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Intracellular Marking of Physiologically Characterized Cells in the Ventral Cochlear Nucleus of the Cat

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ABSTRACT

In the cat ventral cochlear nucleus, separate neuronal classes have been defined based on morphological characteristics; physiologically defined unit types have also been described based on the shape of post-stimulus-time-histograms in response to tone bursts at characteristic frequency. The aim of the present study was to address directly the issue of how morphological cell types relate to physiological unit types. We used intracellular injections of horseradish peroxidase to stain individual neurons after their response characteristics were determined by intracellular recordings. The maintenance of a continuous negative resting potential, the correspondence of the calculated position of the electrode tip at the time of injection to the location of the stained neuron, and the similarity of response properties collected before and after the injection provide evidence that the injected, stained, and recovered neuron corresponds to the functionally defined unit.

In the region around the nerve root in the anteroventral cochlear nucleus, two "primarylike" and one "primarylike with notch" units were "bushy" cells. "Bushy" cells are characterized by primary dendrites arising from one hemisphere of the soma and ramifying repeatedly to produce their bushy dendritic arbor. In this same region, the "chopper" and two "on" units were also "bushy" cells. In the posteroventral cochlear nucleus, the "chopper" unit was a "stellate" cell and the "on" unit was an "octopus" cell.

These results are partially consistent with previous conclusions based on correlations established between the regional distribution of physiological unit types and morphological cell types. More importantly, they confirm and extend recent intracellular marking data (Rhode et al., '83b). If our classification schemes have functional significance, we are left with the conclusion that the distinction between "bushy" and "stellate" cells in the auditory nerve root region of the ventral cochlear nucleus does not correspond in any simple way to distinctions between physiological unit types. More than one morphological cell type can exhibit the same particular response pattern, and the same morphological cell type can exhibit several different response patterns.

Key words: auditory system, cell types, unit types

The cat cochlear nucleus provides a model system for studying how sensory nerve activity produces output discharges in second-order neurons. In a general sense, the role of the cochlear nucleus is to receive incoming auditory nerve discharges and distribute the modified messages to appropriate regions in the brain. The signal transformations that occur between auditory nerve fibers and cochlear

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nucleus neurons are thought to be, at least in part, dependent on the different spatial arrangements of presynaptic endings on the postsynaptic cells (Kiang et al., '65b, '73; Morest et al., '73; Kiang, '75; Tsuchitani, '78). The difficulty in determining synaptic geometry, however, has prompted researchers to seek other morphological features with which to identify neurons, postulate groupings, and predict physiological response properties.

Various morphological classes of cochlear nucleus neurons have been proposed, and the nucleus has been subdivided into regions based on the relative proportions of these neuron types (Osen, '69; Brawer et al., '74; Cant and Morest, '79; Tolbert and Morest, '82). Furthermore, different "unit" types have been electrophysiologically defined on the basis of spontaneous activity and sound-evoked response properties (Rose et al., '59; Kiang et al., '65b; Pfeiffer and Kiang, '65; Pfeiffer, '66; Evans and Nelson, '73; Goldberg and Brownell, '73; Gisbergen et al., '75; Godfrey et al., '75a,b; Bourk, '76; Young and Brownell, '76; Britt, '76; Romand, '78; Ritz and Brownell, '82; Rhode et al., '83a,b). One scheme for classifying unit types was based primarily on the shape of the post-stimulus-time-histograms (PSTH) in response to tone bursts at characteristic frequency (CF, the frequency of tone to which a neuron is most sensitive). Four general categories of unit types were described in the ventral cochlear nucleus: "primarylike," "primarylike with notch," "chopper," and "on" (Pfeiffer, '66; Godfrey et al., '75a; Bourk, '76). Comparisons between the regional distribution of physiologically defined unit types and morphologically defined cell types have provided indirect evidence for structure-function relationships in the ventral cochlear nucleus (Kiang et al., '73; Morest et al., '73; Kiang, '75; Godfrey et al., '75a; Bourk, '76; Tsuchitani, '78). "Primarylike" and "primarylike with notch" units were associated, respectively, with "spherical" and "globular" cell types as seen in Nissl preparations (Bourk, '76). These two cell types are indistinguishable in Golgi preparations and have been placed in the general category of "bushy" neurons (Cant and Morest, '79; Tolbert and Morest, '82). In the anteroventral cochlear nucleus (AVCN), "chopper" and "on" unit types were hypothesized to correlate with the "multipolar" (Nissl) or "stellate" (Golgi) cell category (Bourk, '76). In the posteroventral cochlear nucleus (PVCN), the spatial distribution of "on" units closely coincided with the region occupied by "octopus" cells. The remainder of PVCN contains mostly "multipolar"/"stellate" cells and is further characterized by a predominance of "chopper" units (Godfrey et al., '75a). The evidence for these ideas, however, involves regional correlations between anatomical and physiological characteristics rather than direct demonstrations for indi-

vidual cells. Rhode et al. ('83a,b) began testing these correlation schemes by a direct method, using single cell recording and marking techniques. In the ventral cochlear nucleus, these authors reported unit type/cell type correspondences mostly consistent with the general ideas developed from population data. The proposed correlations of anatomical and physiological categories are summarized in Table 1.

The aim of the present study was to continue the test of hypothesized structure-function correspondences in the ventral cochlear nucleus. Our data are based on the physiological characterization of single units using intracellular recording and horseradish peroxidase (HRP) staining techniques. Technical difficulties in these kind of marking studies are frequently encountered, and strict criteria are needed to prove that the labelled neuron is the same one studied physiologically. Although an obvious limitation is the small yield of data, the strength of this approach is that even a few well-documented cases can support or strongly contradict previous structure-function relationships based on indirect evidence. We sought to address the following specific issues:

- (1) Are all "primarylike" and "primarylike with notch" units "bushy" cells?
- (2) Are all "chopper" units "stellate" cells?
- (3) Are the various types of "on" units in the AVCN "stellate" cells?
- (4) Are "on" units in the PVCN "octopus" cells?

METHODS

The present data were obtained from 30 healthy adult cats with clean external ears, weighing between 1.5 and 3 kg. The surgical preparation was similar to that described previously (Kiang et al., '65a; Bourk, '76). Briefly, the animals were anesthetized with diallyl barbituric acid in urethane solution injected intraperitoneally (75 mg/kg). Supplementary doses were periodically administered in order to maintain areflexia to paw pinches. The posterior cranial fossa was opened and the lateral portion of the cerebellum was aspirated to exposed the cochlear nucleus. During the experiments, the animal was placed in a sound-proof and electrically shielded room. The rectal temperature of the animal was maintained close to 37°C, using a heating pad.

The acoustic stimuli delivery system has been previously described in detail (Kiang et al., '65a). The functional state of the cochlea was monitored by the visual detection level of the compound action potential N_1 recorded with a wire electrode near the round window in response to 100- μ sec-

TABLE 1. Correspondences Between Unit Types and Cell Types Reported for the Ventral Cochlear Nucleus

Cell types		Unit types ¹		
Brawer et al. ('74) (Golgi)	Osen ('69) (Nissl)	Bourk ('76) ² (AVCN)	Godfrey et al. ('75a) ² (PVCN)	Rhode et al. ('83b) ³ (VCN)
	Spherical	PRI		
Bushy	Globular	PRI w N	—	PRI
Stellate	Multipolar	Chopper / on	Dominance of chopper	Chopper / on (PVCN)
Octopus	Octopus	—	On-type L / on-type I	On-type L (PVCN)

¹(PRI) primarylike; (PRI w N) primarylike with notch; (AVCN) anteroventral cochlear nucleus; (PVCN) posteroventral cochlear nucleus; (VCN) ventral cochlear nucleus.

²Population data.

³Cell marking technique.

duration clicks. Noise bursts (25-ms duration) were used as the search stimulus. The physiological characterization of units was based on the shape of the PSTHs obtained in response to 25-ms tone bursts, with a rise and fall time of 2.5 ms and presented 10 times every second, using criteria discussed previously (Pfeiffer, '66; Godfrey et al., '75a,b; Bourk, '76). The PSTHs were computed using a LINC mini-computer with a bin width of 0.25 ms from 600 tone burst presentations. Some of these data were simultaneously recorded on analog tape for off-line analysis. The unit type was determined by the response pattern at CF at 10 or 20 dB above threshold.

The intracellular recording and injection techniques were similar to those developed in this laboratory for auditory nerve experiments (Liberman, '82a,b). Micropipettes were pulled on a Brown and Flaming ('77) microelectrode horizontal puller. The micropipettes were then filled with a 10% solution of horseradish peroxidase (Sigma type VI) in 0.15 M KCl with 0.05 M TRIS buffer (pH = 7.3). The electrodes were bevelled from an initial impedance of 60–120 M Ω in saline to final resistances ranging between 40 and 70 M Ω .

Physiological characterizations of all units in this study were made in the following way. The electrode was advanced with an hydraulic microdrive in steps of 1–3 μ m. Contact with a unit was indicated by a resting potential equal to or more negative than –25 mV; spike activity was triggered using a level detector. The CF and threshold of the unit were determined manually using audiovisual cues. PSTHs were then computed in response to tone bursts at CFs at different intensities; in some cases, the spontaneous discharge rate was computed during a 15-second period without acoustic stimulation. If the resting potential remained stable, injection of HRP was initiated. The intracellular injection of HRP was made iontophoretically by passing positive-current pulses of 1–5 namp (50-ms pulses at 50% duty cycle) through the micropipette. The injection duration was maintained for as long as possible and terminated when the resting potential became less negative than –15 mV. Whenever possible, the physiological properties of the unit were retested after the injection in order to be certain that the electrode had remained in the same cell. For the units reported in this study, intracellular "holding time" ranged from 6 to 30 minutes. With rare exceptions, only one (rarely two) unit(s) in AVCN and one unit in PVCN were injected in each experiment to reduce ambiguity of assigning marked cells to units.

The major obstacle in obtaining stable intracellular recordings from the cochlear nucleus is the presence of brainstem pulsations. To reduce pulsation, a closed recording chamber, consisting of a cylindrical piece of rigid plexiglass, was placed directly above the cochlear nucleus; this chamber abutted the cerebellum medially and was cemented firmly to the cranium laterally. The micropipette was aimed anteriorly in the parasagittal plane and angled 30° from horizontal. The recording chamber was filled with mineral oil and closed with warm paraffin. Nevertheless, in eight of the experiments, pulsations were so severe that they still prevented stable intracellular recordings. We should also like to comment that although recording extracellular spike activity from cochlear nucleus neurons was not difficult, as soon as the pipette tip entered the cell body (indicated by a steady negative DC potential and postsynaptic potentials), spike activity generally stopped. Such units obviously could not be used for this study.

Fifteen to 27 hours after the last unit was injected, the animal was deeply anesthetized and perfused through the heart with a mixture of 0.5% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.3). The head was removed and kept overnight in fixative at 5°C. Either 80- μ m-thick frozen or 60- μ m-thick vibratome sections were taken in the coronal plane. The sections were treated with CoCl₂ and reacted with diaminobenzidine (DAB) using a modification of Adams's technique ('77), counterstained with cresyl violet, and cover-slipped with Permount. The labelled cells were drawn and reconstructed using a light microscope and a drawing tube at a total magnification of $\times 1,250$. The piece-by-piece reconstruction of labelled neurons was performed from serial sections, by aligning cut ends of stained pieces according to their position on the top or bottom surface of the tissue section. Labelled structures such as pieces of dendrites can fit together only on one way. Blood vessels were also used as landmarks to align adjacent tissue sections during the reconstruction process.

