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Projections of the Dorsomedial Nucleus of the Intercollicular Complex (DM) in Relation to Respiratory-Vocal Nuclei in the Brainstem of Pigeon (*Columba livia*) and Zebra Finch (*Taeniopygia guttata*)

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ABSTRACT

Injections of neuronal tracers were made into the dorsomedial nucleus of the intercollicular complex (DM) of pigeons and zebra finches in order to investigate the projections of this nucleus which has long been implicated in respiratory-vocal control. Despite the fact that pigeons are nonsongbirds and zebra finches are songbirds, the projections were very similar in both species. Most descended throughout the brainstem, taking ventral and dorsal trajectories, which merged in the medulla. Those descending ventrally terminated upon the ventrolateral parabrachial nucleus (PBvl), the nucleus infraolivaris superior, a nucleus of the rostral ventrolateral medulla (RVL), and the nucleus retroambigualis (RAm). Those taking a dorsal trajectory via the occipitomesencephalic tract terminated in the tracheosyringeal part of the hypoglossal nucleus (XIIts), the suprahypoglossal region, and nucleus retroambigualis. There were also substantial projections throughout an arc extending between XIIts and RVL rostrally, and XIIts and RAm caudally. Neurons throughout this arc, which include inspiratory premotor neurons at levels straddling the obex and expiratory premotor neurons more caudally (in RAm), were retrogradely labeled from spinal injections. The DM projections were predominantly ipsilateral, but there were distinct contralateral projections to all the homologous nuclei in both species. All but the projections to PBvl and XIIts were reciprocal. In summary, the projections of DM suggest that it is able to influence all the key motor and premotor nuclei involved in patterned respiratory-vocal activity. J. Comp. Neurol. 377:392–413, 1997.

Indexing terms: avian; vocalization; respiration; midbrain; phonation

It has long been known that electrical stimulation of certain regions of the avian midbrain, collectively known as the "midbrain call area," can elicit vocalizations (e.g., Popa and Popa, 1933; Lan, 1958; Brown, 1965, 1971; Murphy and Phillips, 1967; Potash, 1970a,b; Delius, 1971; Peek and Phillips, 1971; Seller, 1980; Jahnke and Abs, 1982; Li and Lan, 1990). The most effective sites, i.e., those where vocalization can be elicited with the smallest amounts of current, correspond to a nuclear component of the intercollicular complex (ICo) in close proximity to rostral levels of the inferior colliculus, known as nucleus mesencephalicus lateralis, pars dorsalis (MLd). A characteristic feature of the behavioral effects of electrical stimulation at these sites is that vocalization occurs not simply as a result of the activation of syringeal muscles, but, even in the fully anesthetised bird, as part of an integrated whole body response involving appropriate, apparently normal activation of inspiratory and expiratory muscles which provide the necessary air stream and postural support for vocalization. The mechanisms by which these

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responses are evoked by electrical stimulation of ICo were studied in considerable detail in the chicken by Phillips and coworkers in the 1970s (reviews in Phillips and Peek, 1975; Phillips, 1983), but although it was shown that the activity of syringeal motoneurons, and neurons ventrolateral to them, could be influenced at short latencies by electrical stimulation of the midbrain (Phillips and Youngren, 1976), a neural pathway linking ICo with vocal and respiratory centers in the medulla was never actually demonstrated anatomically. Indeed, in the canary, such a pathway was originally thought not to exist, because lesions of ICo apparently failed to produce degeneration further caudally (Nottebohm et al., 1976). This, together with the fact that lesions of ICo in canaries appeared to have little effect on normal song production, and the discovery of a direct pathway to vocal motoneurons from the nucleus robustus of the archistriatum in that species, led Nottebohm et al. (1976) to conclude that ICo was relatively unimportant in the control of song. (cf. Phillips and Youngren, 1976). It was not until Gurney (1981) had cause to inject horseradish peroxidase (HRP) into the tracheosyringeal motor nucleus (XIIts) of zebra finches that a direct projection from a specific nucleus of ICo, henceforth called DM (dorsomedial nucleus of ICo), to the vicinity of vocal motoneurons was confirmed (see also Simpson and Vicario, 1990). Similar results were also found in pigeon by Wild and Arends (1987), who showed that the intercollicular nucleus that was retrogradely labeled from injections in XIIts was a distinct, compact nucleus, which, for the sake of interspecific consistency, was also called DM, although it is questionable whether this name is an appropriate descriptor of its actual position (see Discussion). DM in pigeon was also found to project upon nucleus retroambigualis (Wild, 1993a), a nucleus situated in the dorsal central part of the caudal medulla and which extends from XIIts almost to the ventrolateral periphery of the brainstem. It contains neurons which have a respiratory-related rhythm in phase with expiration and which project to regions of the spinal cord containing motoneurons innervating abdominal expiratory muscles (Wild, 1993a). Some projections of DM, at least in a nonsongbird, are therefore such as to confirm its long-suspected role in mediating control of respiratory-vocal activity (Seller, 1980, 1981). However, to date no systematic account of the full complement of the efferent projections of DM in either songbirds or nonsongbirds has been presented and, in view of the long history of the apparent involvement of the midbrain call area in respiratory-vocal mechanisms, is long overdue. A comparison of the projections of DM in songbirds and nonsongbirds is particularly desirable because of the fact that songbirds have telencephalic (archistriatal) projections upon syringeal, laryngeal and expiratory premotor neurons which nonsongbirds such as pigeons do not appear to have (Nottebohm et al., 1976; Gurney, 1981; Wild, 1993b, 1994; Vicario, 1993). It is of considerable interest, therefore, to determine whether the projections of DM in songbirds are to similar nuclei as they are in nonsongbirds, whether they

Abbreviations

Aidm	archistriatum intermedium, pars dorsomedialis
Aivm	archistriatum intermedium, pars ventromedialis
Ang	nucleus angularis cochlearis
AVT	area ventralis Tsai
BCA	brachium conjunctivum ascendens
CA	commissura anterior
Cbd	tractus spinocerebellaris dorsalis
cc	canalis centralis
CP	commissura posterior
CT	commissura tectalis
Cu	nucleus cuneatus
CuE	nucleus cuneatus externus
dh	dorsal horn
DLM	nucleus dorsolateralis anterior thalami, pars medialis
DM	dorsomedial nucleus of the intercollicular complex
DMNX	nucleus motorius dorsalis nervi vagi
FLM	fasciculus longitudinalis medialis
ICo	nucleus intercollicularis
Imc	nucleus isthmi, pars magnocellularis
IO	nucleus isthmo-opticus
IOS	nucleus infraolivaris superior
Ipc	nucleus isthmi, pars parvocellularis
La	nucleus laminaris
LLD	nucleus lemnisci lateralis, pars dorsalis
LLI	nucleus lemnisci lateralis
LLIr	nucleus lemnisci lateralis, pars intermedialis rostralis
lPs	nucleus tractus solitarius, subnucleus parasolitarius
LS	lemniscus spinalis
MC	nucleus magnocellularis cochlearis
MLd	nucleus mesencephalicus lateralis, pars dorsalis
MV	nucleus motorius nervi trigemini
nIII	nucleus nervi oculomotorii
NIII	nervus oculomotorius
nIV	nucleus nervi trochlearis
NV	nervus trigeminus
nVI	nucleus nervi abducentis
NVI	nervus abducens
NVIII	nervus octavus, pars cochlearis et vestibularis
NX	nervus vagus
NXII	nervus hypoglossus
VIIv	nucleus nervi facialis, pars ventralis
XIIl	nucleus nervi hypoglossi, pars lingualis
XIIts	nucleus nervi hypoglossi, pars tracheosyringealis
NC	neostriatum caudale
OI	nucleus olivaris inferior
OM	tractus occipitomesencephalicus
OS	nucleus olivaris superior
Ov	nucleus ovoidalis
PBvl	nucleus parabrachialis ventrolateralis
PL	nucleus pontis lateralis
PLL	nucleus paralemnisci lateralis
PM	nucleus pontis medialis
PrV	nucleus nervi trigemini, pars principalis
PT	nucleus pretectalis
R	nucleus raphe
RAm	nucleus retroambigualis
RPgc	nucleus reticularis pontis, pars gigantocellularis
Rt	nucleus rotundus
Ru	nucleus ruber
RxV	radix nervi trigemini
RVL	nucleus of the rostral ventrolateral medulla
S	nucleus tractus solitarius
SH	suprahypoglossal region
SLu	nucleus semilunaris
SpM	nucleus spiriform medialis
SSp	nucleus supraspinalis
TeO	tectum opticum
TIO	tractus isthmo-opticus
Tn	nucleus taeniae
TPc	nucleus tegmenti pedunculo-pontinus, pars compacta
TS	tractus solitarius
TTD	nucleus et tractus descendens nervi trigemini
UVA	nucleus uvaeformis
v	ventriculus
Vc	nucleus nervi trigemini, pars caudalis
VeD	nucleus vestibularis descendens
VeM	nucleus vestibularis medialis
VeL	nucleus vestibularis lateralis
VS	nucleus vestibularis superior
X	area X of the parolfactory lobe

are more or less extensive, and, because of the need to coordinate respiratory-vocal output from both sides of the brain, whether they are unilateral or bilateral.

MATERIALS AND METHODS

Subjects were 30 homing pigeons (*Columba livia*) of both sexes and 16 male zebra finches (*Taeniopygia guttata*). Each was anesthetised with an intramuscular injection of a mixture of ketamine (50 mg/kg) and xylazine (20 mg/kg) and placed in a David Kopf stereotaxic apparatus with a beak bar designed either for use with pigeons (Karten and Hodos, 1967) or custom made for the zebra finch. DM and other brain nuclei were located for injection of tracers with the aid of the stereotaxic atlas of the pigeon brain (Karten and Hodos, 1967) or an unpublished atlas of the zebra finch brain (Konishi, unpublished). However, interindividual bird variability, coupled with the small size of DM in both species, precluded reliance on stereotaxic coordinates alone. DM was therefore also targeted (in 13 pigeons and 7 zebra finches) with respect to either electrophysiological recordings of click-evoked responses in MLd, or by using electrical stimulation to evoke calling at minimal currents (<50 μA). Tungsten microelectrodes (Frederick Haer, 2–5 MΩ) were used either for recording click-evoked potentials in MLd, or to apply 2-second trains of 100 Hz, 0.5-msec square wave pulses of cathodal current generated by an optically isolated, constant current stimulator (Frederick Haer model 6si). Following the recording of auditory responses, electrical stimulation was applied medial to MLd in the case of zebra finches, or rostral to MLd in pigeons. Once vocalizations had been elicited by minimal currents (15–20 μA in pigeon and 2–10 μA in zebra finch), the electrode was withdrawn and a glass micropipette (outer diameter (OD) 30–50 μm) filled with tracer was lowered to the same coordinate. Tracers injected were either 10% biotinylated dextran amine (BDA, Molecular Probes) in 0.1 M phosphate-buffered saline (PBS) applied iontophoretically (7 seconds on, 7 seconds off, +ve current for 20 minutes; Veenman et al., 1992), or 2% cholera toxin B-chain conjugated to horseradish peroxidase (CTB-HRP; McIlhinney et al., 1988), or unconjugated CTB (List Laboratories) given with the aid of a picospritzer (General Valve).

In order to compare the distribution of DM projections with that of bulbospinal neurons that project to regions of spinal cord containing motoneurons innervating the abdominal expiratory muscles that are involved in producing the air pressures required for vocalization, spinal injections of either wheatgerm agglutinin conjugated to horseradish peroxidase (WGA-HRP; Sigma Chemical Company) or unconjugated CTB (List Laboratories) were made with glass micropipettes (OD 30 μm) attached to a picospritzer. In three pigeons and four zebra finches, multiple injections were made into the ventral quadrant of segments 18–20, i.e., lower thoracic and upper lumbar levels, where motoneurons innervating abdominal expiratory muscles are located (Fedde, 1987; Wild, 1993a). In one of the three pigeons, a CTB injection in the lower thoracic cord was combined with a BDA injection in the contralateral DM.

Retrograde confirmation of the projections delineated by the DM injections was provided by injections of BDA, CTB, or CTB-HRP into the putative brainstem targets of DM in pigeons and zebra finches. In addition, data from these experiments were supplemented by data gathered in several previous studies from this laboratory in which either picospritzer or iontophoretic injections of retrograde tracers were made into various nuclei in the brainstem of pigeons, zebra finches and green finches (Wild, 1992, 1993a,b; Wild and Arends, 1987). In order to assess further the possibility of archistriatal or hypothalamic projections upon DM in pigeon, large picospritzer injections of CTB-HRP were made into the medial archistriatum intermedium (Aim; Zeier and Karten, 1971) in five birds or into large regions of both the medial and lateral hypothalamus in another two.

