

# Cytoarchitectonic Organization and Morphology of Cells of the Field L Complex in Male Zebra Finches (*Taenopygia guttata*)

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## ABSTRACT

The organization of the field L complex, a thalamorecipient auditory region in the telencephalon of birds, was examined in Nissl and Golgi preparations of male zebra finches (*Taenopygia guttata*). The field L complex comprises five cytoarchitectonic subdivisions: L1, L2a, L2b, L3, and L, although the border between L and L2b is not distinct. L2a is a plate extending dorsocaudally from the dorsal medullary lamina in the caudal neostriatum. L1 lies on the anterodorsal border and L3 lies on the posteroventral border of L2a. L, the area designated "field L" by Rose (J. Psychol. Neurol., 1914, 2:278-352), forms the medial and posterior borders of the field L complex. L2b is a thick band that forms the dorsal and dorsolateral boundary of the field L complex and is continuous with L medially. Nucleus interface (Nif) is a nucleus that lies between L2a and L1 near the lateral edge of the complex.

The four types of Golgi stained neurons that occur in the zebra finch field L complex correspond to those described for the European starling (*Sturnus vulgaris*). Additionally, type 3 neurons are subdivided into "unoriented" neurons with spherical dendritic fields and "oriented" neurons with bipolar dendritic fields. Nif contains a distinct class of neurons that have large somata with both thick and thin spiny dendrites. The distribution of Golgi cell types between the subdivisions of the field L complex corresponds to the morphology of cells seen in Nissl material. Type 3 oriented cells are found almost exclusively within L2a. L3 has significantly greater numbers of the largest cells (type 1) and significantly smaller numbers of the smallest cells (type 4) than does L1. There are no significant differences in the distribution of Golgi stained cells between L2b and L. © 1992 Wiley-Liss, Inc.

**Key words:** songbirds, telencephalon, dorsal ventricular ridge, auditory system, Golgi impregnation

"Field L" was recognized by Rose ('14) as a region in the caudomedial neostriatum of birds that was characterized by small, densely packed, darkly stained neurons in Nissl sections. Karten ('68) defined field L in the pigeon (*Columba livia*) as the telencephalic region in which fibers from the thalamic nucleus ovoidalis (Ov) terminate. Bonke et al. ('79a) proposed three subdivisions of field L based both on the distribution of Ov axons and the physiological properties of neurons in field L of Guinea fowls (*Numida meleagris*) (Bonke et al., '79b; Scheich et al., '79). L2 receives the bulk of Ov axons and is bordered anterodorsally by L1 and ventrocaudally by L3. L1 and L3 apparently receive Ov axons, though in much lower density than seen in L2. In addition, Bonke et al. ('79a) defined a subdivision of L2, known as L2a, as the region where fibers from the ventral hyperstriatum (HV) terminate. L2 bulges medially, whereas L2a does not.

Recent studies have identified new regions adjacent to field L that receive thalamic input. In the budgerigar (*Melopsittacus undulatus*), nucleus ovoidalis ventromedialis (OVm) projects to "field L" and adjacent dorsolateral neostriatum (NIDL: Brauth et al., '87). In the pigeon, nucleus semiluminaris pars ovoidalis (SPO), which is located within the tractus ovoidalis (TOv), projects to L2b, a region dorsolateral to L2a (Wild et al., '90). L2a is characterized by high levels of cytochrome oxidase activity, whereas L2b is not in both pigeons (Wild et al., '90) and budgerigars (Brauth, '90). Preliminary results in the zebra finch confirm both patterns of projections: OVm projects to L2b and neurons in TOv project to the caudomedial neostriatum (Fortune and Margoliash, '91). Lastly, there is a small nucleus within the field L complex, nucleus interface (Nif),

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that receives input from the dorsal thalamic nucleus uvaeformis (Nottebohm et al., '82).

In this paper we explore the cytoarchitectural features of the field L complex in Nissl and Golgi material of adult male zebra finches. Each subdivision of the field L complex (L1, L2a, L2b, L3, and L) as well as the nucleus Nif, can be defined in the Nissl material. We find that Rose's field L ('14) is adjacent to, not coextensive with, the subdivisions (L1, L2a, and L3) known to receive input from Ov. In addition, we describe the types and distributions of neurons seen in the subdivisions of field L in Golgi material (see also Saini and Leppelsack, '81). The cytological definitions of the field L complex serve as a framework for studies of the functions of the field L complex, and as a framework for interspecific comparisons.

## MATERIALS AND METHODS

Sexually mature male zebra finches were obtained from Magnolia Bird Farm (CA) and housed in groups of three to six with other zebra finches at the University of Chicago for up to six months. Nine birds were deeply anesthetized with Nembutal and perfused transcardially with 0.9% saline followed by 4%w/v paraformaldehyde in saline (formal-saline). The six birds used in Golgi preparations (Adams, '79) were perfused with a solution of 5%w/v potassium dichromate and 1%w/v chloral hydrate in formal-saline (mordant). Following perfusion the brain of each bird was removed and stored at 4°C in either formal-saline (Nissl preparations) or mordant (Golgi preparations). After three days Golgi brains were rinsed and transferred into 1% silver nitrate where they remained at 4°C for three days. Brains from both preparations were dehydrated in an ethanol series, embedded in celloidin, and cut at 40 µm on a sliding microtome in either the coronal or sagittal planes. Four of the six Golgi brains and two of the three Nissl brains were cut in the sagittal plane. Sections from Nissl preparations were stained with cresyl violet. Sections from both Golgi and Nissl preparations were cleared in 100% alcohol fol-

lowed by xylenes before being mounted and coverslipped on slides with Permount (Sigma Chemical, St. Louis, MO).

Over 30 additional brains, obtained from birds used in physiological and anatomical studies in this laboratory, were also examined. After perfusion, these brains were stored in 30%w/v sucrose in formal-saline overnight. They were frozen and then sectioned (40 µm) in either coronal or sagittal planes. Sections were mounted on gelatin coated slides and stained with cresyl violet.

Both Golgi and Nissl material were analyzed and drawn with the aid of a Zeiss Standard GFL microscope fitted with a camera lucida drawing tube. High power photomicrographs were taken on a Zeiss Axioplan with a camera attachment. A videoprint library of Golgi stained neurons was produced with a Sony (UP-5000) videoprinter attached to a video camera (Javelin JE34622HR) mounted on the Zeiss Axioplan.

## RESULTS

### Cytoarchitecture of the field L complex

The field L complex comprises five cytoarchitectonic subdivisions named L1, L2a, L2b, L3, and L which are seen in both parasagittal and coronal Nissl stained sections. The morphology and arrangement of cells in each of these regions is distinct, except between L2b and L. Finally, Nif is a small cytoarchitectonically distinct nucleus which is partially embedded within the field L complex (Nottebohm et al., '82). The morphology of neurons in Nif is distinct from those of the field L complex.

**Subdivision L2a.** L2a is a lightly staining, fiber rich plate which extends dorsocaudally from the apex of LMD across the caudal neostriatum just ventral to LH. L2a extends medially beyond the apex of LMD, where it loses contact with LMD. The medial and dorsal edges of L2a are surrounded by the densely packed, darkly stained cells of L and L2b, respectively. The lateral edge of L2a is near the lateral edge of Nif. L2a sometimes extends slightly lateral to Nif.

Cells seen in L2a of parasagittal sections are medium-sized to small, ovoid or oblong, with large, distinct nuclei. The major axis of the cell is parallel with the major axis of L2a (see Fig. 6B). Often, these cells are arranged in rows of up to five cells running parallel to L2a. The orientation of cells in L2a and the rows of cells are not visible in coronal sections. L2a also contains both large fusiform and small round cells which are seen in either plane of section.

In parasagittal sections L2a is a thin, lightly staining strip in the caudal neostriatum (Figs. 1, 2). The medial edge of L2a occurs at levels where the posterior bulge of the dorsal ventricular ridge (DVR) separates from the rest of DVR. L2a appears within a thick "halo" of darkly staining cells, which is L (Figs. 1A, 2A). At these levels L2a is roughly parallel to LH. In more lateral sections L2a extends ventrally and becomes continuous with LMD (Figs. 1B,C; 2B,C). At this and more lateral levels, L2a is surrounded by darkly staining cells (L2b) only on its dorsal border and is no longer parallel to LH over most of its extent (Figs. 1B-1E; 2B-2E). Just lateral to where L2a contacts LMD, Nif appears as a small wedge in the corner formed between LMD and L2a (Figs. 1C, 2C). L2a has its greatest dorsoventral extent in sections where Nif is largest (Figs. 1D, 2D). In these sections the dorsal borders of L2a are not always distinct. The lateral edge of L2a, which often corresponds to the lateral edge of Nif, is characterized by a ventral

### Abbreviations

A	archistriatum
APH	parahippocampal area
Cb	cerebellum
CDL	dorsolateral cortex
CIO	internal capsule
DA	dorsal archistriatal tract
DVR	dorsal ventricular ridge
HA	accessory hyperstriatum
Hp	hippocampus
HV	ventral hyperstriatum
HVc	hyperstriatum ventrale, pars caudalis
LAD	dorsal archistriatal lamina
LH	hyperstriatal lamina
LMD	dorsal medullary lamina
N	neostriatum
NC	caudal neostriatum
NI	intermediate neostriatum
NIDL	dorsolateral intermediate neostriatum
Nif	nucleus interface
Ov	nucleus ovoidalis
OVM	nucleus ovoidalis ventromedialis
PA	paleostriatum augmentatum
PP	paleostriatum primitivum
RA	robust nucleus of the archistriatum
RT	nucleus rotundus
SPO	semiluminaris pars ovoidalis
TOv	tractus ovoidalis
X	area X

