Title:
Influence of Inhibitory Inputs on Rate and Timing of Responses in the Anteroventral Cochlear Nucleus

Authors:
Yan Gai\textsuperscript{1,2} and Laurel H. Carney\textsuperscript{1,2,3}

Affiliation:
\textsuperscript{1}Department of Biomedical and Chemical Engineering, \textsuperscript{2}Institute for Sensory Research, \textsuperscript{3}Department of Electrical Engineering and Computer Science, Syracuse University, Syracuse, New York 13244

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Contact information:
Departments of Biomedical Engineering and Neurobiology & Anatomy, University of Rochester, Box 603, 601 Elmwood Ave., Rochester NY 14642
Phone: 585-276-3948. fax: 585-756-5334. Email: laurel.carney@rochester.edu
Abstract:

Anatomical and physiological studies have shown that anteroventral cochlear nucleus (AVCN) neurons receive glycineergic and GABAergic inhibitory inputs. In this study, changes in the temporal responses of AVCN neurons to pure tones and complex sounds after blocking inhibition were analyzed. Blocking inhibition influenced the temporal responses of each type of AVCN neuron. Choppers showed more chopping peaks and shortened chopping cycles after blocking inhibition. Sustained and slowly adapting choppers showed increased regularity throughout the response duration after blocking inhibition, whereas most transient choppers showed increased regularity in the early part of the response. Diverse changes in temporal response patterns were observed in neurons with primary-like and unusual responses, with several neurons showing a large decrease in the first-spike latency after blocking inhibition. This result disagreed with previous findings that onset responses are less affected than sustained responses by manipulating inhibition. Although blocking inhibition had a larger effect on spontaneous activity than on tone-evoked activity, the change in spontaneous activity was less significant because of larger variability. In addition, for relatively high-level masker noises, blocking inhibition had similar effects on responses to noise-alone and noise-plus-tone stimuli, in contrast with previous studies with low-level background noise. In general, inhibition had an enhancing effect on temporal contrast only for responses to amplitude-modulated tones, for which envelope synchrony was enhanced. Results of this study contribute new information about the characteristics, functional roles, and possible sources of inhibitory inputs received by AVCN neurons.

Keywords: iontophoresis glycine GABA regularity latency
INTRODUCTION

The cochlear nucleus (CN) sends major inputs to higher levels of the auditory pathway. Understanding the response properties and information-processing mechanisms of CN neurons can aid the study of higher-level neurons. Established models of auditory-nerve fibers (ANFs, Giguere and Woodland 1994; Robert and Eriksson 1999; Zhang et al. 2001; Zilany and Bruce 2006) provide useful tools to simulate the responses of CN neurons caused by excitatory ANF inputs. However, it is difficult to understand the role of inhibitory inputs because the source of inhibition remains unclear for specific neurons or neuron types.

All principal cell types in the AVCN receive inputs that stain for glycine (Wenthold et al. 1988) and GABA (Saint Marie et al. 1989). Possible glycinergic inputs include projections from the tuberculoventral (vertical) neurons in the dorsal cochlear nucleus (DCN) and D-stellate neurons in the posteroventral cochlear nucleus (PVCN) and AVCN (Arnott et al. 2004; Oertel and Wickesberg 1993; Smith and Rhode 1989; Wickesberg and Oertel 1988, 1990). Vertical cells respond actively to CF tones and weakly to broadband noise (Gibson et al. 1985), whereas D-stellate cells respond weakly to CF tones but actively to broadband noise (Rhode and Greenberg 1994). A third source of glycinergic inhibition is the descending input from the superior olivary complex (SOC). A study in guinea pig using retrograde labeling combined with immunocytochemistry reports glycinergic projections from the lateral (LNTB) and ventral (VNTB) nuclei of the trapezoid body and the dorsal periolivary nucleus to the CN. Most of these projections are ipsilateral (Ostapoff et al. 1997). There are reciprocal connections between the CN and the posteroventral periolivary nucleus (PVPO): the only known input to the PVPO is the ascending projection from the CN, and the only known output of the PVPO is the descending glycinergic projection to the same area of the CN (guinea pig: Helfert et al. 1989; Thompson and
There are also commissural glycinergic projections from the contralateral CN to the ipsilateral CN (Babalian et al. 2002; Wenthold 1987).

Sources of GABAergic inhibition to the AVCN are less clear. The major source of GABAergic inhibition is presumably the descending projection from the SOC. Most of these GABAergic neurons are bilaterally located in the VNTB (Ostapoff et al. 1997). Although there are GABAergic inhibitory interneurons located in the DCN, no connections between these neurons and the VCN have been found. Golgi cells in the superficial granule cell domain are GABAergic (Kolston et al. 1992). It is likely that some AVCN neurons have distal dendrites that receive inputs from these cells (Ferragamo et al. 1998b), since these cells do not project to the VCN itself, but to regions overlying the VCN. However, inhibition is not expected to have a strong effect when the inputs synapse on the distal dendrites of the target neurons. This assumption is confirmed by the fact that GABAergic inhibitory post-synaptic potentials (IPSPs) are insignificant during brain-slice recording (Oertel 1983; Ferragamo et al. 1998a), when inputs from outside the CN are absent.

Early studies that used off-characteristic-frequency (CF) tones detect inhibitory sidebands (Goldberg and Brownell, 1973; Martin and Dickson, 1983; Rhode and Greenberg, 1994) might have yielded misleading results because excitatory and inhibitory areas overlapped in the frequency responses of AVCN neurons. A more direct approach to study the response properties of inhibitory inputs is to make intracellular recordings of IPSPs. The in vivo intracellular study by Paolini et al. (2005) suggested that the difference in discharge regularity between transient and sustained choppers might originate from the relative amount of overlap between excitatory and inhibitory response areas. More specifically, transient choppers have inhibitory response areas that closely match excitatory areas, and this match results in declined firing rate and
regularity compared to sustained choppers. Sustained choppers have more lateral inhibition that is less effective in altering the firing rate and regularity in response to CF tones (Paolini et al. 2005). However, the dominance of excitatory post-synaptic potentials (EPSPs) in response to sound stimuli makes it difficult to detect the presence of IPSPs at any time other than after the tone offset. Moreover, if IPSPs were caused by shunting inhibition, they would be hard to detect because the reversal potential of shunting inhibition is close to the membrane resting potential.

Iontophoretically applying inhibitory receptor agonists or antagonists provides a better test of the effect of inhibition. Caspary et al. (1979, 1994) injected glycine/GABA receptor agonists/antagonists in the AVCN and DCN, and found that inhibition alters the maximum response or near-CF response for the majority of AVCN neurons, which is consistent with findings in the PVCN (Palombi and Caspary 1992) and the inferior colliculus (IC) (Palombi and Caspary 1996), but inconsistent with the hypothesis of lateral inhibition. Unfortunately, because sustained choppers were not differentiated from transient choppers in Caspary et al. (1994), it is not clear whether this finding agrees or disagrees with Paolini et al. (2005), who reported that sustained choppers receive lateral inhibition.

In addition to changes in average rate and frequency response, previous studies also focus on changes in the “temporal contrast” caused by inhibitory inputs. Several features have been observed related to the temporal contrast. First, spontaneous activity is reported to be more suppressed by inhibitory inputs than is sound-evoked activity (Caspary et al. 1979; Ebert and Ostwald 1995a, b). Second, sustained activity is reported to be more suppressed by inhibitory inputs than are onset responses (Kopp-Scheinpflug et al. 2002; Ebert and Ostwald 1995a, b). Third, activity evoked by low-level background noise is reported to be more suppressed by inhibitory inputs than is tone-evoked activity (Ebert and Ostwald 1995a). Fourth, inhibition
enhances synchronization to the stimulus envelope in the PVCN and DCN, especially at low and mid modulation frequencies (Backoff et al. 1999). All these findings suggest that inhibition enhances the temporal contrast of CN responses to simple and complex sounds.

The present study explored the effect of blocking glycinergic and GABAergic inhibition on AVCN responses to short tones, tones in noise, and amplitude-modulated tones. A large number of repetitions of short tones were used in a detailed analysis of the changes in temporal responses. Relatively high-level noise was used to examine the temporal contrast for stimuli at levels similar to those used in psychophysical studies of detection in noise (Costalupes 1983; Zheng et al. 2002). Moreover, the present study injected inhibitory receptor antagonists, whereas some of the observations described above were based on the injection of inhibitory receptor agonists. As pointed out by Ebert and Ostwald (1995b), the steady injection of agonists creates a source of tonic inhibition, which can be qualitatively different from sound-evoked inhibition received by the target neuron. In addition to revealing information about the influence of inhibition on temporal contrast, changes in the responses to tones in noise when blocking inhibition can also help identify the source of the inhibitory inputs. As mentioned earlier, vertical cells and D-stellate cells respond differently to noise and to tones. Therefore, the different effects of inhibition on tones and noise might provide clues about the types of neurons that provide inhibition to different AVCN response types.