RESULTS

The present report describes physiological and morphological properties of neurons in the ventral cochlear nucleus which met all of the following criteria: (1) The physiological characterization in response to tone bursts at CF was made exclusively from spike activity recorded when the electrode was intracellular, as demonstrated by a continuous negative DC resting potential; (2) staining characteristics of the labelled neuron were consistent with its HRP injection parameters (i.e., low current passage correlated with faint labelling); and (3) retrieval of the labelled neuron (cell body or injection site on axon) from histological sections matched the calculated location of the unit at the time of injection. Stained cells which could not be unambiguously matched to their physiological properties or whose morphological characteristics were obscured by extracellular reaction product were discarded from the data base.

After histological processing, HRP reaction product continuously and diffusely filled the neurons, yielding Golgi-like images and obscuring such cytological features as Nissl pattern and nuclear morphology. Consequently, the physiologically defined unit types could only be related to the morphological cell classes originally based on Golgi descriptions. Although the criteria for cell type categorization have been most fully established in immature cats (Brawer et al., '74), cell type descriptions have been expanded by recent studies in adult cats (Cant and Morest, '79; Tolbert and Morest, '82; Tolbert et al., '82; Rhode et al., '83a,b). As a result, we have been able to make reliable distinctions between the classes of "bushy," "stellate," and "octopus" cells. The "bushy" cell shows primary dendrites which arise from one hemisphere or pole of the cell body and then ramify further to produce the characteristic dendritic tuft with spatially clustered dendritic branch points. "Stellate" cells have primary dendrites that radiate in all directions away from the cell body, branching infrequently. "Octopus" cells are found primarily in the central region of PVCN and tend to emit primary dendrites from one hemisphere of the cell body; each primary dendrite is thick and travels some distance before branching. Dendritic branch points tend to be clustered.

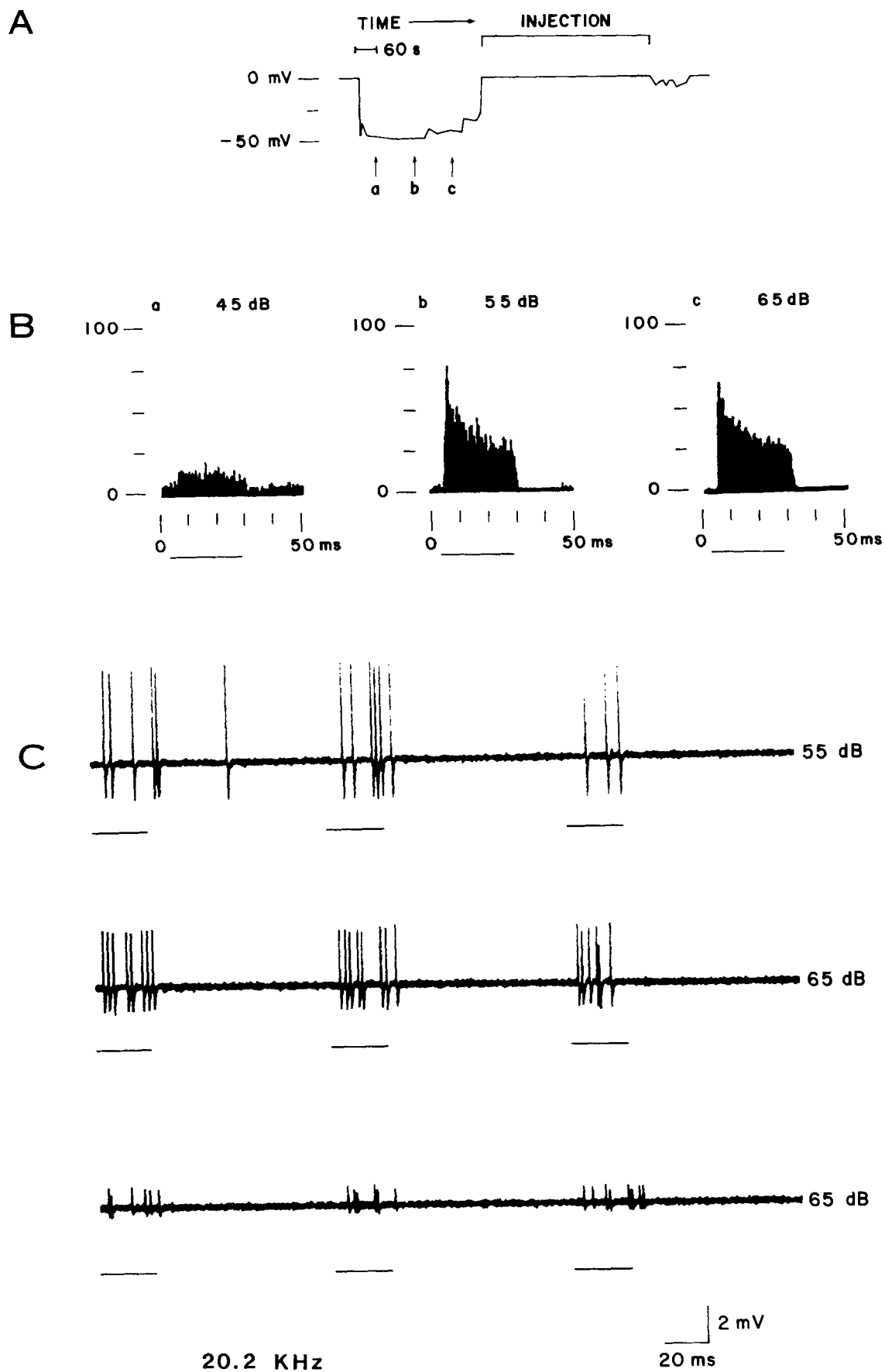


Figure 1

Primarylike units

Bourk ('76) hypothesized that "primarylike" and "primarylike with notch" units were respectively associated with Nissl-defined categories of "spherical" and "globular" cells, both of which presumably correspond to "bushy" neurons seen in Golgi material. In the present marking study, three such units were successfully labelled. The physiological characterization of unit 12-4 is illustrated in Figure 1. The intracellular DC potential was stable at -40 mV (Fig. 1A), but the action potentials were small in amplitude and decreased progressively over time (Fig. 1C). Initially, spike activity could be isolated for the computation of PSTHs in response to tone bursts at CF (20.2 kHz) for three intensity levels (Fig. 1B). The unit is classified as "primarylike" due to the similarity in PSTH shape when compared to those of auditory nerve fibers. After the third PSTH, no detectable spike activity remained, but since the resting potential was stable, iontophoretic injection of HRP was initiated. The injection was interrupted after 440 seconds when the DC potential, monitored on the oscilloscope, suddenly fell to zero.

Abbreviations

A	anterior part of PVCN/multipolar cell area
a	axon
AA	anterior part of the anterior division of AVCN
AN	auditory nerve
AP	posterior part of the anterior division of AVCN
AVCN	anteroventral cochlear nucleus
C	central part of PVCN/octopus cell area
CF	characteristic frequency
CN	cochlear nucleus
dB	decibel
HRP	horseradish peroxidase
kHz	kilohertz
LNTB	lateral nucleus of the trapezoid body
LSO	lateral superior olivary nucleus
ms	millisecond
MSO	medial superior olivary nucleus
mV	millivolt
PD	dorsal part of the posterior division of AVCN
PRI	primarylike
PRI w N	primarylike with notch
PSTH	post-stimulus-time-histogram
PV	ventral part of the posterior division of AVCN
PVCN	posteroventral cochlear nucleus
s	second(s)
SOC	superior olivary complex
SPL	sound pressure level
sp/sec	spikes per second

Fig. 1. Physiological characterization of unit 12-4 ("primarylike"). A. Chart record of the DC resting potential. Abscissa: time, the bar corresponds to 60 seconds. Ordinate: DC potential, negative values toward bottom. During the iontophoretic injection of HRP (2.5 namp for 440 seconds), the resting potential could not be recorded on the chart recorder for technical reasons, but was monitored visually on the oscilloscope. The injection was interrupted when the resting potential suddenly fell to zero. B. PSTHs computed at CF (20.2 kHz) to tone bursts of three different intensities increasing from left to right. Intensity is expressed in dB SPL. Abscissa: time, the length of the abscissa corresponds to 50 ms. The stimulus duration (25 ms) is indicated by the bar associated with the X-axis. Ordinate: number of spikes in each bin. Each PSTH was computed from 600 tone bursts presentations and is associated with a letter (a,b,c) related to the same letters below the DC potential trace, indicating when each PSTH was computed. C. Intracellular record of responses to tone bursts at CF. The upper trace shows the responses to three tone bursts at 55 dB SPL, and in the lower traces to six tone bursts at 65 dB SPL. Note the progressive decrease in the amplitude of spikes as a function of time. The horizontal bars represent the tone burst duration (25 ms). The anatomical reconstruction of the corresponding neuron is shown in Figure 2A.

A camera lucida reconstruction of this neuron is shown in Figure 2A. The HRP reaction product in the cell body was dark but rapidly became faint as it extended into the processes; only those processes which could be traced with confidence are illustrated. The cell body and dendrites of this neuron had smooth surfaces. The primary dendrites and axon arose from the same pole of the cell body, and the clustered distribution of dendritic branch points was characteristic of the general class of "bushy" cells.

The response properties of unit 32-6 are illustrated in Figure 3. A resting potential of -25 mV was obtained and PSTHs were computed in response to tone bursts at CF (2.5 kHz) for three different intensities. After the iontophoretic injection of HRP, the spike activity remained stable and the response properties were retested. The pre- and postinjection PSTHs were similar and indicative of a "primarylike with notch" unit.

The morphological features of the corresponding stained neuron are presented in Figure 2B. The dendritic tree appeared completely labelled and the axon was traced centrally over a distance of 3 mm before it faded along the medial border of the cochlear nucleus. The axon did not branch within the nucleus, but showed a localized distortion (not shown) interpreted as the recording and injection site. The cell body was elongated and smooth. Two primary dendrites arose from one end of the cell and ramify further to form the tuft characteristic of a "bushy" neuron.

The relationship between unit type and neuron class was established for a third case. The unit 27-7 (CF = 3.0 kHz) was electrophysiologically classified as "primarylike" and morphologically defined as a "bushy" cell. The three cases described above support the proposed association of "primarylike" units with "bushy" neurons (Bourk, '76).

Chopper units

"Chopper" units have been correlated with the Nissl-defined category of "multipolar" cells (Godfrey et al., '75a; Bourk, '76). These "multipolar" cells most likely correspond to the class of "stellate" neurons defined in Golgi material (Brawer et al., '74). In the present study, two units showing "chopper" properties were successfully stained by intracellular injection of HRP.

