Intracellular recording reveals temporal integration in inferior colliculus neurons of awake bats

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Abstract:

The central nucleus of the inferior colliculus (IC) is a major integrative center in the central auditory system. It receives information from both the ascending and descending auditory pathways. To determine how single IC neurons integrate information over a wide range of sound frequencies and sound levels, we examined their intracellular responses to frequency modulated (FM) sounds in awake little brown bats (*myotis lucifugus*). Postsynaptic potentials were recorded in response to downward FM sweeps of the range typical for little brown bats (80-20 kHz) and to three FM subcomponents (80-60 kHz, 60-40 kHz, and 40-20 kHz). The majority of recorded neurons responded to the 80-20 kHz downward FM sweep with a complex response. In this response an initial hyperpolarization was followed by depolarization with or without spike followed by hyperpolarization. Intracellular recordings in response to three FM subcomponents revealed that these neurons receive excitatory and inhibitory inputs from a wide range of sound frequencies. One third of IC neurons performed nearly linear temporal summation across wide range of sound frequencies whereas two thirds of IC neurons exhibited nonlinear summation with different degrees of nonlinearity. Some IC neurons showed different latencies of postsynaptic potentials in response to different FM subcomponents. Often responses to the later FM subcomponent occurred before responses to the earlier ones. This phenomenon may be responsible for response selectivity of IC neurons to FM sweeps.
INTRODUCTION

Natural sounds, including animal communication sounds and human speech contain both amplitude and frequency modulations (FM). This modulation creates a temporal structure that is a major source of information for sound recognition including speech (Shannon et al. 1995). Therefore, it is important to understand how the auditory system extracts temporal information from complex sounds.

Numerous studies using FM sounds have shown that the temporal structure of frequency modulation is reflected by neuronal responses in the auditory nerve and in lower auditory brain stem nuclei. At progressively higher levels of the auditory system, neurons become more selective for FM (review: Langner 1992). The inferior colliculus (IC), a large midbrain auditory processing center in vertebrates, contains neurons that are tuned to narrow ranges of modulation frequencies (Casseday et al. 1997; Langner and Schreiner 1988; Schuller 1979; Rees and Moller 1983).

The first systematic study of FM response selectivity in IC neurons was performed in Myotis lucifugus (Suga 1968). Suga found a number of neurons that only responded to FM sweeps but not to pure tones. Similar results were reported later in the IC of other bats (Casseday and Covey 1992; Fuzessery 1994) and rats (Poon et al, 1992).

A few possible neural mechanisms that can underlie FM rate and direction tuning in IC neurons of mustached and pallid bats have been proposed (Brimijoin and O’Neill 2005; Fuzessery et al 2006; Heil et al 1992; Rall 1964; Suga 1965; Suga and Schlegel 1973). Sideband inhibition model proposes that a
directionally selective neuron receives two inputs, one excitatory and the other inhibitory. Typically the best frequencies of these opposing inputs are different. Sweeps in the preferred direction will elicit stronger responses because they stimulate the excitatory area before the inhibitory (Brimijoin and O’Neill 2005; Fuzessery et al 2006; Heil et al 1992; Rall 1964; Suga and Schlegel 1973). By contrast, sweeps in the not preferred direction elicit weaker responses because they activate the inhibitory area before the excitatory. Another model (Serial excitation model) is based on temporally offset, sub-threshold excitatory inputs that are anatomically ordered by best frequency along a dendrite (Rall 1964). An FM sweep that stimulates the inputs in a sequence towards the soma results in summation, whereas stimulation in sequence away from the soma does not. Thus FM selectivity is likely to be a result of the spectrotemporal interaction of excitatory and inhibitory inputs on IC neurons.

IC neurons also showed response selectivity for a number of other stimulus parameters such as interaural intensity difference (Klug et al. 1995; Park and Pollak 1993, 1994), sound duration (Casseday et al. 1994; Covey et al. 1996), sound frequency and sound level (Faingold et al. 1989; Le Beau et al. 1996; Pollak and Park 1993; Vater et al. 1992). These types of response selectivity further suggest that spectrotemporal integrations at the level of the auditory midbrain might (at least partially) be responsible for the response selectivity observed in IC neurons. At present little is known about postsynaptic mechanisms underlying this integration.
Our goal was to study postsynaptic mechanisms underlying spectrotemporal integration in the IC. Intracellular responses were recorded in the IC of the little brown bat in response to different FM sweeps. Bats have proven to be excellent models for studying the processing of FM sounds because these sounds are the most common signals that bats use for echolocation and communication (Simmons and Stein 1980; Simmons et al. 1979).

