Enhancement of Neural Synchronization in the Anteroventral Cochlear Nucleus. I. Responses to Tones at the Characteristic Frequency

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SUMMARY AND CONCLUSIONS

1. Encoding temporal features of the acoustic waveform is an important attribute of the auditory system. Auditory nerve (AN) fibers synchronize or phase-lock to low-frequency tones and transmit this temporal information to cells in the anteroventral cochlear nucleus (AVCN). Phase locking in the AVCN is usually reported to be similar to or weaker than in the AN. We studied phase-locking in axons of the trapezoid body (TB), which is the output tract of the AVCN, and found, to our surprise, that most TB axons exhibited enhanced synchronization compared with AN fibers.

2. Responses from axons in the TB of the cat were obtained with horseradish peroxidase (HRP)- or Neurobiotin-filled micropipettes or metal microelectrodes. A series of short tone bursts at increasing sound pressure level (SPL) was presented at the characteristic frequency (CF) of the fiber and phase-locking was quantified with the vector strength R at each SPL. For each fiber the maximum R value (R_max) was then determined.

3. Low-frequency fibers in the TB showed very precise phase-locking; R_max values could approach 0.99. For the majority of fibers (33/44, 75%) with CF <700 Hz, R_max was ≥0.9 and therefore higher than is ever observed in the AN. We define such fibers as "high-sync." Most of these fibers also entrained to the stimulus, i.e., they fired a precisely timed action potential to almost every stimulus cycle. Some fibers showed perfect entrainment, with maximum discharge rates equaling the stimulus frequency.

4. To exclude the possibility that stimulus paradigms or acoustic and recording equipment were the source of this enhancement, we obtained additional data on low-frequency AN fibers using the same experimental protocol as in our TB experiments. These AN data agree well with published reports.

5. The morphological class of some of the cells studied was identified on the basis of anatomic features revealed by intraaxonal injection of HRP or Neurobiotin. Labeled low-CF axons (N = 7), which were all high-sync, originated from AVCN bushy cells: five were globular and two were spherical bushy cell axons.

6. Spontaneous rate of high-sync fibers covered a range from 0 to 176 spikes/s but were biased toward low values (mean 16 spikes/s). Responses to broadband clicks and sinusoidally amplitude-modulated signals provided additional evidence of improved timing properties.

7. Entrainment and improvement in synchronization at CF can be generated with a model that incorporates 1) convergence of inputs from two or more AN fibers onto an AVCN cell and 2) a postsynaptic cell that requires coincident input spikes before it generates an output spike. The model produces R and average rate values with a realistic dependence on SPL and output discharge patterns that display temporal adaptation.

8. AVCN bushy cells relay signals to binaural comparison circuits in the brain stem. The sharpening of temporal information observed here may be important for the extraction of interaural time differences, which is an important cue for sound localization.

INTRODUCTION

The earliest physiological studies of the auditory system showed its ability to encode temporal features of sound (Galambos and Davis 1943; Kiang et al. 1965; Rose et al. 1967; Tasaki 1954). Temporal information is known to be important in sound localization (Mills 1958; Rayleigh 1876) and may play a role in other perceptual attributes as well (Schouten et al. 1962; Wever 1949). Encoding of temporal information has usually been demonstrated as phase-locking to low-frequency tones. For sinusoidal stimuli up to ~4 kHz, mammalian auditory nerve (AN) fibers preferentially discharge over a restricted range of phase angles and at intervals that are integral multiples of the stimulus period (Johnson 1980; Kiang et al. 1965; Rose et al. 1967).

AN fibers provide the dominant input to cells in the anteroventral cochlear nucleus (AVCN), whose axons course centrally by way of the trapezoid body (TB). With few exceptions (see DISCUSSION), previous recordings from AVCN have indicated that phase-locking in second-order cells is similar to or poorer than in AN fibers (Blackburn and Sachs 1989; Bourk 1976; Goldberg and Brownell 1973; Kettner et al. 1985; Lavine 1971; Palmer et al. 1986; Rhode and Smith 1986; van Gisbergen et al. 1975; Winter and Palmer 1990). For two reasons, data are particularly sparse for cells with low characteristic frequency (CF: frequency at which an increase in firing rate can be elicited at the lowest stimulus level). First, low-frequency stimuli evoke large field potentials that make single-unit recording with low-impedance electrodes difficult and can induce artificial phase-locking (Johnson 1978). Second, there is a problem of cell classification at low CFs. The morphological classes of cells with high CF are well predicted by the shape of the poststimulus time (PST) histogram obtained in response to short tone bursts (Rhode et al. 1983; Smith and Rhode 1987; Smith et al. 1991). At low CFs, however, cells with different morphology fall into the same PST histogram category of “phase-lockers” and other criteria must be used to identify the cell.

In the course of a study of the anatomy of TB fibers with the intra-axonal labeling technique (Smith et al. 1991, 1993) we found that phase-locking was often considerably better than in the AN. The intra-axonal technique circum-
vents the two problems mentioned above. Large phase-
locked field potentials are absent, presumably because of
the distance of the recording site from the AVCN, where
there is a dense packing of cell bodies with the same CF.
Moreover, for fibers injected with the horseradish peroxi-
dase (HRP) or Neurobiotin tracer the morphological cell
class can be identified directly if the cell body is labeled or
can be inferred on the basis of the axonal projection pattern
into the superior olivary complex (SOC) (Smith et al. 1991,
1993). We have found that the TB fibers show both more
precise phase-locking and higher entrainment (response to
every cycle of the stimulus) than AN fibers. Such enhance-
ment occurs in fibers most sensitive to low frequencies, dis-
cussed in this report, as well as in high-CF fibers when stimu-
lated with low-frequency stimuli, discussed in a companion
paper (Joris et al. 1994).

