Analysis of the Role of Inhibition in Shaping Responses to Sinusoidally Amplitude-Modulated Signals in the Inferior Colliculus

R. MICHAEL BURGER AND GEORGE D. POLLAK
Department of Zoology, University of Texas at Austin, Austin, Texas 78712

Burger, R. Michael and George D. Pollak. Analysis of the role of inhibition in shaping responses to sinusoidally amplitude-modulated signals in the inferior colliculus. J. Neurophysiol. 80: 1686–1701, 1998. Neurons in the central nucleus of the inferior colliculus (ICc) typically respond with phase-locked discharges to low rates of sinusoidal amplitude-modulated (SAM) signals and fail to phase-lock to higher SAM rates. Previous studies have shown that comparable phase-locking to SAM occurs in the dorsal nucleus of the lateral lemniscus (DNLL) and medial superior olive (MSO) of the mustache bat. The studies of MSO and DNLL also showed that the restricted phase-locking to low SAM rates is created by the coincidence of phase-locked excitatory and inhibitory inputs that have slightly different latencies. Here we tested the hypothesis that responses to SAM in the mustache bat IC are shaped by the same mechanism that shapes responses to SAM in the two lower nuclei. We recorded responses from ICc neurons evoked by SAM signals before and during the iontophoretic application of several pharmacological agents: bicuculline, a competitive antagonist for γ-aminobutyric acid-A (GABA A) receptors; strychnine, a competitive antagonist for glycine receptors; the GABA A receptor blocker, picrotoxin, and the N-methyl-D-aspartate (NMDA) receptor blocker, (−)-2-amino-5-phosphonopentanoic acid (AP5). The hypothesis that inhibition shapes responses to SAM signals in the ICc was not confirmed. In >90% of the ICc neurons tested, the range of SAM rates to which they phase-locked was unchanged after blocking inhibition with bicuculline, strychnine or picrotoxin, applied either individually or in combination. We also considered the possibility that faster α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors follow high temporal rates of incoming excitation but that the slower NMDA receptors could follow only lower rates. Thus at higher SAM rates, NMDA receptors might generate a sustained excitation that “smears” the phase-locked excitation generated by the AMPA receptors. The NMDA hypothesis, like the inhibition hypothesis, was also not confirmed. In none of the cells that we tested did the application of AP5 by itself, or in combination with bicuculline, cause an increase in the range of SAM rates that evoked phase-locking. These results illustrate that the same response property, phase-locking restricted to low SAM rates, is formed in more than one way in the auditory brain stem. In the MSO and DNLL, the mechanism is coincidence of phase-locked excitation and inhibition, whereas in ICc the same response feature is formed by a different but unknown mechanism.

INTRODUCTION

The central nucleus of the inferior colliculus (ICc) is an auditory region that receives converging inputs from a large number of lower auditory nuclei. The principal projections to the ICc are from the cochlear nucleus, from the lateral and medial superior olives, and from the nuclei of the lateral lemniscus (Bruno-Bechtold et al. 1981; Oliver and Huerta 1992; Ross et al. 1988; Vater et al. 1995; Zook and Casseday 1982b). Many of these projections provide excitatory innervation to the ICc while others provide inhibitory innervation that can be either GABAergic or glycinegic (Glendenning and Baker 1988; González-Hernández et al. 1996; Furbara et al. 1996; Saint Marie and Baker 1990; Semple and Aitkin 1980; Shneiderman et al. 1988; Vater et al. 1992b; Winer et al. 1995).

In this report, we turn our attention to the roles of excitation and inhibition in shaping temporal discharge patterns evoked by “complex” signals in ICc neurons. Specifically, the report is concerned with two principal issues: the degree to which the temporal fluctuations in acoustic signals are preserved in the temporal discharge patterns of ICc neurons and what roles the inhibitory innervation of the ICc play in shaping the temporal discharge patterns of ICc neurons. The temporal fluctuations we focus on are those created by AM, fluctuations that occur naturally in many acoustic signals (Bregman 1990; Gerhardt 1988; Müller-Preuss et al. 1994; Rose and Capranica 1985; Schnitzler 1987; Schuller 1984).

In the laboratory, the natural AM are typically simulated with sinusoidal AM (SAM). These are simple signals composed of only three frequencies, a carrier or center frequency, and two side bands. The frequency of the AM or modulation rate is determined by the frequency separation between the center frequency and side bands.

Previous studies have shown that there is a pronounced difference in the ways that neurons in the auditory nerve respond to SAM signals compared with the ways that neurons in the ICc respond to the same signals. Beginning at the auditory nerve, and continuing in most nuclei below the ICc, the majority of neurons respond to SAM signals with discharges that are phase-locked to the cycles of the modulating waveform, and the phase-locking is evoked by a wide range of modulation rates (Frisina et al. 1990a,b; Huffman et al. 1998; Javel 1980; Joris and Yin 1992, 1998; Rhode and Greenberg 1994; Yang and Pollak 1997). In marked contrast, the vast majority of ICc neurons phase-lock only to low SAM rates, typically <300 Hz (Gooler and Feng 1992; Langner 1992; Langner and Schreiner 1988; Muller-Preuss et al. 1994; Rees and Moller 1983; Reimer 1987; Schreiner and Langner 1988; Schuller 1984), and respond to higher modulation rates either with a response only to the onset of the SAM signal or with sustained discharges that are uncorrelated with the cycles of the modulating waveform.

Given the difference in phase-locking to “high” SAM
rates between ICc neurons and neurons in most lower nuclei, the question arises as to what mechanisms in the ICc transform inputs that largely phase-lock to high SAM rates into outputs that phase-lock only to low SAM rates? Insights into a mechanism that can produce such a transformation are given by studies of the medial superior olive (MSO) and dorsal nucleus of the lateral lemniscus (DNLL) of the mustache bat (Grothe 1994; Yang and Pollak 1997). The MSO and the anterior portion of the DNLL differ from most other lower nuclei in that their neurons phase-lock only to low SAM rates and in this regard are strikingly similar to ICc neurons. Of importance is that the excitatory and inhibitory inputs to both the MSO and anterior DNLL phase-lock to a wide range of SAM rates, as is the case for the inputs to ICc. When blockers of inhibition are iontophoretically applied to MSO or anterior DNLL cells, their discharge patterns change dramatically; when inhibition is intact, they phase-lock only to SAM rates <300 Hz, but when inhibitory inputs are blocked the same cells phase-lock to SAM rates of 500–800 Hz.