We found that majority of IC neurons show postsynaptic responses to a wide range of sound frequencies. The most common response pattern to FM sweep is IPSP-EPSP(spike)-IPSP. About one third of IC neurons perform nearly linear temporal summation across wide range of sound frequencies. This study shows that mechanisms of spectral integration are important for complex sound processing in the IC.
METHODS

Experimental preparation.

Experimental subjects comprised 22 little brown bats, *Myotis lucifugus*. For surgery, the animal was anesthetized via isoflurane inhalation (1.5 - 2.0%, isoflurane administered by a precision vaporizer). After incision of the skin and clearing of the tissues above the skull, a small metal rod was glued to the skull using glass ionomer cement. Following the surgery, animals were allowed to recover for 2-3 days in individual holding cages.

Recordings were made from awake bats. During the recording session the animal was placed inside a single walled sound attenuating chamber (Industrial Acoustics Company, Inc). The metal rod on the bat’s head was secured to a small holder for restraining the animal's head atraumatically, leaving the ears unobstructed for free-field acoustic stimulation. A small hole (~ 50 µm) was then made in the skull overlying the IC through which a recording electrode was inserted to reach the IC. Throughout the recording session, the animal was offered drinking water periodically and monitored for signs of discomfort. After a recording session of 6-8 hours, the exposed skull was covered with sterile bone wax, and the animal was returned to its holding cage. Such experiments proceeded every 2-3 days for a maximum of 2 weeks.

Procedures used in this study were approved by the Institutional Animal Care and Use Committees at the Northeastern Ohio Universities College of Medicine.
**Acoustic Stimulation**

Acoustic stimuli, comprising linear downward FM sweeps, were delivered to the bat via a free field ultrasonic loudspeaker (Ultra Sound Advice US-LS) located 30 cm in front of the bat. The outputs of the loudspeaker were measured with a 1/4-inch microphone (Brüel and Kjaer 4135) and found to be flat ±6 dB between 20 and 80 kHz, the frequency range used in the experiments. The parameters of the acoustic stimuli were controlled by D/A hardware and software from Tucker-Davis Technologies (System III) with sampling rate 200 kHz. FM stimuli that mimicked the bats’ natural FM signal were swept from 80 kHz to 20 kHz in 4 ms (including 0.25 ms rise/fall time) and were termed the *entire* FM sweep. The entire FM sweep was also arbitrarily divided into three equal 20 kHz subcomponents (80 kHz to 60 kHz, 60 kHz to 40 kHz, and 40 kHz to 20 kHz). Each subcomponent had 1.33 ms duration including 0.25 ms rise/fall time. All FM sweeps were presented at a wide range of sound levels, from 0 to 80 dB SPL. Stimuli were delivered at a rate of 4 pulses per second. Unless otherwise specified, for each recorded neuron we presented four different types of FM sweeps (entire FM sweep and three FM subcomponents) at 0, 20, 40, 60, and 80 dB SPL. The entire protocol was repeated as many times as possible while the neuron showed a stable membrane resting potential (fluctuations of the baseline do not exceeded range of a few mV). Only neurons that were tested at least three times with the entire protocol were included in our data analysis.

**Recording procedure**
Microelectrodes were made from 1.2 mm diameter quartz glass (Sutter Instruments Company, Novato, CA) filled with 3 M potassium acetate. Micropipettes, with impedance between 50-120 MΩ were pulled on a Flaming-Brown micropipette puller (Sutter P2000). The electrode was positioned above the IC by means of a precision digital micromanipulator and lowered to the dorsal brain surface. The relative position of each electrode was monitored from the readouts of digital micrometers using a common reference on the skull. Vertical advancement of the electrode was made by a precision microdrive in 2 – 3 µm steps (KOPF Model 660) from outside the sound attenuating chamber. After placement of the electrode on the surface of the IC using a surgical microscope (Leica MZ9.5), the exposure was filled with 4% agar.