METHODS

Animal preparation

The details of the surgical procedure are described in Gai and Carney (2006). Briefly, Mongolian gerbils (*Meriones unguiculatus*) 2 to 4 months of age were anesthetized with
ketamine and xylazine and placed in a double-walled soundproof booth. A patch of skin was removed from the top of the animal’s head, and the skull was glued to a head bar mounted in a stereotaxic instrument (KOPF model 900) using dental acrylic to ensure long-duration single-unit recordings. A plastic sound-delivery tube was coupled to the left meatus after the pinna was removed. A hole in the dorsal and caudal bulla was made to expose the temporal bone, in which a second small hole was made for electrode penetration through the dura to the AVCN. The surgical procedures were approved by the Syracuse University Institutional Animal Care and Use Community.

**Sound stimuli**

Sound stimuli were created digitally with Matlab and converted to analog signals with a programmable Tucker Davis Technologies System III. Linear compensation of levels for frequencies from 70 Hz to 10 kHz was performed for each sound stimulus, based on a calibration table generated at the beginning of each experiment with an ER-7C probe microphone (Etymotic Research). Neurons with CFs outside the range of 0.3 to 9 kHz were not studied.

The sound stimuli were short tones, 100% sinusoidally amplitude-modulated (SAM) tones, and tones in broadband noise. The CF, threshold, and spontaneous rate (SR) were measured using an automated tuning curve (Liberman 1978). Rate-level functions of short tones at CF (25-msec duration, 5-msec cosine-squared ramps, repeated every 100 msec at several levels with a 10- or 15-dB step size) were most frequently used to monitor the iontophoretic effect on neural responses. One hundred repetitions were obtained at each tone level for the categorization of response types according to Blackburn and Sachs (1989) and for the study of details of the temporal responses to tones. The frequency-response area was studied by varying
the frequency and level of 25-msec tones (10 repetitions, 15- or 20-dB step size). At each tone level, the tone frequency swept from below CF to above CF (e.g., 2 octaves below and 1 octave above) in half-octave steps, except when limited by the frequency range of the calibration. Three or four tone levels were studied for each neuron, with one level slightly above threshold, one level at 70 or 80 dB SPL, and one or two intermediate levels.

The carrier frequency of the SAM tone was set at the neuron’s CF. The level of the carrier was approximately 20 dB above the neuron’s pure-tone threshold. The SAM tone had a duration of 600 msec and 5-msec cosine-squared ramps, and was repeated every second for 6 to 8 trials at each of several modulation frequencies, which were arranged from low to high (e.g., from 16 Hz to 1024 Hz) in 1-octave steps.

The tone-in-noise stimulus was either a broadband Gaussian noise (0.1–10 kHz) or the noise plus a CF tone at various tone levels (20–80 dB SPL, 10- or 15-dB step size). The noise spectrum level was 30 dB SPL (overall level of 70 dB SPL). The tone-in-noise stimulus had a duration of 250 msec and 10-msec cosine-squared ramps, and was repeated every 475 sec for 20 to 50 repetitions. Tone levels were randomly arranged in each repetition. The noise was randomly created and frozen for each dataset and thus varied across tests (and before and after blocking inhibition). After each tone-in-noise trial, the same tone was repeated without the noise.

**Recording and iontophoresis**

Neural recordings were amplified by an AC Preamplifier (GRASS P55) through a High Impedance Input Module (GRASS HZP). A voltage-crossing criterion was used to discriminate spikes from background noise, and the times of the peaks of spikes were marked as spike times.
with a resolution of 1 µsec. Only bipolar action potentials were studied, to limit recordings to AVCN cells rather than fibers of passage (Rhode 1998).

Single-neuron extracellular recordings and iontophoretic injections were made with 6-barrel piggyback electrodes (Havey and Caspary 1980). The recording electrode (15–50 MΩ) was attached with epoxy to the injecting barrels, protruding 10–25 µm to their tip. The injecting 5-barrel electrode (World Precision Instruments, 1.2 mm OD x 0.68 mm ID, 10 cm in length) was pulled to a tip; the tip was then manually broken to achieve a diameter of 10–20 µm. Each injecting barrel contained one of the following chemicals: strychnine-HCl (10–20 mM, pH = 3–3.5), a glycine receptor antagonist; bicuculline methiodide (10 mM, pH = 3–3.5), a GABA_A receptor antagonist; gabazine (3–5 mM, pH = 3–3.5), a GABA_A receptor antagonist; glycine (500 mM, pH = 3.5–4); and γ-aminobutyric acid (GABA, 500 mM, pH = 4–4.5). Injecting currents (+20–35 nA per barrel; one or two barrels with the same chemical were used at a time) were generated and monitored by a 4-channel current generator (NeuroPhore BH-2, Harvard Apparatus); a negative holding current (-15 nA) was maintained for each barrel when no injections were being made. To avoid the build-up of net charge due to injected current, a balancing barrel (the center barrel of the 5-barrel electrode) continuously injected a current that was equal to the sum of the currents in the other barrels with inverse polarity. The balancing barrel and the attached recording barrel were filled with 1 M sodium acetate (Kopp-Scheinflug et al., 2002). Sodium was used to be consistent with extracellular ionic concentrations; acetate was chosen to reduce diffusion and transport number during application of hyperpolarizing current (Muller 1992). Occasionally, one of the injecting barrels was filled with 1 M sodium acetate as a control barrel to test the possible effect of electrical current alone.
The multi-barrel electrode was advanced by a manual micropositioner through the dura and cerebellum to the AVCN. Each penetration was constrained within a small range of stereotaxic angles that resulted in penetration of the AVCN (this range of angles was verified in pilot studies to result in recording sites limited to the AVCN), as determined in Gai and Carney (2006). After recording from a neuron, the electrode was moved by at least 150 µm and a delay of at least 30 minutes was imposed before recording from another neuron.

Data Analysis

Neuron response types were categorized based on Blackburn and Sachs (1989): (1) primary-likes (PLs), (2) primary-like-with-notches (PLNs), (3) choppers (including different chopping types), (4) onset, and (5) unusual response types. Unusual response types were those that did not satisfy the criteria for any of the other response categories.

During injections, the SR was computed based on spikes within a 50 to 100 msec window after the onset of the 25-msec tones at the lowest level used for the rate-level function. (The sound-evoked response was always within 40 msec of tone onset, and the lowest tone level was always below threshold.) The SR obtained with this method agreed with the SR computed from the tuning curve program, which was run when each neuron was first encountered.

First-spike latency (FSL) is difficult to measure because of the presence of spontaneous activity. Young et al. (1988) set a cursor by hand at the beginning of sound-evoked activity based on visual examination of the post-stimulus time histogram (PSTH) and discarded all activity before the cursor. Chase and Young (2007) provides a binless algorithm that was used here; this algorithm determines the starting time of the sound-evoked activity by computing the probability of an instantaneous firing rate that can or cannot be generated by spontaneous activity.
Specifically, the algorithm first searches for the time when at least 5 spikes (combined across all stimulus repetitions) have occurred. Then the probability of generating a number of spikes in a certain time interval by spontaneous firing is calculated for a set of intervals between the present spike and each previous spike. The shortest interval includes the previous 5 spikes, and the longest interval includes all previous spikes. When the minimum probability across different lengths of intervals is below a certain criterion (i.e., $10^{-6}$), the time is marked as the start of tone-evoked activity; otherwise, the time point moves to the next spike. In this study, the starting point was used to exclude spontaneous spikes that occurred before the sound-evoked response. The mean FSL was then computed based on the remaining spikes. A $t$-test was used to test the significance of rate changes in response to CF tones after blocking inhibition, and $t$ values were compared across neuron types. In this study, the reported $t$ value is the maximum $t$ value across tone levels unless otherwise specified.

Analysis of drug effects

In the following text, “positive effect” (Caspary et al. 1994) refers to consistently increased neural responses to CF tones based on the rate-level functions, which was frequently repeated during iontophoresis. “Drug effect” (Caspary et al. 1994) refers to an effect on neural responses that was presumably caused by the inhibitory receptor antagonists or agonists, rather than by other unknown factors (e.g., current injection, mechanical effects of the electrode on the neuron, or change in pH). The rate-level function was frequently obtained (approximately every 5 min) to monitor the change of average firing rate and to check the stability of action potentials, which insured that recordings were from the same neuron over time.
Neurons that showed unchanged rate were counted as negative-effect neurons if one of the two following conditions was met --- (1) the injecting and recording tips of the electrode remained close (<25 µm) to each other after the recording session, as judged with a 100x microscope after the recording session, (2) during the same penetration, there were other positive-effect neurons. If neither of these conditions was met, the neuron was not included in the results presented here. In other words, a neuron that showed no change in rate was counted as a negative-effect neuron only if a given electrode was known to function properly.