Survival times were 2–4 days, following which the birds were deeply anesthetised and perfused through the heart with normal saline followed by either 1% paraformaldehyde (PFA) and 1.25% glutaraldehyde in 0.1 M PBS, pH 7.4, for birds receiving CTB-HRP or WGA-HRP injections, or 4% PFA for birds receiving BDA or CTB injections. Brains were blocked in a transverse or parasagittal plane and, together with relevant segments of the spinal cord, were serially sectioned at 40–50 μm on a freezing microtome, and collected in two or three series. To visualize CTB-HRP or WGA-HRP, all sections were treated with 3,3′,tetramethyl benzidine (TMB) according to the method of Mesulam (1978), and one series was subsequently counterstained with neutral red on the slide. BDA was visualized by incubating sections in ExtrAvidin peroxidase conjugate (Sigma Chemical Company; 1:1,000) in 0.3% Triton PBS for 1 hour at room temperature, followed by 5–10 minutes in 0.005% diamino benzidine (DAB) and 0.002% H_2O_2 in PBS. CTB was visualized by sequential incubations of 40-μm sections in a goat anti-cholera B-chain antibody (List Laboratories) at a dilution of 1:30,000 in 0.3% Triton PBS at 4°C for 15 hours, biotinylated rabbit anti-goat IgG (Sigma Chemical Co.) at 1:200, ExtrAvidin peroxidase conjugate at 1:1,000 (the last two at room temperature for 1 hour), and DAB and H_2O_2 at the above concentrations for 2–5 minutes. To visualize BDA and CTB in the same sections, BDA was visualized first, as above, but with the addition of 0.002% $CoCl_2$ to the DAB mixture to color the reaction product black, and then the sections were treated with anti-CTB, etc., as above, with no $CoCl_2$ in the DAB mixture, which left the reaction product brown. At least one series of sections was subsequently counterstained with Giemsa on the slide. All sections were viewed with bright- and darkfield optics; the projections were drawn with the aid of a drawing tube, and these were scanned into a Macintosh PowerMac 7100 computer and traced and labeled by using Photoshop™ (Adobe) and Canvas™ (Deneba) software.

RESULTS

Effects of electrical stimulation

Electrical stimulation at the intensities used (i.e., <50 μA in pigeon and <10 μA in zebra finch) was very effective in eliciting vocalizations, but only from a very restricted set of midbrain sites, having a maximal extent of about 500 μm in the rostrocaudal and mediolateral directions. To the ear, the pigeon coos resembled natural vocalizations, and were accompanied by deep inspirations and large increases in expiratory effort. The elicited chirps of the zebra finch were much more difficult to compare by ear with their natural broad band calls, but, as with the pigeons, they

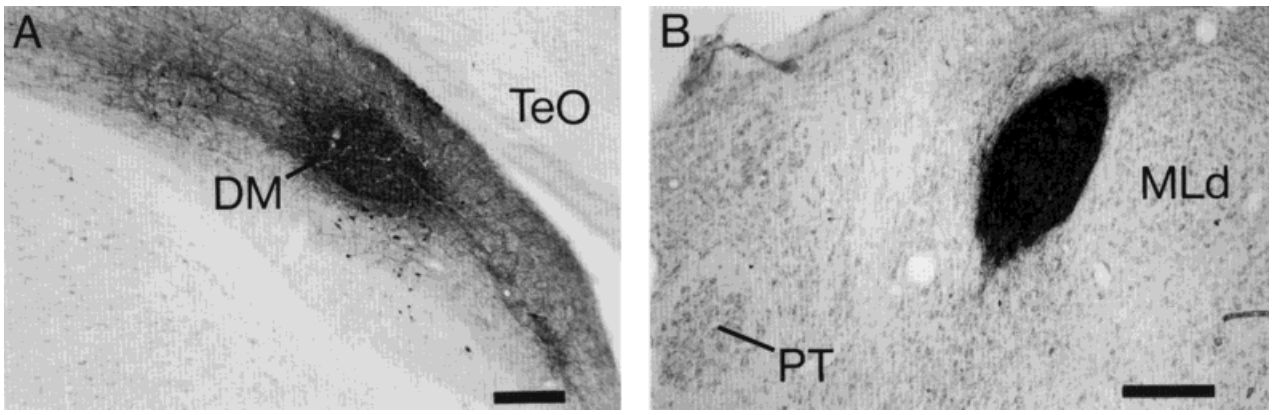

Fig. 1. Photomicrographs showing iontophoretic injections of biotinylated dextranamine (BDA) in DM of pigeon (**A,** transverse plane) and zebra finch (**B,** sagittal plane). Calibration bars = 200 μm.

were accompanied by large increases in inspiratory depth and expiratory effort.

Projections of DM

The projections depicted in Figures 2–4 are based on successful injections in five pigeons and four zebra finches, successful meaning that the injection was centered within DM with minimal spread to surrounding structures. This was most easily achieved by iontophoretic injections of BDA (Fig. 1), but regardless of the type of tracer used, the results were very similar in each of the nine cases, so much so that the following description is applicable to both species. In both pigeon and zebra finch, the great majority of the efferent projections passed caudally, but a minority passed medially to terminate sparsely within the ipsilateral nucleus ruber (Figs. 2A,B and 3A), or to cross to the opposite side and terminate within the contralateral DM (not shown). No ascending projections were noted, although in the zebra finches, labeled fibers were observed in the occipitomesencephalic tract (OM), but these were most likely the axons of retrogradely labeled neurons in nucleus robustus archistriatalis (RA). The descending projections of DM took one of two trajectories, either a ventrolateral one or a dorsal one through the OM, the two tending to merge in the middle and caudal medulla. The former coursed down the lateral side of the pons where there were terminations, first in the ventrolateral parabrachial nucleus (PBvl: Wild et al., 1990; Figs. 2C, 3C, 4A, and 5A,B), and then more caudally in relation to small cells of the nucleus infraolivaris superior (IOS: Wild, 1994), which is wedged between the superior olive and the spinal lemniscus (Figs. 2D, 3D, 4B,C, and 5C). The ventrolateral tract then entered the upper medulla where it gave rise to a prominent terminal field over a loose collection of large, predominently multipolar neurons which has been named the nucleus of the rostral ventrolateral medulla (RVL: Wild, 1993a,b; Figs. 2E, 3E,F, 4B, and 5D–F), pending its further characterization. Labeled fibers then continued caudally throughout the remainder of the medulla and provided terminations to the ventrolateral part of a diagonal terminal field that extended ventrolaterally from XIIts and OM. In the caudal medulla, this terminal field included nucleus retroambigualis.

Fibers taking the dorsal trajectory after leaving DM joined OM as this courses through the dorsal tegmentum of the pons and medulla. Some fibers left OM at rostral levels of its brainstem trajectory and contributed to terminal fields in IOS and RVL, but most began to leave the tract as soon as the tracheosyringeal portion of the hypoglossal nucleus became evident (XIIts), this being at a more rostral level in zebra finch than in pigeon. In both species, medially directed fibers of OM provided terminations throughout the rostrocaudal extent of XIIts, but in neither species were terminations confined to the region of the motoneurons themselves, but were also densely concentrated in the relatively cell sparse neuropil surrounding the nucleus, particularly dorsally and dorsomedially, and in the cellular region dorsal to this, a region we have previously called suprahypoglossal (Wild, 1993b; Figs. 2F–H, 3E–J, 4D, and 6).

Fibers leaving OM ventrally coursed ventrolaterally and provided terminations throughout the diagonal band or arc that extended from XIIts and the suprahypoglossal region towards the ventrolateral periphery of the medulla, and as far caudally as the upper cervical spinal cord (Figs. 2F–H, 3G–J). Outside of XIIts and the suprahypoglossal region, the densest terminations were within the ventrolateral part of the arc at caudal medullary levels, a region that corresponds to nucleus retroambigualis (RAm: Wild, 1993a; Figs. 2G,H, 3H–J, 4C and 7). In neither species were there any terminations in relation to cells of the rostroventrally situated lingual portion of nXII.

The great majority of the DM projections in both pigeon and finch were ipsilateral, but in addition to labeling in the contralateral DM, there were relatively sparse, but none the less distinct, terminations in the contralateral PBvl, IOS, RVL, RAm and XIIts. There were no apparent differences in the density of these contralateral projections in the two species. In the lower medulla, labeled fibers reached the opposite side by crossing the midline between XIIts and the commissural nucleus of the solitary tract, but it could not be established with certainty how the contralateral PBvl, IOS or RVL received their projections from the opposite side.

The remaining cases of injections aimed at DM (eight pigeons and three zebra finches) together comprised an extensive series of "control injections," in that these were not centered within DM, but close to it, with variable amounts of spread or diffusion from the injection site that appeared to involve DM to a greater or lesser degree.

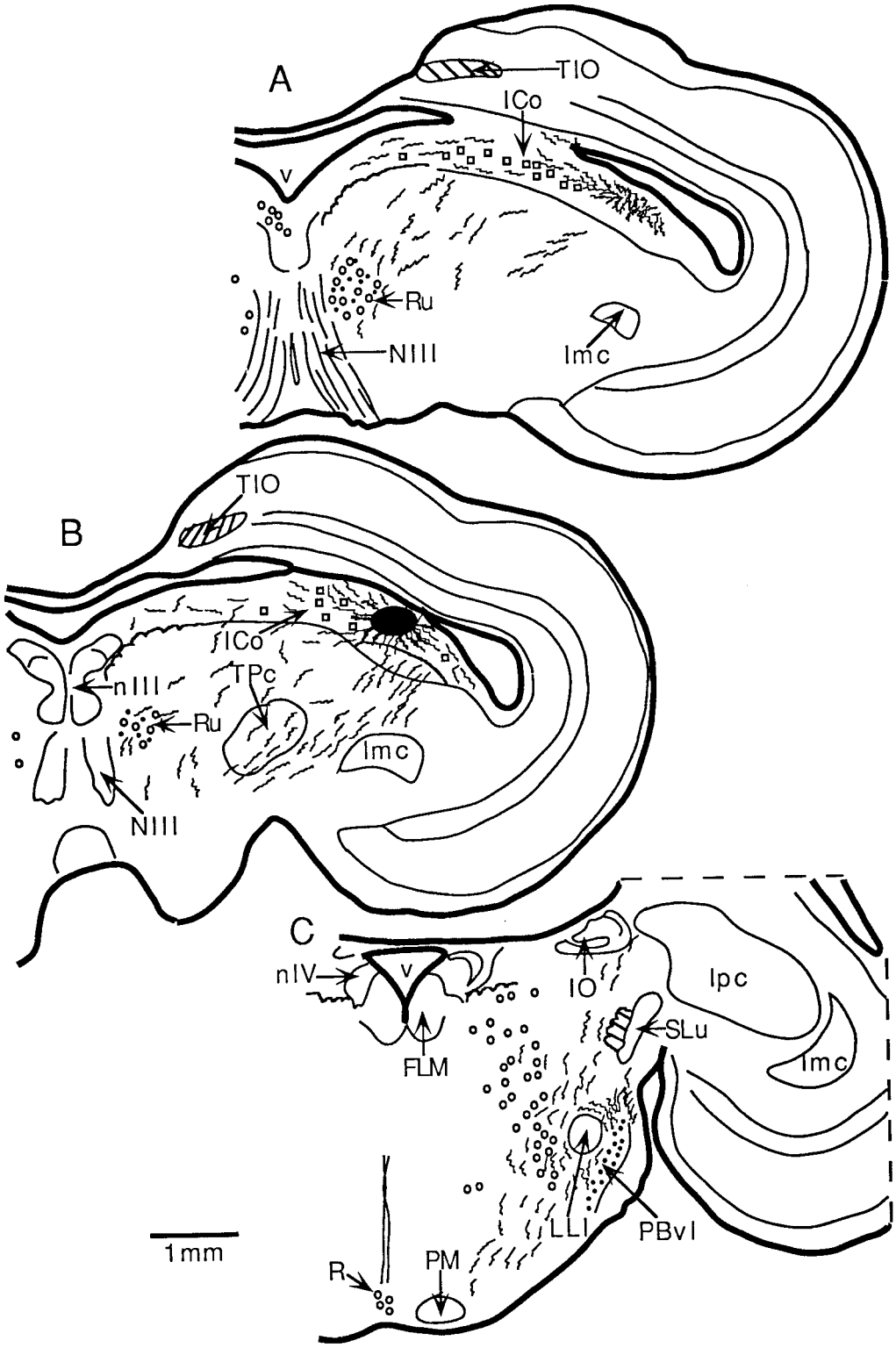

Fig. 2. **A–H:** A rostrocaudal series of schematic transverse sections of pigeon brain showing fiber (wavy lines) and terminal (small filled dots) labeling in the brainstem following an injection of BDA in DM (solid black oval in B). Also shown are retrogradely labeled neurons (open circles) following a spinal injection of cholera toxin B-chain (CTB) in the same bird. Neurons retrogradely labeled from the DM injection are shown as open squares in ICo in A and B.