METHODS
Animal preparation
Our methods for intra-axonal recording and labeling of TB
axons have been described previously (Smith et al. 1991). Briefly,
after induction of anesthesia with an intramuscular injection of a
mixture of acepromazine (0.2 mg/kg) and ketamine (20 mg/kg),
the cat was placed in a soundproof room. A venous cannula al-
lowed infusion of Ringer solution and pentobarbital sodium at
doses sufficient to maintain an areflexic state. Subcutaneous injec-
tion of atropine methyl nitrate (0.08 mg/kg) minimized mucus
secretions. The trachea was cannulated and the basioccipital bone
was exposed via a ventral approach. Both bullae were vented with
30-cm-long polyethylene tubes (0.9 mm ID). The TB was exposed
by drilling a small hole in the basioccipital bone that allowed the
electrode to enter the lateral edge of the pyramidal tract just caudal
to the pontomedullary junction. Both pinnae were removed and
tight-fitting earbars were fitted into the external auditory meati.

Experimental protocol
The acoustic systems in right and left ears were calibrated with
probe tubes close to the eardrum, allowing specification of subse-
quent tona! stimuli in SPL (re 20 μPa). Micropipettes (60–100
MΩ) filled with 5% HRP or 2% Neurobiotin in 0.5 M KCl, were
advanced into the TB. Spike times with respect to stimulus onset
of well-isolated single units were determined with a combination of
a trigger level and peak detecting circuit custom-developed at
the Medical Electronics Laboratory at the University of Wisconsin
and stored digitally to a resolution of 1 μs. A standard pulse was
sent to a unit event timer if the amplified neural signal exceeded a
threshold set by the experimenter, but the pulse was only gener-
ated at the subsequent zero crossing of the signal’s first derivative.
Threshold and CF of single fibers were determined with an au-
tomated tuning curve program. Short CF tone bursts (25 ms every
100 ms, 200 repetitions, rise-fall time = 3.9 ms, starting in sinc
phase) were then presented at increasing SPL, providing on-line
estimates of the PST histogram and vector strength (see below).
Because of the limited time available for data collection during
intra-axonal recordings, only four or five levels were presented,
usually in 20-dB steps. In some fibers phase-locking was also stud-
ied at frequencies below CF in the so-called tuning curve “tail”
(Kiang and Moxon 1974). The latter responses are the subject of
the companion paper.

Successfully impaled fibers were labeled by iontophoretic injec-
tion of HRP or Neurobiotin; anatomic data have been reported
separately (Smith et al. 1991, 1993) and are mentioned in this
report only to corroborate the physiological results. All fibers en-
countered were studied; all data presented here were from fibers responsive to one ear only.

In a limited number of separate (hereafter called “extra-ax-
onal”) experiments intra-axonal labeling was not attempted, which allowed us to study selected fibers over a longer time period.
In these experiments TB recordings were obtained with glass mi-
cropipettes filled with 3 M KCl or with parylene- or glass-coated
tungsten microelectrodes.

Response classification and analysis
TB axons were classified according to the shape of the PST
histogram to short tone bursts at CF. This widely accepted physi-
ological classification scheme for cochlear nucleus (CN) responses
(Bourk 1976; Pfieffer 1966) shows a good correlation (Rhode et
al. 1983; Smith and Rhode 1987; Smith et al. 1991, 1993) with
morphological classifications (Brawer et al. 1974; Olsen 1969).
Spherical bushy cells (SBCs) are associated with the so-called pri-
mary-like (PL) response, which is similar to that of AN fibers;
globular bushy cells (GBCs) have a primary-like-with-notch (PLN)
response, which has a well-timed onset spike followed by a 1-to
2-ms pause and a resumption of sustained activity; and stellate
cells have a chopper PST histogram, which shows several modes
unrelated to the stimulus frequency (Blackburn and Sachs 1989;
Bourk 1976; Pfieffer 1966; Rhode et al. 1983; Rouiller and Ryugo
1984; Smith and Rhode 1987; Smith et al. 1991, 1993; Sproul et
al. 1990; Winter and Palmer 1990). The axonal projections of these three cell classes also differ: only bushy cells have strong pro-
jections to the main nuclei of the SOC. SBC’s project bilaterally
to the medial superior olive (MOs), and GBCs project to the
centralateral medial nucleus of the TB (MNtb) (Friauf and Ost-
wald 1988; Smith et al. 1991, 1993; Sproul et al. 1990; Tolbert et
al. 1982; Warr 1966).