A neuron was considered to show a positive effect only when it satisfied the following two criteria: (1) average rate significantly increased (t test, \( p < 0.05 \)) for at least one super-threshold level (at least 10 dB above threshold), and (2) the rate increase remained significant until the end of the injection and did not disappear immediately upon termination of the injection. (The maximum effect commonly occurred sometime after termination of the injection current.) Some neurons showed significant rate increases occasionally during the injection, but the increase disappeared before or immediately after the injection terminated; these were considered to be negative-effect neurons.

Recovery of the drug effect was indicated when the average discharge rate consistently decreased after termination of the injection by at least 10% of the maximum rate. The recovery process was highly variable across neurons and across inhibitory receptor antagonists. For neurons that did not show signs of recovery within the holding time (between 3 min and 4 hr), the positive effects were still considered drug effects if the above two criteria were met.

RESULTS

I. Responses to short tones at CF
Responses of 89 neurons in 38 gerbils are presented. Table 1 lists neurons that showed positive effects to inhibitory receptor antagonists. Fifty-four out of 89 neurons showed positive effects after injection of either glycine (21/59) or GABA (33/73) receptor antagonists. Of these 54 neurons, 12 were tested with both types of antagonists; these neurons showed a positive effect and full recovery to whichever inhibitory receptor antagonist was tested first for that neuron. Two neurons (2/12, one sustained chopper and one unusual response type) showed positive effects for both antagonists. In general, larger rate changes were observed after blocking GABAergic inhibition than after blocking glycinergic inhibition. (The maximum $t$ values for changes in sound-evoked rate averaged across all neurons were 13.8 and 9.9, respectively.)

Thirty out of 37 negative-effect neurons were tested with both types of inhibitory receptor antagonists, and the other seven negative-effect neurons were only tested with one type of antagonist. As stated earlier, negative-effect neurons sometimes showed significant but temporary rate increases; however, the increased rate either disappeared before or immediately after termination of the injection.

There were 29% to 47% of positive-effect neurons that showed recovery for different inhibitory receptor antagonists (Table 1). Although the percentage of neurons that showed full or partial recovery did not differ substantially for different antagonists (Table 1), it was commonly observed that recovery after injection of bicuculline was relatively quick and complete as compared to the other two antagonists.

Glycine and GABA were only injected during recordings from two and three neurons, respectively. A chopper that showed a positive effect to bicuculline did not change responses in the presence of glycine (a strychnine injection was not available for this neuron). An unusual response type that showed different positive effects to strychnine and bicuculline had a decreased
rate after glycine injection (this neuron will be described in detail below). One PL and one chopper showed positive effects to bicuculline but negative effects to GABA. The responses of the last neuron, a PL, did not change after injection of strychnine, bicuculline, or GABA.

Figures 1–5 show examples of different response types in response to the injection of inhibitory receptor antagonists. Figure 6 shows all 54 positive-effect neurons. General observations for each response type follow.

Primary-likes (PLs) and primary-like-with-notches (PLNs)

Figure 1 shows changes in rate-level functions (left column), PSTHs (middle columns), and interspike intervals vs. time (right columns) for two PLs caused by injecting bicuculline and strychnine, respectively. The PSTHs and interval plots were obtained at the highest tone level (70 or 80 dB SPL). The first neuron showed a maximum change 61 min after the 15-min injection of bicuculline ended, and the positive effect was highly reduced 166 min after the injection ended. For the second neuron, the maximum change occurred 108 min after termination of the 54-min injection of strychnine.

The first PL (Fig. 1A) did not show a change in the shape of PSTH, consistent with most PLs. The onset gradually became earlier and sharper for the second PL (Fig. 1B) as average rate increased. Other neurons also showed this “early peak” during or after injection; this phenomenon will be discussed in more detail below. The coefficient of variation (CV, Blackburn and Sachs 1989) is shown in the right columns (Fig. 1) to quantify the discharge regularity. The CV of the first PL did not change, while the CV of the second PL decreased as the onset peak moved earlier.
Since both PLs had CFs between 1 and 2 kHz, they phase-locked to the tone frequency. No change in the synchronization coefficient was observed with drug injection. In general, blocking inhibition did not vary the synchronization to tone frequency or the phase of the synchronized response for PLs. Diverse effects of inhibition were observed when comparing the rate-level functions and PSTHs for all 18 PLs and 3 PLNs (Fig. 6, the first 21 groups of panels). For example, the shape of the rate-level function and the first-spike latency changed in different ways across neurons.

**Choppers**

Figure 2 shows changes in tone responses after gabazine injection for a transient chopper (Chp-T). In addition to an increased average discharge rate, a systematic change in the PSTH was observed. Before the injection of gabazine, three clear chopping cycles could be identified (Fig. 2, top row, middle column). During the injection, a distinct fourth peak was observed (Fig. 2, middle row, middle column). This neuron showed full recovery 18 min post-injection (Fig. 2, bottom row, middle column); the average rate decreased and the fourth peak disappeared. The right column shows the mean interspike intervals over time that were used to classify chopper types. The intervals were only computed up to 20 msec to avoid end effects (Young et al. 1988; Blackburn and Sachs 1989). This neuron was classified as a transient chopper [also called a transiently adapting chopper (Blackburn and Sachs 1989)] because the intervals increased abruptly over the early part of the response and then slightly decreased later (Fig. 2, top row, right column). During the injection of gabazine, the abrupt increase of intervals at the beginning of the response disappeared (Fig. 2, middle row, right column). This neuron did not show a positive effect to strychnine injection.
Figure 3 shows changes in response to GABA receptor antagonist injection for two slowly adapting choppers (Chp-SAs). These examples, one for gabazine and the other for bicuculline, are presented to show that some basic observations obtained with these two types of GABA receptor antagonists were generally consistent, except the recovery process was usually faster after a bicuculline injection. These two neurons were classified as Chp-SAs since their interspike intervals increased with time up to 20 msec. However, when GABA receptor antagonists were injected, the interspike intervals became relatively constant with time so that the two slowly adapting choppers indeed became sustained choppers. The CV decreased from 0.26 to 0.09, and from 0.25 to 0.15 for the two choppers, respectively. The change of regularity can also be observed from the PSTHs (middle columns), as a larger number of distinct chopping cycles appeared during drug injection. The chopping cycles also decreased correspondingly. The second Chp-SA was also tested with strychnine injection but did not show a positive effect.

Figure 4 shows changes after bicuculline injection for a sustained chopper (Chp-S). The interspike intervals of sustained choppers are nearly constant over time (Blackburn and Sachs 1991). In the present study, various changes to the sustained properties were observed. In the interspike interval plots (Fig. 4, top row, right column), before blocking inhibition, intervals slightly increased as a function of time. Nevertheless, this neuron was classified as a Chp-S because the increase was substantially smaller than for Chp-SA response types (Chp-S responses had CVs $\leq 0.2$ and Chp-SA responses had CVs $> 0.2$). After bicuculline was injected, the intervals became more invariant over time. Similar to the Chp-SA response, more chopping cycles with a faster chopping rate were observed in the PSTH (Fig. 4, middle column) of the Chp-S response. This neuron did not show a positive effect to strychnine injection.
As shown in the population plot (Fig. 6), the change in discharge rate in response to on-CF tones after the injection of inhibitory receptor antagonists was smaller for Chp-S response types (Fig. 6, #22–29) than for Chp-SA (Fig. 6, #36–40) and Chp-T (Fig. 6, #30–35) response types. Previous studies also suggested that Chp-S response types may not receive strong inhibition (Banks and Sachs 1991; Hewitt and Meddis 1993) or that Chp-S response types mainly receive off-CF inhibition (Paolini et al. 2005). However, since Chp-S response types had more regular discharges, the variance in rate was smaller (confirmed by this study), and hence the same amount of rate change can be more meaningful as compared to the other two chopper types. Therefore, the \( t \) value was used to quantify the significance of changes in discharge rate. In fact, the maximum \( t \) values of Chp-S responses across tone levels were comparable to the values of other response types (not shown); it was thus concluded that Chp-S responses to CF tones were also affected by inhibitory inputs.