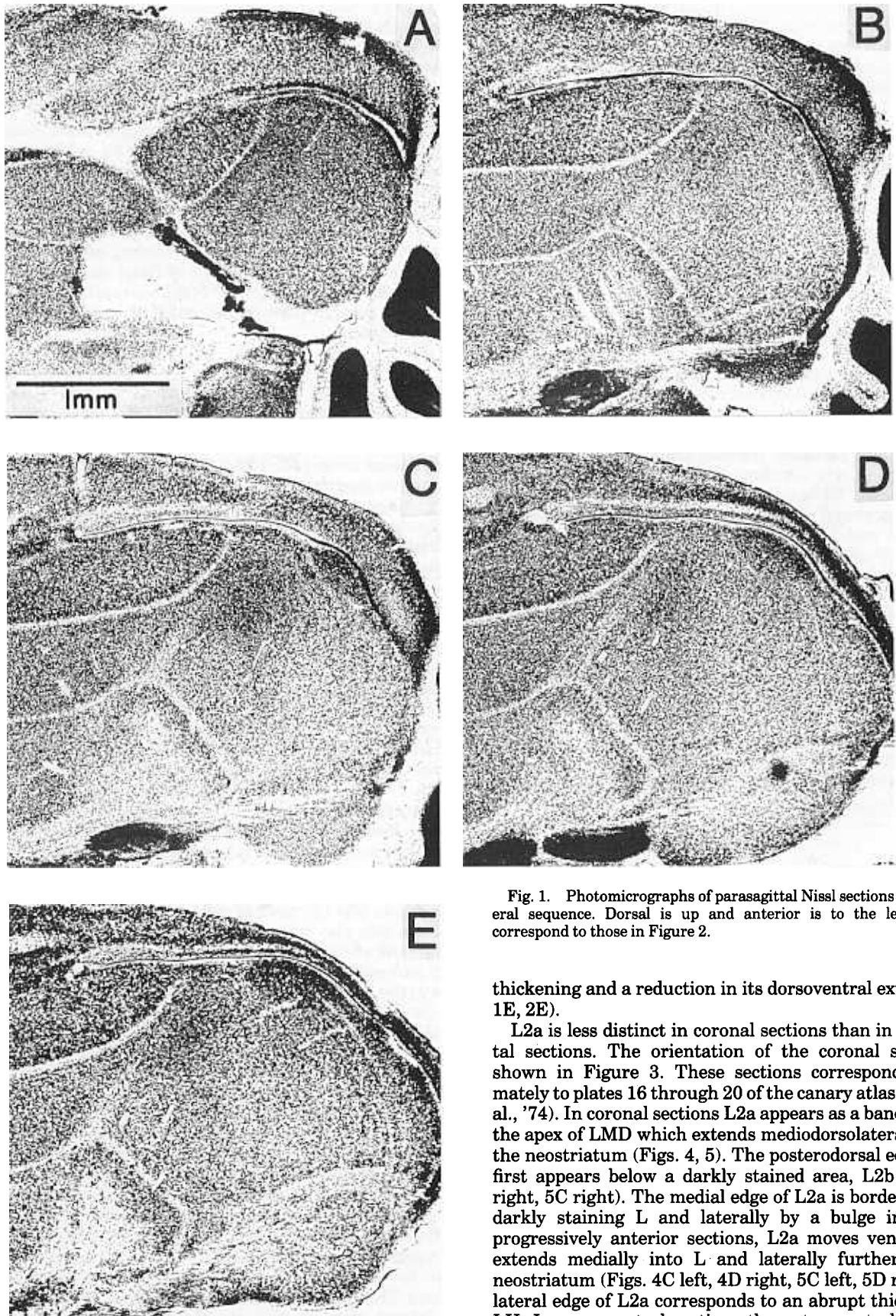


Fig. 1. Photomicrographs of parasagittal Nissl sections in mediolateral sequence. Dorsal is up and anterior is to the left. Sections correspond to those in Figure 2.

thickening and a reduction in its dorsoventral extent (Figs. 1E, 2E).

L2a is less distinct in coronal sections than in parasagittal sections. The orientation of the coronal sections is shown in Figure 3. These sections correspond approximately to plates 16 through 20 of the canary atlas (Stokes et al., '74). In coronal sections L2a appears as a band dorsal to the apex of LMD which extends mediadorsolaterally across the neostriatum (Figs. 4, 5). The posterodorsal edge of L2a first appears below a darkly stained area, L2b (Figs. 4C right, 5C right). The medial edge of L2a is bordered by the darkly staining L and laterally by a bulge in L2b. In progressively anterior sections, L2a moves ventrally and extends medially into L and laterally further into the neostriatum (Figs. 4C left, 4D right, 5C left, 5D right). The lateral edge of L2a corresponds to an abrupt thickening in LH. In more rostral sections the anteroventral border of

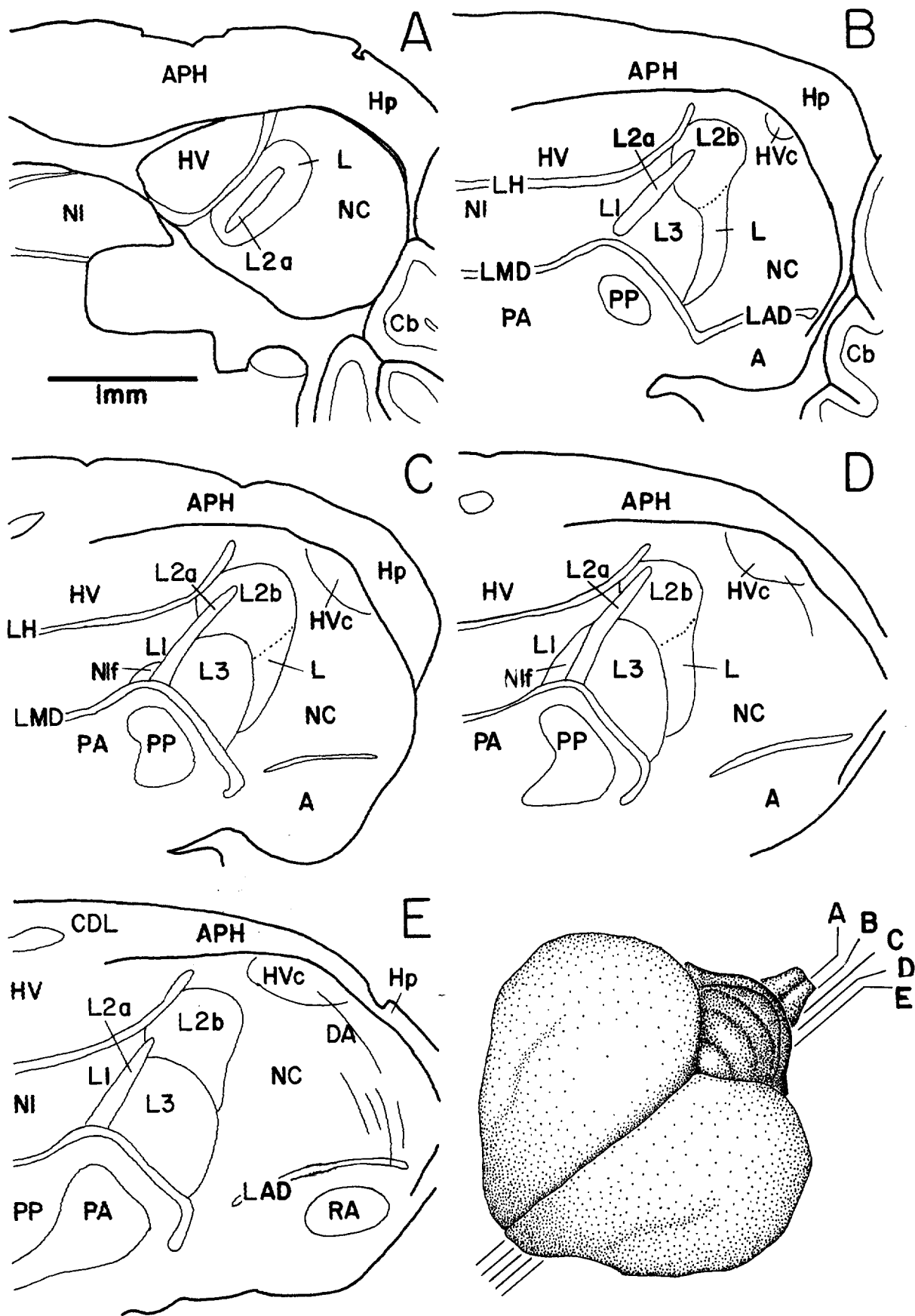


Fig. 2. Outline drawings of parasagittal sections in mediolateral sequence. The dorsal view of the brain in the lower right hand corner indicates the planes of the sections. Dotted lines indicate indistinct borders. Sections correspond to those in Figure 1.

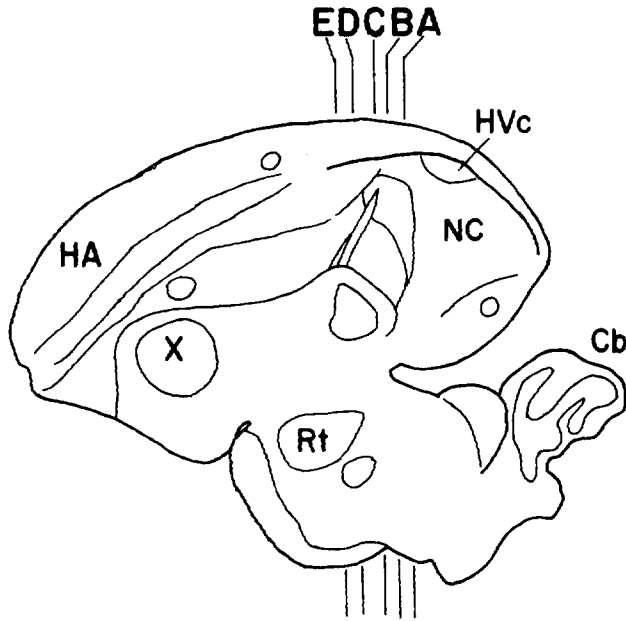


Fig. 3. Diagrammatic parasagittal section. Dorsal is up and anterior is to the left. Lines and letters indicate the planes of the coronal sections in Figures 4 and 5.

L2a becomes continuous with LMD, except medially (Figs. 4D left, 5D left). The portion of L2a which is medial to the apex of LMD fades dorsal to the apex, and thus never comes in contact with LMD.

**Subdivision L1.** L1 lies on the anterodorsal border of L2a and includes all of the neostriatum anterior to L2a, L2b, and Nif between LH and LMD. L1 is bordered medially by L. L1 is similar in appearance with NI, and as a result the anterior and lateral borders of L1 are not distinct in Nissl material.