These findings lead to the proposal that the phase-locking transformations are created by the coincidence of phase-locked excitatory and inhibitory inputs that have slightly different latencies (Grothe 1994; Yang and Pollak 1997). Specifically, the inhibitory inputs have a slightly longer latency than the excitatory inputs. Thus when a SAM signal with a low-modulation frequency is presented, the phase-locked excitatory inputs and phase-locked inhibitory inputs are interleaved, allowing the excitatory inputs to drive the target cell on a cycle-by-cycle basis (Fig. 1). At higher modulation rates, however, the initial excitation leads the inhibition, thereby allowing the cell to respond to the onset of the signal, but the responses to subsequent cycles of the SAM signal are inhibited because the phase-locked excitatory and inhibitory inputs are now temporally coincident. Blocking inhibition unmasks phase-locked excitatory inputs, which the neurons then follow on a cycle-by-cycle basis, even at high SAM rates.

In short, phase-locking restricted to low SAM rates in the MSO and anterior DNLL is similar to the restricted range of SAM rates that evoke phase-locking in ICc neurons. Moreover, all three nuclei receive both excitatory and inhibitory inputs that phase-lock to high SAM rates. It therefore seems reasonable to hypothesize that phase-locking limited to low SAM rates in the ICc might be a consequence of the integration of phase-locked excitatory and inhibitory inputs with slightly offset latencies as was found in the MSO and anterior DNLL.

Here we report on experiments conducted in the mustache bat ICc that tested the above hypothesis. We chose the mustache bat as an experimental subject for two principal reasons. The first is that the previous experiments on SAM coding in the MSO and anterior DNLL were conducted in mustache bats. The second is that the mustache bat brain stem auditory system is fundamentally mammalian in structure, connectivity, and organization (Pollak and Casseday 1989; Ross et al. 1988; Winer et al. 1995; Zook and Casseday 1982a,b). Consequently, features and mechanisms expressed in the mustache bat’s ICc are likely to be present in the ICc of other, less specialized mammals. We tested the hypothesis by recording the discharges from single units in the ICc evoked by SAM signals both before and after blocking GABAergic and glycinergic inhibition with iontophoretically applied drugs. If the hypothesized mechanism is indeed operative in the ICc, then blocking inhibitory inputs should transform ICc neurons from cells that normally phase-lock only to low SAM rates into cells that phase-lock to a much wider range of temporal fluctuations as was found in the previous studies of the MSO and anterior DNLL.

METHODS

Surgical procedures

Seventeen mustache bats Pteronotus parnaellii parnaellii, were used in this study. Surgical procedures were the same as those used in previous studies (Park and Pollak 1993a,b). Briefly, animals were anesthetized with methoxyflurane inhalation (Metofane, Mallinckrodt Veterinary) and 0.02 mg/g neuroleptic, Innovar-Vet
chamber, where it was placed in a restraining cushion constructed funnels formed by the bat’s pinnae and positioned adjacent to the attached to a platform mounted on a custom made stereotaxic of the microphones and wrapped with cellophane tape. The acoustic colliculus, visible through the skull. 200 V DC and driven as speakers. At the start of each experiment, 0.1 M glycine ( in dH2O, pH 3.5 ± 4.0, Sigma ) ; 10 mM bicuculline iontophoresis Acoustic stimulation, processing of spike trains and excitatory / inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, responses evoked by tone bursts and sinusoidal amplitude-modulated Excitatory / inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, Excitatory / inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, Excitatory / inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, Responses evoked by tone bursts and sinusoidal amplitude-modulated Excitatory / inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, Responses evoked by tone bursts and sinusoidal amplitude-modulated Excitatory / inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, Responses evoked by tone bursts and sinusoidal amplitude-modulated Excitatory / inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, Responses evoked by tone bursts and sinusoidal amplitude-modulated Excitatory / inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, Responses evoked by tone bursts and sinusoidal amplitude-modulated Excitatory / inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, Responses evoked by tone bursts and sinusoidal amplitude-modulated

Electrodes

In a few cases, recordings were taken with a single glass pipette filled with buffered 1 M NaCl and 2% Fast Green ( pH 7.4 ). The fast green allowed the electrodes to be easily visualized during placement over the inferior colliculus. Pipettes were pulled to a tip diameter <1 μm with resistances between 10–20 megohms. Most recordings, however, were made with “piggy back” multibarrel electrodes ( Havey and Caspary 1980 ). A five barrel H configuration blank ( A-M Systems, No. 6120 ) was pulled and the tip blunted to 10–15 μm. Recordings were made with a single barrel micropipette, pulled previously, that was glued to the multibarrel array so that the tip of the recording electrode was 10–20 μm from the blunted end of the multibarrel electrode. The recording electrode and the central barrel of the multibarrel electrode were filled with buffered 1 M NaCl and 2% fast green ( pH 7.4 ). The remaining barrels were each filled with one of the following solutions: 0.5 M γ-aminobutyric acid ( GABA ) ( in dH2O, pH 3.5–4.0, Sigma ) ; 0.1 M glycine ( in dH2O, pH 3.5–4.0, Sigma ) ; 10 mM bicuculline methiodide ( in 0.165 M NaCl, pH 3.0, Sigma ) ; 10 mM strychnine hydrochloride ( in 0.165 M NaCl, pH 3.0, Sigma ) ; 75 mM phaclofen ( in 0.165 M NaCl, pH 3.0, Research Biochemicals International ) ; 70 mM ( - )-2-amino-5-phosphonopentanoic acid ( AP5 ) ( in dH2O, pH 7.4, Research Biochemicals International ) . A retention current of 15 nA was applied to drug-containing barrels with opposite polarity for the drug in solution. The barrels of the multibarrel electrode were connected via silver-silver chloride wires to a six-channel microiontophoresis constant-current generator ( Medical Systems Neurophore, BH-2 ) . The central barrel was connected to the sum channel to balance current in the drug barrels and to reduce current effects on the recorded neuron. The recording electrode was connected via a silver-silver chloride wire to a Dagan AC amplifier ( model 2400 ).