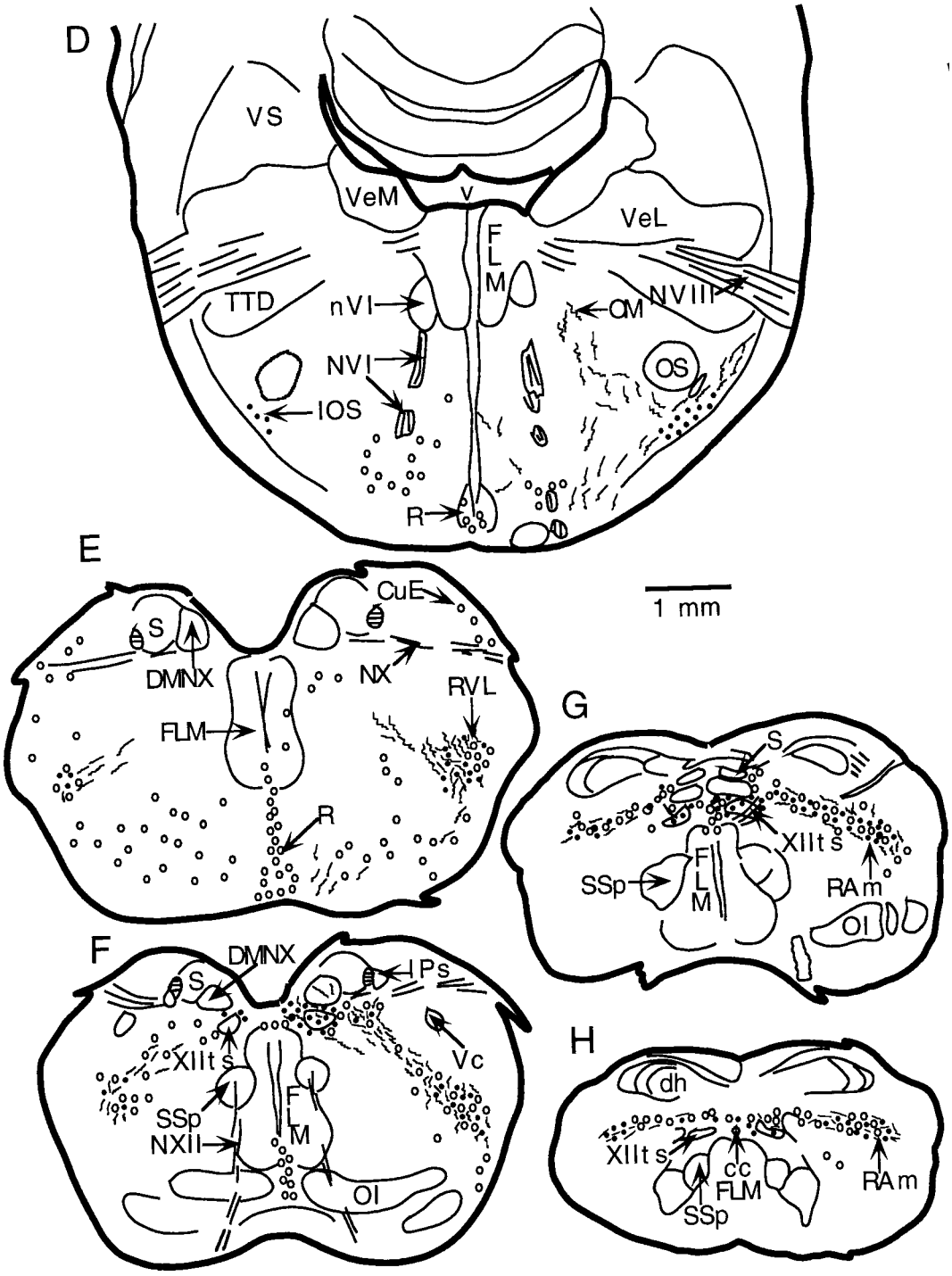

Figure 2 (Continued.)

Despite the apparent partial involvement of DM in these cases, there was no evidence of projections to the lower brainstem.

Distribution of bulbospinal neurons

The distributions of brainstem neurons retrogradely labeled from injections in the lower thoracic and upper lumbar spinal cord of pigeon and finch are shown in Figures 2, 8 and 9. To illustrate the relation of this distribution to the DM projections directly, Figure 2 depicts both retrogradely labeled cells and DM projections in the same pigeon (see Materials and Methods). To facilitate a comparison of the distribution of retrogradely labeled cells in pigeon and finch, Figure 8 depicts the case of another pigeon and a representative case of finch. It is apparent that the distribution of a subset of these bulbospi-

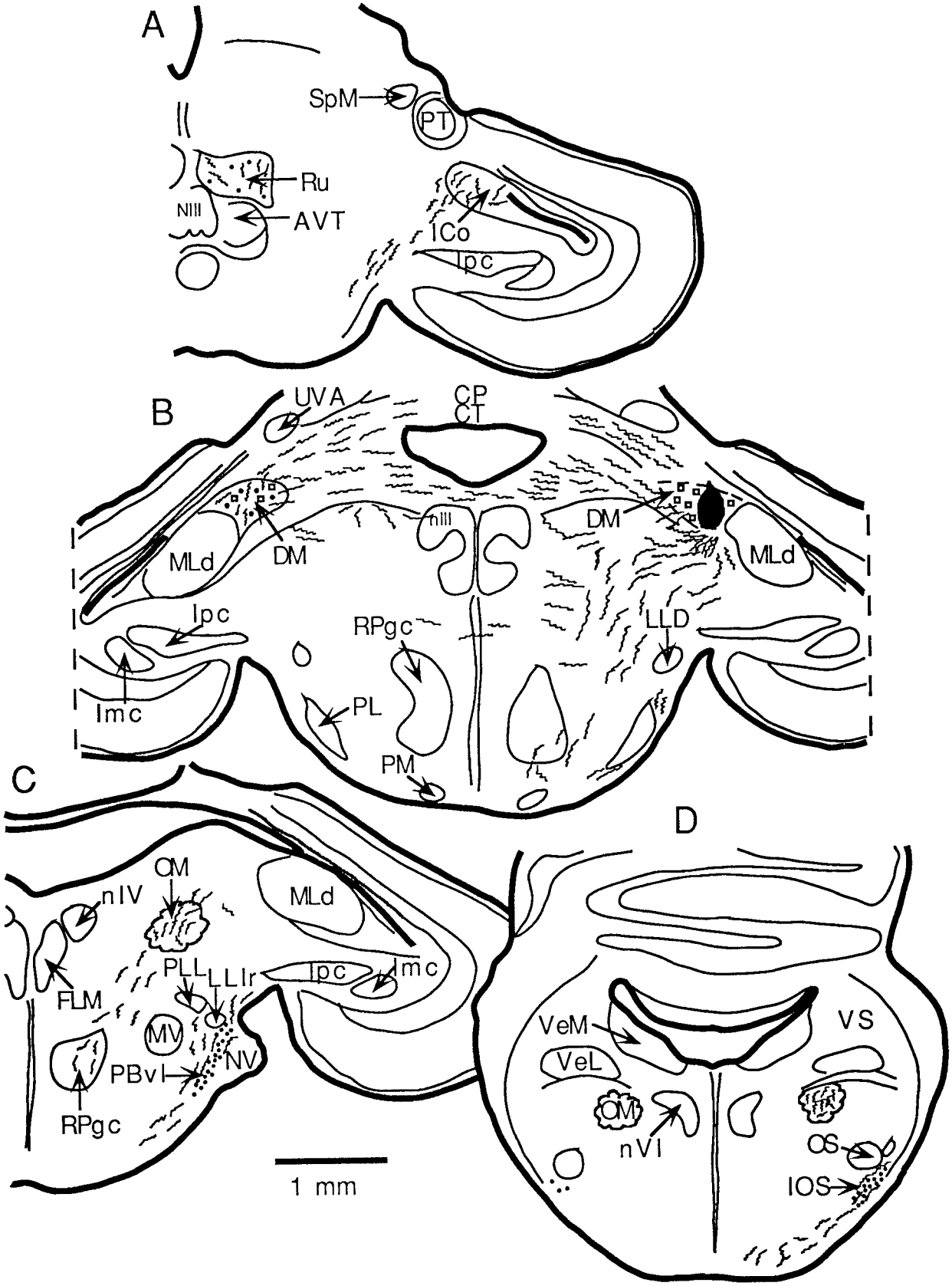

Fig. 3. **A–J:** A rostrocaudal series of schematic transverse sections of zebra finch brain showing fiber (wavy lines) and terminal (small filled dots) labeling in the brainstem following an injection of BDA in DM (solid black in B). Open squares in B represent neurons retrogradely labeled from the DM injection.

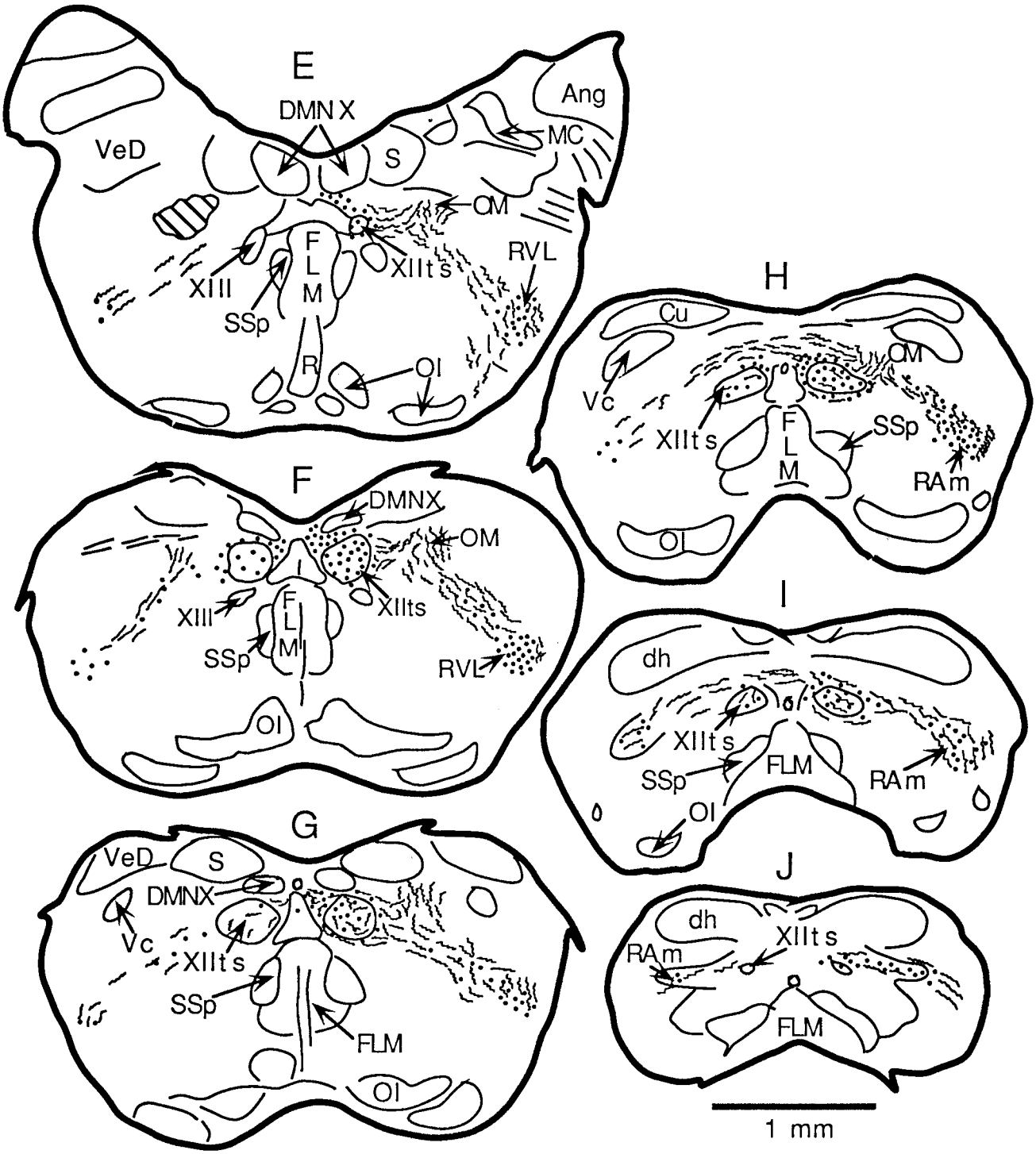

Figure 3 (Continued.)

nal neurons closely corresponds to that of the DM projections (Fig. 9), and that the distribution of bulbospinal neurons in pigeon and finch is basically similar, at least with respect to the zone of DM projections. The general impression of the distribution was one of a diagonal band of medullary neurons stretching from dorsomedial to ventrolateral, this band being more continuous at caudal levels than at rostral levels, where it tended to be divided into dorsomedial and ventrolateral clusters, with a few neurons scattered in between. Thus, at caudal medullary levels there was a dense cluster of labeled neurons within nucleus retroambigualis (Fig. 8D,H), and more scattered labeled neurons in close proximity to XIIts. Just caudal to the obex, and extending rostral to it, the dorsomedial