The cells in L1 are found in small clusters composed of medium and small cells (Fig. 6A). These clusters are often round and densely packed with cells. The cells in the clusters are fusiform or round in shape and range from small to large. In addition, there are isolated neurons which are either large and triangular in shape, or small and round. In some birds the cells in L1 which are near or on the border of L2a stain more intensely than other cells in L1. There appears to be no preferred orientation of cells or clumps within L1.

In parasagittal material L1 first appears medially at levels where L does not surround L2a, which is where L2a begins to extend ventrally towards LMD (Figs. 1B, 2B). L1 is bordered dorsally by LH, dorsocaudally by L2b, caudally by L2a, and ventrally by LMD. At the lateral edge of the field L complex, Nif appears between L2a and L1, forming the caudal border of L1. We were unable to define a distinct border between L1 and NI anterior to it (Figs. 1B-1E; 2B-2E).

In coronal material L1 is dorsal to L2a as L2a moves progressively ventrally away from L2b and LH (Figs. 4C left, 5C left). The lateral extent of posterior L1 is indicated by the lateral edge of L2a ventrally and the thickening of LH dorsally (Figs. 4C left-4D left; 5C left-5D left). As L2a recedes into LMD in more anterior sections, the lateral edge of L1 becomes ambiguous, as the appearance of L1 is

similar to the surrounding neostriatum. The lateral edge of L1 is indistinct but may correspond roughly to the lateral edge of Nif in sections where L2a is not present (Figs. 4E right, 5E right). We have not established cytoarchitectonic criteria which can be used to distinguish NI and L1.

**Subdivision L3.** L3 lies on the posteroventral border of L2a. L3 is a fusiform mass that lies on the dorsal edge of LMD and is surrounded dorsally and caudally by L and L2b, and is bordered on its anterior edge by L2a. L also forms the medial edge of L3.

The cells in L3 are found in large fusiform clusters composed of a few large and several medium-sized or small cells (Fig. 6D). There are also isolated large fusiform or triangular cells and medium-sized fusiform or oblong cells in L3. In general, the cells in L3 are larger and less densely packed than those seen in L1. The large and relatively sparse clusters of L3 give it a punctate appearance at lower magnifications. In some birds the cells in L3 which are near or on the border of L2a stain more intensely than other cells in L3. There appears to be no preferred orientation of cells or clumps within L3.

In parasagittal material L3 first appears medially at levels where L does not surround L2a, which is where L2a begins to extend ventrally towards LMD (Figs. 1B, 2B). L3 emerges between the posterior border of L2a and the dorsal edge of LMD, displacing the darkly staining cells of L, which form the posterior and dorsal borders of L3. Laterally, the darkly staining L2b forms the dorsal border of L3. Also laterally, L recedes dorsally, losing contact with LMD, exposing the caudal border of L3 to NC. L3, which has a punctate appearance, is easily distinguished from NC.

In coronal sections the caudal-most regions of L3 first appear ventral and lateral to the darkly staining regions L2b and L, respectively. Rostrally, L2a forms the dorsal border of L3 (Figs. 4C right-4D right, 5C right-5D right). The ventral border of L3 is ambiguous until LMD appears ventrally (Figs. 4B left, 5B left). The lateral border of L3, however, is distinct and corresponds to the lateral extent of L2b at posterior levels and L2a at more anterior levels (Figs. 4A left-4D right; 5A left-5D right).

**Subdivision L2b and L.** L2b and L are both characterized by their dark, densely packed appearance. They are composed of densely packed, small and medium-sized, round or fusiform cells. Many large clusters containing many cells can be seen in each (Fig. 6C). Cells in L2b stain more darkly and are more densely packed than those in L. A distinct border between L2b and L cannot be drawn on this basis however, as the differences in staining and cell density are not abrupt. Thus, these data are insufficient to determine whether L2b and L should be considered as one or two structures. Together L and L2b form a thick layer over the medial, caudal, and dorsal borders of the other subdivisions of the field L complex. L and L2b also surround the medial and dorsal edges of L2a, respectively. The three-dimensional structure and relationships of L and L2b to the field L complex are shown in Figure 12.

In parasagittal sections L surrounds L2a in the most medial sections in which L2a is visible (Figs. 1A, 2A). In more lateral sections, L is visible only as a band of cells which forms the posterior border of L3 (Figs. 1B-1D; 2B-2D). This band contacts L2b dorsally, which appears as a darkly staining mass around and just caudal to the dorsal edge of L2a. L disappears medial to the lateral edge of field L; L2b is present in sections just lateral to the rest of the field L complex.

In coronal sections L appears as a thick band in the medial neostriatum. L is small anteriorly and progressively enlarges in more posterior sections. At its most anterior edge, L is a dark spot on the ventromedial edge of LH (Figs. 4D left, 5D left). Caudally, L expands in all directions (Figs. 4D right-4A right, 5D right-5A right). L forms the medial border of L1 and surrounds L2a medially (Figs. 4D right, 4C left, 5D right, 5C left). At levels just posterior to L2a, L occupies the medial neostriatum, forming the medial border of L3 (Figs. 4B, 4A left, 5B, 5A left). In these sections a finger of darkly staining cells can be seen extending laterally from the dorsal edge of L; this finger is L2b. L2b follows the course of LH, reaching its most dorsal position directly above the apex of LMD. Laterally L2b fades where the thickening of LH begins. Posterior to L2b, L expands further laterally into the neostriatum before fading into NC (Figs. 4A right, 5A right).

**Nucleus Nif.** Nif is a small, distinct nucleus that extends dorsally from LMD along the anterodorsal border of lateral L2a. The majority of cells in Nif are large, oblong, and have a distinct medium-sized nucleus which is often eccentrically situated (Fig. 6E). Nif cells are rarely found in clusters. These cells stain somewhat more darkly than L2a cells in Nissl stained sections. There appears to be no preferred orientation of cells within the nucleus, but cells that reside on the borders of Nif are often aligned with the border. In addition there are small round or fusiform cells scattered throughout the nucleus. Although cells in Nif generally stain more darkly than cells in the adjacent structures, Nif cells are not densely packed. Thus, when viewed at low power, Nif does not always appear darker than the surrounding tissue.

In medial parasagittal sections (Figs. 1C, 2C), Nif appears as a small wedge of cells located on the dorsal edge of LMD slightly anterior to L2a. In more lateral sections Nif extends dorsally as a thin plate along L2a. At its greatest extent, Nif covers half the length of L2a (Figs. 1D, 2D). Laterally Nif disappears within a few sections. In coronal sections Nif appears as a wedge dorsal to or extending from LMD just lateral to the apex of LMD (Figs. 4E right, 5E right). Nif is visible in only a few coronal sections as it does not have a large rostrocaudal extent. Often a large blood vessel runs through or just lateral to Nif.

### Classes of field L complex neurons in Golgi preparations

The largest cells seen in the Golgi material are type-1 cells (Fig. 7). They have large ( $\approx 12 \mu\text{m}$  diameter), fusiform, or triangular somata, thick dendrites ( $2-3 \mu\text{m}$ ) that are densely covered with spines, and dendritic fields greater than  $200 \mu\text{m}$  in diameter. The largest dendrite is often directed dorsally, though the dendritic field is usually spherical in shape. The density of spines on each dendrite is generally lowest near the soma but increases distally until the dendrite begins to thin, where the density of spines falls.

The majority of cells labelled in our Golgi preparations have medium-sized somata, 8 to  $10 \mu\text{m}$  in diameter. These cells are divided into two groups based on the size and morphology of the dendritic field: type 2 and type 3 cells. The diameter of the dendritic arbors of type 2 cells is large, ranging between 130 and  $200 \mu\text{m}$  (Fig. 8). The somata of type 2 cells are fusiform, oblong, or round. Type 2 cells have dendrites of medium caliber ( $\approx 1 \mu\text{m}$ ) and have a low density of spines. The dendritic arbors of type 2 cells seem

to be more variable than those of the other classes of Golgi cells. Specifically, the dendrites appear to vary more in numbers, thicknesses, and branching.

The diameter of the dendritic arbors of type 3 cells is small, usually below  $100 \mu\text{m}$  (Fig. 9). The somata of type 3 cells are mostly fusiform, though some oval and triangular somata were also seen. Type 3 cells have sparse or irregularly located spines and small varicosities on their dendrites. Two classes of type 3 cells were observed. Type 3 unoriented cells (Fig. 9A) have spherical dendritic arbors. Type 3 oriented cells (Fig. 9B) are defined by an oblong or oval soma with a dendritic arbor that extends primarily in line with the major axis of the soma. In parasagittal material, type 3 oriented neurons are situated such that the soma and dendritic arbor are parallel to L2a. Type 3 unoriented cells are found throughout the field L complex whereas type 3 oriented cells are found only in L2a and in L2b near L2a.

Type 4 cells are very small ( $5-6 \mu\text{m}$  diameter). They have round cell bodies, short stumpy dendrites, and short thin axons (Fig. 10). The dendrites have small varicosities and few spines. The axons of these cells, which have very small varicosities, form small arbors near the neuron. Type 4 cells were found in all of the subdivisions of the field L complex.

Type 5 cells are only found inside and on the borders of Nif. They are large oblong or oval cells with both thick ( $2-3 \mu\text{m}$ ) dendrites and medium ( $1 \mu\text{m}$ ) dendrites (Fig. 11). The thick dendrites are free of spines from the soma to up to  $30 \mu\text{m}$  away from the soma. Spines often appear at the first branching of the thick dendrites. A moderate density of spines covers thick dendrites distally. The thick dendrites on a Nif cell usually emerge from the poles of the cell and can extend over  $125 \mu\text{m}$  from the soma. The thinner dendrites are covered with a moderate density of spines throughout their extent and often do not branch. The spines of type 5 neurons are morphologically similar to those found on the other four types of neurons described above, though a detailed examination of spine morphology has not been conducted. The dendrites of some Nif neurons may extend into L1, though the borders of Nif are difficult to determine in Golgi material.