Acoustic stimulation, processing of spike trains and iontophoresis

Search stimuli were tone bursts presented to the contralateral ( excitatory ) ear at a rate of four per second. The search tones were generated by a sine wave function generator ( Wavetek, Model 136 ) and shaped by a custom made analog switch ( Restek Model 15 ) . Tone-bursts shaped by the switch were 20 ms in duration and had 0.5-ms rise-fall times. Tone-burst frequency was monitored by a frequency counter. Tone bursts also could be generated by the computer as could SAM stimuli having any desired modulation depth and modulation rate. A 24-bit digital interface NuBus card ( National Instruments DIO-24 ) and a digital distributor ( Restek model 99 ) communicated a Power Macintosh 7100/66 computer to a two-channel digital attenuator ( Wilsonics, model PATT ) . The outputs of each independently controlled channel of the attenuator were sent to two 1/4-in Bruel and Kjaer microphones biased with 200 V DC and driven as speakers. At the start of each experiment, speakers, with protection grids attached, were inserted into the funnels formed by the bat’s pinnae and positioned adjacent to the external auditory meatus. The pinnae were folded onto the housing of the microphones and wrapped with cellophane tape. The acoustic isolation with this arrangement was ≈40 dB ( Wenstrup et al. 1986 ) .

Spikes were fed to a window discriminator and then to a Macintosh 7100 computer controlled by a Restek Model 45 real time clock. Peristimulus time histograms ( PST ) and rate-level functions ( SAM ) signals with rates ranging from 50 to 500 Hz. Spike counts ( SC ) evoked by 20 repetitions of each signal together with vector strengths ( VS ) are shown ( right ) . This was an unusual neuron because the vector strengths were ≥0.300 at all SAM rates tested. SAM waveforms ( below each histogram ) schematically represent the signals actually presented. Excitatory/inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, intensity was 30 dB SPL.

FIG. 2. Peristimulus time ( PST ) histograms of phase-locked responses evoked by tone bursts and sinusoidal amplitude-modulated ( SAM ) signals with rates ranging from 50 to 500 Hz. Spike counts ( SC ) evoked by 20 repetitions of each signal together with vector strengths ( VS ) are shown ( right ) . This was an unusual neuron because the vector strengths were ≥0.300 at all SAM rates tested. SAM waveforms ( below each histogram ) schematically represent the signals actually presented. Excitatory/inhibitory ( EI ) neuron, best frequency ( BF ) was 45.1 kHz, intensity was 30 dB SPL.
were generated and graphically displayed. The computer also calculated vector strengths, as described by Goldberg and Brown (1969), as well as spike counts. The vector strength (VS) gives a quantitative value of how well the unit phase-locked to the envelope of an SAM signal. The VS was calculated as follows

\[ \text{VS} = \sqrt{\left(\sum \sin (a_i) \right)^2 + \left(\sum \cos (a_i) \right)^2} / N \]

where \( a_i \) is the phase position of a single spike [i.e., (spike time) \( \times \) SAM rate \( \times 360^\circ \)] and \( N \) is the total number of spikes. A value of 1.0 indicates perfect phase-locking, whereas a value of 0.0 indicates

**FIG. 3.** PST histograms of 3 neurons that phase-locked only to SAM rates <300 Hz. A: unit that responded with sustained discharges to a BF tone burst and with phase-locked discharges to SAM rates <300 Hz. Response pattern to SAM rates >300 Hz were similar to pattern evoked by tone burst. EI neuron, BF was 57.7 kHz, intensity was 40 dB SPL. B: unit that responded phasically to a BF tone burst and phase-locked to SAM rates <250 Hz. At higher SAM rates, it responded only at signal onset. EI neuron, BF was 61.0 kHz, intensity was 60 dB SPL. C: unit that phase-locked to SAM rates <250 Hz but discharged weakly or not at all at higher SAM rates. Monaural neuron, BF was 59.5 kHz, intensity was 40 dB SPL. SCs and VSs are shown (right).
firing is uncorrelated with the waveform. To exclude the contribution of the onset response to vector strength, the first 5–10 ms of the response was excluded from the calculation.

Once a unit was isolated, the frequency to which it was most sensitive (its best frequency or BF), threshold at BF, and binaural type were determined audiovisually. Binaural properties were determined by presenting a BF tone burst, 20 dB above threshold, first to the contralateral ear, then to the ipsilateral ear, and then to both ears simultaneously. When presented simultaneously, the intensity at the ipsilateral ear was increased to determine whether ipsilateral stimulation suppressed or enhanced the response evoked by the signal at the contralateral ear.

After this initial evaluation of neuronal properties, computer-generated tone bursts at the unit’s BF and SAM signals were presented to the contralateral ear. The center frequency of the SAM signal always was set at the unit’s BF. The computer generated tone bursts and SAM signals were 70 ms in duration and presented at 10–30 dB above the BF threshold. All SAM signals were 100% modulated and had modulation rates that ranged from 50 to 500 Hz. After recording the discharges evoked by tones and SAM signals, pharmacological agents were iontophoretically applied and the responses to the same signals were recorded again. The agents applied were bicuculline methiodide, a competitive antagonist for GABA<sub>A</sub> receptors (Borman 1988); strychnine, a competitive antagonist for glycine receptors (Curtis et al. 1971); phaclofen, a competitive antagonist for GABA<sub>B</sub> receptors (Borman 1988; Kerr et al. 1987); and AP5, an N-methyl-D-aspartate (NMDA)-receptor blocker (Davis et al. 1992).