The response characteristics for unit 20-9 are illustrated in Figure 4. This unit had a very low CF (0.3 kHz). As previously mentioned by Bourk ('76), the categorization of low CF units is difficult because of the possibility of phase-locked activity. Although the envelope of the PSTHs at CF was rather irregular, it had three evenly spaced peaks (Fig. 4B). The interval between peaks (5.6 ms) is not equal to the period (3.3 ms) of the stimulus frequency or to a multiple integer of the period, indicating that this response does not correspond to the classical definition for phase-locking. In response to very low-frequency tones, the classical phase-locked units exhibit PSTHs characterized by a sequence of very sharp peaks, regularly distributed across the entire tone burst duration. In the case of unit 20-9, the peaks were prominent only during the first half of the stimulus. The intracellular record of spike discharges further revealed the temporal complexity of the response (Fig. 4C). During the tone burst, some spikes were separated by short intervals which might reflect some phase-locked activity. However, longer intervals unrelated to the period of the stimulus were also present, particularly at the beginning of the tone

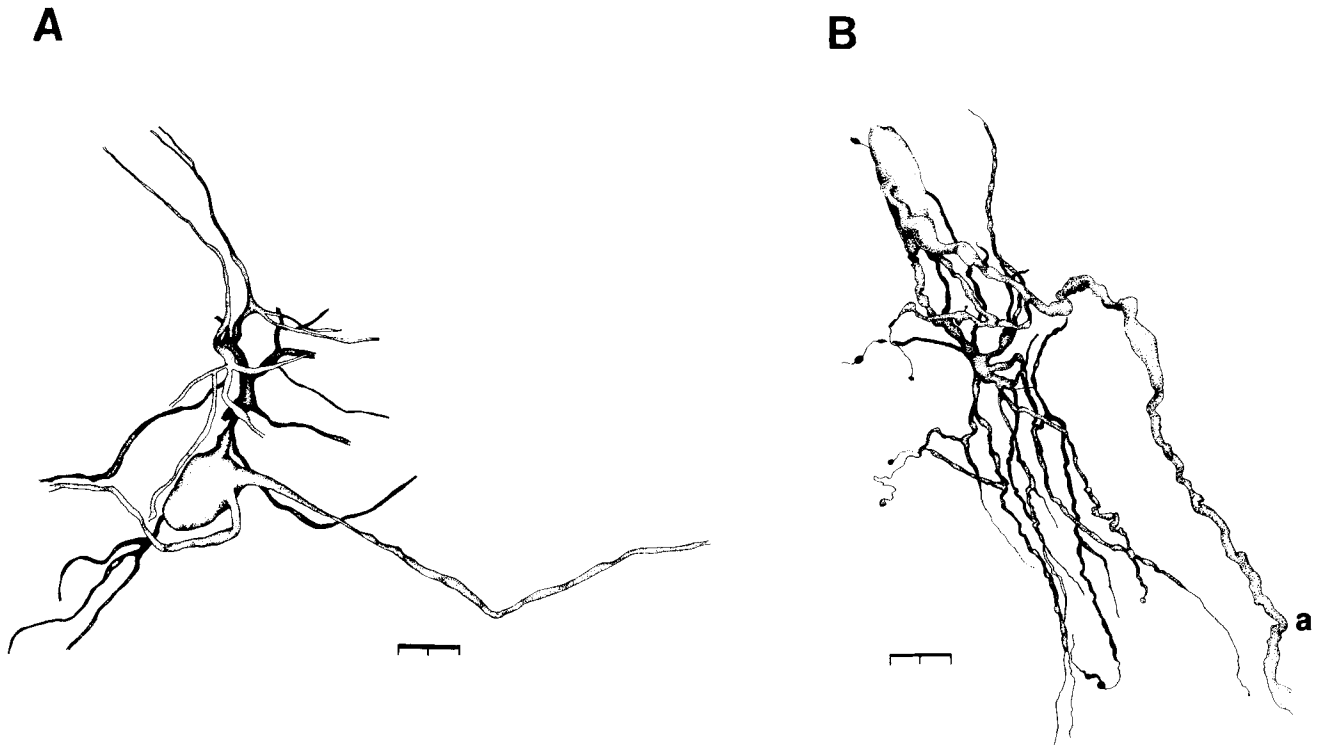


Fig. 2. A. Camera lucida reconstruction of injected unit 12-4 whose physiological responses are illustrated in Figure 1. This "bushy" cell is oriented so that medial is toward right and ventral toward bottom. The scale bar corresponds to 20 μ m. B. Camera lucida reconstruction of injected unit 32-6

whose physiological responses are shown in Figure 3. This neuron is considered a "bushy" cell. Abbreviation: a, axon. See Figure 13 for locations of these cells.

burst: These intervals dominated in the PSTH, giving the chopperlike pattern observed. In comparison to "primary-like" units, "chopper" units are characterized by poor phase-locking. For these reasons, we tentatively categorized this unit as "chopper," although certain other response features were also unusual. For example, there was a diminution of the response with increasing intensities (45–55 dB SPL), and the appearance of greater activity in the second peak of the PSTH computed after the iontophoretic injection. These features may reflect some pathological discharge properties and also partially explain the difficulty in categorizing this unit. In this particular case, there was a small peak in the PSTH (31-ms latency) following the end of the tone burst (at intensities of 45 and 55 dB SPL) caused by a stimulus artifact after the offset of the tone burst.

A camera lucida reconstruction of unit 20-9 is shown in Figure 5A. The reaction product was uniformly dark throughout the neuron, suggesting that the cell body and processes were stained completely. The somatic surface is smooth and without spines. Three primary dendrites and the axon radiate from one side of the cell body. Each dendrite exhibits numerous and clustered branch points, producing the tufted dendritic arbor characteristic of the "bushy" cell class. There are no dendritic appendages and dendritic varicosities are rare. The central projection of this neuron (20-9) is illustrated in Figure 5B. The axon has a diameter of 2 μ m in the immediate vicinity of the cell. It then gradually expands to 3–6 μ m before entering the trapezoid body. The presence of a localized distortion on the

axon, indicative of the injection site, was located approximately 900 μ m from the cell body (see arrow, Fig. 5B). The axon gives rise to two collaterals that course dorsally toward the ipsilateral superior olivary complex. Both collaterals enter the lateral nucleus of the trapezoid body where they ramify and form small terminal boutons before terminating in the lateral superior olivary nucleus as small boutons. The terminal endings were located in the low CF region of each nucleus (Guinan et al., '72; Tsuchitani, '77), consistent with the CF of this neuron (0.3 kHz). As the parent axon continued its trajectory toward the midline, the reaction product gradually faded until it became undetectable.

The response properties of unit 19-15 are illustrated in Figure 6. This unit exhibited a stable DC resting potential of -30 mV (Fig. 6A), but its action potentials were initially small, and decreased over time (Fig. 6C). This unit was classified as a "chopper" on the basis of its multiple-peaked PSTH at its CF of 32.7 kHz (Fig. 6B). The chopper pattern is also evident in the intracellular record, where only the first three spikes are distributed at regular intervals following the onset of each tone burst (Fig. 6C). This response is typical of a "chopper" type-T (transient) response (Bourk, '76).

The camera lucida reconstruction of the recovered neuron is shown in Figure 7A. There is only partial staining of the processes, although the shape of the cell body, the radiation of the primary dendrites in opposite directions, and the absence of clustered dendritic branch points are typical of a

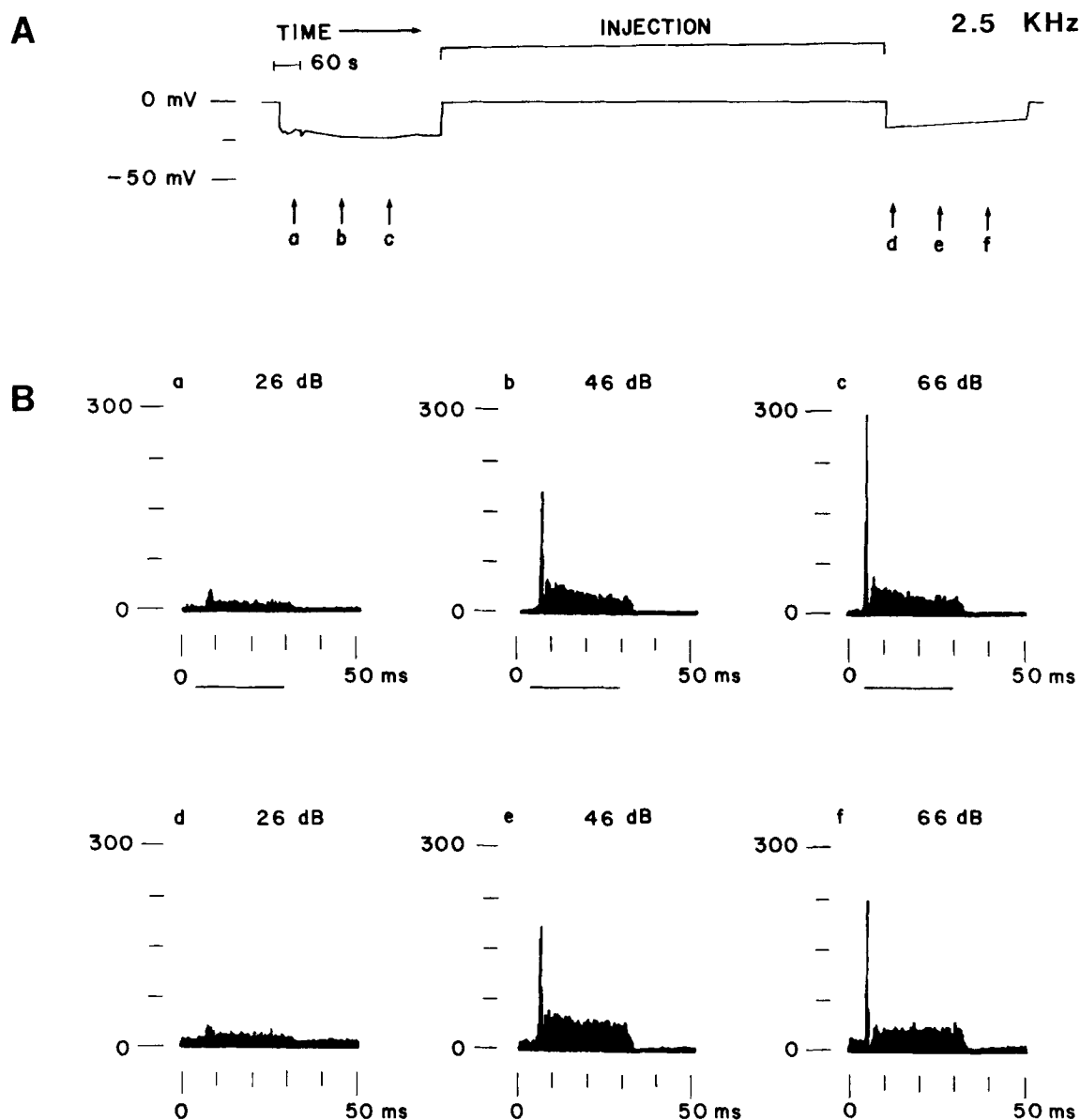


Fig. 3. Physiological characterization of unit 32-6 ("primarylike with notch"). A. DC resting potential record. The injection of HRP was achieved by passing 2 namp of current for 15 minutes through the micropipette. B.

PSTHs obtained in response to pure tones presented at CF (2.5 kHz) before (a,b,c) and after (d,e,f) the injection of HRP. Same conventions as in Figure 1. The morphological properties of this neuron are illustrated in Figure 2B.

"stellate" cell. This particular neuron was located in the multipolar cell area of PVCN (Osen, '69).

From these data, it can be concluded that some "stellate" cells can be associated with "chopper" units. In addition, the unit 20-9 suggests that some "bushy" cells may also exhibit the "chopper" response.

On units in AVCN

"On" units in AVCN were hypothesized to correlate with the "multipolar" cell category as defined in Nissl preparations and the "stellate" cell category as defined in Golgi material (Bourk, '76). The physiological characterization of

unit 24-10 is illustrated in Figure 8. The intracellular penetration produced a steady DC potential of -50 mV, which was maintained for approximately 28 minutes (Fig. 8A). The amplitude of action potentials was large (30 mV) and stable, permitting a retest of physiological characteristics after the HRP injection. The pre- and postinjection PSTHs at CF (2.1 kHz) were very similar (Fig. 8B), and typical of an "on" type-L unit (Godfrey et al., '75a).