### Distribution of Golgi cell types

We classified 566 neurons in the field L complex of six birds. We were unable to classify neurons in regions where the density of stained neurons was high or neurons that had no dendrites either because they were severely damaged or poorly stained. Although we did not count the number of neurons that we could not classify, these probably numbered over 1,000, since the density of staining in one of the Golgi preparations was very high. Owing to the capricious nature of the Golgi technique, we pooled the distributions of Golgi stained cell classes from all six brains (Table 1). The null hypothesis, that the distribution of classes of neurons is independent of location, was tested at the 0.01 level using two-way contingency tables. Type 5 and type 3 oriented neurons, which were found within only one or two subdivisions, respectively, of the field L complex, were not included in the two-way contingency tables.

L2a is the subdivision where majority of type 3 oriented cells were found. All of the type 3 oriented cells that are not in L2a reside near the dorsal edge of L2a in L2b. L2a has significantly greater than random numbers of type 3 ( $X^2 = 11.71, P < 0.01$ ) and type 4 ( $X^2 = 12.96, P < 0.01$ ) cells, and significantly lesser than random numbers of type 2 cells



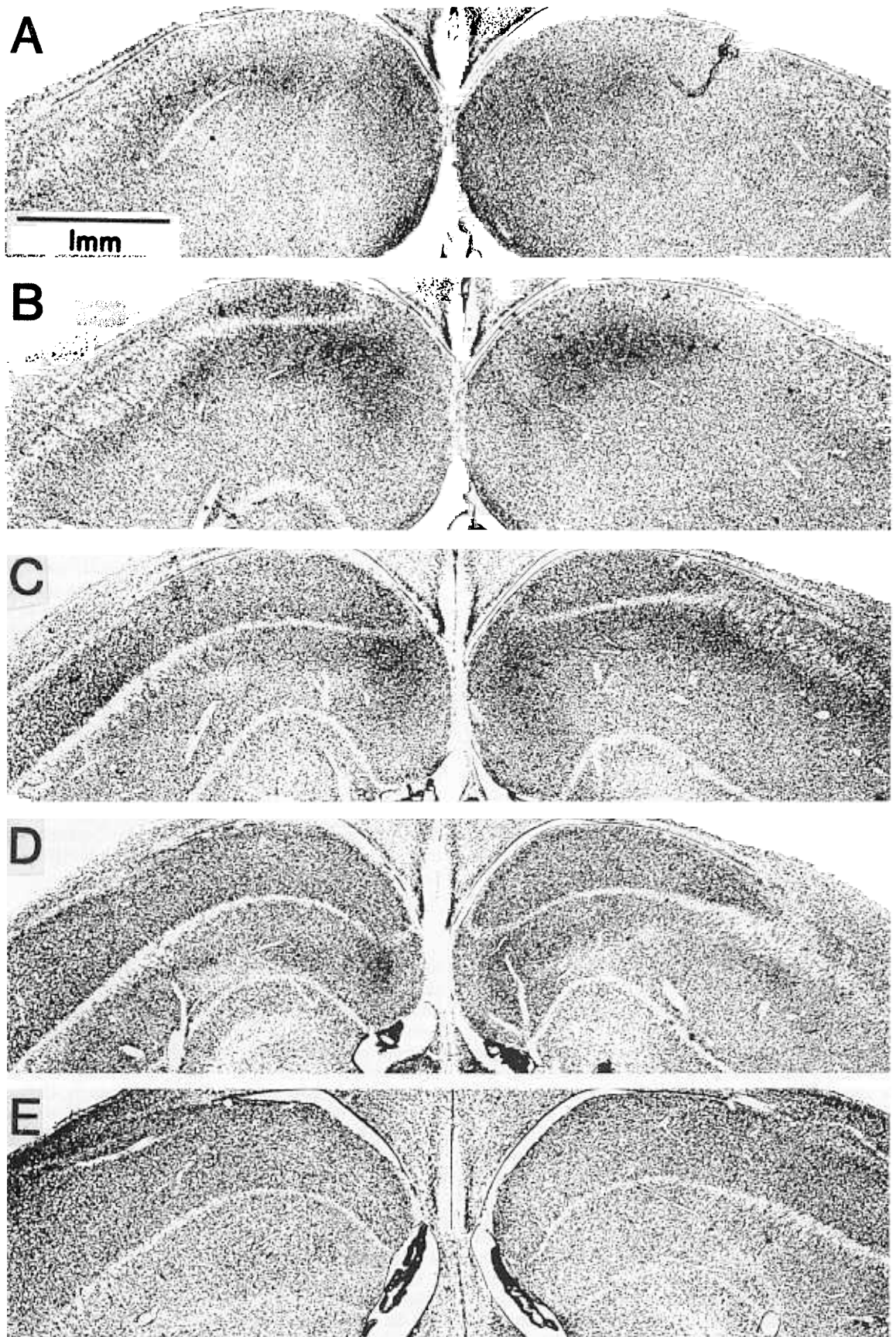


Fig. 4. Photomicrographs of coronal Nissl sections in caudostral sequence. Planes of the sections are illustrated in Figure 3. The plane of section is not perpendicular to the midline: in each section the right hemisphere is somewhat more caudal the left hemisphere. Sections correspond to those in Figure 5.

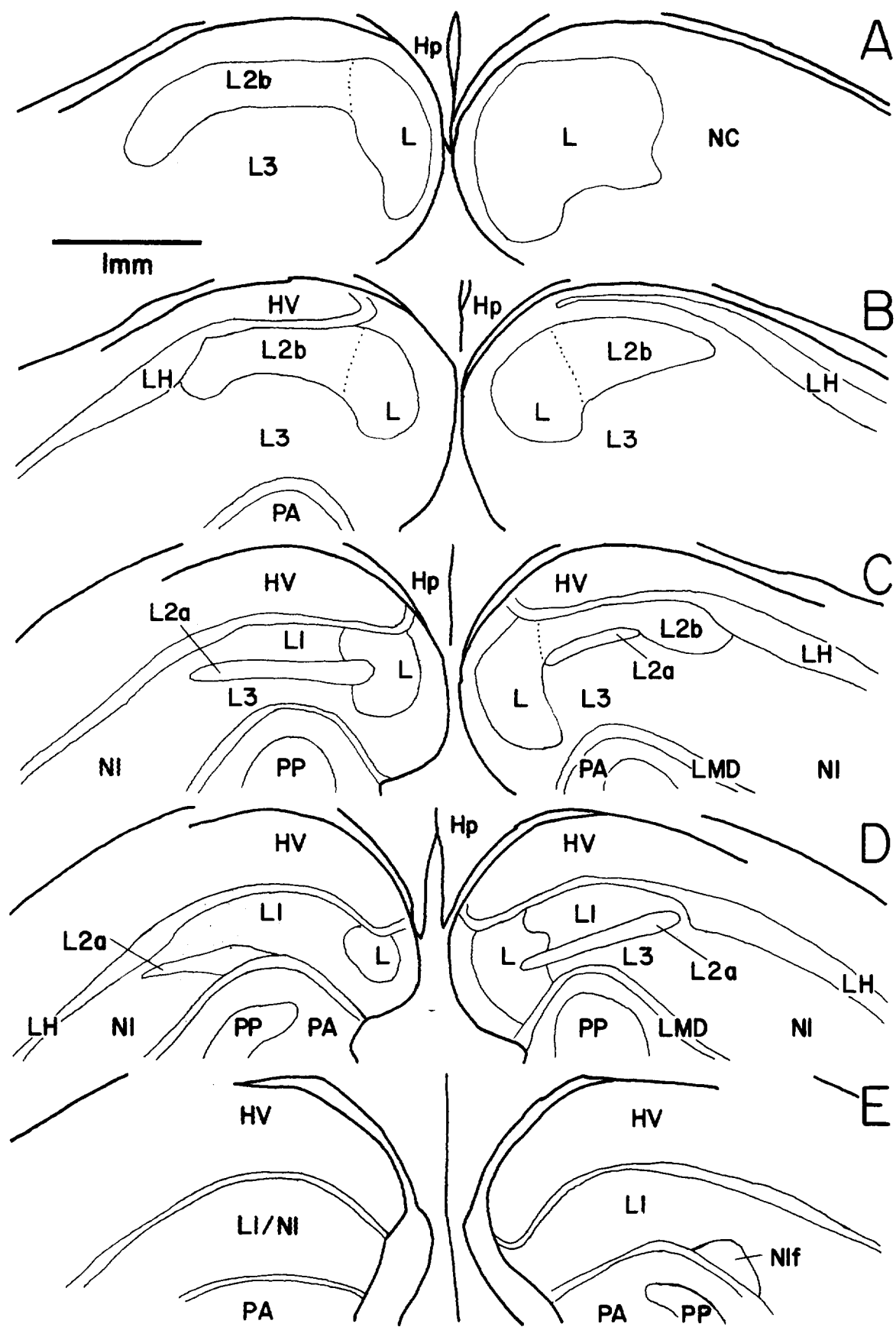


Fig. 5. Outline drawings of coronal sections in caudo-rostral sequence. Sections correspond to those in Figure 4. Dotted lines indicate indistinct borders.