Period histograms with 64 bins were computed by superimpos-
ing the response to tones at CF to individual cycles of the stimulus.
From these the vector strength or synchronization coefficient R
(Goldberg and Brown 1969) was calculated: a uniform distribu-
tion of spikes throughout the stimulus cycle gives an R of 0,
whereas a value of 1 indicates perfect alignment of all spikes in one
bin. The onset response, which was not always in phase with the
sustained response (Bourk 1976), was eliminated by restricting the
analysis to a response window 10–25 ms after stimulus onset.
The lower window limit (10 ms) was chosen to accommodate the
long latencies of the TB fibers with the lowest CFs (see, e.g., Fig. 1
below). Significance of phase-locking (P < 0.001) was evaluated
with the Rayleigh test (Mardia 1972). For each fiber the maxi-
imum R value (Rmax) was determined. To facilitate comparison
with data published for the AN (Johnson 1980) and AVCN
(Bourk 1976), we present some results on an expansive R axis by
plotting [1 – Rmax] on an inverted logarithmic scale (Johnson
1974). Note that, as defined by R, higher phase-locking corre-
sponds to a tighter distribution of the period histogram and not
necessarily to a more “faithful” replica of the sinusoidal stimulus
waveform (as a reference: R computed for a half-wave rectified
sinusoidal waveform = 0.785).

To measure the ability of the fiber to respond with one spike for
every stimulus cycle we calculated an “entrainment index” that
was derived from the interspike interval (ISI) distribution. We
define the entrainment index as the ratio of the number of ISIs falling in a window equaling one stimulus period and centered on
the ISI time axis at 1/CF, divided by the total number of intervals
occurring during the response window. Perfect entrainment (en-
trainment index of 1) is achieved when a single spike occurs with
each stimulus cycle and the ISI distribution consists of a single
FIG. 1. A: comparison of rate-level (•: left ordinate) and synchronization-level (○: right ordinate) functions for an auditory nerve (AN) (top) and trapezoid body (TB) (bottom) fiber. Maximum vector strength or synchronization coefficient ($R_{max}$) values were 0.81 (AN) and 0.97 (TB) and occurred at 50 and 79 dB SPL, respectively. The fibers were chosen for matched characteristic frequencies (CFs) (350 and 340 Hz) and high spontaneous rates (SRs) (69 and 25 spikes/s, • on left ordinate). Filled circles indicate insignificant phase-locking (see METHODS). B: examples of dot rasters for same responses, obtained at indicated SPLs. Dots indicate time of occurrence of action potentials. Each row represents the response to 1 stimulus presentation in order from bottom to top. Stimuli were 25 ms in duration (starting at 0 ms), delivered every 100 ms, with 200 repetitions at each SPL. Rate and synchronization were calculated over a 10- to 25-ms response window.

mode centered at 1/CF. Because of the short stimulus duration (25 ms) and for consistency with other response measures, we used a fixed response window for calculation of the entrainment index: only intervals defined by two spikes occurring within the 10- to 25-ms response window were considered. Use of longer stimuli with inclusion of longer intervals would presumably result in a decrease particularly of the lowest entrainment index values.

Note that phase-locking and entrainment refer to different aspects of the timing of the response. Conceivably a fiber can discharge perfectly in phase ($R = 1$) but always skip one or more stimulus cycles, resulting in an entrainment index of 0.

A limited number of TB fibers were stimulated with broadband rarefaction clicks and sinusoidal amplitude-modulated (AM) tones. The AM signals were 600 ms in duration, were repeated 20 or 40 times every second, and had a modulation depth of 100% and a carrier frequency ($f_c$) equaling CF. Responses were analyzed over a response window of 10–600 ms by calculating $R$ to the modulation frequency.

RESULTS

Synchronization to CF tones

Phase-locking of low-CF neurons in AVCN and AN is similar in several respects (Bourk 1976; Johnson 1980). The responses we obtained in the TB share some basic similarities but also show some clear differences with AN fibers. Figure 1 illustrates rate- and synchronization-level functions for representative low-CF AN and TB fibers. For both fibers average firing rate (Fig. 1A, •) increased monotonically over a 20- to 30-dB range of stimulus level and then saturated. The maximum rate reached by the TB fiber (bottom panel) was higher than that of the AN fiber (top panel) and nearly equaled the stimulus frequency (340 Hz). Synchronization (Fig. 1A, circles) of the response to the stimulus frequency also increased steadily as level was raised above threshold in both fibers. After reaching $R_{max}$, synchronization in AN fibers often shows a small decline (Johnson 1980). In spontaneously active AN and TB fibers $R$ increased at levels below the average rate threshold: the difference in rate and synchronization threshold for the two fibers of Fig. 1 is ~10 dB. A striking difference between AN and TB responses is in the magnitude of $R_{max}$, which was higher for the TB fiber (Fig. 1A). Dot raster histograms of both responses at similar SPLs (Fig. 1B) illustrate that spikes occurred over only a restricted portion of each stimulus cycle in both fibers, but that this region was sometimes much narrower for the TB fiber.

