hypoglossal nerve projections to the principal sensory trigeminal nucleus (PrV) (Wallenberg, 1903; Dubbeldam et al., 1979, 1981; Wild, 1981; Wild et al., 1985; Arends and Zeigler, 1986; Wild and Farabaugh, 1996). Within Bas of barn owls, this auditory projection is tonotopic and in barn owls and budgerigars forms one part of the representation of the whole body (Wild et al., 1997; 2001).

Projections of LLD

In the zebra finch we found a substantial separation of the NA and NL inputs to LLDp and LLDa, respectively (Krützfeldt et al., 2010b), similar to the situation in more caudal regions of VLVp and VLPa in barn owls (Takahashi and Konishi, 1988b). Less clear than in barn owls, however, was a region of LLD in the zebra finch, where the inputs from NA and NL overlap. However, because of their small size and proximity, we were unable to determine on the basis of anterograde tracing whether or to what extent the projections of LLDa and LLDp are separate within the contralateral MLd, but the evidence from retrograde tracing suggests that there is little, if any, regional separation: all our MLd injections resulted in retrogradely labeled cells in both LLDa and LLDp, irrespective of the location of the injection in different parts of MLd. This apparent lack of separation of the LLDa and LLDp projections to MLd is consistent with the lack of a clear separation of the NA and NL terminal fields within MLd (Krützfeldt et al., 2010a), an organization quite unlike that in the chicken and barn owl. In the latter, in particular, NL and LLDa project to the core of ICc, while NA and LLDp project to a shell surrounding the core (Conlee and Parks, 1986; Takahashi and Konishi, 1988a, b; Takahashi et al., 1989; Adolphs, 1993).

In barn owls LLDp is the first site of binaural convergence in the pathway that processes differences in interaural level or intensity (ILD) (Manley et al., 1988; Fujita and Konishi, 1991; Mogdans and Knudsen, 1994). These differences, which are mediated in this species by directionally asymmetrical ears (Payne, 1971), are important for the localization of sounds above 4 kHz in the plane of elevation. LLDp neurons are excited by NA input from the contralateral ear and are inhibited by input from the ipsilateral ear. The source of the ipsilateral inhibition is LLDa, which is connected with its inhibitory partner across the midline via the commissure of Probst (Manley et al., 1988; Takahashi and Keller, 1992; Takahashi et al., 1995). LLDa is part of the interaural time difference pathway, it contains GABAergic neurons (Carr et al., 1989), and, on the basis of retrograde labeling, it projects to the ICc core (Takahashi et al., 1989). Whether it is also connected across the midline with its contralateral counterpart, as LLDa is in the zebra finch, appears not be documented.

Zebra finches, like most other birds, do not have directionally asymmetrical ears, so time and intensity will vary together only in the azimuthal plane. They also have small heads, which may contribute to their poor sound localizing skills (Park and Dooling, 1991). This suggests that, if they have a “time pathway”—which has yet to be demonstrated physiologically—it does not contribute significantly to sound localization. Also, interaural level differences may not be functionally as significant for sound localization, as distinct from sound lateralization, because of the small head width vis-à-vis the comparatively low frequencies that most birds hear. Despite these considerations, NA and NL do have separate inputs to LLD, each LLDa and LLDp is connected with its contralateral counterpart via the commissure of Probst, and both LLD subnuclei may be GABAergic, as in the chicken and barn owl (Müller, 1987; Carr et al., 1989; Manley et al., 1988; Fujita and Konishi, 1991). Yet the evidence from retrograde tracing, at least, suggests that they do not have separate terminal fields within MLd. If so, then it can be asked why a clear separation of the NA and NL inputs to LLDp and LLDa, respectively, is retained in the zebra finch. One answer may be that the lack of separation of the LLDp and LLDa projections to MLd is more apparent than real; these projections, in fact, targeting either different neurons that are intermixed in MLd, or different small clusters of MLd neurons, rather than different and separate territories, as in barn owls. The functional implications of such an anatomical organization, if real, await investigation (cf. Oliver, 2005).

Injections in LLD, like those in LLV (see above) also anterogradely labeled the most caudal part of Bas with distinctively labeled fibers and characteristically large presumptive terminations (cf. Wild and Farabaugh, 1996). Retrograde confirmation of this projection, however, was largely lacking, but our reservations about this lack are similar to those noted above with regard to the lack of retrograde confirmation of the putative LLV projection to Bas.

Both LLDp and LLDa, as well as LLV (see above), were found to project on nucleus ovoidalis (Ov), confirming similar previous findings in pigeons (Wild, 1987), and providing for a direct lemniscal auditory input to the forebrain. The LLDa projection, however, seems to be very sparse and confined to the periphery of Ov. No such projection has been found in mammals.