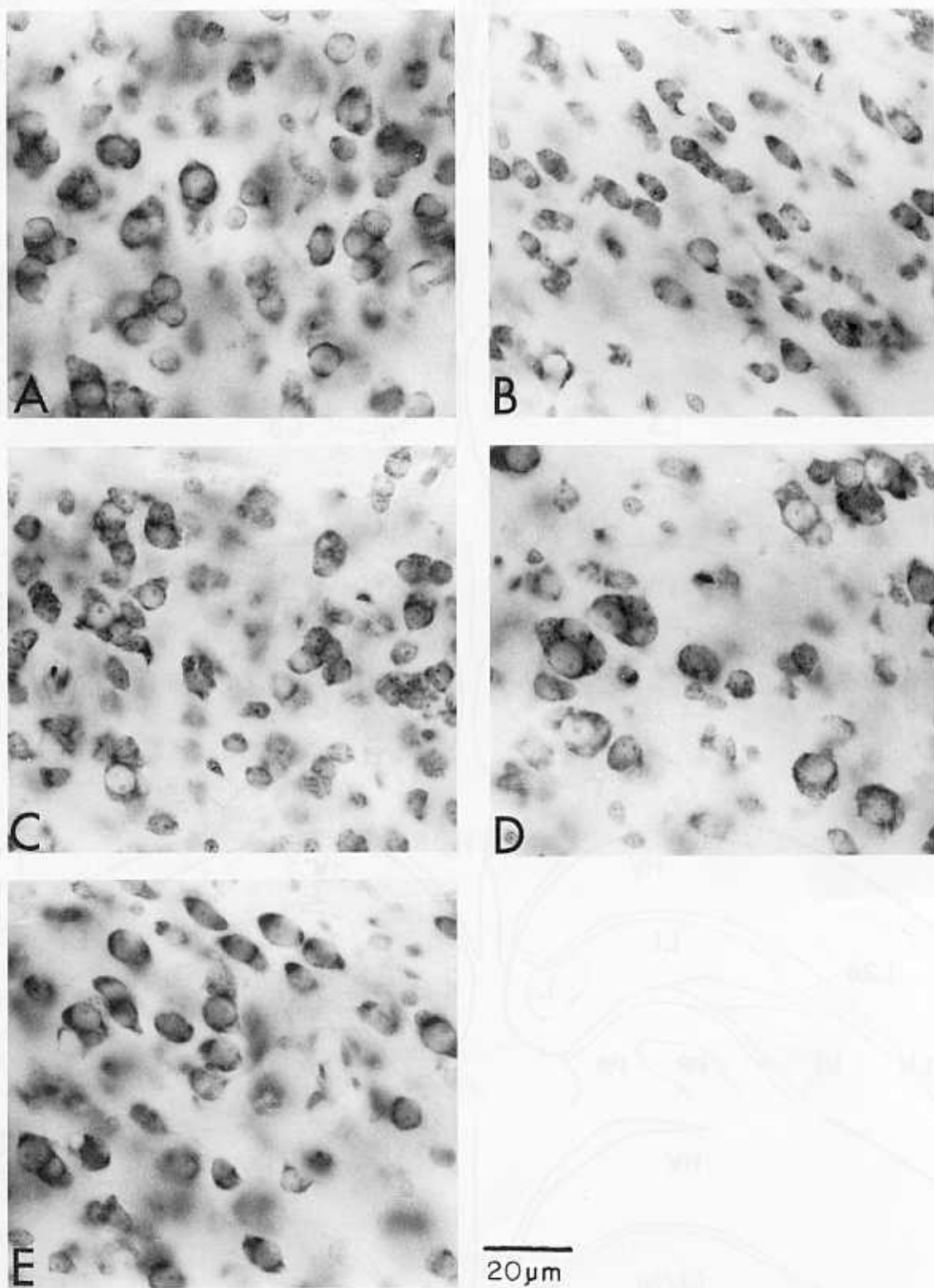


Fig. 6. High power (40 $\times$  objective) photomicrographs of Nissl stained cells. (A) L1; (B) L2a; (C) L2b; (D) L3; (E) NIF.

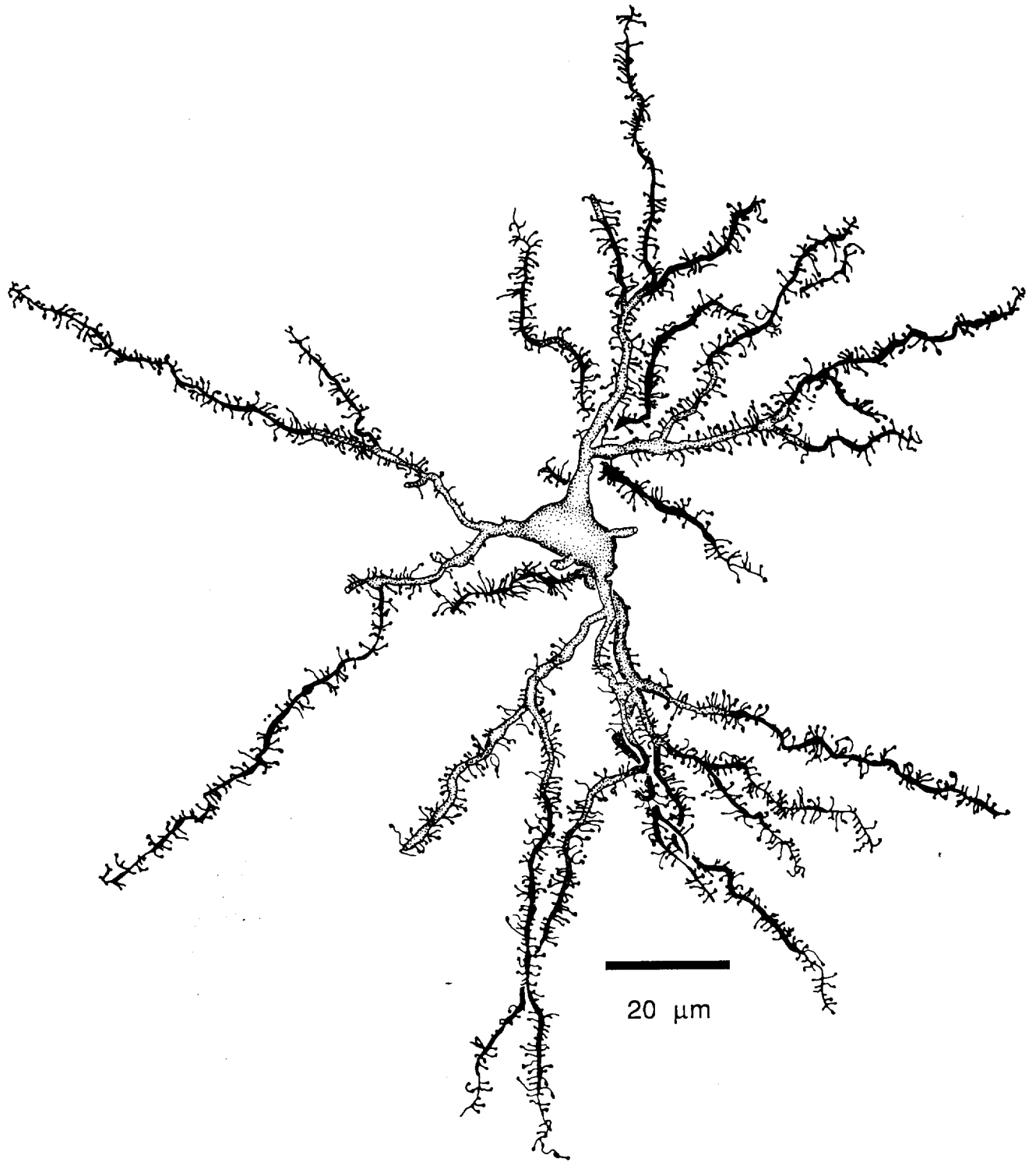


Fig. 7. Camera lucida drawing of a type 1 cell. Dorsal is up. Several dendrites of this neuron are in the subsequent section of the brain and have not been reconstructed for the purpose of clarity.

( $X^2 = 13.50$ ,  $P < 0.01$ ) when compared with the combined total of all of the other subdivisions of the field L complex.

The distribution of cell types in Golgi preparations of L1 and L3 are significantly different when compared with each

other or with the rest of the field L complex. Type 1 cells were found in L3 in significantly greater numbers than random when compared with either L1 ( $X^2 = 9.21$ ,  $P < 0.01$ ) or the rest of the field L complex ( $X^2 = 6.83$ ,  $P < 0.01$ ).

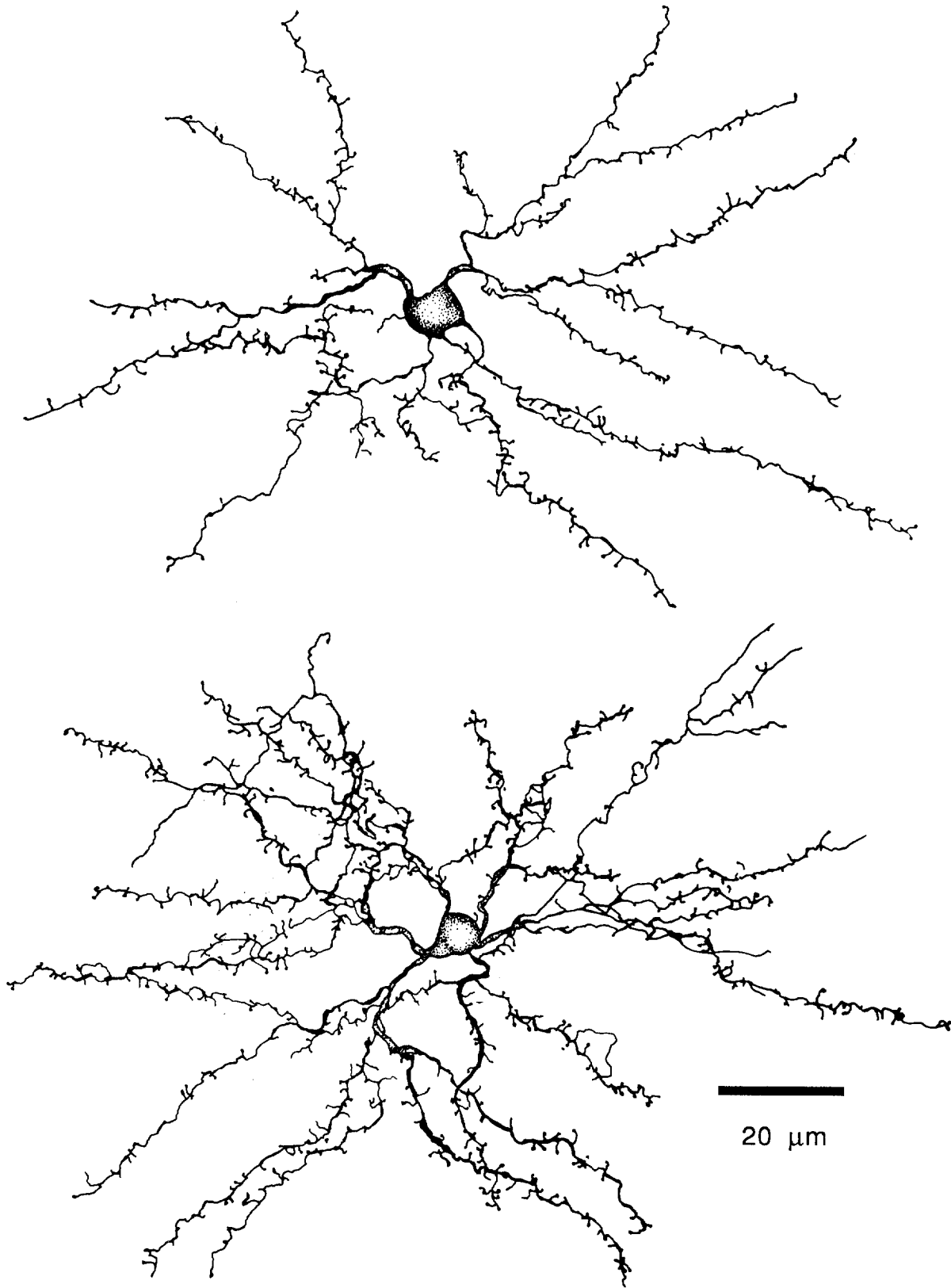


Fig. 8. Camera lucida drawings of two type 2 cells. The cell on the bottom has an unusually small soma for a type 2 neuron.