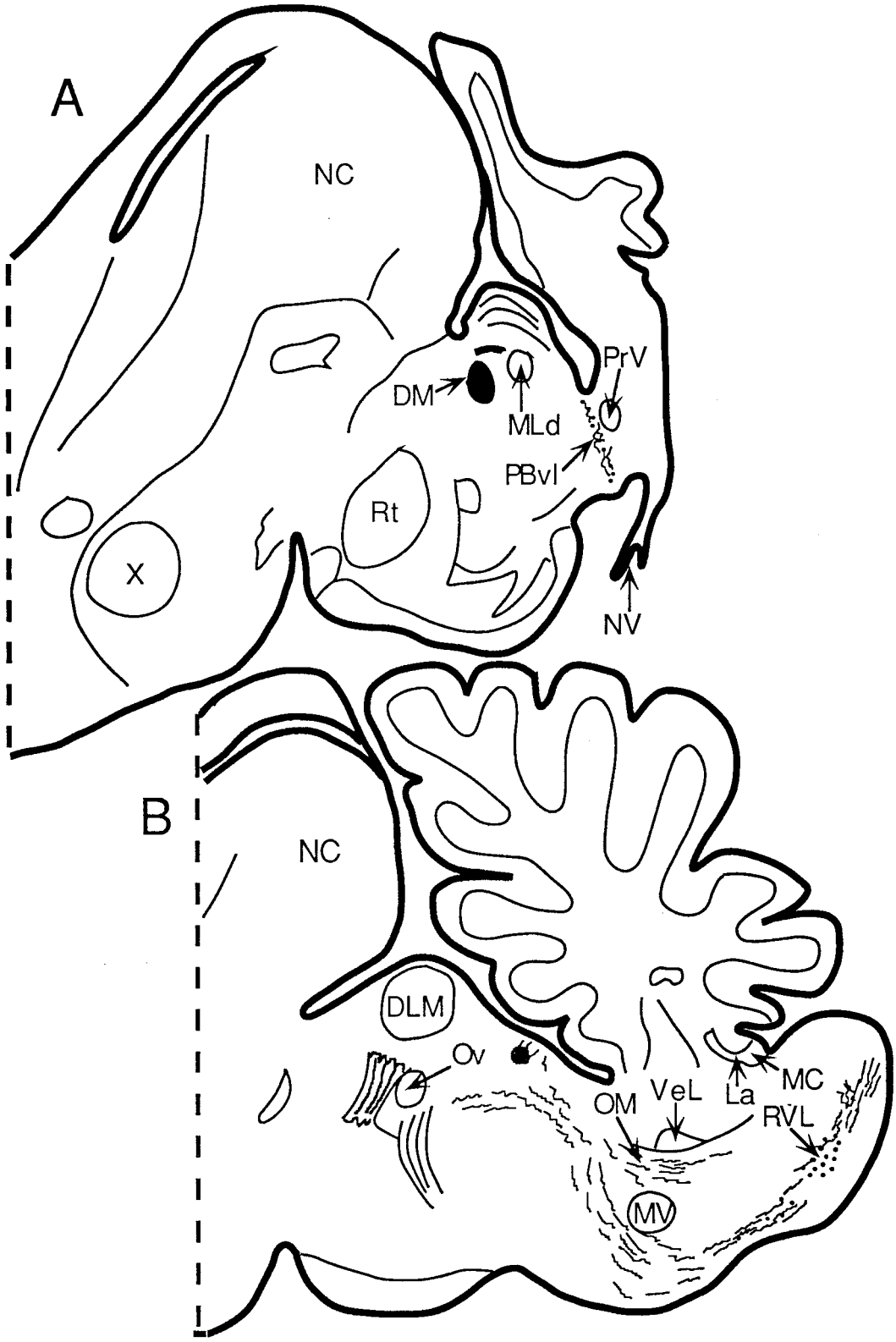

Fig. 4. **A–D:** A lateral-to-medial series of schematic sagittal sections of zebra finch brain showing fiber (wavy lines) and terminal (small filled dots) labeling in the brainstem following an injection of BDA in DM (solid black in A). The open-headed arrow in C points to retrogradely labeled RA axons.

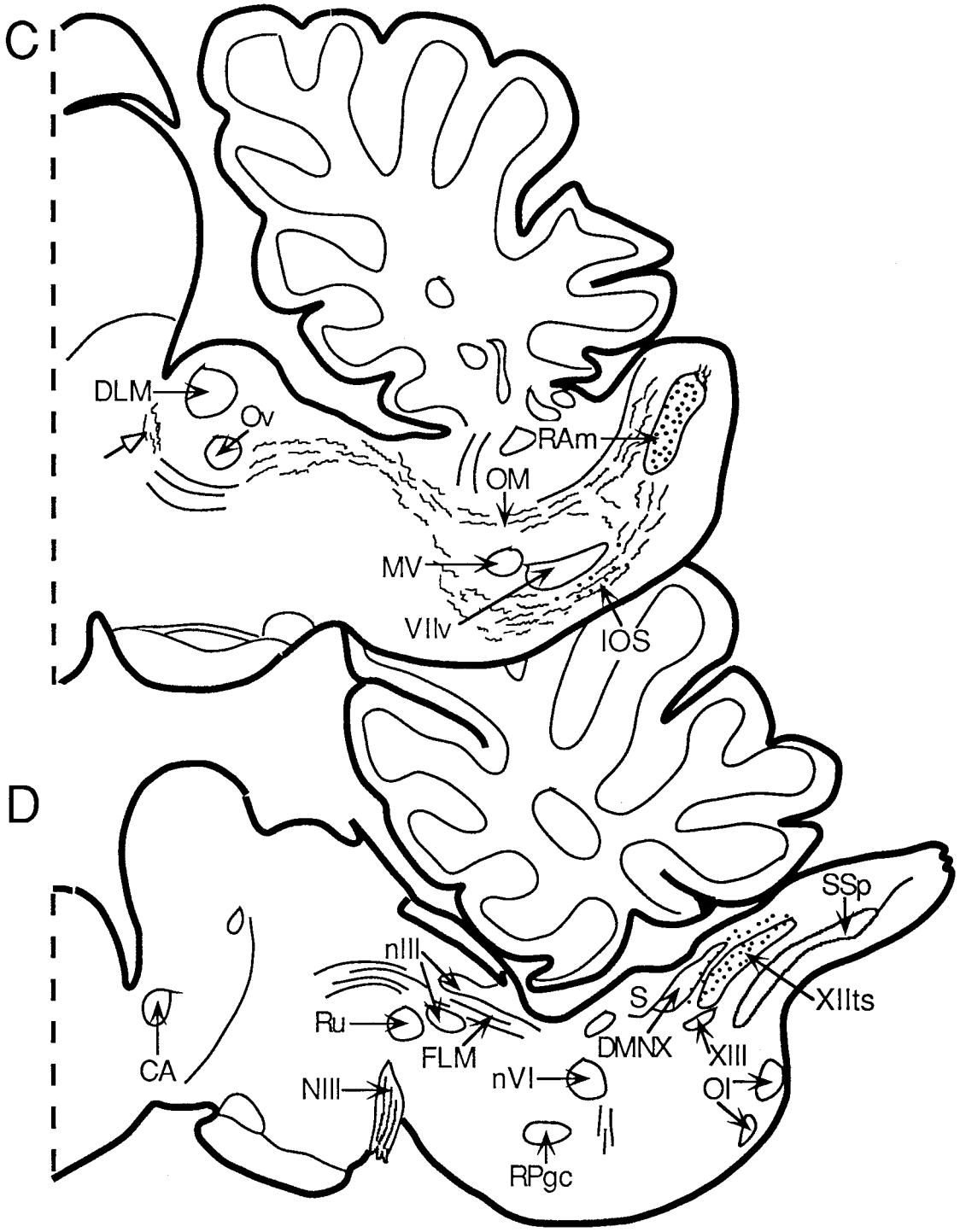

Figure 4 (Continued.)

cluster was located in close proximity to XIIts, and within, and ventral to, the ventral subnucleus of nucleus tractus solitarius (nTS; Fig. 8B,F), whereas the ventrolateral cluster lay within the ventrolateralmost part of the arc of DM terminations. In both pigeon and finch, the bulbospinal neurons were bilaterally distributed, but whereas in pigeon there was a contralateral predominence, in finch this was less obvious, possibly because of the difficulty in confining the injections to one side of the very narrow spinal cord in these small birds.

In all cases, there were many labeled cells in the ventral and ventrolateral tegmentum and in the raphe, vestibular,

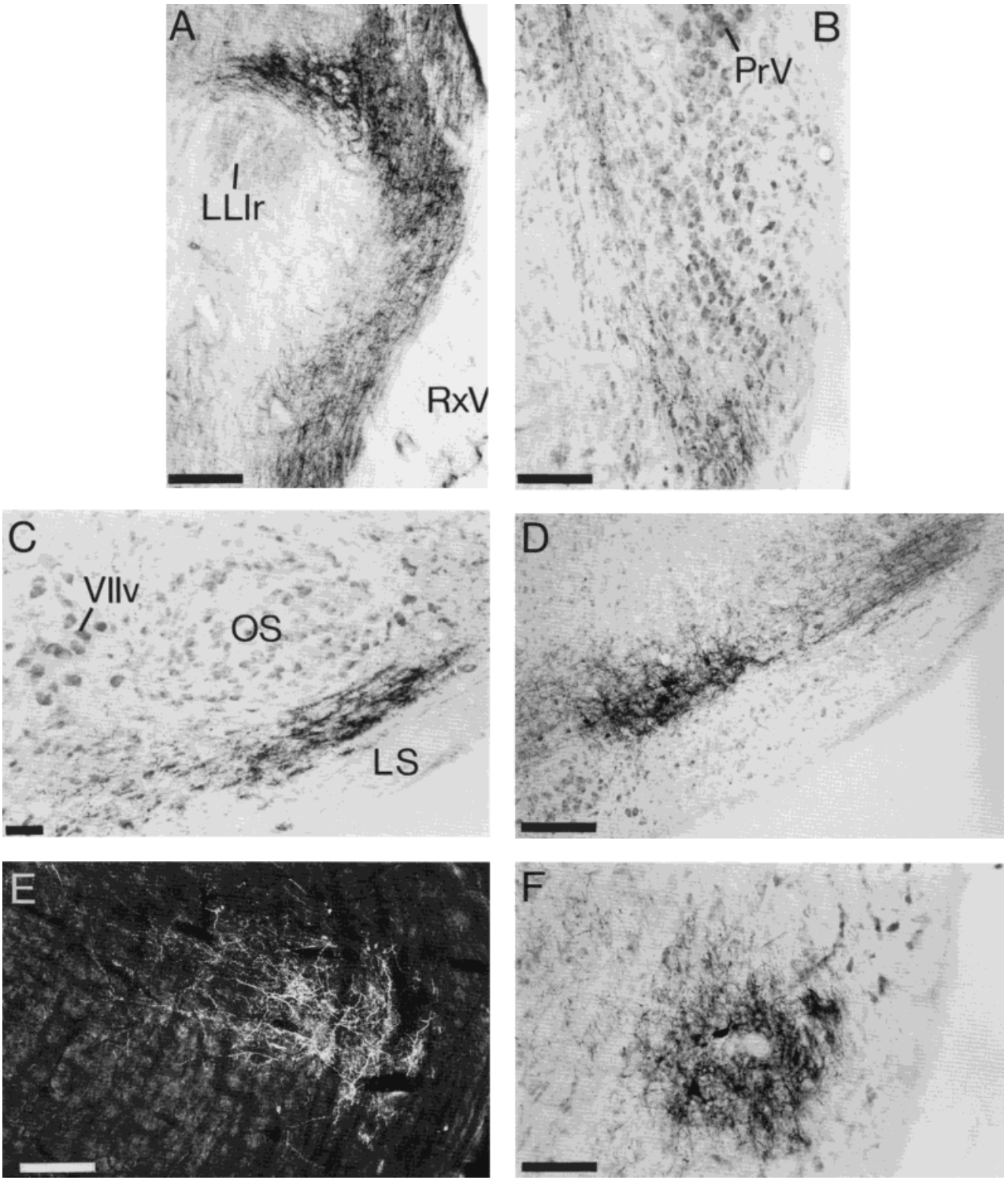

Fig. 5. Photomicrographs of fiber and terminal labeling in pontine and medullary nuclei following injections in DM of pigeon and zebra finch: (**A**) the ventrolateral parabrachial nucleus of pigeon (transverse) and (**B**) finch (sagittal); **C:** nucleus infraolivaris superior of finch (transverse); **D, F:** RVL of finch (sagittal and transverse, respectively); **E:** RVL of pigeon (transverse; darkfield). The labeling depicted in A–D and F is BDA; in E it is wheat germ agglutinin-horseradish peroxidase (WGA-HRP). A is uncounterstained; B–D and F are counterstained with Giemsa. In E and F there also a few retrogradely labeled neurons buried within the terminal field. Calibration bars = 200 μm.