AN

The present TR results derive their significance mainly when contrasted with the auditory nerve (AN). For this purpose we will use 1) published AN data of Johnson (1980); 2) phase-locking data from a previous AN study (Joris and Yin 1992); and 3) data from one additional AN experiment. The general surgical and experimental protocol for AN recordings was similar to the TB recordings. The AN was exposed via a dorsal approach and recordings were made with glass micropipettes filled with 3 M KCl, as described previously (Joris and Yin 1992).
Figure 2 shows a comparison of PST histograms from AN (A) and labeled TB fibers (B and C) with increasing CFs from left to right. The labeled fibers illustrated were chosen at approximately equal CFs (indicated in Hz) and were identified as SBC or GBC axons on the basis of their projection pattern to the SOC (see below). PST histograms depict the response at the SPL at which $R_{\text{max}}$ occurred, except for the three rightmost fibers, which had CFs >5 kHz and insignificant $R$ values at all SPLs. The axons of SBCs and GBCs with CF <700 Hz show more precise phase-locking than AN fibers, reflected in the high probability of firing over a small fraction of each stimulus cycle. A PST histogram of 10 fibers could not easily be classified as phase-locked. Above ~1.2 kHz and at high SPLs, the PST histograms of GBCs begin to show a well-timed onset followed by a notch in the response histogram. The combination of a well-timed onset followed by a notch produces the distinctive PL$_N$ response pattern that allows SBCs and GBCs to be distinguished physiologically. PL$_N$ pattern is not evident in the PST histograms of the high-CF GBC fibers shown in Fig. 2C because in these particular fibers $R_{\text{max}}$ occurred at a level below the SPL required to obtain a clear notch.

Period histograms provide a better visualization of phase-locking, especially at higher frequencies, and are shown for the same fibers in Fig. 3. For ease of comparison all period histograms were shifted along the abscissa to have their mean phase centered at 0.5 cycles and were normalized on the ordinate for the number of spikes. The number above each histogram gives $R_{\text{max}}$. Low-CF TB fibers show narrower period histogram distributions than AN fibers, but fibers with CFs between 2 and 5 kHz show the reverse (4th column).

The dependence of $R$ on SPL is stereotyped within the AN and AVCN population and is reasonably similar in shape between these two populations (Fig. 1, but see below). $R_{\text{max}}$ therefore provides a convenient parameter for comparison of phase locking in the two populations at different CFs (Bourk 1976). Figure 4 shows the overall distribution of $R_{\text{max}}$ for all TB fibers with significant phase-locking encountered in the intra-axonal experiments. The range of values observed differs in two important respects from the range reported for AN fibers, which is indicated in Fig. 4 by the area between the two solid lines (Johnson 1980). First, most low-CF TB fibers had $R_{\text{max}}$ values exceeding the highest values of AN fibers. Of 44 TB fibers with CF <700 Hz, 33 had an $R_{\text{max}}$ ≥0.9, which is higher than is ever seen in the AN: we call such responses “high-sync.” By this criterion, high-sync fibers all had CFs <1 kHz. Note that our definition of high synchronization is a conservative one: the $R_{\text{max}}$ of some TB fibers doesn’t reach the 0.9 criterion but is still above the range of the AN distribution (Fig. 4). Second, at higher CFs the $R_{\text{max}}$ values of TB fibers are generally lower than corresponding values for AN fibers, especially in the range of 2–5 kHz. Neither in the frequency extent over which phase-locking occurs nor in the highest and lowest $R_{\text{max}}$ values does there appear to be a clear difference in the distribution of fibers with PL and PL$_N$ responses, although the highest values between ~1 and 2 kHz tend to come from PL$_N$ responses.

The PST histogram of 10 fibers could not easily be classified as either phase-locked, PL, or PL$_N$. These fibers were assigned to an inhomogenous primary-like (NON-PL) group. Of these fibers six appeared to be low-CF choppers with PST histograms containing multiple modes at non-CF intervals intermixed with phase-locked peaks, especially near stimulus onset. Nondescript sustained PST histograms lacking well-timed features were observed in three fibers and a nonmonotonic rate-level response in the remaining fiber.

Because our primary results hinge on the disparity between TB and AN synchronization it is important to rule out the possibility that differences between the study of Johnson (1980) and our present work account for any of these discrepancies. There are two notable differences in protocol: Johnson (1980) used long (15 or 30 s) tones and did not restrict stimuli to CF. We therefore obtained phase-locking data on low-frequency AN fibers using the same stimulus, recording, and analysis procedures as used in the TB experiments. Our AN $R_{\text{max}}$ values, obtained for re-
FIG. 3. Period histograms and $R_{max}$ values for same responses as Fig. 2. Histograms were constructed over 64 bins, normalized to the number of spikes, and shifted to have a mean phase of 0.5 cycles. $R$, vector strength or synchronization coefficient.

responses to 25-ms tone bursts at CF, show more scatter but are coextensive with the values obtained by Johnson (1980) to 15- or 30-s tone bursts at or near CF (Fig. 5). The good correspondence between the data sets rules out experimental variables as the source of the observed differences between AN and TB.

As is the case for the examples in Fig. 1, $R_{max}$ for high-sync TB fibers tended to occur at higher levels compared with AN fibers. These levels, expressed with respect to the tuning curve rate threshold and therefore called suprathreshold levels, are compared in Fig. 6 for high-sync TB fibers ($N = 46$) and AN fibers ($N = 51$: selected over a similar CF range). The average suprathreshold level was $32 \pm 12.4$ (SD) dB for the high-sync fibers and $16 \pm 7.8$ dB for the AN fibers.

There is some indication of temporal adaptation in the TB responses of Fig. 2 (fibers with CF < 1 kHz in B and C): the first modes of the PST histograms are more narrowly distributed than the later modes. Because there is no evidence for such adaptation in AN responses (Fig. 2A), it provides another example of “non-primarylike” behavior in these fibers. Phase-locking in the AN has been reported to remain approximately constant over time in response to long tones (Johnson 1980), and judging from the shape of PST histograms to short tone bursts the same presumably holds for responses examined on a millisecond time scale.