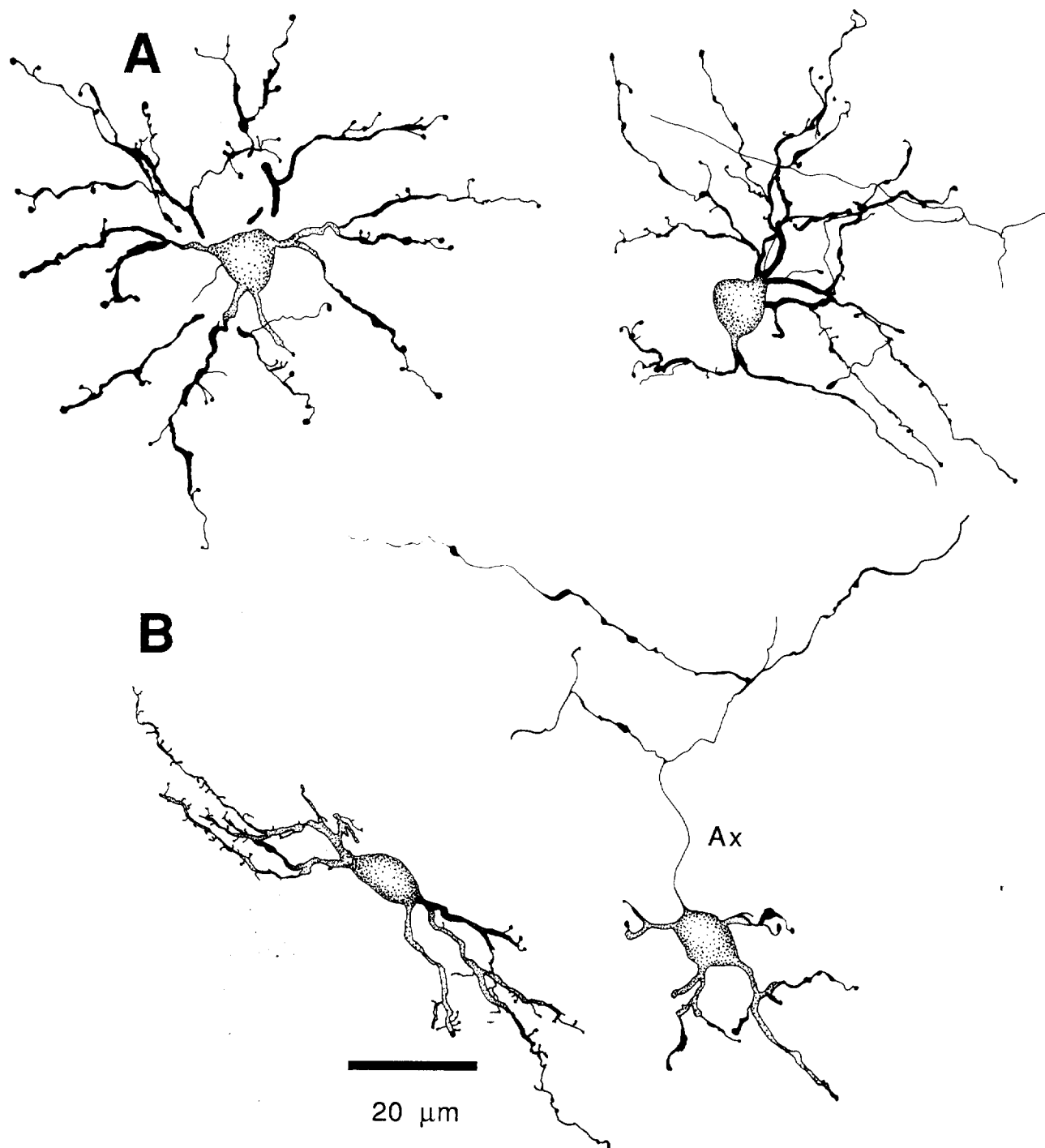


Fig. 9. Camera lucida drawings of type 3 cells. **A:** Examples of type 3 unoriented neurons. **B:** Examples of type 3 oriented cells from parasagittal sections. Dorsal is up and caudal is to the left. (Ax, axon)

Conversely, type 1 cells were found in fewer numbers within L1 when compared to the rest of the field L complex ( $X^2 = 7.75, P < 0.01$ ). The distributions of type 2 and type 3 unoriented cells, when either compared between L1 and L3, or between L1 or L3 and the rest of the field L complex, are not significantly different from random. Type 4 neurons were found more often in L1 than L3, however the differ-

ence is not significant ( $X^2 = 6.22, P < 0.025$ ). When compared to the rest of the field L complex, there were significantly fewer type 4 neurons in L3 ( $X^2 = 7.10, P < 0.01$ ).

The distribution of Golgi cell classes between L and L2b are not significantly different ( $P < 0.01$ ). We found, however, that L had a greater number of type 1 cells in

comparison to L2b. This result is probably a product of sampling error: the border of L and L3, a region which has significantly greater than expected numbers of type 1 cells, is difficult to determine in Golgi stained sections. Thus, some type 1 cells of L3 may have been included in the counts for L.

Type 5 neurons are found only within or on the borders of Nif. They are probably the dominant cell type in Nif.

## DISCUSSION

We have described the cytoarchitectonic organization of the subdivisions of the field L complex, and the types and

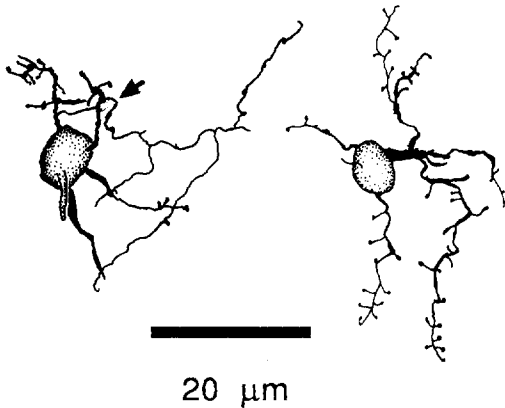


Fig. 10. Camera lucida drawings of two type 4 cells. The arrow indicates the axon.

distributions of cells found in Golgi preparations of these subdivisions. The cytoarchitectonic subdivisions correspond to the functional zones that have been established in both fiber tracing and physiological studies (reviewed by Ulinski, '82; Carr, '91). The region designated "field L" by Rose ('14) is not equivalent to the region which is currently known as field L (Karten, '68).

## Relationship of the field L complex to previous definitions of field L

The current nomenclature for the avian telencephalon results from an amalgamation of terms from Ariéns Kappers et al. ('36), Rose ('14), and some modern, idiosyncratic terms applied to oscine passeriform birds (see Ulinski and Margoliash, '90). Rose ('14) defined fields that were labelled alphabetically from A to S in the forebrains of 23 avian species. Except for field L, Rose's terminology is not commonly used. For example, Rose's field G is commonly known as the neostriatum and field D the ventral hyperstriatum (Ariéns Kappers et al., '36; Karten and Hodós, '67). The term field L was established in the modern literature

TABLE 1. Distribution of Cell Types Across the Subdivisions of the Field L Complex

Sub-division	Cell type				
	Type 1	Type 2	Type 3 unoriented	Type 3 oriented	Type 4
L1	15.5% (13)	45.2% (38)	15.5% (13)	0% (0)	23.8% (20)
L2a	12.9% (8)	11.3% (7)	22.6% (14)	27.4% (17)	25.8% (16)
L2b	20.6% (14)	44.1% (30)	16.2% (11)	4.4% (3)	14.7% (10)
L3	32.4% (101)	43.3% (135)	11.5% (36)	0% (0)	12.8% (40)
L	42.5% (17)	40.0% (16)	7.5% (3)	0% (0)	10.0% (4)