A photomicrograph (Fig. 9A) and camera lucida reconstruction (Fig. 9B) of the recovered neuron are presented. This neuron has three primary dendrites which all arise from the same pole of the cell body, and the dendritic

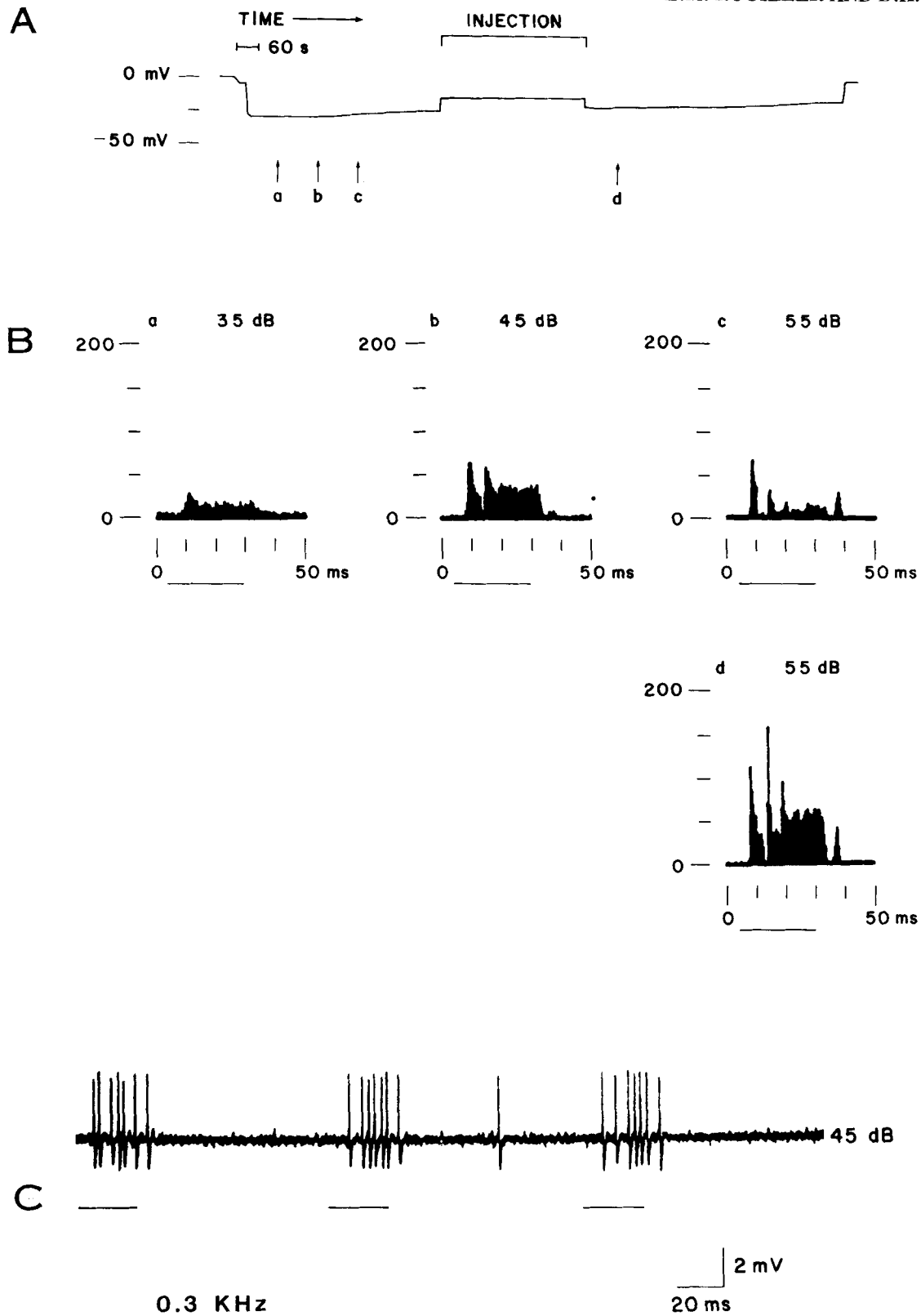


Fig. 4. Physiological characterization of unit 20-9 ("chopper"). A. DC resting potential record. The injection of HRP was made by passing 3 namp for 350 seconds. B. PSTHs in the upper row (a,b,c) were computed from responses to tone bursts at CF (0.3 kHz) at three different intensities (dB SPL) before the injection of HRP. The PSTH in the lower row (d) was

obtained after the injection. C. Intracellular record of responses to three tone bursts at CF presented at an intensity of 45 dB SPL. Same conventions as in Figure 1. The duration of the tone bursts (25 ms) is indicated by the horizontal bars. The morphology of this neuron is shown in Figure 5.

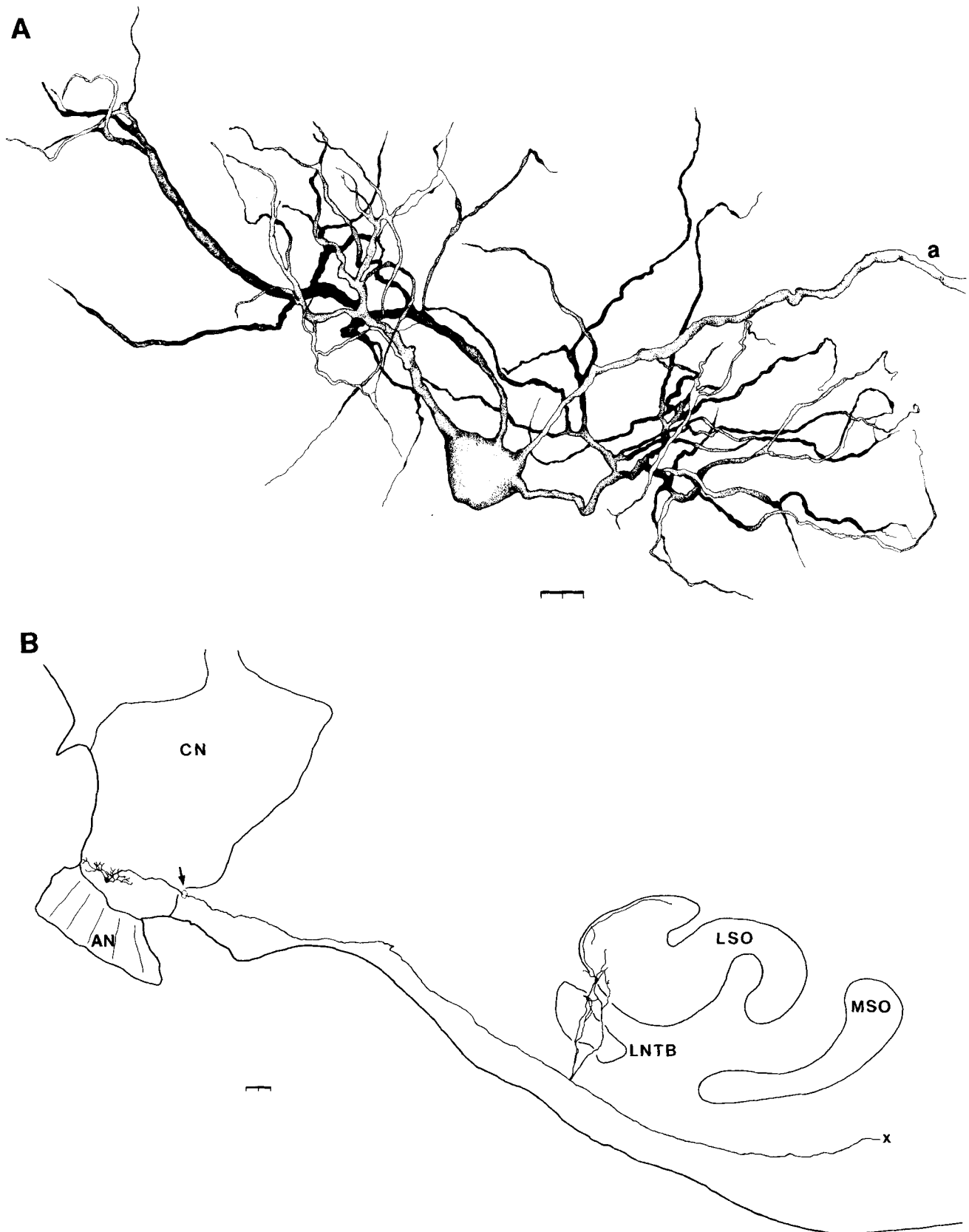


Fig. 5. A. Camera lucida reconstruction of injected unit 20-9 whose physiological responses are shown in Figure 4. This neuron corresponds to a "bushy" cell. Same orientation as in Figure 2. The scale bar corresponds to 20 μ m. Abbreviation: a, axon. The location of the cell is shown in Figure 13. B. Camera lucida reconstruction of the central projection of neuron 20-9,

shown on a coronal section of the ipsilateral half of the brainstem. The arrow indicates the location of the injection site along the axon. The scale bar represents 200 μ m. See list of abbreviations for the identification of the different nuclei. "X" indicates fading of reaction product within the labelled axon.