As shown by the two Chp-SA and one Chp-S types, the regularity of choppers could be affected by inhibitory inputs. Figure 7 shows the CV values before and after the injection of inhibitory receptor antagonists for all choppers that showed positive effects (small CV values indicate regular discharges). The CV is traditionally computed based on discharges in the time window of 12 to 20 msec (Blackburn and Sachs 1989), as shown in Fig. 7, left. Chp-SA response types (Fig. 7, left, plus symbols) showed the largest change in CV after blocking inhibition (three out of five responses actually became sustained choppers). Six out of eight Chp-S response types (Fig. 7, left, right triangles) also showed decreased CV. Although one Chp-T became a Chp-S after injection of GABAergic antagonists, no systematic change in regularity was observed for the other five Chp-T response types (Fig. 7, left, downward triangles). However, as illustrated by the Chp-T in Fig. 2, the change in interspike intervals primarily occurred before 12 msec, which
was excluded by the traditional 12- to 20-msec CV time window. Figure 7, right, shows the CV based on discharges that occurred between 0 and 12 msec after stimulus onset. All Chp-T response types showed decreased regularity within this time window (Fig. 7, right, downward triangles). In summary, irregular chopping patterns can be generated from regular chopping patterns by inhibitory inputs; however, inhibitory inputs do not necessarily account for all irregular chopping patterns. (This finding is revisited in the Discussion.)

Unusual and onset response types

Two onset and 12 unusual response types are presented here. A higher percentage of these neurons showed positive effects compared to the two major response types (Table 1). Four unusual response types showed chopper-like or multimodal patterns, but they did not satisfy the one-spike-per-peak criterion required for categorization as choppers (Blackburn and Sachs 1989).

Figure 5 shows responses of an unusual response type to bicuculline (2nd row), strychnine (4th row), and glycine (bottom row), respectively. In response to CF tones, this neuron had a relatively broad peak at the response onset, followed by low activity that built up in time (top row, middle column). After injecting bicuculline, both onset and sustained activity increased (2nd row, middle column), and interspike intervals decreased (2nd row, right column). The activity increase was maximal during the latter portion of the response and minimal right after onset. This neuron recovered by 64 min post-injection (3rd row). Similar to the Chp-T response type illustrated in Fig. 2, the recovered average rate was slightly lower than the control rate at high tone levels (Fig. 5, 3rd row, left column). This “over-recovery” was reflected in the PSTHs as a less distinct onset and lower activity at the end of the response.
The most interesting change in response to strychnine injection was the appearance of a precisely timed early peak (Fig. 5, 4th row, middle column). This peak was 4.7 msec earlier than the broad peak seen in the original response (top row, middle column). There was also a 1–1.5 msec wide notch after the early peak. The sustained activity showed less increase and was constant over time. The average rate recovered 31 minutes post-injection (5th row, left column). The early peak decreased by half, but did not totally disappear (5th row, middle column). The last row shows the response of this neuron after injection with glycine. Both the early peak and sustained activity decreased drastically, confirming the presence of glycinergic inhibition.

The effects of inhibition on the 11 unusual and 2 onset response types were diverse (Fig. 6, bottom 3 rows; #42 and 47 were the same neuron shown above). Besides the unusual response type described above, another neuron (Fig. 6, #50) showed highly isolated early peaks at times when no discharges were present in the control responses. One example (Fig. 6, #48) showed an enhanced, but not earlier, onset as compared to the control responses. Another example (Fig. 6, #52) had multiple peaks, similar to a chopper (this cell was not characterized as a chopper because it failed the one-spike-per-peak criterion). After blocking inhibition, the timing of these peaks did not change, but an early peak appeared, and the interval between this extra peak and the second peak was approximately the same as the later intervals. On the other hand, two neurons (Fig. 6, #41 and 51) had a distinct extra chopping peak after the primary chopping peaks, but other parts of the response were essentially unchanged.

First-spike latency (FSL) and early inhibition

Figure 8 shows the mean (panels A and B) and standard deviation (panels C and D) of the FSL for all types of responses before and after blocking inhibition. (Glycinergic and GABAergic
inhibitions are not differentiated in the plots because no difference was observed between the two types of inhibition.) The FSL was computed using responses to the highest tone level tested (60–80 dB SPL). The mean FSL decreased significantly for 31 neurons ($p < 0.05$), increased significantly for 8 neurons ($p < 0.05$), and remained unaffected for 15 neurons (Fig. 8A). Figure 8B amplifies the shaded area of Fig. 8A to provide a better view of short FSLs. These two plots show that the mean FSLs of choppers (circles) did not vary substantially after blocking inhibition. The filled symbols in these two plots are 13 cells that had FSLs that decreased by more than 0.8 msec after blocking inhibition (14 symbols are filled in the plots of Fig. 8 because 1 cell showed FSL decrease of more than 0.8 msec for both inhibitory receptor antagonists); all of them were PL, unusual, or onset response types. These units accounted for 44% of all PLs, unusual, and onset types.

Figure 8C and D show standard deviations of the FSL. All but two of the neurons that had FSLs that decreased by more than 0.8 msec (filled symbols) also had decreased standard deviations (Fig. 8C). The FSL standard deviation for all choppers and PLNs was smaller than 1 msec (Fig. 8C, bottom left corner, and Fig. 8D). The FSL standard deviation for all PLs was larger than 1 msec (Fig. 8C).

The inhibition associated with cells that showed FSL decreases of more than 0.8 msec after injecting inhibitory receptor antagonists was called early inhibition. [Note that the 0.8-msec criterion was arbitrarily chosen since no clear boundary existed between early inhibition and later inhibition (Fig. 8A and B).] When applying a $t$ test to the changes in FSL upon blocking inhibition, all FSL decreases were significant for cells with early inhibition. As shown in the mean FSL figure (Fig. 8A and B), there were different degrees of early inhibition. The mean FSLs of some neurons were shortened by approximately 0.8 msec, whereas much larger time
changes were observed for others. It was unclear whether a single underlying mechanism accounted for the large range of FSL shifts. Four out of 13 cells that showed early inhibition required more than 30 min post-injection time to show the maximum effect, and only 1 showed recovery during the holding time. It should also be noted that blocking glycinergic and GABAergic inhibitions both caused large changes in FSLs, although the sources of these two types of inhibitions are presumably different. Potential mechanisms for the influence of early inhibition will be discussed later.

Sound-evoked rate and spontaneous rate

As shown in Table 1, some neurons within each response type showed a positive effect, whereas others did not. Because a positive effect was always associated with an increased discharge rate, the possibility that positive effects were associated with relatively low baseline rates (due to the presence of inhibition) as compared to negative effects was examined (not shown). A large range of discharge rates (from approximately 50 to 600 sp/sec) was observed for both positive- and negative-effect neurons. The five neurons (all of which were choppers) that had the highest rates (>450 spike/sec) did not show positive effects to either glycine- or GABA-receptor antagonists. However, for neurons that had average rates lower than these choppers, positive effects were observed for both high- and low-rate responses. It can thus be concluded that variations in excitatory inputs determined the range of discharge rates across neurons, and inhibitory inputs only modulated the rate within a limited range.

Of the 54 positive-effect neurons (i.e., those for which the increase of sound-evoked rate was significant), 7 showed decreased SR, 16 showed unchanged SR (12 of the 16 had SR = 0), and 31 showed increased SR. Figure 9A shows the mean values of SR before and after blocking
inhibition for both positive-effect (filled symbols) and negative-effect (open symbols) neurons. One interesting observation was that neurons with SR greater than 45 sp/sec before blocking inhibition, which were all PLs or PLNs, never showed positive effects.

Ebert and Ostwald (1995a) computed the percentage of decrease of SR and sound-evoked rate by injecting GABA in the VCN. They found that the SR was significantly more reduced than the sound-evoked rate. In the present study, of the 31 neurons that showed increased SR after blocking inhibition, 27 showed a larger increase of SR than of sound-evoked activity. Figure 9B shows the percentage of SR increase vs. the percentage of maximum sound-evoked-rate increase. Only cells with control SR > 5 sp/sec are plotted, for consistency with Ebert and Ostwald (1995a). Based on this result, which does not take the variance of rate into account, it seemed that inhibitory inputs had a stronger effect on spontaneous activity than on tone-evoked activity.

Statistical tests of changes in SR incorporate the variability in rate. Figure 9C and D shows t values based on changes in SR vs. t values based on changes in tone-evoked rate after blocking glycinergic or GABAergic inhibition for both positive-effect (filled symbols) and negative-effect (open symbols) neurons (D amplifies the shaded area in C). Because the variance of SR was generally larger than the variance of sound-evoked rate, the t values for the change in SR were smaller than the t values for the change in sound-evoked rate for 91% of neurons with positive effects. Therefore, based on this result, inhibitory inputs had a more significant effect on tone-evoked activity than on spontaneous activity.

Although the increase of SR for 33% of positive-effect neurons was significant (t > 1.96, p < 0.05), one must be cautious when evaluating the drug effect on spontaneous activity for three reasons. First, the SRs of some negative-effect neurons also changed (27% showed significantly
increased SR and 13% showed significantly decreased SR). Second, it was observed in at least three positive-effect neurons that SR already varied substantially before the injection of inhibitory receptor antagonists. Third, the recovery of SR was not correlated with the recovery of sound-evoked rate (not shown).