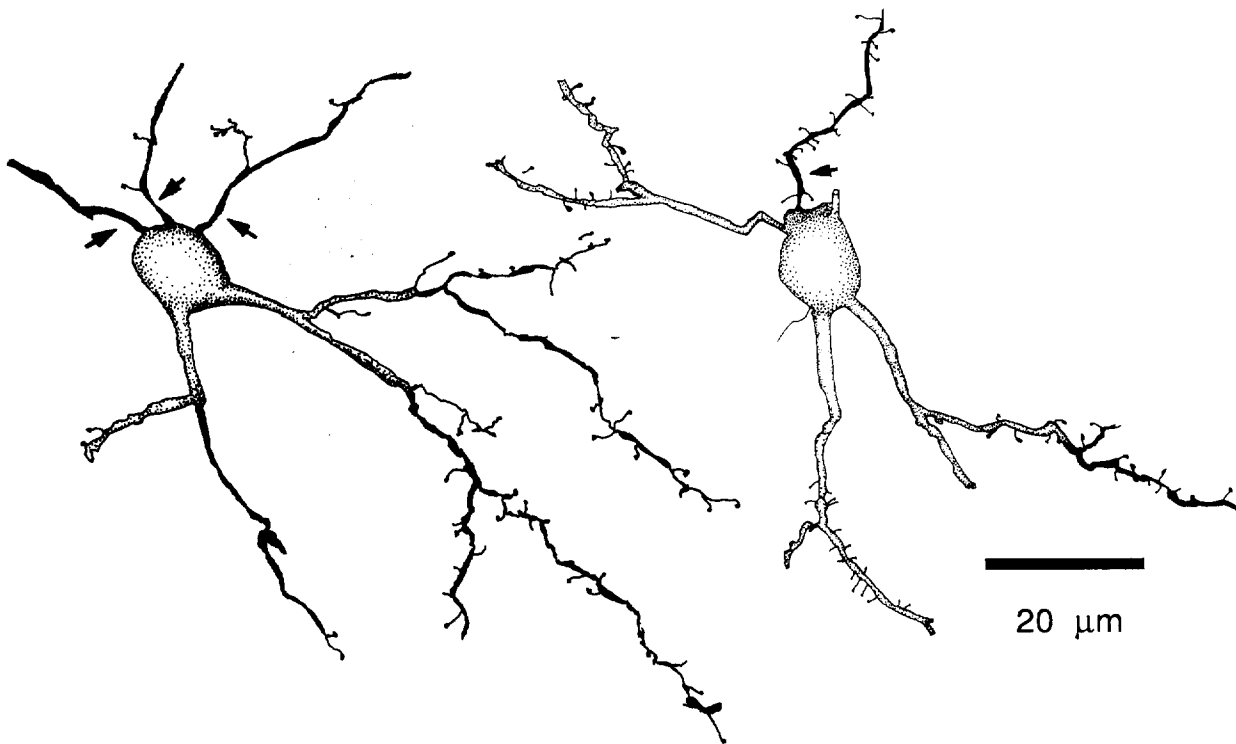


Fig. 11. Camera lucida drawings of two type 5 cells (Nif). The distal portions of the dendrites of these cells were not stained. Arrows indicate thin dendrites.



by Karten ('68). Using Fink-Heimer degeneration methods, he found that efferents of the dorsal thalamic nucleus ovoidalis in the pigeon terminated in "... a sharply localized area ... immediately adjacent to, and continuous with, the CIO;" which, when compared to Nissl stained sections, appeared to be "... localized to a region ... corresponding to Field L of Rose." Most subsequent workers have used Karten's definition of field L, the thalamorecipient zone of ovoidalis efferents, but Kelley and Nottebohm ('79) use a definition which is a compromise between Rose's and Karten's definitions (see below).

The terms L1, L2, and L3, which are subdivisions of Karten's field L, were introduced by Bonke et al. ('79a). Their L2 is equivalent to Karten's CIO, and their L1 and L3 represent the adjacent areas which Karten reported in his study. Bonke et al. ('79a) indicate that their field L is "larger" than the field L of Rose. In addition these authors introduced the term L2a, which is discussed in detail below.

Wild et al. ('90) first described L2b as the "dorsolateral end zone" of field L in the pigeon. L2b is the recipient of efferents from the thalamic nucleus semiluminaris pars ovoidalis. Brauth et al. ('87) also describe a dorsolateral zone of field L in the budgerigar which receives thalamic input from nucleus ovoidalis ventromedialis (OVM). This zone is called dorsolateral intermediate neostriatum, or NIDL. We have chosen to adopt the terminology of Wild et al. ('90) for two reasons. First, the term "L2b" is consistent with the terminology established by Bonke et al. ('79a). Second, the term NIDL describes a position in the budgerigar brain; the orientation of the field L complex, however, differs considerably between species.

In this report the term "L" refers to the area that corresponds to Rose's field L. We compared our sections to those shown in Rose ('14) and found the area which contained "... very dark stained, medium-sized, round densely packed elements ..." in the zebra finch. Rose's field L, now known simply as L, was found to be adjacent to L2a (=CIO), which is the most distinct division of the thalamorecipient zone. Thus, in the zebra finch Karten's field L and Rose's field L are not the same structure.

We have not adopted the term L2 used by previous authors because we could not determine a single cytoarchitectonic definition for L2. Bonke et al. ('79a) show L2 with a bulge on its medial edge, making L2 appear club shaped, whereas L2a, as defined by the projection from HV, does not bulge medially. Two other reports (Karten and Hodos, '67; Kelley and Nottebohm, '79) show a medial bulge on a structure that can be assumed to be L2 (Fig. 2 and Plate A6.25, respectively). The present data suggest that this bulge is L where it surrounds L2a (Figs. 4C,D, 5C,D). We have noticed that in some brains the regions of L that are adjacent to the medial edge of L2a appear to be less darkly stained at low power magnification. The morphology of cells and cell clusters in this less darkly staining region are typical of L, however, and not of L2a.

### Interspecific comparisons

The field L complex is similar in organization but not in its orientation within the brain in different species of birds. The primary difference between species can be explained in terms of the orientation of L2a. In the zebra finch and other oscines (e.g., see Kelley and Nottebohm, '79), L2a appears as an almost horizontal band in coronal sections. In contrast, published descriptions of three Galliform birds, the guinea fowl (Bonke et al., '79a), pigeon (Karten and Hodos,

'67), and the chicken (*Gallus domesticus*) (van Tienhoven and Juhász, '62), show the medial edge of L2a to be ventral to the lateral edge; L2a lies at approximately a 50° angle in coronal sections. This corresponds with a ventromedial rotation of the posterior bulge of DVR that is seen in these species. This rotation also affects HV, which in these species is both dorsal and medial to the field L complex, whereas HV is dorsal to the field L complex in the zebra finch.

The difference in the orientation of the field L complex between species affects its appearance in Nissl material. The sagittal plane is nearly perpendicular to L2a in the zebra finch and in other oscines (personal observations) whereas the sagittal plane is oblique to L2a in non-oscine species (e.g., Karten and Hodos, '67; van Tienhoven and Juhász, '62). Thus in oscines, L2a will be a thin, distinct band in parasagittal sections. The appearance of L2a in coronal sections in oscines depends on the plane of section. In other species, however, L2a will typically be cut obliquely and will appear as a wider, less distinct band. The oriented cells and rows of oriented cells we observed in L2a of the zebra finch will not be visible in sections that are not cut in a plane perpendicular to L2a. This may explain why the oriented cells and rows of oriented cells have not been reported in other species.

This orientation of the field L complex also affects the locations and relationship between L and L2b. As seen in published photomicrographs (Karten and Hodos, '67), there is a darkly staining region adjacent to LH which appears as an almost vertical band that at its dorsal pole arches laterally. Whether or not L and L2b surround the medial and dorsal edges, respectively, of L2a in the pigeon and other non-oscine species cannot be resolved based on previously published accounts.

### Golgi stained cells in the field L complex

The Golgi cell types found in the field L complex of zebra finches are similar to those observed in the DVR of many fathosids (Ulinski, '82). Cell types 1, 2, 3 unoriented, and 4 correspond directly to those found in the auditory neostriatum, which includes field L, of European starlings (*Sturnus vulgaris*) (Saini and Leppelsack, '81). Saini and Leppelsack ('81) reported that type 1 neurons have descending projections and that some type 2 neurons project to HVc. Axons were rarely stained in our material, and those axons which were stained could not be followed for more than 300  $\mu$ m.

Some of the neuronal types seen in the field L complex are similar to those seen in HVc (Nixdorf et al., '89). The "fuzzy neurons" they identify in HVc are larger, but similar in appearance to the type 1 neurons seen in the field L complex. Their "thick dendrite" neurons probably correspond to our type 2 neurons. These authors found that the "thick dendrite" neurons were most variable in their morphologies. Our type 2 neurons were the most variable class of neurons we found, and, using numerical techniques such as those described by Nixdorf et al. ('89), might possibly be further divided into subclasses. Their "short dendrite neurons" probably correspond to our type 3 normal neurons. The similarity between the cell types seen in HVc and those in the field L complex support the recent reinterpretation of HVc as a neostriatal structure (Nottebohm, '87).

Type 5 cells, which are found only in Nif, were stained infrequently, and often the distal arms of the dendrites were not stained. These cells have two unusual features.