During drug application, rate-level functions were monitored while ejection currents of from 10 to 60 nA were applied. For each ejection current, rate-level functions were obtained until the rate-level function, and hence response magnitude, had stabilized. Once responses were stable, the complement of tone bursts and SAM signals was presented again, and the same response features were obtained for comparison with those obtained before the application of drugs.

After acquiring data while drugs were applied iontophoretically, the ejection current was switched off and the cell was allowed to recover. Recovery was complete when both the shape of the rate-level function returned to its predrug form and the maximum spike-count returned to the predrug value. In many cases, however, contact with the unit was lost before recovery was complete. Because recovery times were usually 10–20 min, in these cases we allowed 30 min before searching for another neuron.

FIG. 4. Four units that either failed to phase-lock to any SAM rate or phase-locked weakly to low SAM rates. A–C: responses of 3 units that were primarily onset to tone bursts and to all SAM stimuli. D: neuron that responded with a sustained discharge to tone bursts and did not phase-lock to SAMs. Each unit displayed selectivity for SAM rates by discharging with higher SCs to some SAM rates than to others. SCs are shown at right; VS calculations for D are right of the SCs. A: monaural neuron, BF was 60.8 kHz, intensity was 40 dB SPL. B: EI neuron, BF was 63.6 kHz, intensity was 40 dB SPL. C: monaural neuron, BF was 43.0 kHz, intensity was 40 dB SPL. D: monaural neuron, BF was 63.2 kHz, intensity was 50 dB SPL.
RESULTS

Here we report on the response properties of 195 units recorded from the ICc of the mustache bat. BF, the frequency to which each neuron was most sensitive, ranged from 40 to 65 kHz, although 138 units (70.7%) were tuned to frequencies at or around 60 kHz. The reason for the disproportionate number of 60-kHz neurons was that we targeted the enlarged 60-kHz region of the mustache bat’s ICc (Ross et al. 1988). For each unit, we first determined its BF and then recorded its discharge pattern with BF tone bursts at intensities from threshold to 40 dB above threshold. Of the 195 units, 68% (n = 132) responded with only one or several spikes to the onset of the tone burst (onset pattern) and 32% (n = 63) discharged throughout the duration of the tone burst (sustained pattern). In 135 cells, we also presented BF tone bursts to each ear separately and then simultaneously to determine whether the cell was monaurally or binaurally innervated. Of the 135 cells tested with binaural signals, 53 cells (39%) were monaural, 78 cells (58%) were excitatory/inhibitory (EI) because the discharges evoked by stimulation of the contralateral ear were suppressed by stimulation of the ipsilateral ear, while 4 cells (3%) were excitatory/excitatory and were driven by stimulation of either ear.

Responses of ICc neurons to SAM signals

After this initial evaluation of neuronal properties, we next evaluated the ability of ICc neurons to phase-lock to SAM signals presented to the contralateral ear. A vector strength value of 0.300 was arbitrarily chosen as a cutoff criterion for classifying a response to SAM as being phase-locked. Of the 195 neurons in our sample, 73% (142) phase-locked only to low SAM rates, ≤300 Hz, while 9% (18) phase-locked to SAM rates >300 Hz. Of the 18 cells that phase-locked to higher SAM rates, only 9 (4.5%) locked to rates as high as 500 Hz, the highest SAM rate that we tested. An example of a unit that locked to rates as high as 500 Hz with a vector strength of ≥0.300 is shown in Fig. 2.

As noted above, the majority of ICc neurons phase-locked only to SAM rates ≤300 Hz; at higher modulation rates, the neurons either responded to the SAM signals as they did to a tone burst or did not respond at all, even to the onset of the SAM signal. Two examples of neurons that phase-locked only to low modulation rates and continued to discharge to higher SAM rates with tone burst like responses are shown in Fig. 3, A and B. The onset unit shown in Fig. 3C phase-locked to low rates of modulation but responded weakly or not at all at higher modulation rates.

![Graph showing neuronal responses to SAM signals](image-url)

**Fig. 5.** Neuron illustrating that the range of SAM rates that evoked phase-locked discharges was unchanged by the application of bicuculline and strychnine. **Left:** responses evoked by SAM signals before application of bicuculline or strychnine. **Middle:** responses during bicuculline application. **Right:** responses while strychnine and bicuculline were applied simultaneously. In each case, phase-locked responses were only observed at SAM rates ≤200 Hz. SCs evoked by 20 repetitions of each signal together with vector strengths are shown (right of each histogram). BF was 60.5 kHz, intensity was 30 dB SPL. Ejection currents for each drug are shown at top.
Not all ICc neurons phase-locked to SAM signals. Thirty-five (18%) of the neurons either failed to phase-lock to any SAM signal or responded weakly with an occasional phase-locked discharge at low modulation rates. Four examples are shown in Fig. 4. Depending on their discharge pattern to tone-bursts, these neurons responded with either onset or sustained responses to SAM signals.

Although the cells in Fig. 4 displayed little or no phase-locking, their spike counts changed systematically with SAM rate. The spike counts of some cells were highest at low SAM rates (Fig. 4A), some at high SAM rates (Fig. 4B), whereas the spike counts of others were highest at intermediate rates (Fig. 4C).

Effects of blocking inhibition on neurons that phase-locked to low SAM rates

We next tested the hypothesis that a delayed phase-locked inhibition interacts with a phase-locked excitation to limit the phase-locking of the majority of ICc neurons to low SAM rates. If the hypothesis is correct, then blocking inhibition by the iontophoretic application of antagonists for GABA_A (bicuculline) and glycine (strychnine) receptors should un-mask an underlying excitation that is phase-locked even to high modulation rates, as seen in previous studies of the MSO (Grothe 1994) and DNL (Yang and Pollak 1997).