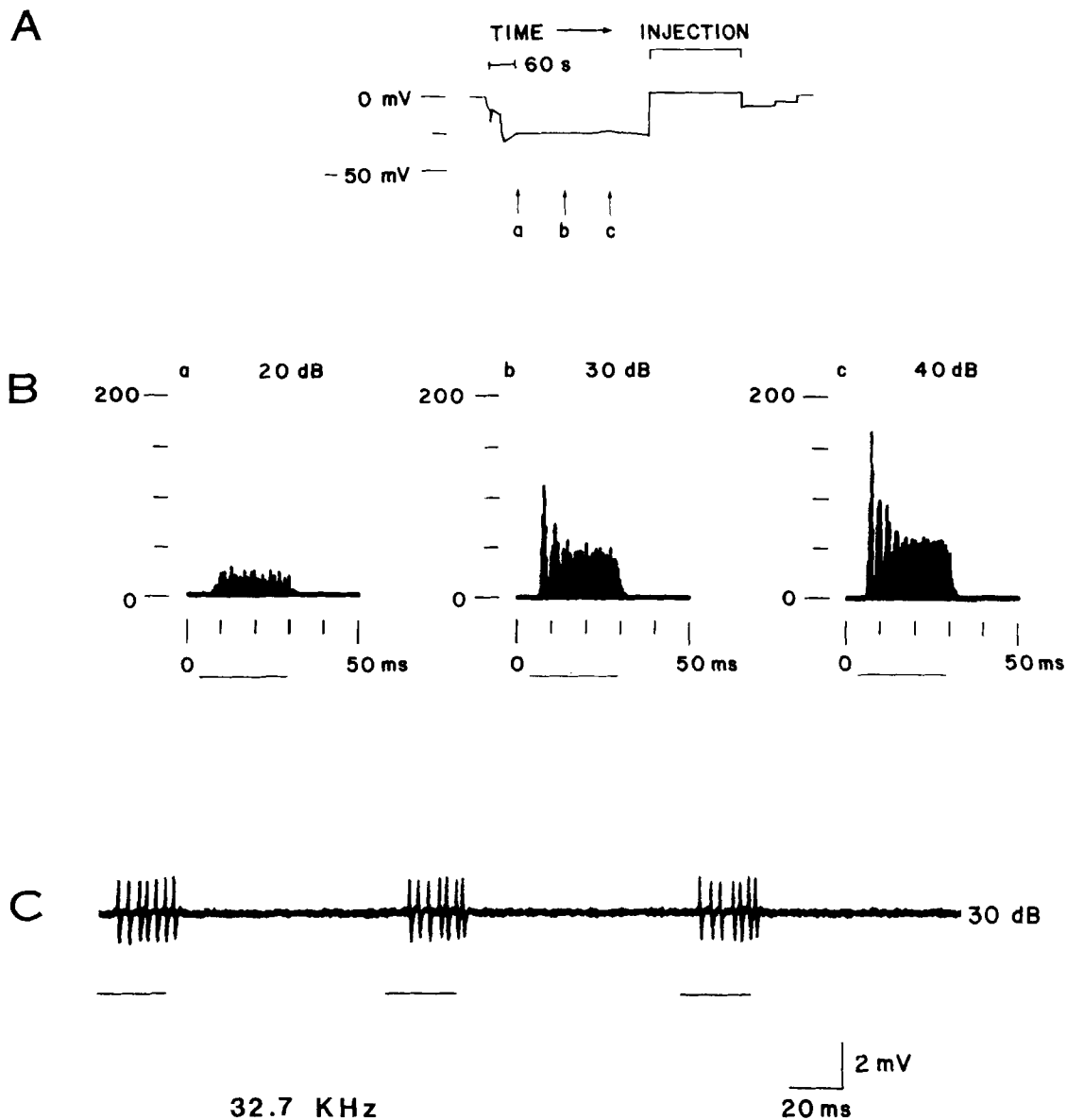


Fig. 6. Physiological characterization of unit 19-15 ("chopper"). A. DC resting potential record. The injection of HRP was made by passing 2.5 namp for 200 seconds. B. PSTHs computed from responses to tone bursts at CF (32.7 kHz) of three different intensities. C. Intracellular record of re-

sponses to three tone bursts at CF presented at an intensity of 30 dB SPL. The duration of the tone bursts is indicated by the horizontal bars. Same conventions as in Figure 1. A camera lucida reconstruction of this neuron is shown in Figure 7A.

processes appear stained all the way out to their tips. This neuron exhibits dendritic tufts formed by numerous and spatially clustered branch points, indicative of a "bushy" cell. It is further distinguished by the presence of somatic spines (Fig. 9A) and numerous dendritic varicosities. The apparent injection site, characterized by a prominent swelling along the axon, is indicated (arrow, Fig. 9A,B). The axon diameter ranges between 2 and 6 μm and could be traced through the trapezoid body nearly to the midline. Little information about the central projection of this neuron is available. The axon runs medially and gives rise to one collateral directed toward the ipsilateral superior oli-

vary complex. Both the axon and its collateral, however, faded some 100 μm beyond the axonal branch point.

The classification of unit 30-29 on the basis of its response to tone bursts at CF (1.8 kHz, Fig. 10B) is equivocal. The PSTH of this unit is not uncommon, and illustrates the difficulty in distinguishing a "primarylike with notch" unit from an "on-type L" unit. There is an initial peak in the histogram which is separated from later sustained activity by a notch. As such, the unit could be considered as "primarylike with notch" and shares some properties with unit 32-6 (Fig. 3). However, as illustrated by unit 32-6, following the initial peak, a "primarylike" response is further char-

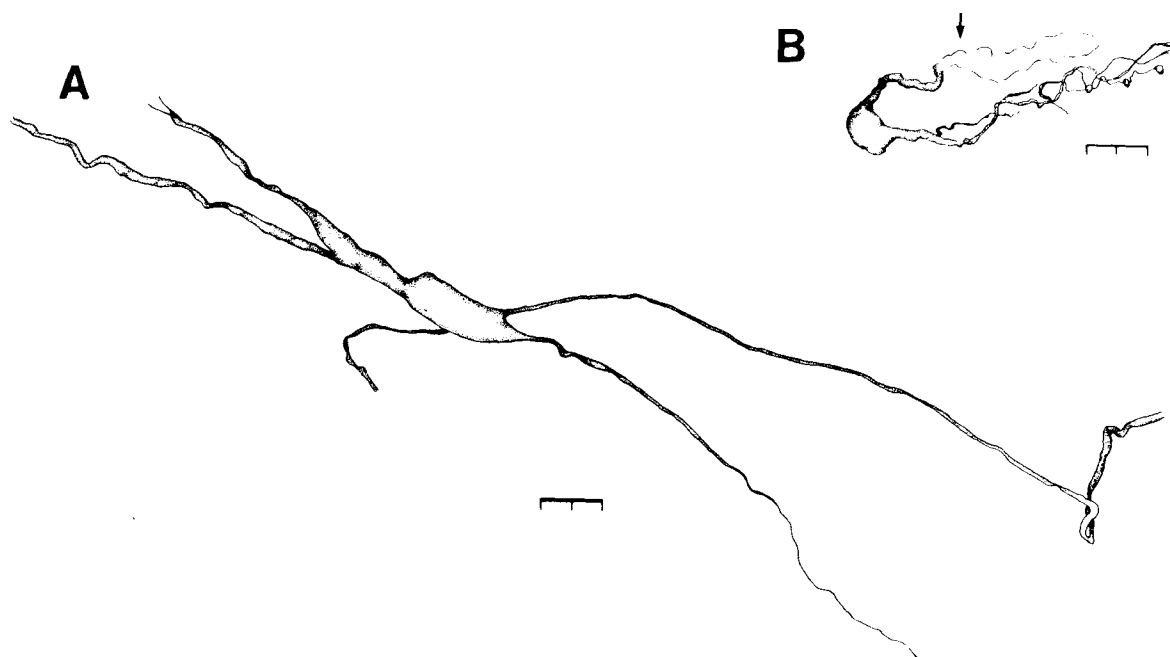


Fig. 7. A. Camera lucida reconstruction of injected unit 19-15 whose physiological responses are shown in Figure 6. This neuron corresponds to a "stellate" cell. B. Camera lucida reconstruction of unit 36-19 whose phys-

iological record is illustrated in Figure 12. This neuron belongs to the "octopus" cell category. Same orientation as in Figure 2. The scale bar corresponds to 20 μ m. The locations of these cells are shown in Figure 13.

acterized by stable and sustained activity over the duration of the tone burst. Unit 30-29 does not meet this criterion: Following the notch, the sustained activity rapidly decreases to a very low level well before the end of the tone burst. Because this response is mostly restricted to the initial portion of the tone burst, we consider the unit to be more characteristic of the "on" class rather than "primary-like with notch." Furthermore, it is not unusual to see "on-type L" responses characterized by the presence of a notch following the initial peak. We acknowledge, therefore, that the categorization of this unit could be debated.

The recovered HRP stained neuron is represented in Figure 11A. The polarized distribution of the five primary dendrites and the clustered dendritic branch points support the classification of this cell as a "bushy" cell, although this particular neuron has a relatively small number of dendritic branch points for its class. The somatic and dendritic surfaces are smooth. The central projection of this cell (30-29) is shown in Figure 11B. The axon ranges between 2 and 6 μ m in diameter and could be traced well into the trapezoid body. The location of the injection site was determined by the presence of a localized distortion on the axon, found some 600 μ m from the cell body (arrow, Fig. 11b). The axon gives rise to one collateral in the vicinity of the ipsilateral superior olivary complex, which ramifies further. One major branch reverses direction and projects back toward the region of the axonal branch point, whereas the others course toward the caudal portion of the lateral nucleus of the trapezoid body and the lateral superior olivary nucleus. The collaterals in the lateral nucleus of the trapezoid body terminate in small boutons but the other

branches progressively fade before their termination, as does the parent axon close to the midline.

These two cells illustrate that "on" units in AVCN do not always correspond to "stellate" cells. The present data demonstrate that "on" units can be "bushy" cells. Therefore, "bushy" cells do not exclusively exhibit "primarylike" responses.

On units in PVCN

On the basis of population data, Godfrey et al. ('75a) associated the "on-type I" and "on-type L" units of PVCN with "octopus" cells. In the present study, one "on" unit located in PVCN was electrophysiologically characterized and labelled with HRP. The unit exhibited unstable spike activity after impalement. Nevertheless, before the unit became physiologically inactive, 2 PSTHs were computed (Fig. 12B) in response to pure tone at CF (4.6 kHz). The responses observed are typical of an "on-type I" unit (Godfrey et al., '75a). This unit was sensitive only to the onset of the tonal stimulus and, like most cells of this category, was not spontaneously active in the absence of sound.

A camera lucida reconstruction of the corresponding stained neuron (36-19) is shown in Figure 7B. The cell body was darkly labelled, but only a small portion of the processes are visible. One of the two dendrites appears to have exploded, as shown by a small amount of extracellular reaction product nearby; this presumably corresponds to the injection site (see arrow). The general configuration of the cell body, the thick primary dendrites gathered on one side of the soma, and the clustered dendritic branch points allow tentative categorization of this neuron as an "octo-

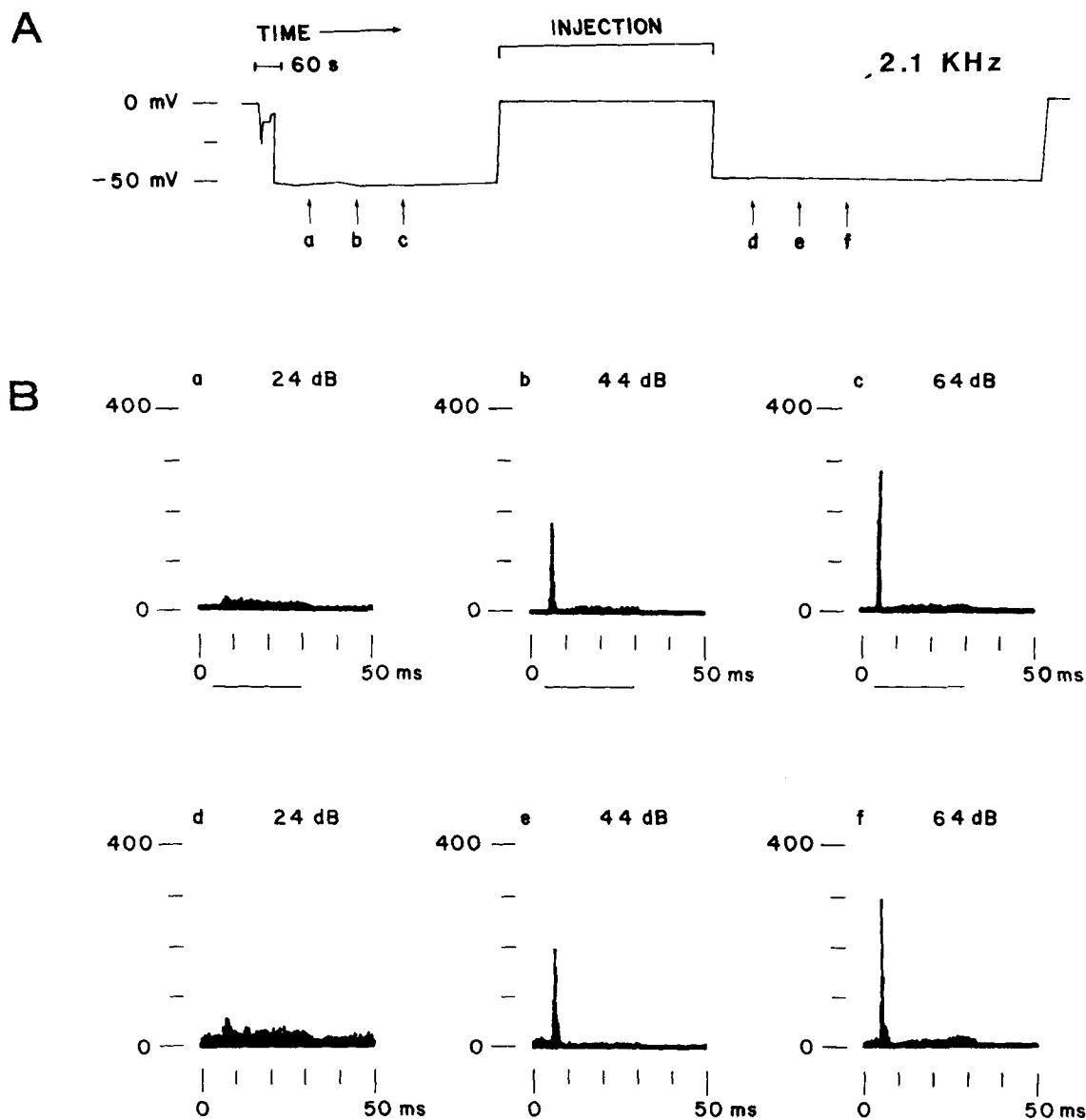


Fig. 8. Physiological characterization of unit 24-10 ("on"). A. DC resting potential record. HRP was injected by passing 3 namp for 450 seconds. B. PSTHs computed at CF (2.1 kHz) for different intensities. Beneath the DC

record, PSTHs were computed before (a,b,c) and after (d,e,f) the injection. Same conventions as in Figure 1. The morphology of this neuron is illustrated in Figure 9.

pus" cell; it is a small octopus cell, located in the octopus region (see Fig. 13).