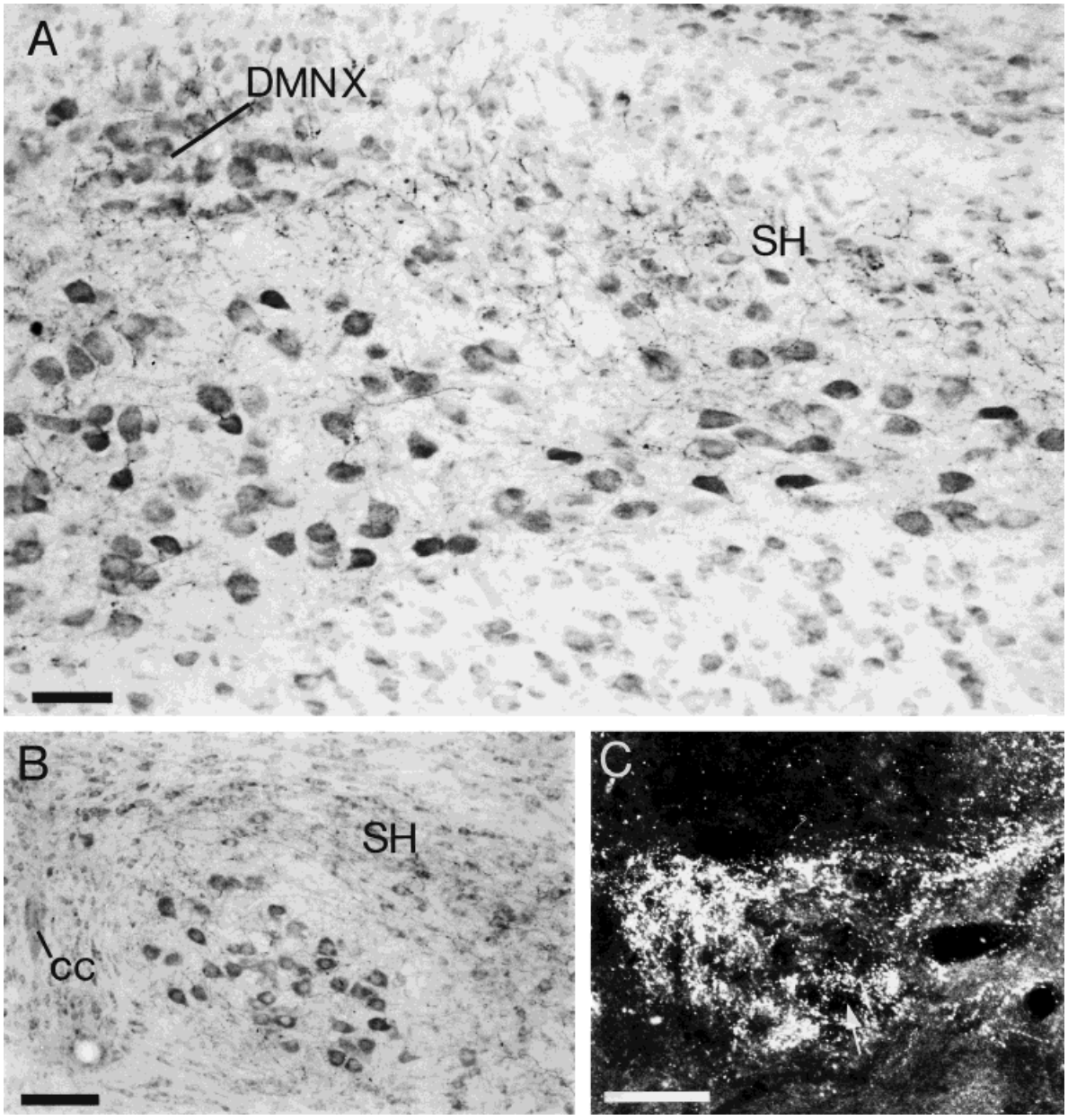

Fig. 6. **A, B:** Photomicrographs of fiber and terminal labeling in XIIts and the suprahypoglossal region (SH) following an injection of BDA in DM of zebra finch. A is a sagittal section, rostral to the left; B is a transverse section through the right XIIts. Both sections were counterstained with Giemsa. **C:** Darkfield photomicrograph of terminal labeling in the right XIIts of pigeon following an injection of WGA-HRP in DM. The arrow points to one of the motoneurons of this nucleus. Note that densest labeling is in the neuropil dorsomedial to the nucleus. Calibration bars = 100 μm.

rubral and hypothalamic nuclei, and locus coeruleus and subcoeruleus, but these are not of central concern in the present context.

Retrograde confirmation of the DM projections

In pigeon and finch, injections of any of the tracers used in the present study (BDA, WGA-HRP, CTB-HRP or CTB alone) in any of the putative targets of DM (e.g., XIIts, RAm, RVL, PBvl) all produced retrograde labeling of DM neurons, bilaterally but predominantly ipsilaterally. In pigeon, irrespective of whichever nucleus was injected, these neurons formed the same tightly clustered, ovoid group within the rostral part of the intercollicular region, immediately ventral to the medial tip of the tectal ventricle (Fig. 10A). The rostrocaudal level corresponds to one where the third nerve roots begin to be visible ventral to the oculomotor nucleus, and just rostral to the auditory nucleus mesencephalicus lateralis pars dorsalis (MLd; but see Discussion). In a few cases a few labeled neurons were also found slightly more caudally, wedged between the dorsal border of MLd and the overlying stratum griseum

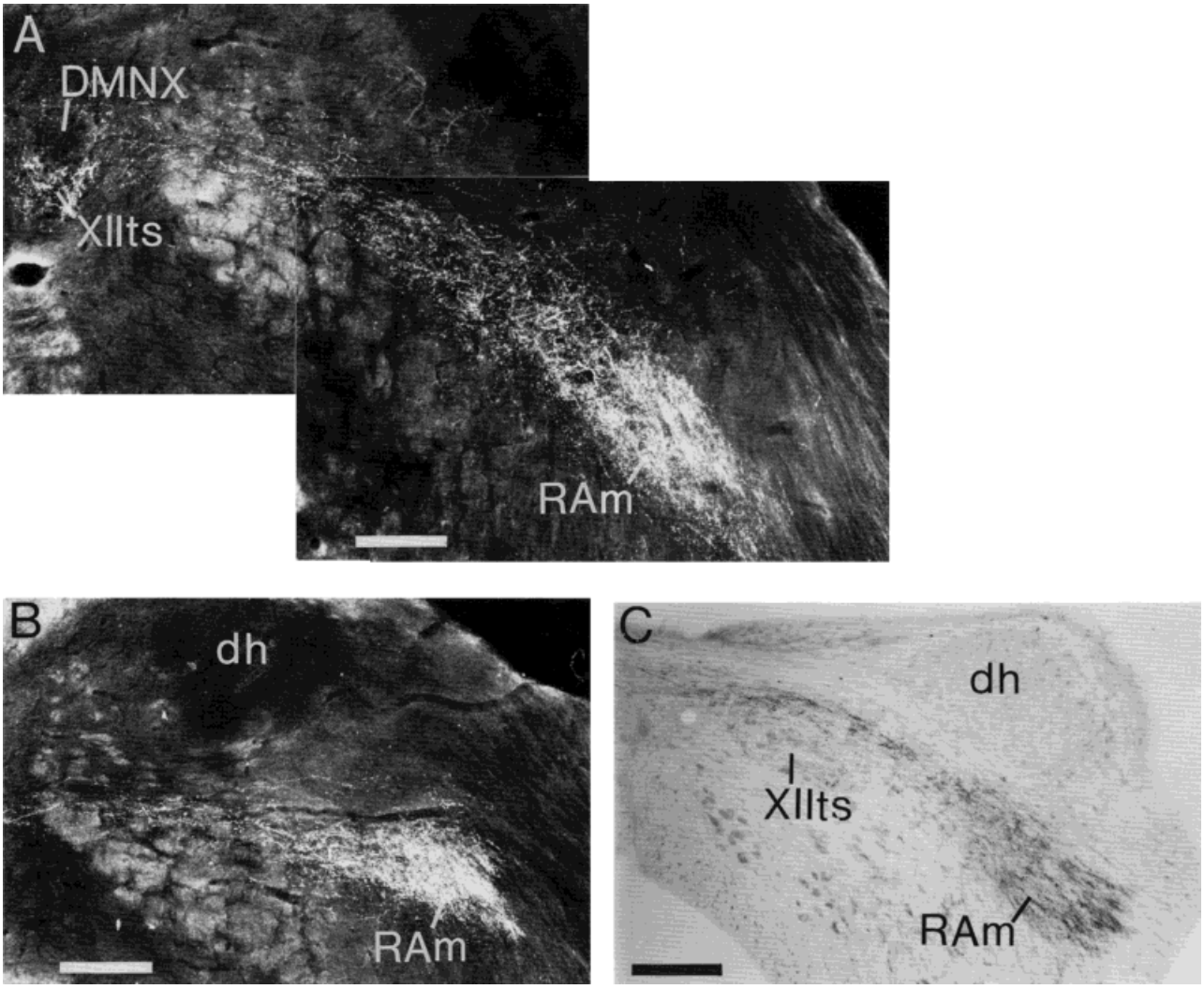

Fig. 7. **A, B:** Darkfield photomicrographs of fiber and terminal labeling in the right XIIts and RAm of pigeon following an injection of WGA-HRP in DM. A is at the level of the caudal pole of XIIts; B is further caudal, at the level of the spinomedullary junction. In B note the medially directed fibers destined to cross to the opposite side. **C:** Fiber and terminal labeling in XIIts, the suprahypoglossal region, and RAm of zebra finch following an injection of BDA in DM. Calibration bars = 200 μm.

periventriculare (SGpv). The presence of these latter neurons did not appear to correlate with any particular one of the DM target nuclei injected.

In the zebra finch, the retrogradely labeled neurons forming DM were located medially adjacent to the medial aspect of MLd, there being a sharply defined border between the two nuclei (Fig. 10B). The medial border of DM, however, was not as well defined, and in some cases retrogradely labeled neurons extended more medially than in others, suggestive of a possible topography of DM projections in the zebra finch. The detailed analysis of such a topography was, however, beyond the scope of the present study. As with the pigeon, there were in some cases a few labeled cells wedged between the dorsal border of MLd and SGpv.

Injections of either CTB-HRP or CTB alone, both of which tracers are known to produce retrograde labeling of dendrites as well as cell bodies (e.g., Wild, 1990; Altschuler et al., 1994), gave no indication that the dendrites of DM neurons in either species extended any appreciable distance outside the nucleus.

Sources of afferents to DM

Although the inputs to DM were not the major concern of the present study, some of them were nevertheless indicated by retrograde labeling from the DM injections, or by anterograde labeling produced by injections in putative target nuclei. In the pigeon, where sources of descending inputs to DM have not been identified (Wild et al., 1993), injections were placed either in the archistriatum or hypothalamus in order to investigate the possibility of a projection to DM.