ICc neurons that displayed changes in phase-locking after inhibition was blocked were rare. Of the 43 units that phase-locked to low SAM rates and to which we applied bicuculline, strychnine, or both drugs, only 3 (7%) increased the range of SAM rates to which they phase-locked by ≥150 Hz. In the other 40 units (93%), neither the application of bicuculline nor of strychnine substantially changed the range of SAM rates to which the neurons phase-locked. We illustrate the ineffectiveness of blocking inhibition on phase-locking with the units shown in Figs. 5–7. For the unit in Fig. 5, clear phase-locked discharges were evoked in the predrug condition by SAM rates of 50 and 100 Hz, whereas 150 Hz evoked only occasional phase-locked spikes. Higher SAM rates evoked responses largely at the onset of the signals. The application of bicuculline increased response magnitude, an effect of bicuculline commonly seen in this and previous studies (Faingold et al. 1989; Le Beau et al. 1996; Pollak and Park 1993; Vater et al. 1992a), but phase-locked discharges still were evoked only by SAM rates ≤150 Hz. When strychnine was applied together with bicuculline, no

FIG. 6. Another neuron in which the range of SAM rates that evoked phase-locked discharges was not changed when inhibition was blocked by application of strychnine or bicuculline. Left: predrug condition where phase-locked responses were evoked only by SAM rates <200 Hz. Middle: responses during application of strychnine. Right: responses during application of strychnine and bicuculline. SCs and VSs are shown to the right of each histogram. EJ neuron, BF was 60.3 kHz, intensity was 40 dB SPL. Ejection currents for each drug are shown at top.
additional effect was obtained either on response magnitude or on range of SAM rates that elicited phase-locking.

The phase-locking of the unit in Fig. 6 was also unaffected when inhibition was blocked. In this case, the effects of strychnine alone initially were evaluated. The principal effect of strychnine was to increase the response magnitude, but it had little or no effect on the range of SAM rates that evoked phase-locking. Bicuculline then was applied with the strychnine and did not appreciably change the range of SAM rates that evoked phase-locked discharges.

We also considered the possibility that the failure of the antagonists to allow phase-locking to higher SAM rates may have been due to the application of an insufficient dosage of drugs. We therefore evaluated phase-locking in 10 units by applying drugs with increasingly higher ejection currents. Increased dosages, however, did not substantially change the range of SAM rates that evoked phase-locking in any of the units tested. An example is shown in Fig. 7. In this unit, we first documented responses to SAM signals when bicuculline was applied with an ejection current of 30 nA and then with higher ejection currents of 40 nA (not shown) and 60 nA.

Although response magnitude increased in a dose-dependent manner, there was little change in the range of SAM rates that evoked phase-locking from that observed in the predrug condition.

In summary, blocking inhibition in the vast majority of cells that phase-locked to low SAM rates did not unmask an underlying excitatory drive that was phase-locked to all SAM rates tested. The results provide little support for the hypothesis that phase-locking limited to low SAM rates is due to inhibitory innervation.

Effects of blocking inhibition on neurons that did not phase-lock to SAM

The results of blocking inhibition in 11 neurons that displayed no phase-locking to SAM signals were only slightly different from those obtained with neurons that phase-locked to low SAM rates described above. In 4 of the 11 units, SAM signals evoked no phase-locked responses either before or during the blockage of inhibition. In only 2 of the 11 neurons was there a pronounced change in phase-locking. One is

![FIG. 7. Range of SAM rates that evoke phase-locking was not changed by increasing dosages of bicuculline. Left: responses evoked by SAM rates before application of bicuculline. Also shown are responses to same signals evoked while bicuculline was applied with an ejection current of 30 nA (middle) and then with a current of 60 nA (right). Regardless to current level, bicuculline caused an increase in response magnitude but did not cause neuron to phase-lock to higher SAM rates than it did before bicuculline was applied. SCs and VSs are shown to the right of each histogram. EI neuron, BF was 60.1 kHz, intensity was 40 dB SPL.](http://jn.physiology.org/Downloaded from http://jn.physiology.org/ by 10.22.33.1 on July 8, 2017)
shown in Fig. 8. Strychnine was not applied to this cell so we are uncertain as to whether additional changes would have occurred had glycinerigic inhibition been blocked as well.

Five other units had less substantial changes than the unit in Fig. 8. In at least some of those neurons, the small changes occurred when both bicuculline and strychnine were applied. Small changes in phase-locking are illustrated by the neuron in Fig. 9. In the predrug condition, this cell responded almost exclusively with an onset response to SAM signals and had a much greater spike count to low SAM rates than to higher rates. Occasionally phase-locked discharges were evoked by 50- and 100-Hz rates, but these were rare. Bicuculline caused an overall increase in response magnitude. In addition, it seemed to block a weak sustained inhibition previously evoked by SAM rates of 50 and 100 Hz because the size of the phase-locked discharge peaks appeared to increase to a somewhat greater degree than did the onset responses. However, onset discharges were evoked almost exclusively by SAM rates of $\geq 150$ Hz. The addition of strychnine transformed the response pattern evoked by tone bursts from an onset to a sustained pattern. Now 50-Hz SAM signals evoked a strong response. However, the phase-locking was not very precise (the vector strength was 0.323) because the unit responded throughout the duration of each modulation cycle rather than to a specific portion of the phase of each cycle. Surprisingly, SAM rates of 100 and 150 Hz also evoked sustained discharges, but the phase-locking was poor (the vector strength was $\leq 0.300$) and the peaks were of lower amplitude than at 50 Hz. At SAM rates of $\geq 200$ Hz, only weak sustained discharges were evoked that were not phase-locked.

To summarize, blocking inhibition in neurons that did not phase-lock to SAM signals resulted in enhanced phase-locking to low modulation rates (i.e., $\leq 250$ Hz) in only a few neurons. In most of these cells, blocking inhibition caused only a small enhancement in phase-locking to modulation rates of 50 or 100 Hz, although response magnitude often increased markedly. In none of these cells did the application of bicuculline or strychnine allow the cell to phase-lock to SAM rates of 500 Hz, as was found for neurons that did not phase-lock to SAM in a previous study of the mustache bat DNLL (Yang and Pollak 1997).