To compare the time course of phase locking over a short time scale in AN and TB fibers we obtained three measures from each phase-locked peak of the PST at the SPL where $R_{max}$ was obtained. Examples of these measures as a function of time are shown in Fig. 7 for an AN (A) and high-sync TB (B) fiber with CF of 380 Hz. Number of spikes (top panels), $R$, and spike time SD ($\sigma$) (bottom panels) were calculated over a response window equal to the stimulus period and centered on the average response phase measured over 10–25 ms. The analysis window was shifted on the PST axis by one period until all the peaks of the PST had been analyzed. There is no evidence of adaptation in either the discharge rate (top panel) or timing (bottom panel) for the AN fiber response (Fig. 7A). Even when rate adaptation occurred at higher SPLs (not shown), $R$ remained at approximately the same value throughout the response, as reported for long tones by Johnson (1980).

By contrast, in the high-sync fiber (Fig. 7B) discharge rate and precision of synchronization decreased with time after stimulus onset. $R$ and $\sigma$ vary in a mirror image fashion, but the $R$ metric is compressive at high values, so changes in phase-locking over time are more apparent with the $\sigma$ metric. The TB fiber entrained maximally in the first four response cycles where there was one spike per cycle at every stimulus repetition (Fig. 7B, top panel). Some fibers showed no rate adaptation and entrained over the whole response duration (entrainment is more fully described below). The PST histogram clearly shows the second peak to be best timed: $\sigma$ and $R$ for this peak were 28 $\mu$s and 0.998, respectively. The subsequent peaks become progressively wider ($\sigma$ and $R$ at last peak are 118 $\mu$s and 0.961) and approach the peak width of AN fibers, which only rarely show $\sigma < 100$ $\mu$s. We presented longer-duration tones (600 ms) at CF to a few high-sync fibers and, despite the temporal adaptation near or after stimulus onset, the strength of phase-locking can remain high ($R \approx 0.9$) for the whole stimulus duration.

**Entrainment to CF tones**

Besides the enhancement in timing we also looked for improvement in the degree to which low-CF TB fibers entrain, i.e., discharge at each cycle of a CF tone. Figure 8 compares entrainment in an AN and high-sync TB fiber of similar CF (425 and 400 Hz, respectively). Figure 8B shows the ISI histogram at the SPL yielding the maximum
enlarged synchronization in cochlear nucleus

0.99

PHL  * m PL

m PLN A

m SBC .

m GBC A

X

E

K

0.9

0.8

0.7

0.6

0.5

25 ms duration

1 long duration

(Johnson, '80)

FIG. 4. Comparison of $R_{max}$ in AN and TB fibers. Solid lines: upper and lower boundaries of the range of maximum vector strengths reported for AN fibers (Johnson 1980). TB fibers are represented by a single symbol per fiber. Filled symbols: anatomically labeled SBC ($N = 16$) or GBC axons ($N = 13$). Other symbols indicate unlabeled fibers classified on the basis of their PST histogram: phase-locked (PHL: $N = 60$), primary-like (PL) ($N = 34$), primary-like with notch (PLN) ($N = 40$), and NON-PL ($N = 10$). All data points shown (total $N = 176$) had significant phase-locking for $\pm 2$ SPLs. Extra-axonal data not included. The ordinate designates ($1-R_{max}$) on a logarithmic scale to provide an equal variance axis (Johnson 1974).

entrainment index, calculated over a window equal in length to one period of the stimulus. This window, indicated by the bar below the abscissa, was centered on the interval equal to the stimulus period. There are no response intervals falling outside of the window in the high-sync TB fiber, resulting in an entrainment index of 1 and a firing rate equal to the stimulus frequency. In most AN and TB fibers the increase of the entrainment index with SPL (Fig. 8A, *) closely followed the increase in average rate (•). The SPLs for maximal entrainment were not necessarily the same.

Previous studies have also shown that even at high SPLs AN fiber responses to low-frequency stimuli show multimodal ISI histograms (Kiang et al. 1965; Rose et al. 1967), indicating that discharges skip stimulus cycles or, at very low frequencies, that several spikes per stimulus cycle can occur. In contrast, TB fibers can discharge a well-timed spike to every stimulus cycle for frequencies up to $\sim 700$ Hz, resulting in ISI histograms with a single high-amplitude, narrow mode centered at the stimulus period.

In general, fibers that showed high synchronization (Fig. 3) also showed high entrainment. Because there are limita-

FIG. 5. Comparison of AN $R_{max}$ values for tones of long duration (+: Johnson 1980, $N = 324$) and 25-ms tone bursts at CF ($\diamond$: $N = 114$). Note absence of $R_{max}$ values $\geq 0.9$.

FIG. 6. Suprathreshold (re tuning curve threshold) levels at which $R_{max}$ values were obtained in AN (+) and high-sync TB fibers (symbols for TB fibers as in Fig. 4).
rarely exceeded 300 spikes/s (Rhode and Smith 1985). With high entrainment the average rate of TB fibers approached or just exceeded the stimulus frequency (cf. Figs. 1 and 7): the upper boundary of the distribution of the high-sync fibers' maximal discharge rate followed a slope of 1. This behavior seemed to break down at the same frequencies where the drop in entrainment occurred (>700 Hz; Fig. 9, top panel).