**Frequency-response area**

The response area was used to evaluate whether the frequency tuning of inhibitory inputs was aligned with that of excitatory inputs. Caspary et al. (1994) found that there are more cells with on-CF inhibition than off-CF inhibition for both primary-likes and choppers. In general, the present study also observed more on-CF inhibition (including broad inhibition) than off-CF inhibition (25 on-CF or broad inhibition cells as compared to 16 off-CF inhibition cells). A unique finding here was that most of the off-CF inhibition was exhibited by PLs or PLNs (n=9), as shown by the example in Fig. 10A. The majority of choppers (12/14) showed on-CF (especially broad) inhibition, as shown by the example in Fig. 10B. The hypothesis in Paolini (2005) that sustained choppers receive lateral inhibition and transient choppers receive on-CF inhibition was not supported by the present study. Four and two out of six sustained choppers received on-CF and off-CF inhibition, respectively; four out of four transient choppers received on-CF inhibition.

II. Responses to complex sounds

*Responses to sinusoidally amplitude-modulated (SAM) tones*

Forty-eight positive-effect (based on pure-tone responses) neurons were further tested with SAM-tone stimuli (19 and 30 neurons for glycine- and GABA-receptor antagonists,
respectively). Figure 11 shows changes in the rate (r-MTF) and sync (s-MTF) modulation-transfer functions after blocking inhibition for different response types. PLs (n=17) and PLNs (n=2) generally showed flat r-MTFs and relatively flat s-MTFs. Choppers showed low-pass (n=9), high-pass (n=6), band-pass (n=2), or flat (n=1) r-MTFs. Of course, these shapes can depend on the range of modulation frequency tested with respect to the neuron’s individual characteristics (and the choice of the range of modulation frequency was limited by frequency boundary in the calibration table). Half of the choppers showed band-pass s-MTFs and the other half showed low-pass s-MTFs, with maximum synchronies higher than those of primary-likes. The r-MTFs and s-MTFs of unusual and onset response types had more diverse shapes than the basic shapes described above (Fig. 11).

After blocking glycinergic or GABAergic inhibition, 16 out of 19 and 28 out of 30 neurons showed significantly increased rate ($p < 0.05$; Fig. 11, shaded areas in r-MTFs). Different degrees of synchrony reduction were observed (Fig. 11, shaded areas in s-MTFs; neurons were not classified into decreased or unchanged synchrony groups because it was difficult to test the significance of synchrony reduction). For example, the first two examples in Fig. 11 had large rate increases but relatively small reductions in synchrony, as compared with the second neuron in the second row, which had a smaller change in rate but a large reduction in synchrony. For the 18 choppers, the correlation coefficient between maximum rate increase and maximum synchrony decrease was significant (0.70, $p < 0.05$); the correlation was insignificant for the 19 PLs/PLNs and the 12 unusual/onset response types (0.16 and 0.27, respectively). Blocking GABAergic inhibition generally had a larger effect on synchrony than blocking glycinergic inhibition when the effect on average rate was the same (not shown).
Changes in the phase of the phase-locked response to the SAM envelope after blocking inhibition can indicate the temporal relationships between excitatory and inhibitory inputs. Figure 12 shows the change in phase at $f_m = 32$ Hz, a relatively low modulation frequency, for all neurons studied. Cells that showed increased phase after blocking inhibition received inhibition that affected the later part of the response more than the early part of the response in each cycle. In Fig. 12A, filled symbols indicate phase changes after blocking glycinergic inhibition and open symbols indicate phase changes after blocking GABAergic inhibition. After injection of GABA receptor antagonists, all 12 choppers showed increased phase (open circles, increase of 0.38–31.0°); other neurons showed mixtures of increased and decreased phase.

Of the 13 neurons that showed early inhibition (an increase of FSL more than 0.8 msec), 11 were tested with SAM tones. Fig. 12B marks the phase changes for cells with early inhibition with filled symbols (glycinergic and GABAergic inhibition were not differentiated here). Eight of the 12 filled symbols (1 unusual response type was tested for both inhibitory receptor antagonists) showed decreased phase after blocking inhibition. Thus, for these neurons, the inhibitory response generally discharged earlier than the excitatory response throughout the sustained response to the SAM tones. Four neurons with this early inhibition showed invariant or increased SAM phase after blocking inhibition. For these four cells, the early inhibition affected the onset, but inhibition later in the response did not necessarily precede excitation within each modulation cycle.

Inhibition that was stronger at certain stimulus phases than at others within a modulation cycle was referred to as phasic inhibition. Relatively constant inhibition was referred to as tonic inhibition. Note that tonic inhibition did not necessarily mean that the inhibitory interneuron discharged constantly all the time. Inhibitory inputs were likely to be tuned and phase-locked to
the modulation frequency, since at low modulation frequencies (e.g., $f_m = 32$ Hz), the phase of the SAM-tone responses changed after blocking inhibition for approximately 85% of cells studied (Fig. 12). However, for cells that receive dendritic inhibitory inputs, the dendrites of the target neuron may have smoothed the phase-locked inhibition enough to obscure a phasic effect. An intracellular study by White et al. (1994) characterized the low-pass-filtering effect of dendrites for VCN choppers. Based on the extracellularly recorded spike times in the present study, no clear low-pass-filtering property could be identified.

To further explore the tonic or phasic effects of inhibition, simulated tonic inhibition was added to the SAM-tone responses recorded after blocking inhibition. Specifically, a constant value was subtracted from a period histogram so that the remaining number of spikes was equal to the number of spikes before blocking inhibition for a particular modulation frequency. Note that this simulation approach assumed that the effect of inhibition was linear on firing rate, which has been suggested to describe somatic shunting inhibitory inputs (Koch 1999). While this may not be strictly the case in reality, the simulation provides insight concerning the rate changes that were observed. Because the rate increase caused by blocking inhibition can differ across modulation frequencies, the subtracted constant value was varied with modulation frequency, based on the assumption that the tonic inhibition was tuned to modulation frequency. Figure 13A shows the SAM-tone responses of a PL before (thin solid line) and after (thin dotted line) blocking GABAergic inhibition. After subtracting out a simulated tonic inhibition, the rate difference disappeared, whereas the synchrony difference remained (Fig. 13A, middle, thick dotted line). Thus, constant inhibition did not predict the observed change in synchrony for this PL neuron. In contrast, for the chopper in Fig. 13B, constant inhibition predicted most of the difference in synchrony except at high modulation frequencies (Fig. 13B, middle, thick dotted
Comparing the period histograms at $f_m = 32$ Hz (Fig. 13, bottom row), it was clear that the shape of the period histogram for the PL changed after blocking inhibition, whereas the period histogram for the chopper had parallel shifts in rate without a shape change in the period histogram. Of course, this simulated tonic inhibition can also create an increase in synchrony (8 cells), as shown in Fig. 13C; the increase in synchrony was also an effect of phasic inhibition.

Figure 14 shows the effect of adding simulated tonic inhibition on the difference of synchrony between control responses and responses after blocking inhibition. Note that the maximum synchrony change could occur at either low or high modulation frequencies; thus by using the maximum synchrony, the result can be biased depending on the corresponding best modulation frequency ($f_m^*$). Therefore, in Fig. 14, the difference of synchrony was the average value at all modulation frequencies lower than $f_m^*$. Symbols close to the diagonal indicate neurons for which there was little or no change of synchrony by adding simulated tonic inhibition. Symbols under the diagonal indicate neurons for which the simulated tonic inhibition was able to more or less account for the synchrony difference.

For all but one chopper, the difference of synchrony between control responses and those after inhibition was blocked can be more or less accounted for by subtracting constant activity, especially for choppers injected with GABA receptor antagonists (open circles). Combining the results from Fig 14 with Fig. 13A, which shows increased phase at $f_m = 32$ Hz for all choppers after blocking GABAergic inhibition, the following conclusions can be made. GABAergic inhibition received by choppers was smoothed and probably delayed. (The “delay” could be either a synaptic delay or a smoothing effect.) At very low modulation frequencies, phasic properties were observed. At mid and high modulation frequencies, the inhibition appeared to be tonic. The effect of glycinergetic inhibition on choppers was unclear because the sample size was
small (n=6) and the observations were varied. For PL, PLN, unusual, and onset types, the symbols are closer to the diagonal (with some exceptions of unusuals) (Fig. 14).