The relationships between morphological features and response properties for our results are summarized in Table 2. The anatomical location of each individually labelled neuron is shown in Figure 13, where locations are represented on appropriate coronal sections of the block model atlas for the cochlear nucleus (Kiang et al., '75).

DISCUSSION

Functional significance of morphological distinctions

The cochlear nucleus is a conceptually convenient but technically difficult place to study input-output relation-

ships of second-order sensory neurons. Primary sensory information is provided by the central extension of auditory nerve fibers, characterized by a variety of endings and distributed throughout the different regions of the cochlear nucleus. It has generally been assumed that every ramification of individual auditory nerve fibers carries nearly the same information. If true, then the modification of the messages taking place in the cochlear nucleus is likely to be dependent on events related to the neurons and their synaptic organization. A corollary of this hypothesis is that morphological differences among cochlear nucleus neurons may be related to physiological response properties.

In the present study, Golgi-like images were obtained by intracellular marking of cochlear nucleus neurons with

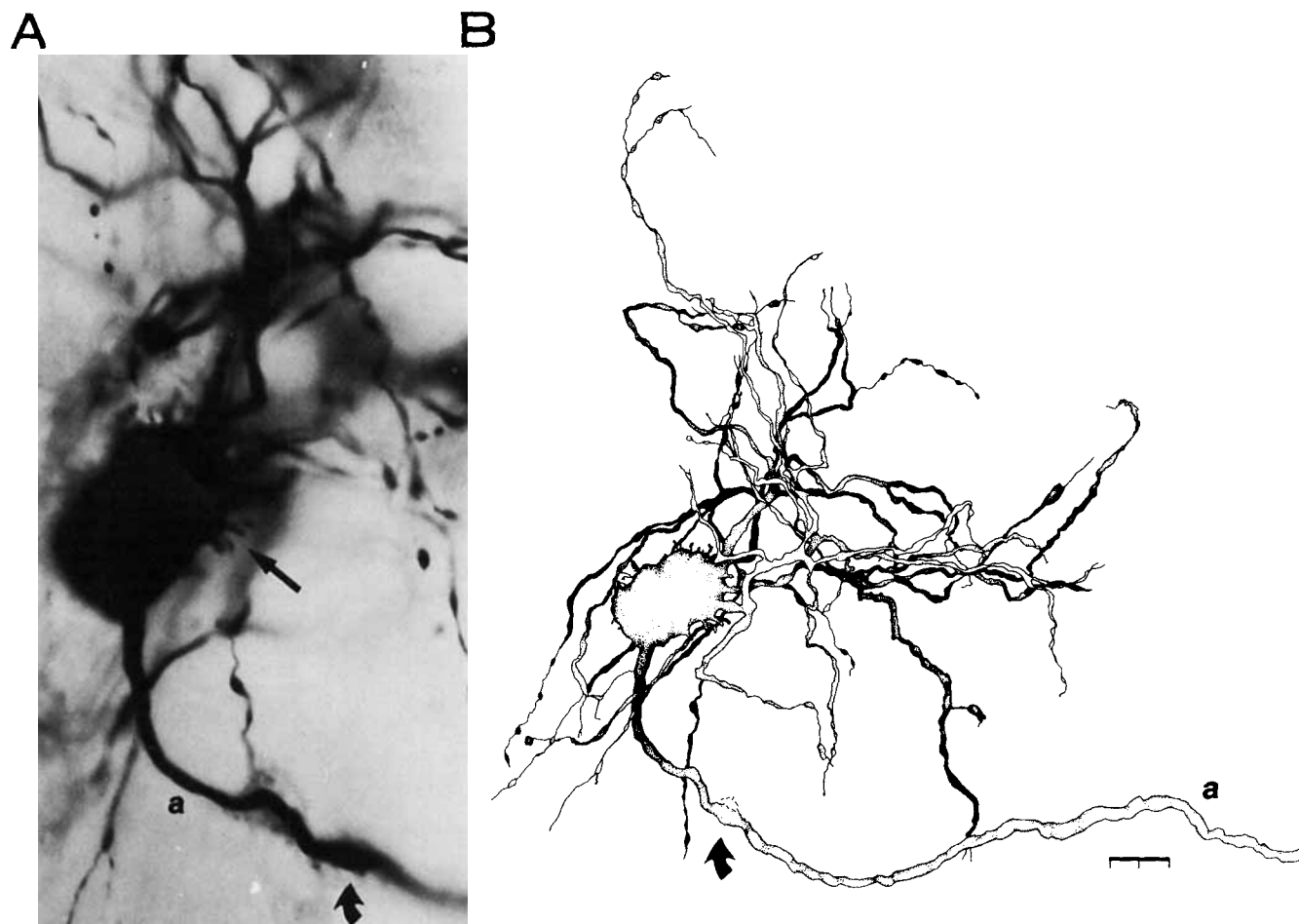


Fig. 9. A. Photomicrograph of injected unit 24-10. The bottom arrow shows the swelling along the axon interpreted as the injection site. The upper arrow shows the presence of somatic spines. B. Camera lucida reconstruction of injected unit 24-10. This neuron conforms to the "bushy" cell

category. Same orientation as in Figure 2. The scale bar corresponds to 20 μ m. The arrow indicates the location of the presumed injection site. Abbreviation: a, axon. This unit's physiological record is shown in Figure 8 and its location is shown in Figure 13.

TABLE 2. Summary of Physiological and Morphological Properties of Ventral Cochlear Nucleus Neurons

Unit	CF (kHz)	Unit type ¹	Spontaneous rate (sp/sec)	Threshold ²	Cell type	No. of primary processes ³	Soma surface	Soma size (μ m) min * maj axis
AVCN								
12-4	20.2	PRI	—	45	Bushy	3	Smooth	14 * 21
27-7	3.0	PRI	—	20	Bushy	2	Smooth	19 * 27
32-6	2.5	PRI w N	130	26	Bushy	3	Smooth	10 * 29
20-9	0.3	Chopper ⁴	21.5	35	Bushy	4	Smooth	22 * 28
24-10	2.1	On (L)	—	24	Bushy	4	Spines	18 * 28
30-29	1.8	On (L) ⁴	5	24	Bushy	6	Smooth	12 * 31
PVCN								
19-15	32.7	Chopper	—	20	Stellate	5	Smooth	9 * 42
36-19	4.6	On (I)	0	35	Octopus	2	Smooth	8 * 12

¹PRI = primarylike; PRI w N = primarylike with notch.

²The thresholds, indicated in dB SPL, were determined manually as rapidly as possible using audiovisual cues, since a unit's "holding time" was limited. Therefore, they are probably at least 5-10 dB higher than the actual thresholds, if carefully determined by PSTHs computed from 60 second sequences of data at different intensities.

³The number of primary processes includes the axon, because for some neurons characterized by incomplete HRP staining, the axon could not be safely distinguished from the dendrites.

⁴The categorization of these units presented a degree of uncertainty as mentioned in the text.

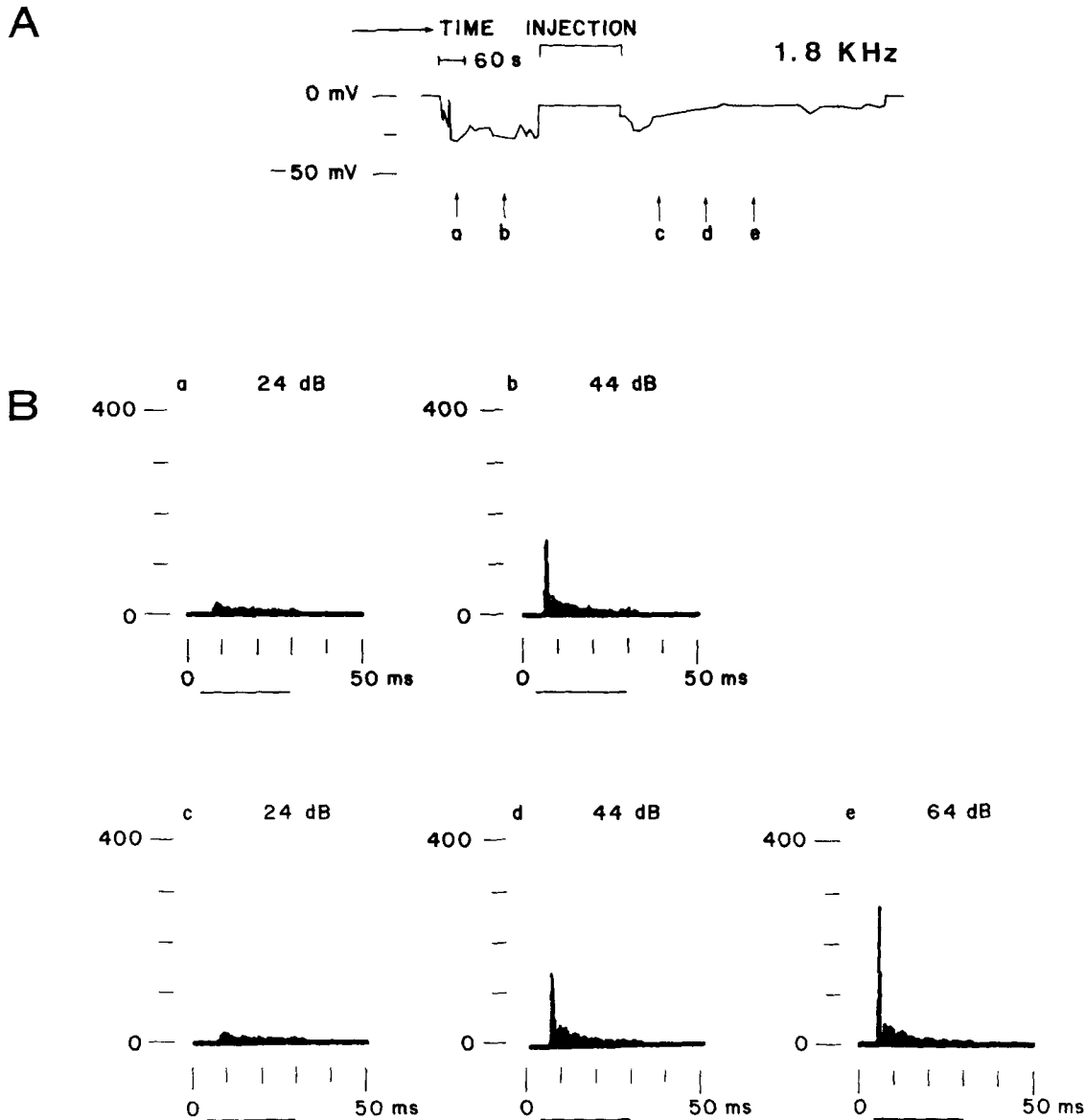


Fig. 10. Physiological characterization of unit 30-29 ("on-L"). A. DC resting potential record. HRP was injected by passing 4.5 namp for 170 seconds. B. PSTHs at CF (1.8 kHz) obtained before (a,b) and after (c,d,e) the

injection of HRP. Same conventions as in Figure 1. This neuron is shown in Figure 11.