**Results of blocking GABA$_B$ receptors**

The results presented above indicate that the ability of ICc neurons to phase-lock only to low SAM rates is not a feature shaped by either GABA$_A$ or glycine receptors. Although these are the principal inhibitory receptors in the ICc (Fubara et al. 1996), evidence from both receptor binding
FIG. 9. PST histograms showing slight enhancement of phase-locking while inhibition was blocked for a neuron that, in the predrug condition, did not phase-lock to SAM. SCs and VSs are shown to the right of each histogram. EI neuron, BF was 60.8 kHz, intensity was 40 dB SPL. Ejection currents for each drug are shown at top.

(Fubara et al. 1996) studies and pharmacological studies (Faingold et al. 1989) indicate that at least some ICc neurons also possess GABA_B receptors. In contrast to GABA_A and glycine receptors, which are directly gated ligand receptors, GABA_B receptors act through G proteins (Nicholl 1988) and are thus slower than the GABA_A receptors. It seems possible, therefore, that the release of GABA from inputs that follow even high SAM rates with phase-locked discharges could activate GABA_B receptors on ICc neurons that, in turn, would generate a more sustained inhibition than that generated by the directly gated receptors. If this were the case, then blocking GABA_A and glycine receptors with bicuculline and strychnine would leave the putative slower GABA_B-mediated inhibition unaffected. In this hypothesis, GABA_B receptors would be the main agents responsible for preventing phase-locking to high SAM rates.

To test this hypothesis, we blocked GABA_B receptors in eight neurons that phase-locked only to low SAM rates. In each neuron, we first applied the GABA_B receptor blocker, phaclofen, and then added both bicuculline and strychnine to block both the GABA_A as well as the GABA_B and glycine receptors. The hypothesis was not confirmed in any of the eight cells tested. A representative example is shown in Fig. 10. The application of phaclofen caused a small increase in response magnitude in this cell, as was typical, whereas bicuculline and strychnine caused a much larger increase in response magnitude. However, the range of SAM rates that evoked phase-locking was unchanged by the application of phaclofen or by the combined application of phaclofen, bicuculline, and strychnine.

Results of blocking NMDA receptors

We next considered the possibility that slower excitatory receptors might be mediating the response features evoked by SAM signals. If both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors contribute to the excitatory response of ICc cells, one explanation for some units might be that although the faster AMPA receptors follow high temporal rates of incoming excitation, the slower NMDA receptors follow only lower rates. Specifically, in units that responded with more sustained but nonphase-locked responses to higher modulation frequencies, it seems possible that each cycle of a high-frequency SAM signal could generate a phase-locked AMPA receptor discharge that was smeared by a ‘‘longer lasting’’ discharge due to NMDA currents. If this hypothesis is correct, then blocking inhibition should increase response magnitude but should have little effect on the range of SAM rates to which the neurons phase-lock because the excitatory response of the cell is itself smeared by the NMDA component of excitation. On the other hand, blocking both inhibition and NMDA receptors should unmask a phase-locked AMPA response that is neither ‘‘contaminated’’ by NMDA currents nor suppressed by GABAergic inhibition.

To test the NMDA hypothesis, we recorded from seven neurons that phase-locked only to low SAM rates and blocked NMDA receptors by the iontophoretic application of AP5 together with bicuculline. The hypothesis predicts that after blocking NMDA and GABA_A receptors, an uninhibited underlying AMPA response should be revealed that phase-locks even to high SAM rates.
FIG. 10. Neuron showing that blocking of \( \gamma \)-aminobutyric acid-B (GABA\(_B\)) receptors with phaclofen as well as blocking GABA\(_A\) and glycine receptors with bicuculline and strychnine fails to increase range of SAM rates that evoke phase-locking. 

Left: strong phase-locked responses were elicited only by SAM rates \(<\)200 Hz before drugs were applied. Middle: when GABA\(_B\) receptors were blocked by phaclofen, response magnitude increased but phase-locked discharges were still evoked only by SAM rates \(<\)200 Hz. Right: when bicuculline and strychnine were applied together with phaclofen, there was a further increase in response magnitude but there was no increase in the SAM rates that evoked phase-locking. 

SCs and VSs are shown to the right of each histogram. EI neuron, BF was 61.2 kHz, intensity was 30 dB SPL. Ejection currents for each drug are shown at top.

The NMDA hypothesis, like the hypothesis we proposed for GABA\(_B\) receptors, was not confirmed. In none of the seven cells did the application of AP5 by itself, or in combination with bicuculline, cause a significant increase, or any increase at all, in the range of SAM rates that evoked phase-locking. An example is shown in Fig. 11. In this unit bicuculline was first applied and caused an increase in discharge magnitude but did not cause the cell to phase-lock to higher SAM rates than it did before bicuculline was applied. Adding AP5 to the bicuculline caused a depression of response magnitude that presumably was due to the blockage of NMDA currents. A depression of evoked responses by AP5 was typically seen when AP5 was applied alone or in combination with bicuculline. The significant feature, however, was that the range of SAM rates to which the neuron discharged with phase-locked responses was unchanged by either bicuculline or by AP5 in combination with bicuculline.