**Responses to tones in noise**

Forty-seven neurons that showed positive effects were further tested with tone-in-noise stimuli (n = 19 and 30 for glycine- and GABA-receptor antagonists, respectively; 2 of 47 showed positive effects for both inhibitory receptor antagonists). The example shown in Fig. 15A represented the observations made for most AVCN neurons: after blocking inhibition, the average rate increased by a similar amount in response to different tone levels in the presence of a 30-dB SPL (spectrum level) noise (thick lines). This consistent change in the response across tone levels was called a “parallel change” (Caspary et al. 1993). Filled squares indicate that, at a given tone level, the change in average rate between the response to the tone-plus-noise and that of the noise alone had a $d' \geq 1$ (Gai and Carney 2006). For the chopper illustrated in Fig. 15A, the detection threshold (lowest tone level for which $d' = 1$) based on the neural responses was 70 dB SPL both before and after blocking GABAergic inhibition. Occasionally, the detection threshold based on average rate changed, as shown in Fig. 16B.

Ten out of 49 neurons showed changes in detection threshold$. Specifically, after blocking inhibition, 4 out of 31 PL, PLN, unusual, and onset response types showed threshold increases from the highest level tested to unmeasurable thresholds, 5 out of 18 choppers showed thresholds decrease from the unmeasurable thresholds to the highest level tested, and 1 out of 18 choppers showed a threshold increase from 60 to 70 dB SPL. Because of the small sample size, it was not possible to determine whether the difference in direction of the response changes for different response types was a general result.
Figure 15A and B also show the average rate in response to long-duration pure tones (250 msec; thin lines). If an inhibitory interneuron responded actively to broadband noise but weakly to tones (as expected for D-stellate cells; Rhode and Greenberg 1994), blocking inhibition should increase responses to noise more than to tones. On the contrary, if an inhibitory interneuron responded weakly to broadband noise but actively to tones (as expected for vertical cells, Gibson et al. 1985), blocking inhibition should increase responses to tones more than to noise. Because tone responses were recorded at several tone levels, the maximum response was used for comparison to noise-alone responses. Figure 15C shows rate increases to noise-alone (N) vs. to tone-alone (T) stimuli after blocking glycinergic (filled symbols) or GABAergic inhibition (open symbols). Twenty-four neurons (49%) showed an increase in rate of at least 10% in response to noise after blocking inhibition (above the diagonal), 2 (4%) showed stronger responses to tones (below the diagonal), and 23 (47%) showed less than 10% rate increase for either stimulus (close to the diagonal). No obvious difference was observed for different types of antagonists. For glycinergic inhibition, the results suggested a higher possibility of these neurons receiving inhibitory projections from D-multipolar neurons (neurons that respond stronger to noise than to tones). This issue will be discussed further below.

Gai and Carney (2006) tested several temporal metrics (related to stimulus fine structure or envelope and to discharge reliability) on tone-in-noise responses of AVCN neurons and made comparisons to changes in the average discharge rate and to psychophysical detection thresholds. In the present study, detection thresholds based on these temporal metrics were not consistently altered by blocking inhibition (not shown).
DISCUSSION

Average discharge rate in response to CF tones

Although all neurons with positive effects showed significant increases in average rate for at least one sound level, blocking GABAergic inhibition generally had a larger effect on average rate than blocking glycinergic inhibition. Whether a neuron received inhibitory inputs was unrelated to its maximum tone-evoked rate, similar to findings in the IC (Le Beau et al. 1996). An interesting finding was that the presence or absence of inhibition was more related to the original SR (Fig. 9A); neurons that had an original SR > 45 sp/sec, which were all PLs or PLNs, never showed a positive effect after blocking inhibition.

Although rate saturation was frequently observed for PL response types (Fig. 6), all control rate-level functions monotonically increased with tone level, whereas Kopp-Scheinpflug et al. (2002) report non-monotonic rate-level functions for half of their neurons with pre-potentials (five of ten units). After blocking inhibition, that study reports that rate-level functions for two of the five units became monotonic, whereas the others remained non-monotonic. In the present study, the shape of rate-level functions for PLs did not change as dramatically as observed in their study. Because a change in the shape of the rate-level function was also not observed for long-duration tones (250 msec, interleaved with the tone-plus-noise stimuli), the difference between the two studies was not caused by different stimulus durations (tone duration was 100 msec in the previous study). The difference between studies may have been caused by different recording sites. All recordings in Kopp-Scheinpflug et al. (2002) have pre-potentials, whereas pre-potentials were never observed for the neurons included in the present study. This difference suggested that the recording sites in the present study were relatively caudal in the
AVCN and did not include the rostral region where bushy cells receive large endbulbs of Held (Pfeiffer 1966; Shofner and Young 1985).

**Inhibition influences temporal responses of choppers**

Changes in the temporal response patterns in the PSTH after blocking inhibition were observed for AVCN choppers and some of the observations agreed with the findings reported for 15 PVCN choppers and 4 AVCN choppers after injecting GABA (Ebert and Ostwald 1995b). For example, the presence of inhibition reduced the number of chopping cycles and slowed the chopping frequency. Ebert and Ostwald describes results for 50-msec tone bursts, including the regularity for the first 20 msec and the last 20 msec. The regularity of sustained choppers decreases most during the last 20 msec, and the sustained choppers become more similar to transient choppers. The regularity of transient choppers, whose chopping patterns only exist within the first 20 msec, decreases most during the first 20 msec; i.e., transient choppers become more transient.

Based on these findings, Ebert and Ostwald conclude that (delayed) inhibitory inputs might be responsible for creating the transient pattern, which supports the hypothesis in a modeling study of choppers (Banks and Sachs 1991). However, as briefly mentioned in the Introduction, the use of inhibitory receptor agonists to study inhibitory inputs may have some shortcomings, which might be the major cause of different conclusions between that study and the present study. The injected constant (tonic) inhibition can differ from the actual inhibition received by the neuron, and the effect of the “excessive” inhibition can differ from that of the actual inhibition. Also, the modeling study of Hewitt and Meddis (1993) shows that by varying the parameters of excitatory synapses (e.g., location and strength), transient chopping patterns
can be created without inhibitory inputs. Therefore, although adding more inhibition to transient choppers can further reduce the chopping regularity at the early part of the response, the possibility that the later part of the irregular response was created by excitatory inputs cannot be ruled out. Using a more direct method of injecting inhibitory receptor antagonists, the present study showed that for all but one transient chopper, blocking inhibition only extended the chopping for several milliseconds. For these neurons, excitatory inputs were presumably responsible for creating the transient pattern. Only one transient chopper became a sustained chopper after blocking inhibition. In fact, slowly adapting choppers were more likely to be generated by sustained choppers receiving inhibition; three out of five slowly adapting choppers became sustained choppers after blocking inhibition. Therefore, the present study concluded that although inhibitory inputs are sometimes able to convert chopping response types, excitatory inputs determine the basic chopping patterns.

Changes in mean FSL for choppers were small (< 0.8 msec), although this change was sometimes significant due to the small variation in FSL for choppers. Palombi and Caspary (1992) report that 75% of PVCN neurons (mostly choppers) have decreased FSL with an average change of 0.5 msec after blocking GABAergic inhibition. In the IC, a small number of sustained and unclassified neurons also show FSL reduction (Le Beau et al. 1996). The present study found both increased and decreased FSL for choppers after blocking inhibition (Fig. 8). For choppers, the FSL is related to the integration time across a large number of inputs (van Gisbergen et al. 1975; Young et al. 1988). The presence of inhibition can only delay the integration time; therefore, the variation of FSL by inhibition is limited. To account for the decreased FSL by blocking inhibition, one possibility was that the inhibition arrived at the same time or earlier than the excitation. This conclusion was counterintuitive since, in previous brain-
slice studies, electrically shocking ANFs always caused delayed IPSPs (e.g., Oertel 1983; Ferragamo et al. 1998a). Other possibilities include some source of spontaneously active inhibition that was present for the in vivo experiments, such as inputs from outside the CN, and inhibition that had a lower threshold than the excitation.

Labeling studies have shown that both glycinergic and GABAergic endings tend to be located on the dendrites of stellate cells (Wenthold et al. 1988; Saint Marie et al. 1989) and on the somas of bushy cells. Correspondingly, as stated above, the effect of inhibition on chopper responses was expected to be smooth as a function of time. Prolonged chopping cycles in the presence of inhibition indicated that the temporal summation of many small EPSPs to reach discharge threshold was delayed by a relatively constant hyperpolarization. This hyperpolarization might build up over time to change sustained choppers into slowly adapting choppers. The SAM-tone responses of the choppers also supported this hypothesis. The enhancement of inhibition for these choppers could be largely predicted by simulated tonic inhibition (Fig. 14), especially for synchrony changes caused by blocking GABAergic inhibition.

In addition, changes in the frequency-response area after blocking inhibition indicate that all chopping response types were dominated by on-CF or broad inhibition. This observation was not consistent with the hypothesis by Paolini et al. (2005) that the regularity of choppers is determined by the overlap of excitatory and inhibitory areas and that sustained choppers thus receive lateral inhibition.