HRP. Previous descriptions of these neurons are mostly derived from Golgi studies in immature animals. Therefore, caution must be exercised in trying to relate HRP-stained neurons from adult cats to Golgi-stained neurons from kittens (Ryugo and Fekete, '82). For example, there is a wide variety in the form of neurons fitting the general descriptive category of "bushy" cell (Table 2), a conclusion shared by others studying these cells (Rhode et al., '83b; Tolbert et al., '82). Dendritic arbors can range from sparse tufts with little branching (Fig. 11A) to dense, highly ramified configurations (Fig. 9B). There is also considerable variation among "bushy" cells in the number of primary dendrites (one to five), contrasting sharply with the zero to one pri-

mary dendrites reported for kittens (Brawer et al., '74). The Golgi criteria used to distinguish different cell types of immature animals are therefore not strictly applicable to HRP-stained neurons in adult cats. Nevertheless, we propose that the cases presented here are not marginal and their morphological categorization as "bushy" or "stellate" is unequivocal despite the variability within each category.

Structure-function relationships in the ventral cochlear nucleus

Our data are consistent with some of the previous structure-function relationships reported in the literature. In PVCN the correspondence of a "stellate" cell to a "chopper"

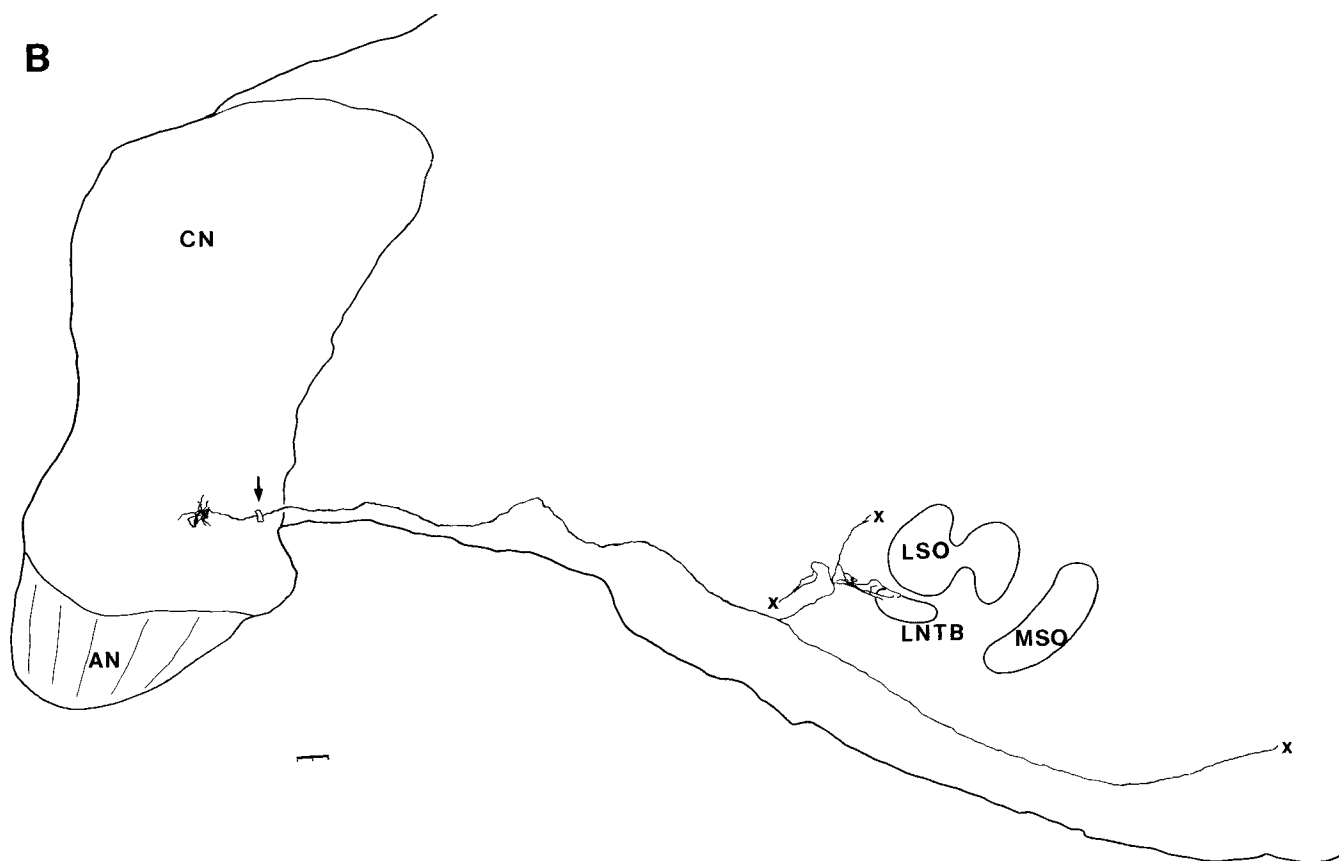
A**B**

Fig. 11. A. Camera lucida reconstruction of injected unit 30-29 whose physiological record is shown in Figure 10. This neuron corresponds to a "bushy" cell. Same orientation as in Figure 2. The scale bar corresponds to 20 μ m. Abbreviation: a, axon. The location of this cell is shown in Figure 13. B. Camera lucida reconstruction of the central projection of neuron 30-

29 shown on a coronal section of the ipsilateral half of the brainstem. The presumed recording and injection site is indicated by the arrow. The scale bar is 200 μ m. "X" indicates fading of the reaction product within the labelled processes.

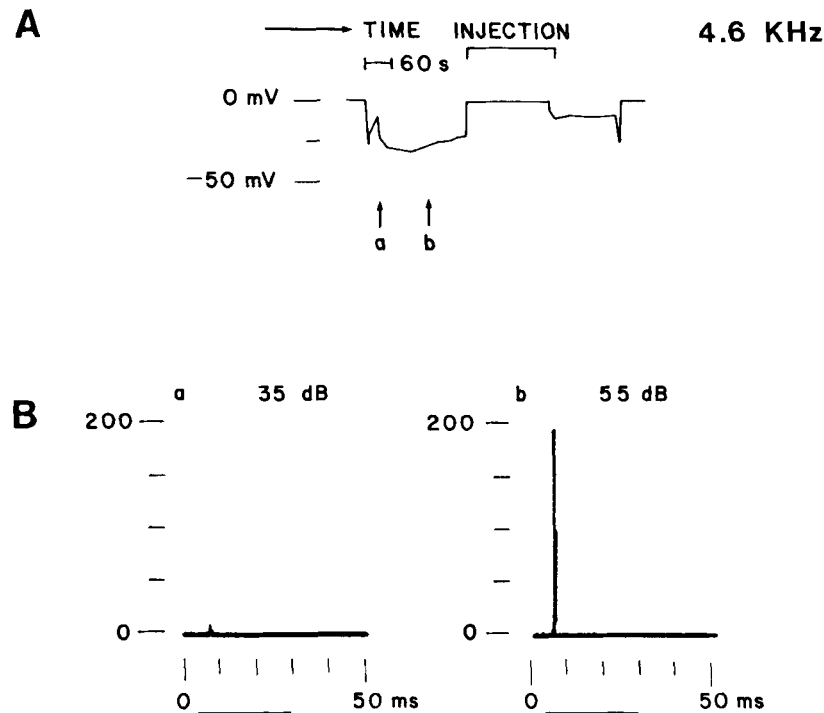


Fig. 12. Physiological characterization of unit 36-19 ("on-I"). A. DC resting potential record. HRP was injected by passing 3 namp for 160 seconds through the micropipette. B. Response patterns to tone bursts at CF (4.6 kHz)

presented at two different intensities. Same conventions as in Figure 1. The anatomical reconstruction of this neuron is shown in Figure 7B.

unit is supported by the unit 19-15. The indirect evidence of Godfrey et al. ('75a) that "octopus" cells of PVCN exhibit "on" responses at CF 10 or 20 dB above threshold is consistent with unit 36-19 of the present study. In AVCN, our marking data support the previous association of "primarylike" and "primarylike with notch" units with "bushy" neurons (Bourk, '76). Recently, Rhode et al. ('83b) reported similar results from neurons intracellularly stained with HRP. Both their results and our own indicate that a particular physiological unit type can be associated with more than one morphological cell type. "Stellate" cells in PVCN may correspond to "chopper" units as well as "on" units. The present study demonstrates that this is also true in AVCN for the general category of "bushy" cells, which could be physiologically classified as "primarylike," "primarylike with notch," "on," and "chopper" (Table 2). It can be concluded that the general morphological distinction between "bushy" and "stellate" cells in the auditory nerve root region of the ventral cochlear nucleus does not account for the distinction of "primarylike" units from "on" and "chopper" units.

Although our data base is admittedly limited, it seems that unit type categories are insufficient for relating functional properties to certain morphological features of "bushy" cells, such as the presence or absence of somatic spines or the number of primary dendrites. An "on" unit (24-10) and a "chopper" (20-9) unit can have the same number of primary dendrites. One "on" cell (24-10) is characterized by the presence of somatic spines whereas another "on" cell (30-29) has a smooth cell body. The representation of HRP-labelled neurons in the present study is only a

projection of the cell onto a plane, suggesting that some particular spatial features of the neurons might be missed. It is possible that a three-dimensional reconstruction and rotation of the stained neurons might provide useful information in these structure-function studies. In the ventral cochlear nucleus, such difficulties were not unexpected because of the conspicuous variability of dendritic morphology within the category of "bushy" neurons in the adult cat. There could also be a CF effect on dendritic morphology. An interesting correlation has been reported in the nucleus laminaris of the chicken, where a gradient of dendritic size followed the tonotopic gradient of CF (Smith and Rubel, '79).