Teich and Khanna (1985) analyzed the pulse-number distribution of AN responses and showed that average rate in AN fibers increases in both its mean and its variance as a function of SPL, so that there is generally little change in the mean-to-variance ratio. Although we did not perform such an analysis on TB fiber responses, the combined presence of high phase-locking and entrainment and concomitantly increased discharge rate and reduced variance should result in higher mean-to-variance ratios of discharge rate than are present in the AN.

Morphology and other properties of high-sync units

A variety of anatomic techniques have demonstrated a high correlation between morphological cell type and axonal projection pattern in the SOC (Smith et al. 1991, 1993; Spirou et al. 1990; Tolbert et al. 1982; Warr 1966). We labeled seven fibers with CF <700 Hz; these cells were all high sync and showed one of two projection patterns characteristic of bushy cells. Two medium-sized fibers crossed the midline in the dorsal component of the TB (Fig. 10A) and showed a heavy projection to the MSO, and were therefore derived from SBC's (Smith et al. 1993). Five large-diameter fibers crossed the midline in the ventral component of the TB (Fig. 10B) and showed a large calyceal ending in the MNTB, and were thus derived from GBCs (Smith et al. 1991). High-sync responses can thus originate from either SBCs or GBCs, although our small sample consists mainly of GBCs.

As mentioned above, at low CFs physiological criteria generally do not allow one to distinguish between the two bushy cell classes. Previous anatomic/physiological correlative studies have shown a differential distribution of spontaneous rate (SR) in populations of labeled bushy cells; SR is on average high for SBCs and low for GBCs (Fig. 11B; Smith et al. 1991, 1993). Although few data are available, this difference seems particularly marked at low CFs. Figure 11B shows that in the phase-locking range, below ~4 kHz, there are only two labeled GBCs with a high SR, whereas there are no SBCs with low SR. Both high and low SRs are found in the population of unlabeled high-sync fibers (Fig. 11A), consistent with the anatomic evidence that both types of bushy cells contribute to the high-sync population. The bias toward low SRs suggests that we recorded mostly from GBC axons, as was the case for our labeled sample.

To study whether the enhanced timing properties of high-sync fibers applied to more broadband impulsive and sustained stimuli we obtained responses from a limited set of fibers to clicks (N = 5) or AM tones (N = 6). PST histo-

![Figure 7](image-url)

**FIG. 7.** Temporal adaptation in an AN (A) and high-sync TB (B) fiber. **Top panels:** PST histogram (ordinate indicates number of spikes per bin, number of bins = 400) and discharge rate averaged over a window equaling the stimulus period (×). **Bottom panels:** spike time SD (σ) (+, in ms) and R (○). Symbols are positioned at center of response windows over which values were computed. SPLs and Rmax (over 10–25 ms) values were 44 dB and 0.85 for AN fiber and 60 dB and 0.97 for TB fiber. The shorter latency of the TB fiber relative to the AN fiber reflects the higher SPL used.
grams of responses of low-frequency AN fibers to clicks (Fig. 12, top) show multiple peaks separated by intervals of 1/CF (Kiang et al. 1965). High-sync TB fibers also showed multipeaked responses but, as was the case for their responses to pure tones, the peaks were narrower (Fig. 12, bottom; σ of 3rd peak = 26 μs).

Cells with PL or PL₁ responses show slightly better phase-locking to the envelope of AM stimuli than AN fibers do (Frisina et al. 1990). Figure 13 compares $R_{max}$ values for phase-locking at the carrier (abscissa) and envelope (ordinate) frequency in AN and high-sync TB fibers with CF < 1 kHz. Carrier phase-locking always yields larger $R_{max}$ values than envelope phase-locking, both in AN and TB fibers, because all data points are below the line indicating equal values (dotted line). Of six high-sync TB fibers tested with AM stimuli, four showed much higher envelope phase-locking with $R_{max}$ values exceeding those of the AN (Fig. 13, *). These fibers all had low SRs, whereas the two remaining TB fibers had a high SR. An SR-based difference in envelope phase-locking was also noted in the AN (Joris and Yin 1992).

The six TB fibers of Fig. 13 were classified as high-sync on the basis of their $R_{max}$ values to short-duration tone bursts. In four of these fibers the responses to long-duration (600 ms) AM stimuli, when binned on the $f_c$ (abscissa), also show $R_{max}$ >0.9. This illustrates that enhanced phase-locking is not just a transient phenomenon observed to short-duration stimuli. For two fibers $R_{max}$ of the long-duration responses dropped below the high-sync criterion, even though the criterion was met by the short tone burst responses (Fig. 13, dashed line). Modulation by itself did not seem to affect carrier phase-locking because virtually identical $R$ values were obtained in the few instances where responses to unmodulated and modulated long-duration tones were available for the same fiber (not shown).

Coincidence model of synchronization enhancement and entrainment

Stimulus manipulations that result in altered spatiotemporal discharge patterns across the AN array but do not affect the response rate of any given AN fiber can influence the response rate of GBCs (Carney 1990). This sensitivity and other response properties of GBCs have been interpreted as the outcome of a cross-correlation or coincidence detection performed by the cell on its inputs (Carney 1992). Intuitively, the requirement to have coincident firing of a number of AN inputs to elicit a postsynaptic spike should reduce the stimulus phase range over which the postsynaptic cell fires and thus result in increased $R$ values to a low-frequency pure tone. We tested this idea on a low-CF AVCN model by comparing $R$ and entrainment to CF tones over a range of SPLs for pre- and postsynaptic components.