Inhibition influences temporal responses of PL, PLN, unusual, and onset types

More diverse changes in the short-tone PSTHs were observed for PL, PLN, unusual, and onset types after blocking inhibition. Some neurons exhibited relatively constant inhibition over
time, and others changed more dramatically at certain time points. It was difficult to describe a
general effect of inhibitory inputs for these neurons.

Neurons such as spherical bushy cells receive relatively fewer excitatory and inhibitory
inputs, and their membrane properties prevent temporal summation (Oertel 1983; Manis and
Marx 1991). As mentioned earlier, when neurons exhibited phase-locking to tone frequency,
blocking inhibition did not significantly change the phase of the response. Therefore, temporal
summation must have been minimal in these neurons, and the role of inhibition was not to
influence integration time, which is the short time required for the membrane potential to reach
the firing threshold. Instead, the inhibition was more likely to suppress individual post-synaptic
spikes than to delay their timing. The observed diversity in the changes of responses caused by
blocking inhibition also indicated that the role of inhibition might be quite different from neuron
to neuron even within the same cell type.

The SAM-tone responses of the PL, PLN, unusual, and onset types showed that both
increases and decreases in phase occurred at low modulation frequencies after blocking
inhibition. In contrast, choppers were more frequently observed to have increased phase.
Moreover, results with simulated tonic inhibition suggested that the change of synchrony after
blocking inhibition for these neuron types was less likely to be explained by smooth inhibition
(hyperpolarization) as compared to choppers (Fig. 14). Combined with the minimal temporal
summation described above, the effect of inhibition on bushy cells was less uniform over time.

Cells that showed a decrease in FSL of more than 0.8 msec were categorized in this study
as having early inhibition. These long FSLs have not been explicitly reported in previous studies.
Blocking inhibition sometimes revealed a precisely timed early onset that was not present in the
control response. A strong, constant (tonic) inhibition was not likely to account for this
phenomenon because it would have also blocked most of the later responses. Changes in membrane potentials and ion-channel activity may have also affected the FSL. For example, *in vitro* intracellular recordings from DCN principal cells show that the FSL can be highly varied by changing the steady hyperpolarization (Manis 1990). That is, holding the membrane potential hyperpolarized before a depolarizing current occurred increased the FSL of a cell in response to the depolarizing current. In the present study, if an AVCN neuron had similar membrane properties and the hyperpolarization was related to inhibitory channels, blocking the inhibitory receptors could have contributed to the decrease of FSL. Future *in vitro* study is required to test whether a similar phenomenon would be observed for AVCN PL/unusual neurons. However, our present extracellular study was inadequate to identify the specific mechanism that accounted for the change of FSL.

Another general difference between PL/unusual neurons and choppers was that more than half of neurons in the first group showed off-CF inhibition, while the majority of choppers showed on-CF inhibition. If one possible role of inhibition was to enhance frequency contrast, then this role was more prominent for PL/unusual neurons.

AVCN bushy cells (PLs and PLNs) send major ascending inputs to the binaural nuclei. At low and mid CFs, bushy cells generally show enhanced synchronization to tone frequency (Joris et al. 1994) compared to ANFs, which is reasonable since fine-timing information is critical for the low-frequency binaural system. However, findings related to FSL in the present study suggest that at least some bushy cells might possess a more complex information-processing mechanism.
Inhibition vs. temporal contrast

The present study did not find global enhancement of temporal contrast in AVCN responses. First, although spontaneous activity had a larger change than tone-evoked activity in terms of percentage of rate change, the significance of the change (t values) in spontaneous activity was generally smaller than the significance of the change in tone-evoked activity. More importantly, the change in the spontaneous activity was weakly correlated to the drug effect. Second, blocking inhibition did not always have a larger effect on sustained activity than on onset activity. On the contrary, the FSL for a number of neurons varied substantially. Instead of showing a less distinct onset peak after blocking inhibition, as suggested by previous studies (Kopp-Scheinpflug et al. 2002; Ebert and Ostwald 1995a, b), a number of neurons showed a more distinct onset. Palombi and Caspary (1992) also report that the mean value and variability of the FSL is reduced after application of bicuculline for PVCN neurons. Although the underlying mechanism for changing the FSL was unclear, inhibitory inputs did seem to affect the onset activity more than the sustained activity for some AVCN neurons.

Third, in the presence of a relatively high-level background noise, inhibitory inputs did not have a stronger suppressive effect on responses to noise alone than on responses to tones plus noise. After blocking inhibition, the majority of cells had rate increases that were similar in size for noise-alone and noise-plus-tone stimuli at different tone levels. When comparing responses to noise-alone and tone-alone stimuli, the majority of AVCN neurons showed greater rate changes in response to noise stimuli than in response to pure tones (Fig. 15C). That is, inhibition seemed to have a stronger effect on noise responses than on pure-tone responses. However, this differential effect was not strong enough to facilitate the detection of tones in noise based on discharge rate. The finding by Ebert and Ostwald (1995a) that inhibition has a strong suppressive
effect on background-noise activity but a minimal effect on responses to a tone added to the noise might be related to the low noise level they used. (The tone level was approximately 25 dB above discharge threshold; the overall noise level was 10 dB below the tone level, e.g., 0–10 dB SPL spectrum level; and the noise bandwidth was approximately 20 kHz.) Moreover, that study injected inhibitory receptor agonists. As mentioned before, applying inhibitory receptor agonists creates a source of constant inhibition, which presumably differs from the actual inhibition received by neurons. For example, if an inhibitory interneuron does not respond actively to noise, no suppression of the background-noise activity of the target neuron should be observed. On the contrary, applying inhibitory receptor antagonists might generate more “meaningful” responses, i.e., the behavior of a neuron with only excitatory inputs. Applying inhibitory receptor antagonists removes the effect of inhibition only when the inhibition is active.

Instead, the present study agreed with the findings of Caspary et al. (1993), who compared broadband-noise responses at different noise levels to CF-tone responses at different tone levels for spherical bushy cells using inhibitory receptor antagonists and agonists. They reported that inhibition has similar effects on noise responses and pure-tone responses, which did not support the idea of inhibition enhancing temporal contrast. (Tone-in-noise stimuli were not used in that study.)

Lastly, analysis of responses to the SAM tones in the present study agreed with the previous study in the PVCN and DCN (Backoff et al. 1999) and found that inhibitory inputs generally enhanced the synchronization to amplitude modulation and thereby enhanced the temporal contrast. However, the enhancement did not always exist, as different degrees of synchrony reduction were observed for different neurons after blocking inhibition. The present study also found that GABAergic inhibition was more effective in enhancing synchrony than
glycinergic inhibition. Enhanced synchronization was not always caused by a cycle-by-cycle
shaping effect of the inhibition (phasic inhibition), but was also caused by a constant reduction of
activity (tonic inhibition). Although tonic and phasic inhibition were analytically separated in
this study, in reality there may not be a clear boundary between these two inhibitory profiles. In
fact, it was hypothesized that most inhibitory interneurons were synchronized to amplitude
modulation, since 85% of AVCN neurons showed changes in phase at low modulation
frequencies after blocking inhibition. However, the smoothing effect of dendrites that received
inhibitory inputs may have prevented observation of synchrony in the inhibition, especially at
mid and high modulation frequencies where the smoothing was effectively longer than a
modulation cycle. By analyzing phase changes after blocking inhibition in responses to SAM
stimuli with a low modulation frequency (32 Hz), it was found that the majority of choppers with
positive effects in response to inhibitory receptor blockers showed increased phase after blocking
GABAergic inhibition. This result indicates that the inhibition was synchronized to amplitude
modulation and was effectively delayed with respect to excitation (Fig. 12).

Possible sources of inhibition

By comparing the effect of inhibition on responses to broadband noise and pure tones,
this study provided useful information about the identity of inhibitory neurons that projected to
AVCN neurons. In this study, 49% of cells showed stronger responses to noise after blocking
inhibition, while only 4% showed stronger responses to tones. This result suggested that for
glycinergic inhibition, there were possibly fewer vertical neurons (which respond more strongly
to tones) than D-stellate neurons (which respond more strongly to noise) that projected to the
AVCN neurons studied here. However, for the 47% of cells that showed similar increases in rate
for noise-alone and tone-alone stimuli, it was likely that a mixture of inputs from D-stellate and vertical neurons compromised each other’s effects. This result represents a challenge for models of AVCN neurons because of the difficulty in determining the proportions or strength of inhibitory inputs from each type of inhibitory neurons. For bushy cells, different types of models with different sources and functions of inhibitory inputs would be required to explain the observed diversity in the PSTHs. In contrast, models for stellate cells can be relatively homogeneous, because the general effect of inhibition was similar to a constant hyperpolarization.