Comparative comment

Grothe (2000) suggested that a complete nucleus by nucleus comparison of brainstem auditory nuclei in birds and mammals simply cannot be made 1) because birds do not possess several of the auditory brainstem structures present in mammals, e.g., a dorsal cochlear nucleus, a medial nucleus of the trapezoid body, or a lateral superior olive; 2) because structures such as NL and its presumptive functional equivalent in mammals, namely, the medial superior olive (MSO), have not been shown to be homologous; and 3)

because the independent but near-simultaneous evolution during the Triassic period of tympanic ears in frogs, stem reptiles (and hence birds), and mammals suggests the absence of a common ancestor of birds and mammals capable of hearing airborne sounds (Clack, 1997; Grothe, 2003). With regard to (2), Grothe (2003) pointed out the very different roles served by inhibition in MSO in mammals and NL in birds, the former being feedforward and glycinergic from the medial nucleus of the trapezoid body and the latter being feedback and GABAergic from OS, a difference that has important implications for ITD processing in the two animal classes. Other obvious differences between the auditory brainstem projections of birds and mammals are the facts that one of the lateral lemniscal nuclei (LLI) in birds does not project to the IC, but instead to the frontal telencephalon (Arends and Zeigler, 1986; Conlee and Parks, 1986; Takahashi and Konishi, 1988a; Wild and Farabaugh, 1996; Wild et al., 2001; Krützfeldt et al., 2010a), and that LLD and/or LLV in birds project directly to the auditory thalamus and other diencephalic nuclei, as well as to the ICc. In addition, the projection of LLD to MLd in the zebra finch seems to be almost totally contralateral, whereas that from DNLL to the IC in mammals is bilateral.

Considering possible similarities between avian and mammalian auditory pathways, the large spherical bushy cells of the AVCN in mammals are generally considered morphologically and functionally similar to NM neurons in birds (Jhaveri and Morest, 1982; Sullivan and Konishi, 1984; Cant and Benson, 2003). They project ipsilaterally and contralaterally to the MSO in a similar manner to the ipsilateral and contralateral projections of NM neurons to NL in birds. Like NM neurons, they do not project to the inferior colliculus, but do project to auditory brainstem nuclei other than MSO (e.g., VNLL; Cant and Benson, 2003), unlike NM neurons, which do not project to any nucleus other than NL.

Multipolar neuronal types have been described in NA of pigeons and barn owls (Häusler et al., 1999; Soares and Carr, 2001) that have similar counterparts in the ventral cochlear nuclei of mammals (Doucet and Ryugo, 1997). However, the majority of cells in NA (“stubby cells”) are quite dissimilar to any of the multipolar neurons in the ventral cochlear nuclei of mammals (Soares and Carr, 1999). Some NA cells have similar firing patterns to those of the ventral cochlear nucleus of mammals, e.g., “choppers” (Köppl and Carr, 2003), as well as similar projections that include the OS/olivary complex, the LLV/VNLL, the ICc, and, to a lesser extent of similarity, the dorsal nucleus of the lateral lemniscus (Cant and Benson, 2003). In barn owls and zebra finches the LLD has two distinct parts, anterior and posterior, which receive separate projections from NL or NA, respectively (Takahashi and Konishi, 1988b; Krützfeldt et al., 2010b). In mammals, in contrast, DNLL is described as a single, binaural nucleus that receives relatively sparse projections from the cochlear nuclei (Glendenning et al., 1981; Huffman and Covey, 1995; Schofield, 2005).

One significant point of similarity between mammalian and avian auditory brainstem pathways, however, is the massive input from LLV and VNLL to the IC of birds and mammals, respectively (e.g., Benson and Cant, 2008; present results). In mammals this input is a major source of both GABAergic and glycinergic inhibition on the IC (Schofield, 2005). There is some evidence in chickens that LLV possesses glycinergic neurons (Westerberg and Schwartz, 1995), and there is evidence from barn owls and possibly pigeons and zebra finches that LLV is GABAergic (Carr et al., 1989; Veenman and Reiner, 1994; Wild and Krützfeldt, unpubl. obs.). VNLL neurons are thought to be involved in the recognition of temporal patterns and the processing of complex sounds (Benson and Cant, 2008). If LLV neurons in birds have similar functional attributes, and if these attributes are conveyed to the ICc by way of inhibitory signals, they could possibly contribute to the processing of complex auditory stimuli, such as that involved in feature extraction and the recognition of conspecific song (Woolley and Casseday, 2004, 2005; Logerot et al., 2009).

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Figure 1.

A–H: Fiber and terminal labeling in the contralateral OS (H) and in the ipsilateral and contralateral LLV and LLIr (E, F), LLDp (C, D), and MLd (A, B) following an injection of BDA in the left OS (G, counterstained). **I–O:** Alternating counterstained and noncounterstained sections showing terminal labeling throughout the entire rostrocaudal extent of LLI following the injection of BDA in the ipsilateral OS shown in G. All sections were cut in the transverse plane. PLL: para-lateral lemniscal nucleus (Wild and Farabaugh, 1996); PrV: principal sensory trigeminal nucleus. Scale bars = 200 μm in G, H; 300 μm in C–F, I–O; 400 μm in A, B.

Figure 2.