**Discussion**

The principal goal of this study was to test the hypothesis that coincidence of phase-locked excitation and inhibition is the underlying mechanism that limits phase-locking to low rates of SAM stimuli in ICc neurons. Coincidence is employed widely by the auditory system for determining a variety of monaural and binaural response features. Among these are the coding of interaural disparities in the superior olivary complex (Joris and Yin 1995; Park et al. 1996), the shaping of phase-locked discharges in the mustache bat’s MSO and DNLL (Grothe 1994; Yang and Pollak 1997), the tuning for signal duration in the ICc (Casseday et al. 1994; Ehrlich et al. 1997), and the delay tuning in the ICc, medial geniculate body, and cortex of bats (Mittmann and Wenstrup 1995; Olsen and Suga 1991; O’Neill and Suga 1981). The acoustic stimuli that generate the coincidences are different in each of these cases, but the process is the same. Thus we reasoned that if a common mechanism limits phase-locking to low SAM rates in three successively higher regions of the auditory pathway, the MSO, DNLL and ICc, it would provide additional evidence that coincidence is a common strategy employed by the mammalian auditory system for shaping a wide variety of response features, including temporal discharge properties evoked by “complex” signals. It is for
FIG. 11. PST histograms from a neuron in which the range of SAM rates that elicited phase-locked responses was unchanged by simultaneously blocking NMDA receptors with AP5 and GABA<sub>A</sub> receptors with bicuculline. Left: responses evoked by SAM rates before AP5 or bicuculline was applied. Middle: when bicuculline was applied by itself, it caused an increase in response magnitude with little change in the range of SAM rates that evoked phase-locked discharges. Right: responses change little in range of SAM rates that evoked phase-locking during application of AP5 and bicuculline. SCs and VSs are shown on the right of each histogram. BF was 60.1 kHz, intensity was 40 dB SPL. Ejection currents for each drug are shown at top.

these reasons that we were surprised to find that the blockage of inhibitory receptors, whether GABA<sub>A</sub>, glycine, or GABA<sub>B</sub> receptors, had virtually no effect on the range of SAM rates that evoked phase-locked discharges in the vast majority of ICc neurons. Given the difference in the results that we expected from those we observed, we consider, in the following sections, some potential explanations for this disparity.

**Did the antagonists fail to reach appropriate receptors?**

The first potential explanation is that coincidence of phase-locked excitation and inhibition is, in fact, the mechanism shaping responses to SAM in the ICc but that the application of antagonists failed to reach the appropriate receptors and thus did not block the critical inhibitory synapses. The possibility that drugs failed to reach the appropriate receptors is an inherent problem with in vivo iontophoretic studies, but the weight of evidence makes this explanation unlikely. One piece of the evidence arguing against this explanation is that the application of bicuculline or strychnine typically caused a substantial increase in response magnitude, showing that the antagonists were effective in blocking at least some inhibitory receptors. Moreover, these antagonists were applied continuously for periods of 10–30 min, and in several neurons, more than one injection current was applied that caused a dose-dependent increase in response magnitude without changing the SAM rates that evoked phase-locking. Because there are no high-affinity uptake mechanisms for bicuculline or strychnine, the effects of antagonists last minutes or tens of minutes after application. These periods of effectiveness allow the antagonists to diffuse up to several hundred micrometers from the ejection electrode. We know this because, in some cases, contact with a unit was lost during application of the antagonist, and search for another unit was initiated immediately thereafter. When a second neuron was recorded several hundred micrometers from the previous unit, it was found that the second unit’s activity was super normal and that its activity would recover progressively within 5–20 min, suggesting that the antagonists applied at the site of the first neuron had diffused several hundred micrometers to block inhibition at the second neuron. These observations suggest that the spread of the antagonists used in this study was sufficient to allow the agents to block receptors on most of the dendritic trees of the cells to which they were applied. When the features above are considered together with the paucity of units whose phase-locking to SAM changed after application of antagonists, it seems to us that a failure to block critical receptors is an unlikely explanation for the results we obtained.
Do ICc cells simply follow the temporal synchrony of their inputs?

A second potential explanation is that most excitatory inputs to ICc cells phase-lock only to low rates of SAM, and the assumption that the predominance of excitatory input to ICc cells phase-lock to high SAM rates is incorrect. In this explanation, the phase-locking of ICc cells simply follows the phase-locking of their inputs.

This may well be the explanation for some cells but almost surely is not the explanation for most. The reason is that, with one exception, the majority of nuclei that provide excitatory projections to the ICc have neurons that phase-lock to SAM rates well >400 Hz, and most phase-lock to rates substantially higher. This has been reported for the types of cochlear nucleus cells that project to the ICc, multipolar, and fusiform cells (Frisina et al. 1990a,b; Rhode and Greenberg 1994), almost all cells in the LSO (Joris and Yin 1998), and for most cells in the intermediate nucleus of the lateral lemniscus (Huffman et al. 1998). The exception is the mustache bat MSO. The mustache bat MSO is unusual in that it receives only sparse projections from the ipsilateral cochlear nucleus and is innervated largely by the contralateral cochlear nucleus and medial nucleus of the trapezoid body (Covey et al. 1991; Grothe et al. 1992). As mentioned previously, MSO cells phase-lock only to low SAM rates due to the interactions of phase-locked excitation from the cochlear nucleus and phase-locked inhibition from the medial nucleus of the trapezoid body (Grothe 1994; Grothe et al. 1992). MSO projects heavily onto the ICc in the mustache bat (Ross and Pollak 1989; Ross et al. 1988) and thus could be a source of excitatory inputs to some ICc cells that phase-lock only to low SAM rates. However, given the large number of excitatory inputs that phase-lock to high rates, it seems unlikely that MSO inputs alone can explain the phase-locking to low SAM rates that predominate in the ICc.