Intracellular responses of IC neurons were amplified through a single channel amplifier (CYGNUS Technology Inc. model IR183A) and monitored on a digital oscilloscope (YOKOGAWA DL1640). Intracellular waveforms from IR183A and sound stimuli from Tucker Davis system were digitized and then stored on a computer hard drive using EPC-10 digital interface and PULSE software from HEKA at bandwidth of 100 kHz. In order to detect cell impalement, small (5-100 nA) current pulses of 100 ms duration were delivered through the microelectrode. A sudden negative DC shift and the presence of synaptic potentials indicated an intracellular impalement, which was often verified by passing positive current to evoke action potentials. Stable intracellular impalements were signaled by a prolonged (> 3 min), stable drop (>40 mV) in the DC level. Intracellular recordings typically lasted 3 – 5 min (maximum 40 min). During intracellular
recording cell membrane resting potential usually fluctuated not more than a few mV. Successful intracellular recording was always accompanied by the presence of postsynaptic potentials and in almost all our recordings by large action potentials (> 30 mV). Each of our recorded cells satisfied all of the above criteria for being studied with our experimental protocol. Sudden decrease in membrane resting potential was typical just before a recorded neuron was lost. Intracellular recordings were performed for IC cells located at depths of 200 µm to 1200 µm in order to predominantly collect responses from cells in the central nucleus of the IC. Different angles of electrode penetrations were used in different experiments for the same animal to avoid local damage of the IC. Therefore, recording depth indicated for neurons presented in this manuscript does not give an exact location of a recorded neuron in the IC along dorsoventral axis.

*Data analysis.*

During intracellular recording we often observed response variability from stimulus presentation to another. Therefore an algebraic average for all traces recorded at each stimulus parameter was calculated (Fig.1). A baseline value was first calculated by determining the mean value for all samples of each waveform for a time window at least 200 ms (sampling rate = 10 microseconds). Since spikes and/or large PSPs could alter the mean value we eliminated all values that exceeded one standard deviation from that mean. Then a new baseline mean value was calculated. PSPs were defined as depolarizing or
hyperpolarizing fluctuations on the averaged traces that exceeded two standard deviations (95% confidence limits) from the baseline and occurring within 50 ms time window after stimulus on-set. PSPs latencies were calculated to the crossing the two standard deviations threshold.

Off-line data analysis and statistical analysis were made using custom software written by Olga Galazyuk.

RESULTS

*Complex postsynaptic responses of IC neurons*

Intracellular responses of 117 IC neurons to FM stimuli (80-20 kHz, 4 ms duration, and 0.25 ms rise/fall time) were recorded in 22 awake little brown bats.

Neurons in the IC displayed a variety of synaptic responses to acoustic stimulation that reflected both excitatory and inhibitory inputs. The most common response pattern (73/117 units or 62%) was hyperpolarization followed by depolarization with or without spike followed by hyperpolarization [IPSP-EPSP(spike)-IPSP]. Only two neurons from this population did not exhibit any spikes in their responses. Typical responses contained one or two spikes (67/73), but much less often 3 or 4 action potentials (4/73) were produced. Sound level did not change the general response pattern of these neurons; depolarization was preceded and followed by hyperpolarization with or without spikes.
Intracellular responses of a representative neuron from this population are shown in Figure 2A-D. In response to the 80-20 kHz FM sweep, this unit exhibited a relatively stable response pattern (IPSP-EPSP(spikc)-IPSP) which was independent of sound level. In response to the FM sweep presented at a subthreshold level for spikes (10 dB SPL) a small initial IPSP occurred followed by an EPSP (Fig.2A), whereas at supra-threshold sound levels (40 dB SPL and 60 dB SPL) this unit exhibited the IPSP-EPSP-spike-IPSP response pattern (Fig.2B,C). In the waveform shown in Figure 2C an action potential was generated at a level below the resting membrane potential. In spite of that, we believe that this spike was generated by an EPSP which did not reach the resting membrane voltage because it occurred during an IPSP. Responses of this neuron to lower and higher sound levels showed clear depolarization (Fig.2B,D). At the sound level of 80 dB SPL this neuron did not discharge but it retained the IPSP-EPSP-IPSP response pattern (Fig.2D). Thus, the vast majority of IC neurons in response to FM sweeps show IPSP-EPSP-IPSP response pattern with one or two spikes.