A simple “shot-noise”-type model is used for the coincidence detecting neuron (Carney 1992; Colburn et al. 1990; Young et al. 1993). The model cell (Fig. 14) receives input discharge times, on a trial-by-trial basis, from $n$ inputs. Input spikes are provided by a model that simulates the temporal response properties of low-frequency AN fibers (Carney 1993). The influence of input discharges on the voltage of the postsynaptic model cell are represented by simple decaying exponential waveforms with a time constant ($τ$) of 0.5 ms and maximal amplitude (w), expressed as a fraction of threshold, of 0.8. Overlapping waveforms summate;
The sensitivity of the model cell to the coincidence of its inputs depends on several parameters. In general, for a cell to act as a coincidence detector, it must receive more than one input \((n > 1)\) and the amplitudes of the individual inputs must be below the threshold level \((w < 1)\). The summation of several near-simultaneous inputs, each having a probability density function with a finite rise time, results in a more quickly rising probability density function for the combined effect of the inputs. The shorter rise time for the summed inputs, in combination with refractoriness, results in a driving function that elicits discharges with a lower temporal variance than the input discharges.

The \(\tau\) of the decaying exponential in response to each input plays a role in determining how sensitive the cell is to the temporal relationship between its inputs. If the influence of the input decays very rapidly, that is when \(\tau\) is small, the inputs must occur almost simultaneously with one another to bring the cell to threshold. This has the effect of both increasing \(R\) and lowering SR. For longer values of \(\tau\), increases in \(R\) could occur, but were accompanied by unphysiologically high SRs. Although the value of \(\tau\) is very short compared with most membrane time constants, it includes the effect of the low-threshold M-like conductance, present in bushy cells, that reduces the membrane time constant by a factor of 4–10 (Manis and Marx 1991). The \(\tau_{\text{off}}\) of the postsynaptic cell also contributes to the high-sync responses and entrainment in this model. By preventing inputs from accumulating during the refractory period (simulating afterhyperpolarization of the cell), the cell is effectively prevented from discharging again until the next phase of the low-frequency stimulus. Thus, within a cycle, there can only be a single, well timed, postsynaptic spike. At the highest SPLs, the average rate of the model cell (Fig. 15A) nearly equals the stimulus frequency, and the cell is thus entrained.

**DISCUSSION**

Our data constitute the first population study of phase-locking in the TB and show that the precision of phase-locking in a substantial proportion of TB fibers is much higher than in the AN. The majority of low-frequency TB fibers show both more precise phase-locking and entrainment than AN fibers. Temporal information after the first processing stage of the auditory system is therefore much more accurate than has heretofore been appreciated.

**Comparison with previous studies**

A striking difference from results of previous studies is in the degree of phase-locking. Isolated examples of high phase-locking in morphologically unidentified cells have been mentioned in a few studies where recordings were made from the CN itself (Carney 1990; Rhode and Kettner 1987; Rhode and Smith 1986; Rose et al. 1974), but previous studies have generally emphasized the similarity of phase-locking of presumed bushy cells and AN fibers (Blackburn and Sachs 1989; Bourk 1976; Goldberg and Brownell 1973; Kettner et al. 1985; Lavine 1971; Palmer et al. 1986; Rhode and Smith 1986; Smith and Rhode 1987;
van Gisbergen et al. 1975; Winter and Palmer 1990). For instance, one of the most extensive studies states that “when the stimulus frequency is below 800 Hz, the period histograms of most units in AVCN are largely indistinguishable from those of AN units” (Bourk 1976), whereas in our study of TB responses this is the region where the largest differences are seen. The least-squares fit of Blackburn and Sachs (1989) to their data from PL and PLN fibers mismatches our data both at low CFs, where their fit is too low (although they have very little data at CFs <1 kHz), and at high CFs, where it is too high. Phase-locking measures for CFs <1 kHz were not reported in previous studies of TB fibers (Brownell 1975; Friauf and Ostwald 1988; Spirou et al. 1990).

The frequency dependence of phase-locking in the cat peripheral auditory system can be described by a low-pass filter function, characterized by a corner frequency at 2.5 kHz and attenuation near 100 dB/decade (Weiss and Rose 1988). Consistent with previous studies (Blackburn and Sachs 1989; Bourk 1976; Rhode and Smith 1986), we find that phase-locking in bushy cells degrades at lower frequencies than AN fibers, equivalent to a lower corner frequency (Fig. 4). In contrast to these previous studies, however, we found almost no overlap between the distribution range for AN and TB fibers for CFs above ~2 kHz.