The possibility of relationships between unit types and neuronal classes based on Nissl criteria remains viable. The existence of two types of "multipolar" cells (Cant, '81) presumably represents separate subsets of the "stellate" cell class; as such, they become strong candidates to account for the "on" and "chopper" unit types associated with the "stellate" cell type. The "bushy" neurons as defined by Golgi criteria are reported to include both "spherical" and "globular" cell classes as defined by Nissl preparations (Brawer et al., '74). The present Golgi categories are not sufficient to predict unit types since we found that "bushy" cells were associated with examples of four different physiological types. Given the wide variability among neurons within the same morphological and/or physiological class, it would seem that a reexamination of the classification schemes is in order. It could be hypothesized that differences in the synaptic inputs are most important in determining unit discharge patterns, implying that postsynaptic

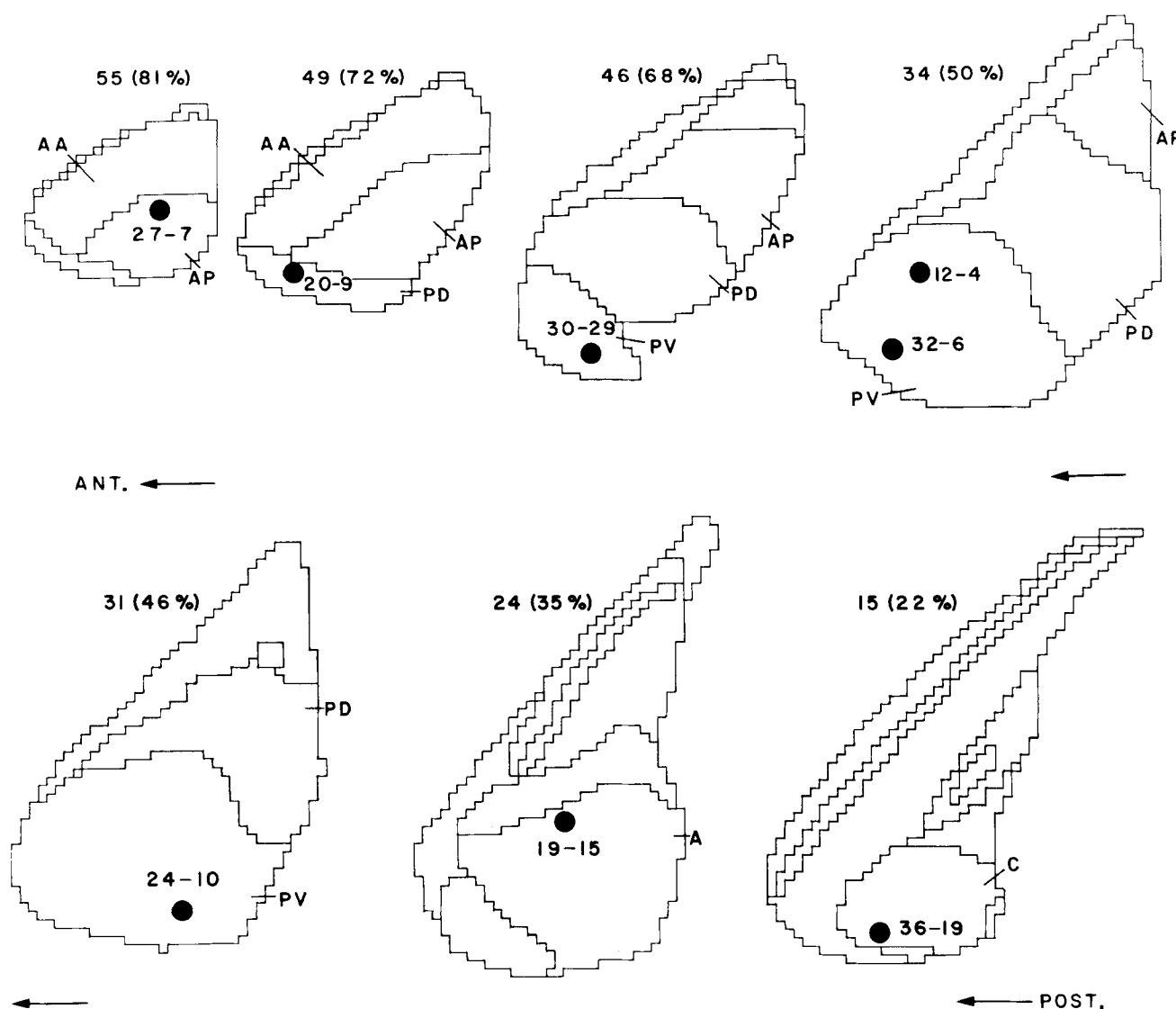


Fig. 13. Coronal atlas through the cochlear nucleus block model (Kiang et al., '75), indicating the boundaries of anatomical subdivisions and the location of the HRP-labelled neurons. The number above each section indicates its serial order in the block model. The most posterior section of the cochlear nucleus is 1 whereas the most anterior section is 68. The corre-

sponding percentage intervals through the cochlear nucleus are enclosed by parentheses, where 0% is anterior and 100% is posterior. The location of each neuron is indicated by ● and identified by its unit number. The principal anatomical subdivisions are indicated by abbreviations as defined in the text.

cell morphology alone would not necessarily be related to unit type. It is also possible that membrane properties of the cells not detectable in the gross description of the neuronal shape might also be a determining factor for response characteristics. In this context, it should also be noted that the dichotomy between stellate and pyramidal cells in visual cortex fails to account for the simple and complex receptive field classifications (Gilbert and Wiesel, '79).

The cell 30-29 (see Figs. 8, 9) reflects the complexity of structure-function relationships. The general shape of the PSTHs is consistent with its categorization as an "on" unit. However, the initial peak is followed by a notch when the stimulus intensity is increased to 44 and 64 dB SPL. In this respect, the unit shares some physiological properties with

the "primarylike with notch" unit (Fig. 3). The presence of a notch in the PSTH is interpreted as reflecting the refractory period of the first spike precisely time-locked to the onset of each tone burst. This case illustrates a common difficulty we encountered in distinguishing "primarylike with notch" units from "on-type L" units. The distinction between "primarylike" and "primarylike with notch" was generally unequivocal; in contrast, we found a continuous gradation in unit types ranging from the prototypical "on-type L" to the prototypical "primarylike with notch" unit. The refinement of criteria necessary to distinguish these two (or more) types should now be considered. Furthermore, these two cells (30-29, 32-6) are morphologically comparable: The cell bodies are elongated and the dendritic trees,

spatially restricted to one hemisphere of the cell body, show only a few branch points.

Intracellular synaptic potentials

The present study was essentially focused on the issue of physiological and morphological correspondences. Therefore, the cellular mechanisms that underlie the generation of the different response types were not specifically addressed during these experiments. For optimal physiological characterization of the units, we sought intracellular recordings from axons which provided more stable spike activity. It is for this reason that we assume that the majority of our recordings were characterized by the absence of postsynaptic potentials. Nevertheless, when the electrode was presumably in the cell body, we noticed the presence of a sustained depolarization during the tone bursts for the "chopper" units and we never saw a sustained depolarization for "primarylike" units, in agreement with observations by Romand ('78).

Technical considerations in intracellular recording and cell marking

Our data can be divided into two separate categories on the basis of the stability of the intracellular spike activity and the appearance of HRP-stained neurons. For one category of units (24-10, 20-9, 30-29, 32-6), very stable spike activity could be intracellularly recorded for up to 30 minutes. The corresponding labelled neurons showed apparently complete filling of their processes with HRP. The axon could be traced for a distance exceeding 5 mm. A typical swelling or interruption of the axon was found, and was interpreted as the recording and injection site. A second category of units (12-4, 19-15, 27-7, 36-19) was characterized by spike activity whose action potential amplitude progressively decreased over time. This diminution of spike amplitude began immediately after impalement with the micropipette, so that only 2-3 PSTHs at CF could be computed before the spike activity totally disappeared. Because the resting potential remained stable, these units were injected with HRP. The result, however, was only a partial staining of the corresponding neurons. We hypothesize that the electrode was in the cell body or dendrite, although the presence of an injection site could only be demonstrated in one case (36-19). A swollen and partially exploded dendrite, surrounded by a small amount of extracellular HRP reaction product, was evidence of the injection site. This interpretation is consistent with the idea that the intracellular presence of the electrode near or in the cell body might compromise the spike generator, thus resulting in a progressive decrease in spike size. If the cell is physiologically inactivated by the electrode, it might not fill completely with HRP, since the transport of HRP has been reported to be enhanced by neural activity (Mesulam, '82). If the cell is killed by the electrode, a similar explanation for partial filling would be suggested.

The successful physiological characterization from intracellular recordings and optimal staining for morphological identification of the injected neurons occurred most often when the axon was impaled. This situation permitted a physiological examination of the unit before and after the injection of HRP. The close similarity of response properties collected before and after the injection provides, with the monitoring of a continuous negative resting potential, the most convincing evidence that the injected neuron corresponds to the functionally defined unit. After histological

processing, additional evidence is provided by the strict correspondence between the calculated position of the electrode tip at the time of injection and the location of the cell body or axonal injection site. Independent confirmation was provided by matching the CF of each unit with the tonotopic map of the ventral cochlear nucleus (Rose et al., '59; Bourk et al., '81). These precautions ensure that structure-function analyses can be conducted with confidence at the cellular level. Intracellular recordings from axons do not allow the study of postsynaptic events. They have the advantage, however, of minimizing the effect of the intracellular electrode on the spike generator. One must always be concerned that penetration by a microelectrode sufficiently affects the cell so that its PSTH changes. This is precisely the case in the dorsal cochlear nucleus, where the intracellular electrode penetration changes "pauser" units into "chopper" units (Rhode et al., '83a). In the ventral cochlear nucleus, fortunately, there is no evidence to suggest that intracellular recordings alone alter PSTH categories, irrespective of whether the recordings are somatic or axonal (Rhode et al., '83b; present results).

The small amount of data presented here reflect the technical difficulty of these kinds of studies in the cochlear nucleus. A similar conclusion can be drawn from the recent reports of Rhode et al. ('83a,b). The movements of the brainstem, presumably related to the cardiac pulsations and respiratory movements, in most cases limit the intracellular recording to just a few seconds. Even when a stable negative resting potential can be obtained, there is usually a progressive loss of spike activity within a minute or less, so that the unit cannot be physiologically characterized. Finally, in many of these experiments there was an unexplainable failure to stain and recover the neurons after ostensibly successful recordings and injections.

Directions for future work

The present study demonstrates that "bushy" and "stellate" neurons do not easily correlate with the different unit types in the nerve root region of the ventral cochlear nucleus. Certainly, the correspondence between structure and function needs to be established for more neurons before it can be decided which combination of anatomical features might provide most reliable indicators of unit type, and vice versa. Future directions for this kind of work require an electron microscopic analysis of the stained neurons in order to study their synaptic organization. Such studies might also yield important information about the Nissl pattern of the injected cell.

We also demonstrated for four units (20-9, 24-10, 30-29, 32-6) that this approach of intraaxonal recording and marking techniques reveals useful information about the central axonal projections of individually characterized cochlear nucleus neurons. This technique, even without the physiology, could provide important data as a straight anatomical study of specific cell type projections. None of these four labelled axons branched within the cochlear nucleus to give rise to local-circuit or "recurrent" collaterals. Each axon (20-9, 24-10, 30-29) gave rise to at least one collateral which ramified within the ipsilateral superior olivary complex, but beyond that, the parent axon gradually faded as it approached the midline. For survival times ranging between 15 to 27 hours, the axon could be traced centrally over a distance of approximately 6 mm. This observation is consistent with previous data obtained from intracellular

HRP injections of cat Purkinje neurons (Bishop and King, '82). More data are needed to determine whether this distance represents the limit of the method or whether a longer survival time would allow the anterograde transport of HRP for a longer distance. A possible approach would be to record from axons of ventral cochlear nucleus neurons, not in the cochlear nucleus itself, but in the trapezoid body where axons are more densely packed. In this way, the number of intraaxonal penetrations might be increased, although the problem of brainstem pulsation remains to be solved. Such an approach might also allow the tracing of axons all the way to their recipient target region, especially in the contralateral side of the brainstem.

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