A smaller population of IC neurons (25/117 or 21%) showed a quite different response pattern. In these neurons, FM stimuli evoked membrane depolarization without any sign of hyperpolarization. A representative neuron from this population in response to 80-20 kHz FM sweeps exhibited an EPSP that increased in duration with increasing sound level (Fig.2 E-H). When sound level was increased from 10 dB SPL to 40 dB SPL, 60 dB SPL, and 80 dB SPL EPSP durations increased from 6 ms to 13 ms, 24 ms, and 30 ms,
respectively (Fig.2E-H). The number of spikes in the response also increased from two at 10 dB SPL (Fig.2E) to 4 and 5 at 40 and 60 dB SPL, respectively (Fig.2F, G) and finally to 7 spikes at 80 dB SPL (Fig.2H). Sound level did not alter the general response pattern of these IC neurons. However, response spike count and duration of these neurons increased with sound level. Thus, there is a major difference between these neurons and the neurons exhibiting IPSP-EPSP(spike)-IPSP response pattern.

Response pattern of the remaining 19/117 neurons (17%) could not be classified according to any of the two categories of IC neurons described above. Nine of them responded with IPSPs only (see Fig.5 for reference). The remaining 10 neurons showed either an IPSP- EPSP-spike (3 units) or EPSP-spike-IPSP response pattern (4 units) or their response pattern was unstable and varied from one presentation to another (3 units).

Responses to sub-components of the entire FM sweep

As described above, 62% of IC neurons responded to the 80-20 kHz FM sweep with complex multi-component postsynaptic responses: IPSP-EPSP(spike)-IPSP. This response pattern may be evoked by convergence of different frequency-dependent inputs to each IC neuron. Furthermore, these inputs should arrive within a limited time window to be integrated. We hypothesized that complex multi-component postsynaptic responses in the IC are the result of spectro-temporal integration. In order to test this hypothesis
we compared postsynaptic responses of IC neurons to the entire FM sweep and to each of three arbitrarily but equally divided FM sub-components: 80-60 kHz, 60-40 kHz, and 40-20 kHz (1.33 ms duration, and 0.25 ms rise/fall time). Unless otherwise specified, all FM sweeps were presented at the same sound level, usually at 10 dB or 20 dB above a unit’s threshold for spikes.

**Spectral integration**

Intracellular responses of 41 out of 73 IC neurons exhibiting the IPSP-EPSP(spike)-IPSP were studied in response to the entire FM sweep and also to three FM subcomponents. Postsynaptic responses (PSPs with or without spikes) in response to the first (80-60 kHz), second (60-40 kHz), and third (40-20 kHz) FM subcomponents were evident in thirteen of 41 neurons. Postsynaptic responses to the first (80-60 kHz) and the second (60-40 kHz) FM subcomponents were displayed by 24 of 41 neurons. Two of 41 neurons showed postsynaptic responses to the second (60-40 kHz), and third (40-20 kHz) FM subcomponents. Finally, two of 41 neurons showed postsynaptic responses to the first (80-60 kHz) subcomponent only. Thus, a vast majority of IC neurons (39/41) responded to at least two FM subcomponents that corresponds to the frequency range of 40 kHz.

Action potential response thresholds to the entire FM sweep often were different from the thresholds to individual FM subcomponents. Twenty six percent (11 of 41) of units fired spikes in response to the entire FM sweep
whereas they showed only postsynaptic potentials (excitatory and/or inhibitory) without spikes in response to FM subcomponents presented at the same sound level (Fig.3). Data from the unit shown in Figure 3 demonstrate a complex response pattern (IPSP-EPSP-spike-IPSP) to stimulation with the entire FM sweep (Fig.3A). Truncating the signal to the initial 80-60 kHz FM and the second 60-40 kHz FM subcomponents resulted in a postsynaptic response pattern similar to that received for the entire FM sweep, but below the unit’s spike threshold (Fig.3B,C). In response to the 40-20 kHz FM it showed a small EPSP followed by a small IPSP (Fig.3D).

11% (5/41) of these neurons showed lower spike threshold to individual FM subcomponents presented alone than to the entire FM sweep. These neurons fired in response to usually one out of three FM subcomponents while they showed no action potentials in response to the entire FM sweep presented at the same sound level. However, when sound level was increased these 5 units fired to both the entire FM sweep as well as to some of three FM subcomponents presented alone. Thus our data suggest that response of a substantial number of IC neurons is a result of spectral integration across different frequencies. It is possible that this number is underestimated, because the sound level increment which we used for these experiments (10 dB or 20 dB) was too large to make definitive judgments about fine differences in the response thresholds.
Temporal integration