The dominance of inputs that phase-lock to high SAM rates is reflected in the responses of neurons in the DNLl, the auditory nucleus immediately below the ICc. Because numerous features of the DNLl are similar to portions of the ICc, it is instructive to compare the phase-locking of neurons in the mustache bat’s DNLl with those in the ventromedial region of the mustache bat’s ICc. Both regions are dominated by excitatory/inhibitory neurons (Ross and Pollak 1989; Wenstrup et al. 1986; Yang et al. 1996) and both receive a similar complement of projections: from the ipsilateral MSO, from the LSO bilaterally, from the contralateral DNLl through the commissure of Probst, and from the ventral and intermediate nuclei of the lateral lemniscus (Ross and Pollak 1989; Vater et al. 1995; Yang et al. 1996). Although the binaural properties and complement of afferent projections are similar in the two regions, their phase-locking to SAM and effects of blocking inhibition on phase-locking are vastly different. The mustache bat DNLl is divided into a larger posterior division and a smaller anterior division (Yang and Pollak 1997; Yang et al. 1996). Posterior DNLl cells discharge with sustained patterns to tone bursts and routinely phase-lock to SAM rates of 600–800 Hz or higher. Because DNLl cells are predominately GABAergic (Adams and Mugniani 1984; Vater et al. 1992b; Winer et al. 1995) and project bilaterally to the ICc, these cells almost certainly provide phase-locked inhibitory innervation to the ICc. Anterior DNlll cells are different. They discharge with onset patterns to tone bursts and phase-lock only to low SAM rates, features that are virtually indistinguishable from most onset ICc cells. Unlike ICc cells, however, blocking inhibition unmasks an underlying phase-locked excitatory input that allows anterior DNLl cells to phase-lock to SAM rates of 500–600 Hz and display response properties closely resembling posterior DNLl cells (Yang and Pollak 1997). Because the DNLl and EI region of the ICc receive similar inputs, it seems unreasonable to argue that DNLl receives excitatory innervation that phase-locks to high SAM rates while the same innervation provides EI cells in the ICc with excitation that phase-locks only to low SAM rates.

In summary, although there are some excitatory inputs to ICc that phase-lock only to low SAM rates, the majority of excitatory inputs and at least some inhibitory inputs phase-lock to a wide range of SAM rates. Moreover, two regions, the DNLl and EI region of the ICc, receive a similar complement of inputs yet display pronounced differences in their responses to SAM and blocking inhibition. Some observations made by Covey et al. (1996) are also relevant in this regard. They showed, with whole cell recordings, that at least some IC neurons receive subthreshold excitatory inputs that follow high stimulus modulation rates although those rates did not evoke phase-locked discharges. These features, then, provide little support for the hypothesis that the phase-locking of ICc neurons is simply a reflection of inputs that lock only to low SAM rates but rather suggest that many, if not most, excitatory inputs to ICc cells phase-lock to high SAM rates.

Are “other,” “slower” receptors involved?

Another potential explanation that we considered previously is the involvement of slower receptors, such as GABAA or NMDA receptors. These receptors might not be able to follow the higher phase-locked inputs and thus would produce “smeared” responses at the higher SAM rates. However, phase-locking to higher SAM rates did not occur when these receptors were blocked or when they were blocked together with GABAA or glycine receptors. These results, then, do not support the hypothesis that “slower” receptors are the principal agents that prevent ICc neurons from phase-locking to higher SAM rates.

Are intrinsic membrane properties different in the ICc than they are in lower nuclei?

If the explanations discussed above are unlikely, then what explanation might be advanced to account for the results presented here? One possibility is that the complement of voltage-sensitive channels in ICc neurons acts to restrict the phase-locking to low SAM rates. There is now a wealth of studies showing that the particular complement of voltage-sensitive channels plays a critical role in determining the response properties of neurons in a variety of auditory nuclei (Fu et al. 1996; Manis 1990; Oertel et al. 1988; Perney and Kaczmarek 1997; Reyes and Spain 1994; Reyes et al. 1996; Rhode and Smith 1986). However, there is no direct evidence that confirms the hypothesis that voltage-sensitive
channels limit phase-locking to low SAM rates in ICc neurons, although the results from a study by Fortune and Rose (1997) are consistent with this hypothesis. They obtained “whole cell” recordings from midbrain neurons in electric fish evoked by amplitude- and frequency-modulated signals. They found that in some neurons, active conductances were prominent and contributed significantly to the low-pass filtering of those cells. It should be noted, however, that the modulation frequencies they employed ranged only from 2 to 30 Hz and were much lower than those used in this and other studies of AM coding in the mammal auditory system. An unpublished intracelluar study of slices from rat IC neurons by Peruzzi and Oliver (personal communication) is more germane. They found that cells that had particular voltage-sensitive potassium channels discharged on a one-to-one basis to brief, depolarizing current pulses with frequencies ≤110 Hz, whereas cells that lacked those channels could only follow frequencies <50 Hz.

It should be noted that neither the results reported by Fortune and Rose (1997) nor the results from the Peruzzi and Oliver study can account for the features that we found in the bats’ IC. Rather those studies only suggest that voltage-sensitive channels may contribute to the low-pass filtering of IC neurons in mammals. Consequently, the suggestion that inherent membrane properties might explain our results is not based on strong supporting evidence from studies of channel properties in ICc neurons but rather is proposed because channels, together with innervation patterns, shape neuronal discharge patterns and because other explanations can either be ruled out or are unlikely.

Concluding comments

Although we can only offer a speculation of the mechanism that limits phase-locking in the ICc, there is a significant, yet puzzling feature of the auditory system revealed by the results of this study; specifically, the mechanisms that limit phase-locking in the ICc are different from the coincidence detection of excitation and inhibition that limits phase-locking in the MSO and DNL. These are puzzling features because they make us wonder why the auditory system recreates what is seemingly the same response property at least three times in successively higher order nuclei, and why neurons in the ICc employ a different mechanism from the mechanism employed in the MSO and DNL to recreate the same response property? Our interpretation of these features is that the mechanisms underlying restricted phase-locking in ICc are not designed for processing AM per se. Rather they are reflective of a broader processing strategy utilized by the ICc for the integration of temporally patterned excitation and inhibition and are more complex or at least are partially different from the processes employed by lower auditory nuclei. What the full ramifications of these mechanisms are for information processing of naturally occurring complex signals at the ICc is, at the moment, unclear, but their clarification represents one of the principal challenges for auditory neuroscience.

We thank C. Resler for designing and implementing the electronics for data acquisition. We also thank B. May, E. Bauer, A. Klug, T. Park, and L. Hurley for critical comments.

This work was supported by National Institute of Deafness and Other Communications Disorders Grant DC-00268.

Address reprint requests to G. D. Pollak.

Received 20 April 1998; accepted in final form 23 June 1998.

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