Commissural inhibition from the other cochlear nucleus was not considered a major source of glycinergic inhibitory activity in the present study because sound was not delivered to the contralateral ear. In addition, Wenthold (1987) shows that neurons in the contralateral CN with commissural projections are large stellate cells, which usually have low or medium spontaneous activity (Blackburn and Sachs 1989; Smith and Rhode 1989). Therefore, even if there were commissural projections to the neurons described in the present study, these inputs would not have provided significant sound-driven or spontaneous IPSPs.

Response properties of other inhibitory interneurons, especially those located in the SOC, are unclear. Neurons in the SOC that project bilaterally to the IC receive descending inputs from the IC, and descending pathways from high auditory levels to the CN can be formed (Schofield and Cant 1999). As stated in the Introduction, the major GABAergic inputs were assumed to come from the SOC. GABAergic inhibition generally had greater effects on both average rate and temporal responses of AVCN neurons; therefore, descending GABAergic inputs apparently play an important role in information processing by AVCN neurons. Identification of the
inhibitory inputs from the SOC and knowledge of their response properties would facilitate future studies of the effects of inhibition on responses of AVCN neurons.
TEXT FOOTNOTES

1The GABAergic inhibition described here only refers to GABA_A-receptor-mediated inhibition, not GABA_B, although both are present in the VCN (Altschuler et al. 1986). Ebert and Ostwald (1995a) report that injecting GABA_A receptor antagonist muscimol had significant effects on neurons that responded to GABA injection, while injecting the GABA_B-receptor antagonist baclofen on the same neurons showed small effects that were not consistent with the GABA effect.

2The 100x microscope became available after the first nine positive-effect neurons (from experiments g340–g354) had been studied. Before the introduction of the microscope, neurons without a positive effect were excluded from this study unless the same penetration yielded other positive-effect neurons.

3One PL showed a threshold increase from 70 to 75 dB SPL. This was not considered to be a real threshold increase, since the testing tone levels were mistakenly offset by 5 dB after blocking inhibition.
ACKNOWLEDGMENTS

We acknowledge the generous contribution of advice and expertise of Donald Caspary, who instructed us in the application of the iontophoresis technique. Steven Chase provided an efficient algorithm for computing the first-spike latency. Shig Kuwada and Lorraine Pawson provided advice and assistance with the piggyback electrodes. Susan Early provided editorial assistance. We also thank Kristina Abrams, Bill Dossert, and the staff of the LAR for help with the animals and experimental setup. This study was supported by NIH NIDCD-01641.
REFERENCES


FIGURE LEGENDS

Fig. 1 A, pure-tone responses of a PL response type (g396u1) before and after injection of bicuculline. Total duration of bicuculline injection was 15 min. Left column, rate-level functions for CF tones (CF = 1808 Hz). Middle and right columns, PSTHs and mean interspike intervals as a function of time in response to a 70 dB SPL tone at CF. The top row shows responses before injection. The middle and bottom rows plot responses after injection. CV, coefficient of variation (Blackburn and Sachs, 1989). B, PL, g342u4, total duration of strychnine injection was 54 min. CF = 1103 Hz (same format as A). Note the appearance of early peaks in the middle panels of the bottom two rows.

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Fig. 3 A, Chp-SA, g381u2, total duration of gabazine injection was 12 min. CF = 1547 Hz. B, Chp-SA, g362u2, total duration of bicuculline injection was 23 min. CF = 543 Hz (same format as Fig. 1). Note the faster chopping and extra cycles in the 2\textsuperscript{nd} row, middle panel of A and in the 2\textsuperscript{nd} row, middle panel of B.

Fig. 4 Chp-S, g362u1, total duration of bicuculline injection was 16 min. CF = 1476 Hz (same format as Fig. 1). Note the faster chopping in the 2\textsuperscript{nd} row, middle panel.

Fig. 5 Unusual response type, g368u2, total duration of drug injection was 23 min, 60 and 7 min for bicuculline, strychnine, and glycine, respectively. CF = 3544 Hz (same format as Fig. 1). Note the early peaks in the middle column, 2\textsuperscript{nd}, 4\textsuperscript{th}, and 5\textsuperscript{th} rows.

Fig. 6 Rate-level functions (odd-numbered columns) and PSTHs (even-numbered columns) in response to CF tones for all neurons before and after blocking glycinergic (Gly) or GABAergic (Gab) inhibition. In the rate-level plots, thick lines are average rates before injection, and thin lines are maximum average rates during or after injection. In the PSTH plots, top panels are PSTHs before injection, and
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Fig. 9 *A*, spontaneous rate (SR) before and after blocking glycinergic or GABAergic inhibition. *B*, percentage of increase of SR vs. percentage of increase of the maximum sound-evoked rate across tone levels after blocking inhibition. Only neurons with control SR > 5 sp/sec are plotted. *C* and *D*, change in spontaneous rate compared to change in sound-evoked rate after blocking inhibition in terms of *t* values. *D* is the shaded area in *C*. Open/filled symbols indicate neurons with negative/positive effects.

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Fig. 11 Rate (odd-numbered columns) and synchrony (even-numbered columns) modulation transfer functions (MTFs) for all neurons before and after blocking glycinergic (Gly) or GABAergic (Gab) inhibition. For rate MTFs, shaded areas indicate increased average rate after blocking inhibition. For sync MTFs, shaded areas indicate decreased synchronization to modulation frequency after blocking inhibition. The y-axis of the rate plots was from 0 to a value slightly larger than the maximum rate.

Fig. 12 Change of phase vs. change of rate for SAM tones with *f*<sub>m</sub> = 32 Hz. The x-axis is the maximum *t* value for a change in rate across all modulation frequencies. The y-axis is the increase of phase in degrees. In *A*, filled symbols indicate changes after blocking glycinergic inhibition and open symbols
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Fig. 13 Responses to SAM tones of three neurons before and after blocking GABAergic ($A$ and $B$) and glycinergetic ($C$) inhibition. The top and middle rows plot r-MTF and s-MTF, respectively. The bottom row plots the period histogram at $f_m = 32$ Hz (two identical cycles are shown for the purpose of illustration). The thin solid and dashed lines represent responses before and after blocking inhibition. The thick dotted line represents responses with simulated tonic inhibition (the r-MTF with simulated tonic inhibition is the same as the r-MTF of the control). CF = 1031, 1476, and 1103 Hz for the neurons in $A$, $B$, and $C$, respectively.

Fig. 14 Synchrony decrease with simulated tonic inhibition vs. synchrony decrease after blocking inhibition. First, the modulation frequency that resulted in a maximal decrease in synchrony after blocking inhibition ($f_m^*$) was identified. Then the average change of synchrony at all modulation frequencies lower than and equal to $f_m^*$ was computed. Filled symbols indicate responses studied after application of glycine antagonists; open symbols indicate application of GABA antagonists.

Fig. 15 $A$ and $B$, average discharge rates for tones in noise (T+N) and same-duration tones (T) before and after blocking GABAergic inhibition for two choppers. Small shifts were added to the plotted tone levels to avoid overlap of error bars. The responses to noises alone are marked as X on the abscissa. The filled squares indicate increases of average rate compared to the response to noise alone with $d' > 1$ (Gai and Carney 2006). $C$, percentage of rate increase for the maximum tone-alone responses across tone level (T) vs. percentage of rate increase for noise alone (N). Filled symbols indicate responses studied with application of glycine antagonists; open symbols indicate application of GABA antagonists. One unusual response that showed 237% (504%) rate increase to T (N) and one chopper that showed 254% (279%) rate increase to T (N) are not plotted because since the rate changes numbers were so large (both were injected with bicuculline). CF = 1476 and 1579 Hz for the neurons in $A$ and $B$, respectively.
Table 1 Summary of positive effects for all recorded neurons

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>PL</th>
<th>PLN</th>
<th>Chp-S</th>
<th>Chp-T</th>
<th>Chp-SA</th>
<th>Unusual</th>
<th>Onset</th>
<th>Overall</th>
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<tr>
<td>Strychnine</td>
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<td>8</td>
<td>5</td>
<td>8</td>
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<tr>
<td></td>
<td>Positive Effect</td>
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<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&gt;10% Recovery</td>
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<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
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<tr>
<td>Bicuculline</td>
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<td>2</td>
<td>1</td>
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<tr>
<td></td>
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<tr>
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<td>0</td>
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<td>0</td>
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<td>0</td>
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<td>4</td>
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<tr>
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</tbody>
</table>

PL, primary-like; PLN, primary-like-with-notch; Chp-S, sustained chopper; Chp-T, transient chopper; Chp-SA, slowly adapting chopper.
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