Injections in DM led to retrograde labeling in several of the targets of DM; i.e., the projections were reciprocal. This was the case for the contralateral DM, and for IOS, RVL and RAm bilaterally (e.g., Fig. 5E,F). Injections in RVL and RAm also produced terminal labeling in DM, which was more easily detected contralaterally than ipsi-

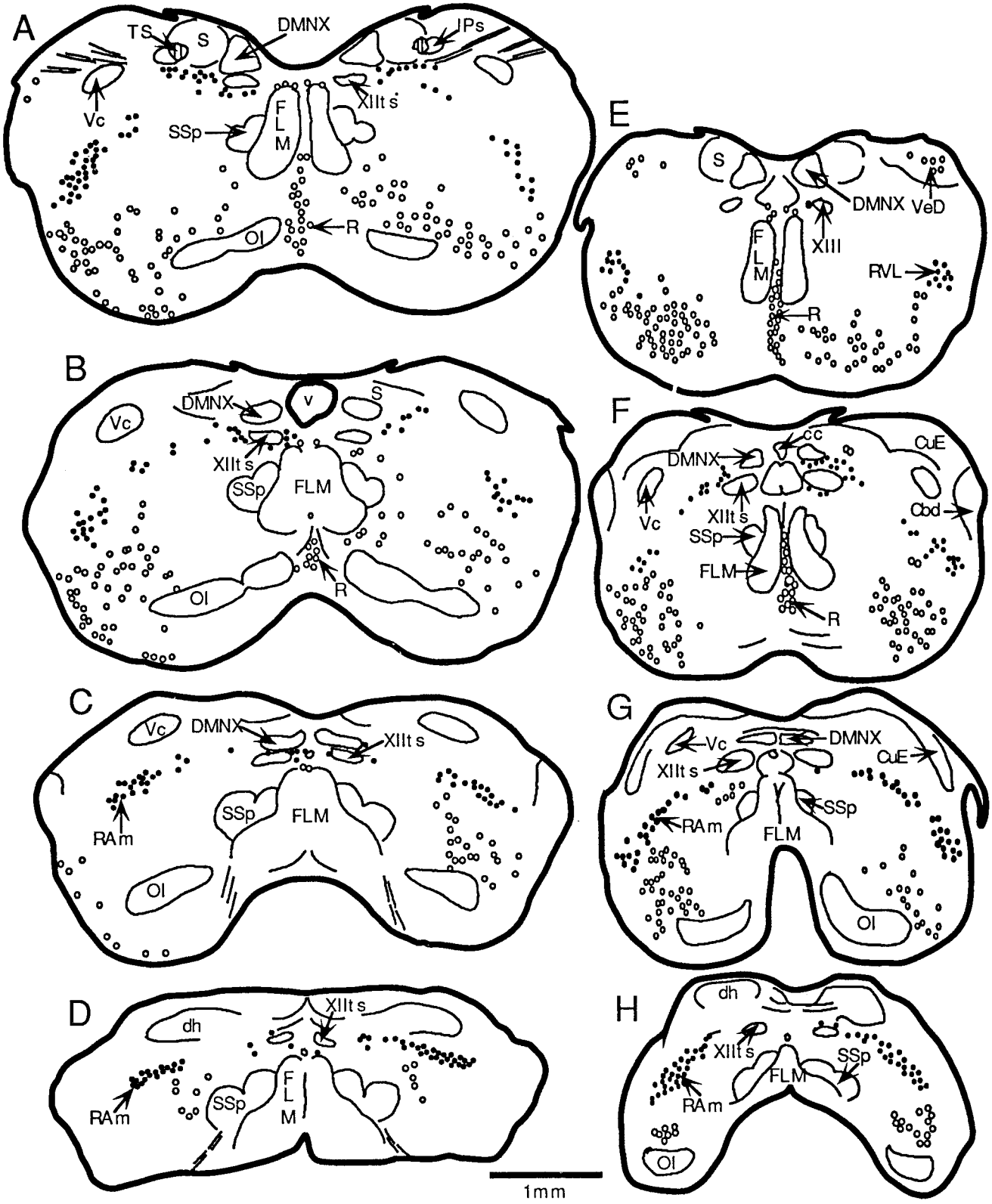

Fig. 8. A rostrocaudal series of schematic transverse sections of pigeon (**A–D**) and finch (**E–H**) medulla showing the distribution of retrogradely labeled neurons following injections of WGA-HRP into the lower thoracic spinal cord. Filled dots are labeled neurons considered to be in the path of DM projections as determined from other cases. Open circles are labeled neurons considered to be outside the path of DM projections.

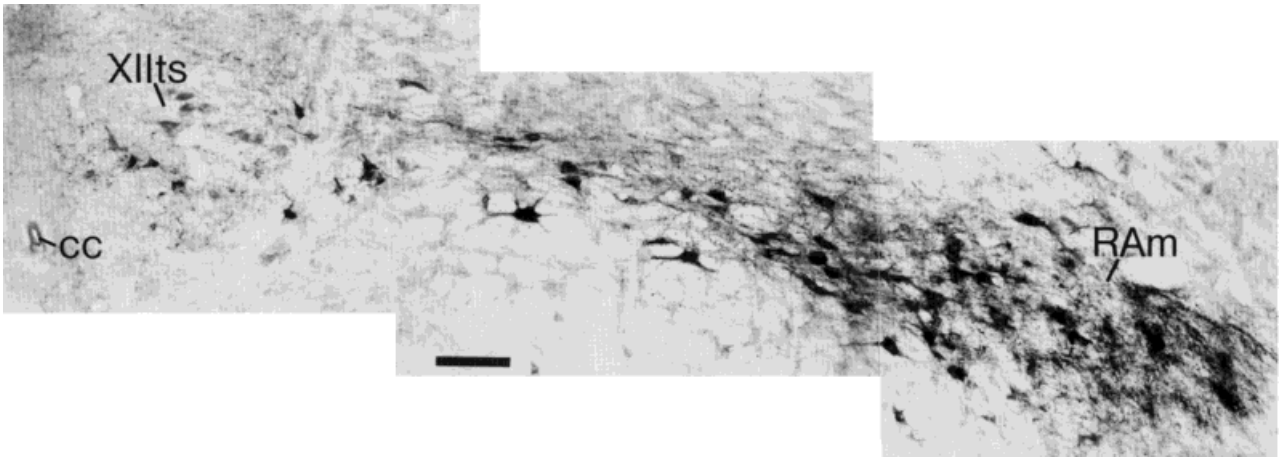

Fig. 9. Photomicrographic montage of fiber and terminal labeling near XIIts and in RAm of pigeon (following an injection of BDA in DM) in relation to neurons retrogradely labeled by an injection of CTB in the lower thoracic spinal cord. Calibration bar = 100 μm.

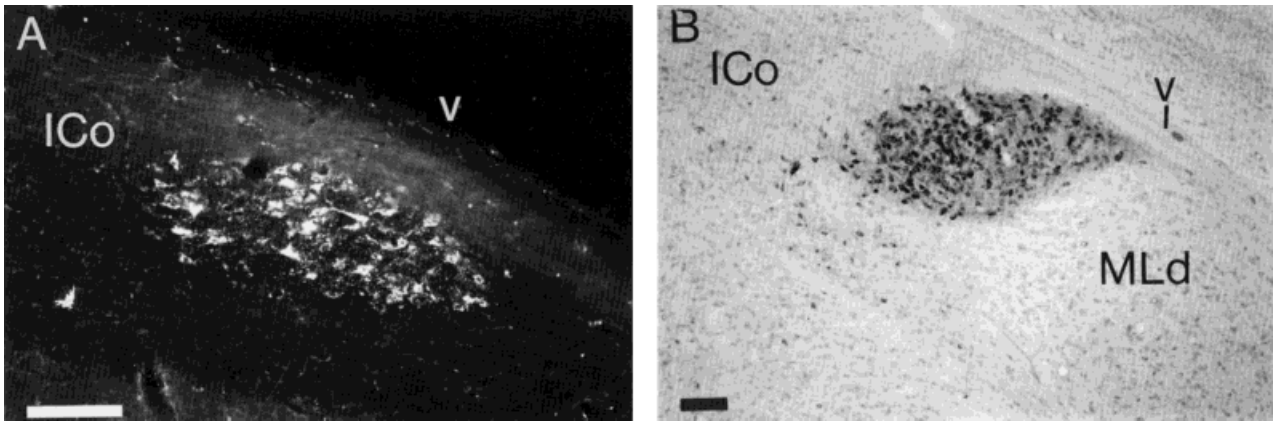

Fig. 10. Photomicrographs of transverse sections showing retrogradely labeled neurons in DM of pigeon (**A,** darkfield) and zebra finch (**B**) following RVL injections of either WGA-HRP or CTB, respectively. Similar patterns of retrograde labeling are produced by injections in any of DMs targets; see text. Calibration bars = 100 μm.

laterally, because it was not obscured by the many fewer retrogradely labeled neurons in the contralateral DM than in the ipsilateral DM.

In pigeon, injections largely confined to DM also retrogradely labeled many neurons within the intercollicular region itself, particularly medial to the injection site (Fig. 2A,B). Where the injection was not confined to DM, however, but invaded the surrounding intercollicular region, retrogradely labeled cells were also found in the dorsomedial and ventromedial parts of the ipsilateral archistriatum intermedium (Aidm and Aivm; Zeier and Karten, 1971; Wild et al., 1993; Fig. 11). In such cases, many labeled cells were also found in the hypothalamus and in the preoptic area. Nevertheless, large injections of CTB-HRP into the hypothalamus (n = 2), or of CTB, CTB-HRP or BDA into the whole of the medial archistriatum (n = 5) of male pigeons (sex identified by postmortem dissection) failed to provide any evidence that these regions projected specifically upon DM. Instead, anterograde labeling from such injections was distributed to parts of ICo adjacent to DM, but not within it. In effect, DM in such cases was defined by exclusion. These results replicate those of Wild et al. (1993), Berk (1987) and Berk and Butler (1981), and for this reason are not shown.

In the forebrain of zebra finches, injections confined to DM gave rise to labeled neurons confined to nucleus robustus archistriatalis (RA), predominantly ipsilaterally. Labeled neurons were distributed predominantly in more dorsal regions of the nucleus, but there were some labeled neurons in ventral regions as well (Fig. 12). Injections not confined to DM, especially those with some involvement of the overlying tectum, also labeled cells in archistriatal regions laterally and dorsolaterally adjacent to RA (not shown).

DISCUSSION

Identification and location of DM

The "midbrain call area" of birds, i.e., the region from which vocalizations have frequently been reported to be elicited by electrical stimulation, is very much larger than

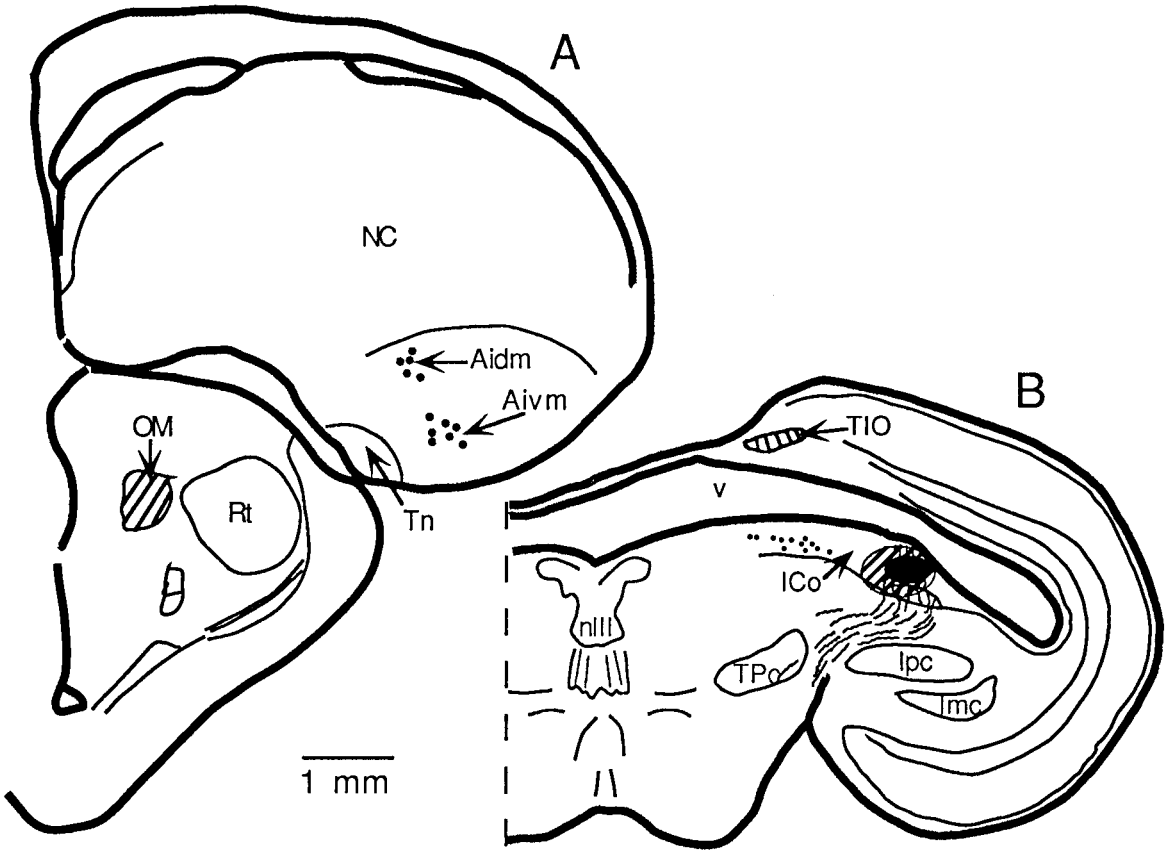

Fig. 11. Schematic transverse sections of pigeon brain showing the location of retrogradely labeled neurons in the archistriatum (dots in **A**), following an injection of WGA-HRP centered on DM (solid black in **B**), but not confined thereto (hatched).