The hypothesis that temporal summation across different frequency bands can produce the response to the entire FM sweep was tested in 16 IC neurons. For each neuron, the algebraic sum of all postsynaptic responses to the three FM subcomponents was compared with its response to the entire FM sweep. In order to sum the individual postsynaptic potentials, responses to the FM subcomponents were realigned to account for their position within the entire FM sweep. In other words, before summation the PSP responses to the second and third FM subcomponents were shifted +1.33 ms and +2.66 ms, respectively. The summated potential then was normalized to the unit’s resting membrane potential. A correlation coefficient was used to determine how well the summated potentials matched unit responses to the entire FM sweep. Because all neural responses were collected with the same sampling rate (100 kHz), we were able to calculate correlation coefficients between the event amplitudes using a high resolution data set (within a 50 ms time window beginning from stimulus onset 5000 points were compared between responses). Action potentials could potentially contaminate the correlation coefficient values. Therefore, data points during action potentials (1.5 ms) and correspondent points on the other trace were excluded from analysis.

For all 16 IC neurons studied during these experiments, the correlation values were first calculated for responses measured at sound levels 10-20 dB above each unit’s threshold for action potentials. Five out of 16 neurons had correlation coefficient values between 0.6 and 0.85. A representative neuron
from this population is shown in Figure 4. All stimuli for this neuron were presented at a sound level 20 dB above action potential threshold. The response to the first FM subcomponent was similar to that elicited by the entire FM sweep (compare Fig.4A and B). In response to the second FM subcomponent (60-40 kHz) this neuron showed a bimodal IPSP containing a maximum point of depolarization in the middle (Fig.4C). Finally, in response to the 40-20 kHz FM subcomponent this neuron showed an EPSP (Fig.4D). The algebraic sum of postsynaptic responses to the three FM subcomponents correlated well ($r = 0.69$, $p<0.001$) with the unit's response to the entire FM sweep (Fig.4 bottom panel).

Another neuron from this population had an unusual response pattern because it did not fire action potentials to FM sweeps (Fig.5). Therefore, this neuron gave us an opportunity to directly compare its postsynaptic potentials in response to the entire FM sweep with the summated postsynaptic potential to the three FM subcomponents. All stimuli were presented at a sound level 20 dB above threshold for PSP (0 dB SPL). To the entire FM sweep this unit showed a complex IPSP containing two (early and late) components (Fig.5A). Responses to the first, second, and third FM subcomponents presented alone were IPSPs with different latencies and durations (Fig.5B-D). The summated response to all three FM subcomponents was well matched to the unit's response to the entire FM sweep ($r = 0.71$, $p<0.001$) (Fig.5E). In order to determine the contribution from each of the three FM subcomponents we compared the postsynaptic responses to each of these subcomponents to the
response elicited by the entire FM sweep (Fig. 5F, G, H). Response to the 80 kHz-60 kHz subcomponent provided a major contribution to the entire response \((r = 0.73, p<0.001)\) (Fig. 5F). The response to 60 kHz-40 kHz subcomponent had longer latency revealing a contribution to the later part of the initial IPSP \((r = 0.64, p<0.001)\) (Fig. 5G). Finally the response to the 40 kHz-20 kHz subcomponent contributed to the later phase of the response to the entire FM sweep \((r = 0.29, p<0.001)\); this is evident in the later bimodal IPSP (Fig. 5H). Thus, responses of this unit to each of the three FM subcomponents made a contribution to the unit’s response to the entire FM sweep.

For the remaining 11/16 units the correlation values were either low (5/16 units; from 0.3 to 0.6) or close to zero (6/16 units; from 0 to < 0.3).

Thus, one third of IC neurons performed temporal summation across a wide range of sound frequencies in a nearly linear manner. The remaining two thirds of neurons performed nonlinear temporal summation with different degrees of nonlinearity.

**Sound level and temporal summation**

The influence of sound level on postsynaptic responses was studied in 14 IC neurons. For about one third of these neurons (5 of 14) summated postsynaptic potentials in response to three FM subcomponents were highly
correlated with responses to the entire FM sweep at sound levels from 10 to 20 dB above unit’s threshold for spikes. At high sound levels however (60, 80 or 90 dB SPL) this correlation was noticeably reduced.

Postsynaptic responses of a representative neuron from this population are shown in Figure 6. This figure demonstrates the effect of sound level on the relationship between postsynaptic potentials in response to the entire FM sweep and the summated response to the FM components. The correlation coefficient values are shown for five different sound levels (0, 20, 40, 60, and 80 dB SPL). Analysis of the data reveals a low correlation between the summated PSPs and the response to the entire FM sweep at 0 dB SPL (see Fig. 6B for reference). Poor correlations at 0 dB SPL were expected, because this neuron showed very little if any response at this sound level. At 20 dB SPL this neuron showed a subthreshold postsynaptic response to the entire FM sweep. At this sound level the correlation coefficient was maximal (see Fig.6C for the reference). When the sound level was further increased to 40, 60, and 80 dB SPL correlation coefficients declined to 0.5, 0.3, and 0.07, respectively (Fig.6A; see Figs. 6D, E, and F for reference).