Thus our data obtained from TB fibers are less “nerve-like” than data obtained from AVCN recordings both in the gain and in the upper limit of phase-locking, and the question arises as to the origin of this discrepancy. Although we have no data directly bearing on this issue, the most likely explanation to us seems to be sampling bias: cells with high phase-locking in AVCN may be hard to record from, e.g., because of their size, spatial location or strong field potential, and/or their axons in the TB may be particularly easy to record from, e.g., because of a larger fiber diameter. There are alternative explanations that we feel are less plausible. Recordings from the CN may compromise some aspect(s) of the cellular environment critical for high-sync phase-locking, e.g., through aspiration of cerebellum or use of large metal electrodes, whereas this environment is left intact during axonal recordings. Interestingly, Stopp and Whitfield (1963) reported effects on SR of

FIG. 10. Examples of the identification of labeled axons on the basis of the position of the axon at the midline [top panels; outlines show TB and pyramidal tract (PT) in frontal sections] and the dominant collateral projections (bottom panels). A: SBC axons cross the midline in the dorsal component of the TB and show a heavy innervation of the ipsilateral and contralateral medial superior olive (MSO). B: GBC axons cross the midline in the ventral TB component and have 1 or 2 large calyx ending(s) in the contralateral medial nucleus of the trapezoid body. Arrows near labeled axons in top panels indicate orthograde direction. The SBC projection to MSO is a computer-generated (EUTECTICS) frontal plane reconstruction of collaterals to the ipsilateral MSO, which is shown as an outline. Scale bar of micrograph in B is 25 μm. CFs were 200 Hz (A) and 450 Hz (B).
TB fibers by insertion of glass microelectrodes in the ventral CN. Another possibility is that the discharge statistics at an axonal point several branchpoints away from the soma differ from those recorded near the soma (Chung et al. 1970). Inconsistent with these explanations is the fact, mentioned above, that high-sync responses have been observed in some AVCN recordings. Finally, we want to emphasize again that by recording from axons in the TB, rather than from cells in the AVCN, contamination of the recordings by large phase-locked field potentials or by recordings from AN fibers is avoided.

Interestingly, second-order auditory neurons in the barn owl show enhanced phase-locking, although the difference is much less marked (Sullivan and Konishi 1984). A de-
Mechanisms of synchronization enhancement and entrainment

We hypothesize that the neural mechanism for generating the enhanced synchronization requires 1) convergence of inputs from two or more AN fibers onto a CN cell and 2) a postsynaptic cell that requires coincident input spikes before it generates an output spike. Evidence for convergence and coincidence derives from the observation that cells with high synchronization in the AVCN also show sensitivity to spatiotemporal patterns across AN fibers, as demonstrated with Huffman sequences (Carney 1990). Moreover, intracellular recordings from GBCs show large and fast subthreshold excitatory postsynaptic potentials (EPSPs) (Smith and Rhode 1987), indicating the need for coincidence. Also, coincidence detection can explain the presence of low SRs in low CF GBCs (Fig. 11; Spirou et al. 1990) despite the high AN convergence ratios on these cells (Liberman 1991; Spirou et al. 1990).

At a cellular and subcellular level bushy cells and their inputs show many specializations that may enhance coincidence detection: fast synaptic potentials and a short membrane time constant (Wu and Oertel 1984) that is effectively reduced by an M-like current (Banks and Smith 1992; Manis and Marx 1991). In addition, fast kinetics of the postsynaptic receptor have been found in cells of the avian homologue of the AVCN, the nucleus magnocellularis (Raman and Trussell 1992).

Temporal adaptation, as observed in high-sync and not AN responses (Figs. 2 and 7), is also present in the responses of the coincidence model (Fig. 15). The coincidence mechanism is sensitive to input rate, because increases in the number of inputs and input spike rate cause an increase in \( R \) (measured over 10–25 ms); it appears that this mechanism can transform the peristimulus rate adaptation of the AN inputs into a temporal adaptation on the postsynaptic side. Other factors may contribute to the temporal adaptation. Inhibitory inputs hyperpolarizing the cell may increase the postsynaptic cell superthreshold, resulting in tightened phase-locking. Our modeling results suggest a similar mechanism in bushy cells of the AVCN of the cat (see below).

Some cells in the posteroventral cochlear nucleus (PVCN) of the cat exhibit properties of phase-locking and entrainment much like the high-sync fibers reported here (Rhode and Smith 1986; Rhode and Kettner 1987). Such responses, classified as onset/O\(_{16}\), are prevalent in the octopus cell area of the PVCN (Rhode and Smith 1986), suggesting they are derived from multipolar cells. Multipolar cells from this region of the PVCN project centrally by way of the intermediate acoustic stria (Warr 1969) and it is therefore unlikely that their axons contributed to our high-sync population.

Functional relevance

Response transformations between the peripheral and central auditory system, such as sideband inhibition and increased dynamic range, are often viewed as mechanisms that have evolved for sharpening of rate information, and it is tempting to similarly regard the enhancement of phase-locking as a sharpening in the temporal domain. The best evidence for the use of temporal cues by the auditory system is provided by the psychophysics and physiology of
FIG. 15. A: responses of a model AN fiber (top) and bushy cell (BC, bottom) analyzed and plotted as in Fig. 1A. The stimulus parameters are as in Fig. 1, with the Cs for all 15 AN fibers fixed at 350 Hz. The AN response is from 1 of the input fibers to BC, taken from the same simulation. The thresholds of the AN model were adjusted to approximately match the response of Fig. 1A. B: PST histograms taken from the responses in A at $R_{max}$. Model parameters are: absolute refractory period ($\tau_{refr}$) = 1.5 ms, time constant ($\tau$) = 0.5 ms, $n = 15$, maximal amplitude ($w$) = 0.8. The model was run in time increments of 10 μs. Binwidth is 0.1 ms.

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