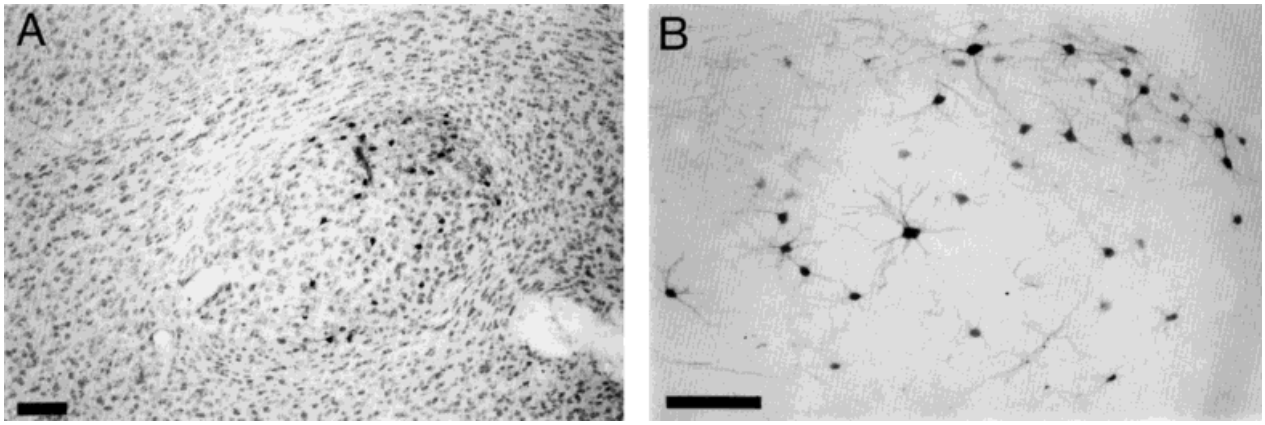

Fig. 12. **A, B:** Photomicrographs of retrogradely labeled neurons in RA of zebra finch following an injection of BDA in DM, such as is shown in Figure 1. Both are sagittal sections, rostral to the left. A is counterstained with Giemsa, B is uncounterstained. Calibration bars = 100 µm.

the only midbrain nucleus (viz. the dorsomedial nucleus of ICo) observed in the present study to give rise to projections to XIIts and the respiratory premotor nuclei. Although this is consistent with the finding that in some studies there is a direct relationship between lowest effective current intensity and proximity to the nucleus here identified anatomically as DM (e.g., Potash, 1970b; Brown, 1971; Seller, 1980), it is apparently not consistent with the fact that vocalization can be elicited, even with small electrodes and small currents, from regions of the midbrain at some distance from DM. Scheich et al. (1983), for instance, show stimulation sites at the level of caudal MLd in the guinea fowl that were effective in eliciting natural calls at well below 50 µA. These sites would be at

least 1 mm from DM, if DM in the guinea fowl is located in a similar place to DM in pigeon. An important difference between studies such as those described by Scheich et al. (1983) and the present study, however, is that the guinea fowl in the former study were not anesthetised. The much greater level of excitability of neurons in the awake bird, therefore, could well have led to the effective stimulation of DM or its efferent pathways, even though the stimulating electrode was at some distance from DM. Outside DM, other sites frequently reported to be effective in eliciting vocalizations when stimulated are those within the nucleus mesencephalicus lateralis, pars ventralis (MLv), a broad region ventral to ICo through which courses the dispersed efferent outflow of DM as shown in the present study. Many DM axons also course immediately dorsal to the nucleus isthmi parvocellularis (Ipc), and some through the nucleus tegmenti pedunculo-pontinus, pars compacta (TPc), both nuclei previously thought to have a role in vocal control (Seller, 1980; Scheich et al., 1983).

In the zebra finch, DM was identified by Gurney (1981) as the larger celled, cytoarchitectonically distinctive component of ICo which lies medial to MLd, beneath the anterior limb of the tectal ventricle, and on the basis of its receipt of projections from nucleus robustus of the archistriatum, originally shown by Nottebohm et al. (1976). Since Gurney (1981) then found that cells in this same location were specifically retrogradely labeled by medullary injections of HRP that included XIIts, DM was also identifiable on the basis of its descending projections to the medulla. In nonsongbirds, it is not yet possible to identify DM on the basis of its descending afferent inputs because, if there are any, they have not yet been identified, despite claims to the contrary in the dove (Cohen, 1983; see below). Furthermore, a definition of DM in pigeon without recourse to retrograde labeling is decidedly problematic, because the cells which are retrogradely labeled by injections of HRP into XIIts do not lie in exactly the same location relative to MLd, as DM does to MLd in songbirds. In pigeon, DM does *not* lie in the same rostrocaudal plane as any portion of the classically defined MLd, such as appears in the atlas of Karten and Hodos (1967). It lies rostral and *lateral* to the dorsomedial component of MLd that receives its input from nucleus laminaris, and dorsal to the rostral pole of MLd as defined by the rostral extent of projections from the cochlear nucleus angularis (Wild, 1995). This difference in placement of DM in songbirds and nonsongbirds is potentially a source of confusion. Deviche and Güntürkün (1992), for instance, compared the pattern of immunohistochemical labeling in DM of a songbird with the pattern in what they claim to be DM of pigeon, but their DM of pigeon does not correspond at all with DM as defined in the present and a previous study on the basis of retrograde labeling (Wild and Arends, 1987). In pigeon, DM does *not* extend rostroaudally from A3.5 to A2.75 (Karten and Hodos, 1967 coordinates) as Deviche and Güntürkün (1992) say it does (it lies rostral to A3.5), and it certainly does *not* project upon "n. glossopharyngeus pars tracheosyringealis" (sic) because there is no such nucleus. The region said by Deviche and Güntürkün (1992) to be DM in pigeon is in fact the medial part of rostral MLd that receives a projection from nucleus laminaris (see also Puelles et al., 1994).

Efferent projections of DM

The compact nature and relatively small cross-sectional area of DM in pigeon (diameter = 0.5 mm), its location immediately ventral to the tectal ventricle, and the considerable interindividual variability in the head size of the homing pigeons used in the present study, conspired to make it very difficult to target the nucleus accurately or consistently, despite injections being made only at coordinates where electrical stimulation evoked calling with relatively small currents (<20 μA); in only 5 out 13 cases was the injection centered precisely on DM. Diffusion from other injections adjacent to DM often appeared to include DM, but in none of these cases was there any evidence of labeling in the medulla. These negative results, combined with the invariant results of the five positive cases, testify to the validity of the projections outlined here in pigeon as being specifically those of DM. This conclusion is given additional support by two factors: (1) injections into any of DMs putative targets retrogradely labeled the same small, tightly packed cluster of cells in the midbrain which we have identified as DM, the only other cells labeled in the midbrain being a few lodged between the dorsal border of MLd and the tectal ventricle, caudal to the main cluster of DM cells; and (2) the projections of DM in pigeon as outlined here were in all respects very similar to those in the zebra finch.

Thus, in both pigeon and zebra finch the main targets of DM are the ventrolateral nucleus of the PBvl; the narrow region sandwiched between the superior olive and the spinal lemniscus, i.e., IOS (Wild, 1994); RVL; XIIts and its immediate surrounds; RAm; and the diagonal band or arc between XIIts and RVL rostrally, and between XIIts and RAm caudally. Projections to all these nuclei were predominantly ipsilateral, but there were distinct contralateral projections to all targets, including the contralateral DM. No difference in the density of these contralateral projections between pigeon and finch was obvious to the eye.

Functional considerations related to recipient nuclei

The pattern of descending projections of DM in pigeon and finch is entirely consistent with its having a role in respiratory-vocal modulation or control, as suggested by others (e.g., Seller, 1980, 1981). Its first target, the PBvl, itself projects upon all the same lower brainstem nuclei as does DM, including XIIts (Wild and Arends, 1987; Wild et al., 1990). It receives a projection from the nucleus parasolitarius lateralis (lPs) of nTS, which, in turn, receives a primary afferent projection from the lung via the vagus nerve (pigeon: Katz and Karten, 1983; songbirds: Wild, unpublished). It is assumed that PBvl thereby receives information related to respiratory phase by virtue of the intrapulmonary CO_2 (and possibly mechano-) receptor axons traveling in the vagus nerve to lPs. As a population, vagal fibers innervating CO_2 receptors increase their firing rate during inspiration (Gleeson, 1987), probably inhibiting lPs neurons as they do so (Fortin et al., 1994), but the effects of this inhibition on PBvl neurons are unknown; i.e., it is not known whether PBvl is active during inspiration or expiration, or even whether it has a respiratory rhythm at all. Until this question is resolved, the effects of DM activation on PBvl during vocalization must remain speculative.

Little is known about the IOS except that it appears to be part of the same respiratory-vocal network as the other nuclei labeled in this and previous studies (Wild, 1993a,b). There are some large facial motoneurons situated between the superior olive and the spinal lemniscus, these having their axons in the facial nerve and innervating extrinsic

tongue or head muscles, muscles that could conceivably be involved in movements associated with respiration and/or vocalization; but the DM terminations do not appear to be related to these, but to the much smaller cells of IOS sandwiched between these motoneurons and the spinal lemniscus.

The nucleus of the RVL lies at the periphery of the medulla and appears to be continuous caudally with the ventrolateralmost component of the diagonal band of neurons that extends from XIIts into the ventrolateral medulla. It is distinguishable from this band, however, by virtue of its neurons being larger and arranged loosely in circular fashion (as seen in transverse section; in reality RVL is a column of neurons). The present study confirmed that some RVL neurons project to spinal levels where abdominal expiratory motoneurons are located (Wild, 1993a). RVL also projects upon XIIts and nucleus retroambigualis (Wild, 1993a), and preliminary evidence suggests that its neurons have a respiratory rhythm in phase with expiration (Wild, unpublished; Reinke and Wild, 1996). If confirmed, this would suggest that RVL is involved in providing part of the total drive to expiratory and vocal motoneurons. However, it may also project upon other respiratory motoneurons in the lower brachial and upper thoracic regions of the cord (Reinke and Wild, 1996).

The diagonal band or arc of neurons extending from XIIts into the ventrolateral medulla is also major target of DM projections. It is composed primarily of two clusters of neurons, one located dorsomedially and the other ventrolaterally, with a scattering of neurons in between that is most evident at levels caudal to the obex. The functional analysis of these clusters of neurons in the avian medulla, some of which have distinct topographic, connectional and functional similarities to respiratory related nuclei in mammals, is in its infancy (Wild, 1993a; Reinke and Wild, 1996), and the following comments should be regarded as preliminary. At rostral levels, just in front of the obex, the dorsomedial cluster does not include any neurons of IPs, which form a small, tightly packed group situated laterally adjacent to the tractus solitarius. Thus pulmonary afferents, which project upon lPs (Katz and Karten, 1983), do not appear to be a source of direct input to bulbospinal neurons in birds. The dorsomedial cluster includes both large neurons scattered within and ventral to the ventral subnucleus of nTS (Katz and Karten, 1983), and smaller ones lying more medially, in close proximity to XIIts. The ventral subnucleus of nTS (subnuclei medialis ventralis, pars posterior and pars caudalis; mVp, mVc; Katz and Karten, 1983) receives vagal sensory projections via axons that travel in the recurrent esophageal nerve, which, although described by Katz and Karten (1983) as innervating the thoracic esophagus, may also innervate the trachea, as the recurrent laryngeal nerve does in mammals. If so, it is possible that the dorsomedial cluster of bulbospinal neurons, some of which have a respiratory rhythm in phase with inspiration (Reinke and Wild, 1996), receive an input from airway receptors in the trachea.

The ventrolateral cluster at rostral levels (i.e., at levels straddling the obex) occupies a similar position to that occupied by RVL more rostrally. Unlike RVL neurons, however, most of the bulbospinal neurons in this ventrolateral cluster have a respiratory related rhythm in phase with inspiration (Reinke and Wild, 1996). They can be retrogradely labeled from injections in either the lower brachial and upper thoracic spinal cord, where inspiratory motoneurons innervating the principal inspiratory muscle, M. scalenus, are located (Fedde, 1987; Reinke and Wild, 1996), or in the lower thoracic spinal cord, where some of the inspiratory intercostal motoneurons are located (Wild, 1993a; Reinke and Wild, 1996).

In contrast to the neurons of the rostral ventrolateral cluster, those in the ventrolateral cluster at caudal medullary levels are bulbospinal expiratory premotor neurons that make up nucleus retroambigualis (Wild, 1993a). Since DM has projections to both the rostral and caudal ventrolateral clusters, it appears that it may terminate on both inspiratory and expiratory premotor neurons. This is consistent with a role in organizing the total respiratory pattern during vocalization, which includes not only expirations, which form the basis of sound production, but inspirations as well. In fact, in some songbirds, inspirations occur not only between phrases, but also as "mini-breaths" between rapidly repeated notes or elements (Calder, 1970; Hartley and Suthers, 1989), but whether DM has a special role in "mini-breathing" in songbirds is not known. Since laryngeal motoneurons are also located just dorsomedial to the ventrolateral cluster at levels straddling the obex (Wild, 1981, 1993b), DM may also be involved in the control of laryngeal aperture during respiratory-vocal activity.