A–D: Anterograde fiber and terminal label in the left (ipsilateral) NA, NM, and NL at four rostrocaudal levels following the injection of BDA in OS shown in Fig. 1G. Some retrograde labeling of cell bodies and their processes is also present in NA, particularly laterally (C, D). **E–J:** Anterograde fiber and terminal labeling in the left (contralateral) and right (ipsilateral) OS (E, J) and in the (right) ipsilateral NA, NM, and NL (F–I, representing four rostrocaudal levels) following an injection of BDA in the right LLV shown in Fig. 4A (see also Fig. 3). Scale bar = 300 μ m.

Figure 3.

A–C: Labeling in the left NM, NL, and NA following an injection of BDA in the ipsilateral OS (see Fig. 1G). Note the punctate labeling along the medial (internal) and lateral (external) processes of NL neurons in B, the cell bodies of which appear also to receive terminations. Note also the retrograde labeling of cell bodies in NA (C). **D–F:** Labeling in the right NM, NL, and NL following an injection of BDA in the ipsilateral LLV (see Fig. 4A). The transverse sections are uncounterstained, but cell bodies can faintly be seen as a result of the heavy metal (CoCl_2) intensification of the DAB reaction product (see Materials and Methods in Krützfeldt et al., 2010a). Scale bar = 100 μm .

Figure 4.

A: An injection of BDA in the right LLV. **B–I:** Terminal labeling in the ipsilateral structures identified. E shows a higher-power view of the area boxed in D. Bas: nucleus basorostralis; DM: dorsomedial nucleus of the intercollicular region; E: entopallium; H: hyperpallium; LSt: lateral striatum; N: nidopallium; OM: occiptomesencephalic tract; Ov: nucleus ovoidalis; TeO: tectum opticum; Uva: nucleus uvaeformis. Scale bars = 300 μ m in A–C, D, G, I; 150 μ m in F; 75 μ m in E; 1 mm and 100 μ m in H.

Figure 5.

A–F: Anterograde labeling in the structures identified following an injection of BDA centered on the left LLDa shown in **G, H**. The direct projection to MLd is predominantly, if not entirely, contralateral (F and the boxed higher magnification in E), whereas the projection to the ipsilateral MLd (D) is assumed to have arisen, via somatopetal and somatofugal transport, from neurons in the contralateral LLDa shown in G (right side) and **J** that project across the midline via Probst's tract, as shown in G and schematically in **I** (see text). **K:** A CTB injection centered on LLDa, but with greater involvement of LLDp than the injection of BDA shown in H. **L:** Retrogradely labeled cells in the contralateral LLDp as well as the contralateral LLDa resulting from the injection in K (see text). Scale bars = 250 μm in A, G; 400 μm in B, D, F; 75 μm in C; 100 μm in E; 300 μm in H, K; 200 μm in J, L.

Figure 6.

A–D: Retrograde (and anterograde) labeling in the left OS (A, B) or left LLV (C, D) following injections of BDA in the ipsilateral NA or NL, respectively. **E:** Retrogradely labeled cells in the left LLV following an injection of CTB in the contralateral OS. **F:** Retrogradely labeled cells (and anterograde fiber and terminal labeling) in the right OS following an injection of BDA in the ipsilateral LLV. Scale bar = 100 μ m.

Figure 7. Retrograde labeling from MLD injections. An injection of CTB in the left MLd is shown schematically in the insert between **A**, **B**. Its center is solid black and the spread is shown by a dotted surround. Retrogradely labeled cells in a ventral part of the contralateral LLDa and in dorsal and ventral parts of the contralateral LLDp are shown in **B**. **A**: Anterograde labeling in the ipsilateral LLD (predominantly in LLDa) is assumed to have arisen via collaterals of the cells shown in **B**, via Probst's tract (at the arrow, and see text). **C–F**: Retrogradely labeled cells in the ipsilateral and contralateral LLV (**C**, **D**) and OS (**E**, **F**). Scale bar = 100 μ m.

Figure 8.

A: Labeled cells in the left LLDp and few in LLDa following an injection of BDA into the ipsilateral Ov (noncounterstained transverse section). **B:** Labeled cells in the left LLV following an injection of BDA in the ipsilateral Ov in another case (transverse section counterstained with cresyl violet). Scale bar = 250 μ m.

Figure 9. Schematic summary diagram of the projections of NA (A), NM and NL (B), and OS (C). The nuclei of origin of the projections are shown as gray and the relative densities of projections to the various targets are indicated by the weight of the lines and the size of the arrowheads.

Figure 10.

Schematic summary diagram of the projections of LLV (A), LLI (B), and LLD (C). The nuclei of origin of the projections are shown as gray and the relative densities of projections to the various targets are indicated by the weight of the lines and the size of the arrowheads. In C the commissure of Probst is enclosed by a dashed oval. In B the LLI projections to n. basorostralis in zebra finches were defined by Wild and Farabaugh (1996) and the crossed interconnections of MLd and the projections of Ov to L2 (thalamorecipient lamina of the telencephalic auditory area) in zebra finches have not been described herein, but exist as unpublished observations of Krützfeldt and Wild.