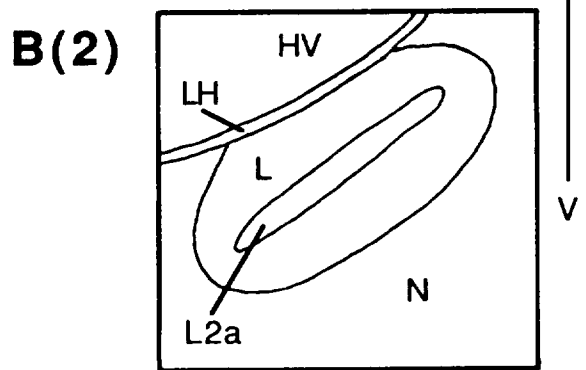
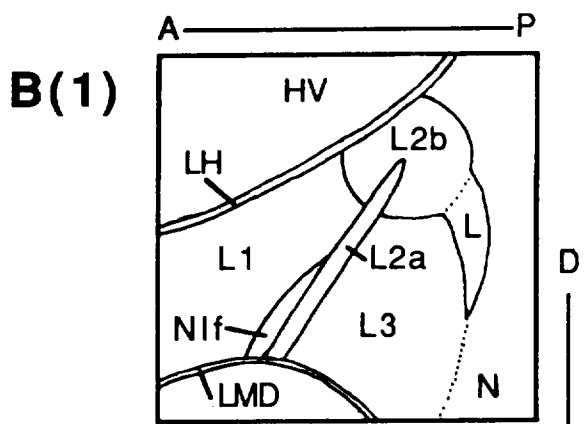
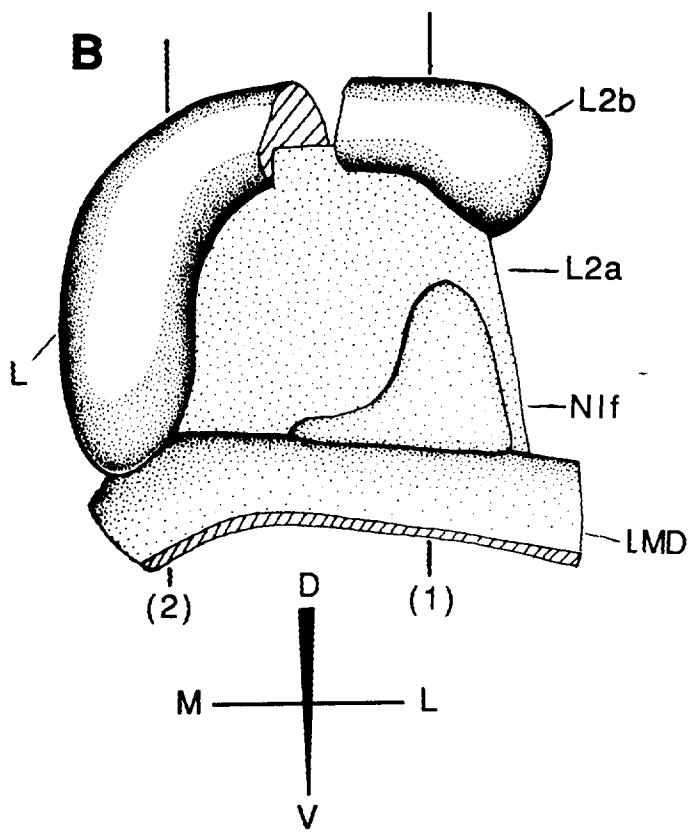
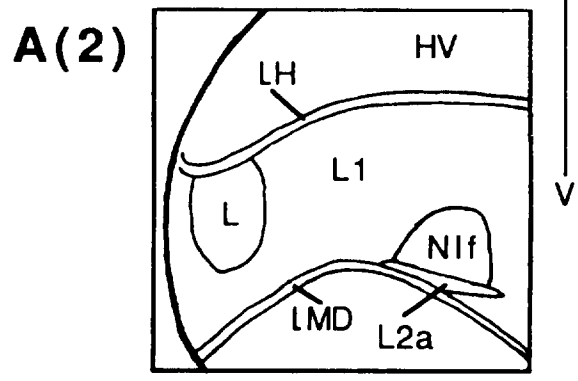
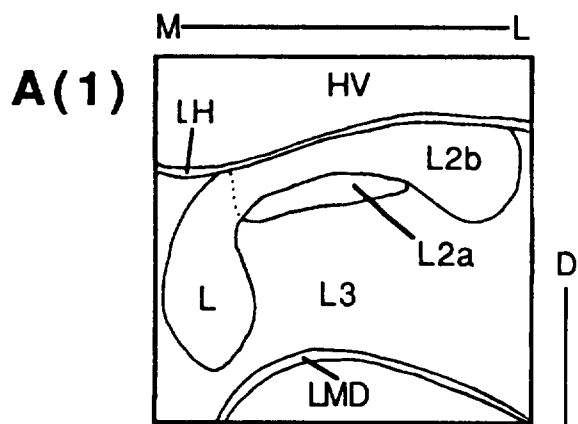
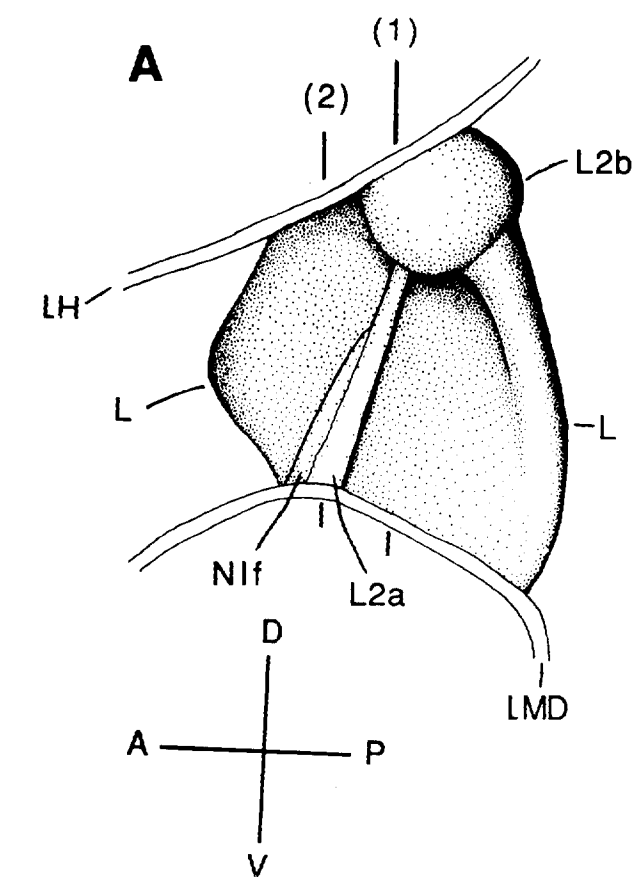


Figure 12

First, they have two classes of dendrite. Second, thick dendrites are smooth proximally and have spines distally. The two types of dendrites may receive separate functional inputs. Nif itself has not been described in non-oscine species. This characteristic cell type may be useful for identifying a possible homologue of Nif in non-passerine birds.

### Relationship between Golgi and Nissl material

The distributions of cell types between the subdivisions of the field L complex in Golgi material correspond to the morphology of cells in Nissl material. L2a is characterized in Nissl material by the presence of ovoid or oblong, medium-sized cells which are often arranged in rows of up to five cells. Type 3 oriented cells are found almost exclusively within L2a, which suggests that the rows of cells in L2a are type 3 oriented cells. Nomarski microscopy of L2a in parasagittal Nissl stained sections reveals that axons travel through L2a parallel to the rows of cells in L2a (personal observations). This indicates that the dendritic arbors of type 3 oriented cells are also parallel to the axons in L2a.

L3 is characterized in Nissl material by its large clumps of medium-sized and large cells, whereas L1 has smaller clumps and more medium-sized cells. Type 1 cells, the largest type of Golgi stained cells in the field L complex, were found in significantly greater numbers in L3 and fewer numbers in L1. Type 4 cells, the smallest type of Golgi stained cells in the complex, were found in significantly fewer numbers in L3. Thus, L3 both has bigger clusters than L1 and has a greater number of large cells than L1.

L and L2b are both characterized by large clumps of many small and medium-sized cells. Cells in L2b appear to stain more intensely and may be more densely clumped than those in L. In Golgi preparations the border between L2b and L is not visible. Thus, assigning Golgi stained neurons to L or L2b is problematic. Although the distribution of Golgi stained cells that we observed in these two subdivisions is not significantly different, the possibility therefore remains that a significant difference exists.

### Functional zones of the field L complex

We have introduced the term "field L complex" to reflect the addition of L2b and the identification of L as an entity which is separate from L1, L2a, and L3. The subdivisions of the field L complex share two common features. First, they all appear to have auditory responses (Bonke et al., '79b; Scheich et al., '79; Müller and Leppelsack, '85; Margoliash, '86; Rübsamen and Dörrscheidt, '86). Second, they all appear to receive direct input from either OV or nuclei associated with OV (Karten, '68; Bonke et al., '79a; Brauth et al., '87; Wild et al., '90; Fortune and Margoliash, '91).

Physiological studies suggest that the field L complex is composed of at least four functional units. Neurons in "L2" have stronger responses to tone bursts than neurons in L1 or L3 (Bonke et al., '79b); the L2 of that paper includes L2a and part of L. Conversely, neurons in L1 and L3 have more complex response properties (Scheich et al., '79). Heil and Scheich ('91a,b) also divided field L into three functional zones, L1, L2, and L3, based on differences in latencies and frequency bandwidth responses to tones. They did not recognize a distinct L2b. These authors report, however, a systematic increase in the broadness of tuning of both "off" and "on" responses in the dorsal regions of L2 and parts of L1 and L3. This dorsal area may correspond to L2b, as Wild et al. ('90) reported that neurons in L2b of the pigeon are generally more broadly tuned than the rest of field L. Similarly, Rübsamen and Dörrscheidt ('86) found that they could not classify the auditory responses in a small region of dorsolateral field L, which may also be L2b.

Whether or not L2b and L are parts of one or two separate functional units is an unresolved issue. Since L has not previously been recognized as an independent entity, we cannot compare the auditory responses in L and L2b. Preliminary evidence in zebra finches shows that L receives input from neurons in TOv and L2b receives input from Ovm (Fortune and Margoliash, '91). These projections either represent parallel pathways from TOv and Ovm, respectively, or a single topographic projection from a combined complex composed of TOv and Ovm. The shapes and relationship between L and L2b is complex (see Fig. 12). Both structures surround L2a: L surrounds medial L2a and L2b surrounds dorsal L2a. L and L2b meet near the dorsomedial corner of L2a (Fig. 12B). L also has anteromedial and posterolateral extensions. The dorsal edge of the posterolateral extension of L borders L2b (Fig. 12A).

The field L complex is a probable source of auditory input to the song system of oscine species. Using anterograde proline autoradiography, Kelley and Nottebohm ('79) observed that neurons within field L project to a region ventromedial to HVc and that neurons anterolateral to field L project directly into HVc. These authors defined field L on the basis of anterograde staining of OV efferents as "... an oval shaped region in the medial neostriatum ...". An examination of field L as depicted in their Figure 2 suggests that they restricted field L to include only the area where L2a is surrounded by L, which is a combination of Rose's ('14) and Karten's ('68) definitions of field L. Thus, their definition of field L includes only the most medial regions of the field L complex, and excludes L1 and L3. Kelly and Nottebohm ('79) reported that injections lateral to field L anterogradely labelled HVc. These lateral injections may have included L1, and therefore L1 may be a direct source of auditory input to HVc.

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Fig. 12. Space-filling schematics of the field L complex with representative sections. Schematic sections are shown to the right. A: Lateral is above the plane of the page and medial is below the plane of the page. L1, which is in the space anterior to L2a and Nif, and L3, which is in the pit formed by L and L2b caudal to L2a, are transparent. B: Anterodorsal is above the plane of the page and posteroventral is below the plane of the page. LH has been removed: the unstippled region on L and L2b is where LH contacts these structures. L1, which covers L2a, Nif, LMD, and the portions of L and L2b below where LH contacts L and L2b, is not depicted. A small piece of L2b has been cut away to show how L2a invades L2b and L.

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