The DM terminations upon the tracheosyringeal motoneurons themselves have the most direct influence on vocalization. But the activation of these neurons without a closely coordinated activation of expiratory premotor neurons would be ineffective as far as vocalization is concerned (Hartley, 1990). It may well be, therefore, that single DM neurons project upon both XIIts motoneurons and respiratory premotor neurons in order to effect this coordination.

As was noted in the Results, the DM projections upon XIIts are not confined to the motoneurons themselves, but are concentrated in the neuropil that surrounds XIIts, especially dorsally and dorsomedially, and in the cellular region dorsal and dorsolateral to this neuropil. The significance of this pattern of terminations in what we have called the suprahypoglossal region (Wild, 1993b) is not as straightforward as it might appear. The neuropil is densely filled with the processes of XIIts motoneurons (Wild, 1993b), upon which DM axons presumably terminate, but there are also cell bodies of nonmotor neurons in this region, some of which project to the spinal cord (Wild, 1993a; present study), and some of which may be local interneurons. This could be significant in the context of bilateral vocal control, in that neurons in close proximity to XIIts can be retrogradely labeled bilaterally by unilateral injections into XIIts (Wild, unpublished observations). These findings imply that there are interneurons in close proximity to XIIts that have axonal projections to the vocal motor nucleus of both sides, and thus may be an important element in bilateral coordination of vocal production. These neurons could receive terminations from the predominantly ipsilateral DM projections and thereby influence the activity of XIIts motoneurons contralaterally as well as ipsilaterally. This scenario is consistent with the relatively fewer projections of DM to the contralateral XIIts and respiratory nuclei, and may indicate that any role DM may have in bilateral coordination of vocal production is effected in close proximity to the vocal motoneurons themselves.

In pigeon and finch, a sparse projection of DM upon the ipsilateral red nucleus was identified, and in pigeon this was confirmed following a close inspection of retrograde labeling produced by rubral injections of CTB-HRP made

in a previous study (Wild, 1992). The functional significance of this projection seems likely to be related to the fact that vocalization in birds is often accompanied by the adoption of distinct postures, some of which may form part of an elaborate, ritualized visual display, and since the avian ruber projects to all levels of the spinal cord (Wild et al., 1979), it is a most appropriate motor nucleus to mediate control of body and limb muscles in the service of vocalization.

Afferent connections of DM

In the zebra finch, the only source of descending afferents upon DM was RA, but although the cells retrogradely labeled from DM injections were found predominantly in the dorsal parts of the nucleus, many were present in other parts of the nucleus as well. This may be important because of the role different regions of RA are thought to play in respiratory-vocal control (Vicario, 1991; Vates and Nottebohm, 1995). Vicario (1991) has shown, for instance, that the ventral two-thirds of RA projects upon the different functional groups of XIIts motoneurons, whereas the dorsal "cap" projects upon DM. In the present study, it may be objected that the cells labeled in ventral parts of RA from DM injections could have been caused by the injection pipette causing damage to, and thus uptake of tracer by, RA axons in the OM that were destined for XIIts, OM lying at the ventral edge of the hemisphere and through which a vertically oriented pipette must pass to reach DM. Such an objection may be countered, however, by noting that RA axons occupy only the most medial part of OM as it leaves the hemisphere (Wild, 1993b), and that in order to target DM, the pipette must pass through OM more laterally, and did so in the present experiments. It thus appears that there may be cells in ventral RA that project upon DM, which would suggest that the functional segregation of RA may not be clear-cut.

In the pigeon, injections centered on DM, or close to it, also retrogradely labeled cells in the archistriatum, i.e., in Aivm and Aidm, and in the medial hypothalamus. The projections of Aivm have previously been charted and do not terminate upon DM but upon adjacent regions of ICo (Wild et al., 1993; present results). The position of Aidm within the pigeon archistriatum, however, is similar to the position of RA within the songbird archistriatum, although in pigeon, Aidm hardly warrants nuclear status, in the sense of being a collection of neurons cytoarchitecturally distinguishable from surrounding regions. Nevertheless, from the comparative point of view, its position would appear to make it a strong candidate for a source of projections upon DM, but we have not been able to convince ourselves that this is the case, despite several attempts to do so using large injections of a variety of tracers that cover all parts of the medial archistriatum. We stress, however, that such a nucleus in the archistriatum of pigeon is still a possibility, despite our negative findings, because it could be that it is very small and difficult to target accurately, even more so than DM, where in the present study it was very clear that the injection had to be *within* the nucleus in order to label its efferent projections. This is also the case with injections in RA, which is a much larger nucleus than the pigeon DM (Wild, 1993b). There is also evidence from electrical brain stimulation studies of various parts of the forebrain of nonsongbirds, including the diencephalon and archistriatum, that suggests the midbrain call area might receive a descending projection via the occipitomesencephalic tract, a tract that originates in the archistriatum and traverses the diencephalon (Zeier and Karten, 1971; Phillips and Youngren, 1974; Seller and Armitage, 1983; Scheich et al., 1983).

The question of an "RA-type" nucleus in nonsongbirds is an important issue because of the apparent de novo origin of a telencephalic vocal control system in songbirds. Alternatively, the finding of a direct archistriatal projection upon DM in a nonsongbird, especially of one that also had projections upon XIIts, could suggest that the vocal control system of all birds evolved from a common ancestor (with the possible exception of psittacines; Brenowitz, 1991; Striedter, 1994). Cohen (1983) implied the presence of such a nucleus in the dove, but the archistriatal neurons that were retrogradely labeled from injections of HRP into XIIts were located rostrolaterally in the archistriatum, and lesions that included that region did not produce terminal degeneration within XIIts, but in a region laterally adjacent to it. We feel, therefore, that the evidence presented by Cohen (1983) is not sufficient to prove the presence of a "robustus-type" nucleus in the archistriatum of a nonsongbird. Moreover, his results are inconsistent with those of Zeier and Karten (1971) in pigeon, who did not find any archistriatal projections upon XIIts, and with those in pigeon and finch showing the origin, course, and termination of lateral archistriatal projections upon premotor regions of the medulla, rather than upon cranial nerve motor nuclei themselves (Berkhoudt et al., 1982; Wild and Farabaugh, 1996).

In the absence of direct archistriatal projections upon DM cell bodies in nonsongbirds, indirect projections may exist. Perhaps the archistriatal projections upon ICo terminate upon the dendrites of DM neurons that extend beyond the borders of the nucleus itself, but in the present study this idea was not supported by the evidence. (The apparent lack of extensively radiating dendrites of DM neurons, in both pigeon and zebra finch, also does not support the idea that contact with MLd neurons is made by this means, if any auditory contact is made at all). Alternatively, it could be that Aidm and Aivm project upon ICo neurons that, in turn, project upon DM. In support of this idea, injections that were centered on DM in pigeon retrogradely labeled cells in ICo medial to DM, but whether these cells actually project upon DM, or upon other more lateral parts of ICo via fibers that pass near or through DM, remains to be determined. Large hypothalamic injections in pigeon also failed to label DM, although they did confirm a massive projection upon ICo that defined DM by exclusion, as shown by Berk (1987; see his Fig. 3B; and Berk and Butler, 1981).

In both zebra finch and pigeon, DM received reciprocal projections from several of the brainstem nuclei to which it projected, excluding PBvl and XIIts. This is indicative of a complex interplay of feedforward and feedback control of respiratory-vocal activity.

Respiratory-vocal control in songbirds and nonsongbirds

In pigeon and finch, each of the respiratory-vocal nuclei in the brainstem projects upon successively more caudal nuclei, in cascade-like fashion (Wild, 1994). In songbirds, however, this cascade begins in the archistriatum, whereas in nonsongbirds such as pigeon, it seems to begin in the midbrain (Wild, 1994). Thus, all the nuclei targeted by DM in both pigeon and zebra finch, except nucleus ruber and

PBvl, are the same ones that in songbirds also receive a projection from RA, and via the same brainstem trajectories (cf. Wild, 1993b). In songbirds, therefore, DM seems to be on a sideline of the main trunk from archistriatum to XIIts and respiratory premotor nuclei, whereas in nonsongbirds such as pigeon, the main trunk seems to originate in DM. The explanation of this difference is usually associated with the fact of learned song in songbirds and of (largely) unlearned calls in nonsongbirds, song-learning presumably being the responsibility of the telencephalon, the output of which from somatic-sensorimotor regions of the archistriatum, including RA, descends to brainstem nuclei (Zeier and Karten, 1971; Wild, 1993b). Songbirds, however, often incorporate calls into their song which may be learned or unlearned, and thus differ between the sexes. Simpson and Vicario (1990) and Vicario and Simpson (1990) exploited this sex difference in the vocalization of male and female zebra finches to show that DM lesions did not abolish song, but merely altered its temporal features, whereas lesions of RA had severe effects on the acoustic and temporal structure of song, but left the unlearned long call of the nonsinging female intact. The suggestion was, therefore, that in both songbirds and nonsongbirds, DM is concerned with the production of unlearned calls, the duration, repetitive nature and relatively simple structure of which in many species, are controlled by the projections of DM upon respiratory and vocal nuclei. This suggestion is supported by the effects of electrical stimulation of DM in zebra finches and other songbirds (Seller, 1980; Vicario and Simpson, 1995; present study). Learned song, however, is controlled by the high vocal center (HVC) and RA, the descending projections of which are somehow able to override the basic midbrain vocal control mechanism to produce the total pattern of respiratory-vocal output. How this is accomplished at the synaptic level, when RA, DM and PBvl (and other brainstem nuclei) all have pronounced inputs to the same respiratory-vocal nuclei, is an intriguing problem awaiting future resolution. An unanswered question in this context relates to the function of the RA projections upon DM itself. Is DM co-opted by RA during singing, or is DM held in obeyance for the duration? In nonsongbirds, what is the precise role of DM in the control of the acoustic structure of calls, some of which, such as those of the guinea fowl, are considerably more complex than the simple nonsyringeally modulated long call of, for instance, the female zebra finch (Scheich et al., 1983; Simpson and Vicario, 1990). And in both songbirds and nonsongbirds, what is the functional significance of the projections of DM upon PBvl, the one respiratory-vocal nucleus that in songbirds does not appear to receive a projection from RA? Given the assumed input to PBvl originating from pulmonary CO_2 receptors during inspiration, perhaps DM has a special role in controlling the effects of this input during vocalization.

Comparison with other species

Vocalization is a complex motor act involving coordination of diverse muscle groups of the vocal (laryngeal or syringeal), respiratory and articulatory (jaw, pharynx, tongue) systems. A vocal control system is present in a wide variety of vertebrates, from fish to primates, and although they may have very different effector structures, there are some basic similarities in the components of the control network at brainstem levels (e.g., Bass, 1985, 1989; Wetzel et al., 1985; Holstege, 1989; Schmidt, 1992; Jürgens, 1994). In addition, in many animals there are descending projections upon this network originating in the midbrain, in mammals, for instance, from the periaqueductal grey (PAG), although these projections do not for the most part access the motoneurons involved in vocalization directly (Holstege, 1989; Jürgens, 1994). The function of PAG in vocalization is not entirely clear, but there are good reasons for thinking in terms of a vocal trigger mechanism rather than a pattern generator (Jürgens, 1994). In contrast, in birds, the direct projections of DM and RA onto vocal motoneurons as well as respiratory premotor neurons undoubtedly mediate the very close coordination that exists between the production of calls and song on the one hand, and respiratory muscle activity on the other (Gaunt et al., 1982; Hartley and Suthers, 1989; Hartley, 1990; Suthers et al., 1994). It is noteworthy, however, that, as in mammals, there do not appear to be direct projections from the midbrain vocal center directly upon either jaw or tongue motoneurons (Wild and Zeigler, 1980; present study), even though jaw movements are an integral part of vocal production in birds, as they are in mammals (Hausberger et al., 1992; Westneat et al., 1993; Suthers et al., 1996). The coordination of jaw movements with those of the respiratory and vocal systems during vocalization must, therefore, be effected via interneurons, the location of some of which in close proximity to XIIts is consistent with a possible input from DM (Berkhoudt et al., 1982; present study).

The projection in songbirds (and parrots) from the archistriatum upon the respiratory vocal network of the brainstem appears to have no clear parallel in other vocal vertebrates (Sutton and Jürgens, 1988). In the squirrel monkey, where much of the work on vocalization has been performed, long descending vocalization pathways originate in the anterior cingulate region, but they do not appear to reach the medulla directly, but relay in the PAG (Jürgens and Pratt, 1979). Furthermore, RA cannot readily be compared with those parts of the mammalian amygdala that also project upon the PAG, despite the (misleading) terminology. Rather, RA seems more likely to be part of the avian archistriatum that has been considered comparable with deep layers of somatic sensorimotor isocortex, than with more caudal parts of the archistriatum that make up parts of the avian amygdala proper and project largely upon the hypothalamus (Zeier and Karten, 1971). By virtue of its control over learned performance of vocalization, RA may be considered analogous to those parts of motor cortex in the human that are involved with the control of language performance, but there are no clear comparative grounds for the establishment of any homologous relationship between them.

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