Figure 7 shows the population data from 14 IC neurons. This population shows a similar trend for the relationship between the correlation coefficient and sound level to that demonstrated for the neuron shown in Figure 6. The highest correlation coefficient values occur between 20 and 40 dB SPL (close to neuron spike threshold) and the correlation coefficients decreased at higher sound levels. During our experiments the large incremental step in
sound level (20 dB) made it nearly impossible to determine the exact range of sound levels where peak correlation coefficients occurred for each neuron. Nevertheless if we take into consideration that normally the PSP responses of IC neurons have much lower thresholds than those for spikes we can roughly estimate a 20 dB range over which correlations are high. It suggests that within this 20 dB range IC neurons perform a better integration across frequencies than at very high sound levels.

*Time course of inputs determines neuron responses to FM sweeps*

Our data demonstrate that vast majority of IC neurons (39 of 41) responded to more than one FM subcomponent. It suggests that these neurons integrate information across a wide range of sound frequencies. If convergence occurs at the IC, the timing of these inputs to IC neurons would be important for defining the response to FM stimuli. In order to address this question we studied intracellular responses of eight IC neurons when the three FM subcomponents were presented at different inter-component time delays (0ms, 1ms, 2 ms, 4ms, 8ms, 16ms, and 32ms). All FM subcomponents were always presented in the same sequence as they appear within the entire FM sweep. One limitation to this approach is that each of the three FM subcomponents has its own rise/fall time of 0.25 ms. In other words, when all three FM subcomponents are combined in a train with 0 ms inter-component time intervals the result is a 4 ms FM sweep (the entire FM sweep) with two
embedded notches. Such a stimulus has less energy than the entire FM sweep and a different spectrum. Therefore, in order to determine how these differences might modify neurons’ response patterns, responses from all 8 IC neurons to the entire FM sweep were compared with responses to a train of three FM subcomponents with 0 ms inter-subcomponent time interval. For all of these neurons we found little differences between responses to these two types of FM stimuli (Fig. 8).

For three out of eight neurons studied the time interval between FM subcomponents was critical for their responses. Firing in response to the FM train was dramatically suppressed at particular inter-subcomponent intervals. The size of the interval that produced the suppression varied slightly from one neuron to another (from 2 to 4 ms). A representative IC neuron from this population is shown in Figure 9. We first studied this neuron’s intracellular response to the entire FM sweep and to three FM subcomponents presented alone. This neuron responded to the entire FM sweep with a common response pattern IPSP-EPSP-spike-IPSP (Fig. 9A). In response to the first FM subcomponent (80 kHz-60 kHz) presented alone it showed an EPSP-spike-IPSP response pattern with an EPSP latency of about 12 ms (Fig. 9B). To the 60 kHz-40 kHz FM subcomponent it exhibited a large IPSP with latency about 6.5 ms (Fig. 9C). At the given sound level this neuron did not show any response to the third FM subcomponent (see averaged traces in Fig. 9D). Analysis of PSP latencies to different FM subcomponents suggested that, a shorter-latency inhibitory input elicited by the second FM subcomponent
(occurring 1.33 ms later than the first FM subcomponent) arrived before an excitatory input elicited by the first FM subcomponent.

In response to a train of three FM subcomponents with 0 ms inter-subcomponent delay this neuron response was very similar to the response which was evoked by the entire FM sweep (compare Fig. 9E with Fig. 9A). However, when the delay was increased to 2 ms we observed a distinct suppression of neural firing from four spikes down to one (Fig. 9F). At this delay an IPSP in response to the second FM subcomponent occurred before and partially overlapped with the later EPSP that was evoked by the first FM subcomponent. At 4 ms delay this neuron showed a weak EPSP with no action potentials probably because the IPSP and EPSP were coincident (Fig. 9G). However, when the delay was increased to 8 ms the IPSP to the second FM subcomponent was shifted just beyond the EPSP permitting a partial return of neural firing (Fig. 9H). Increasing the inter-subcomponent interval to 16 ms further delayed the IPSP so that this neuron fired four action potentials similar to the 0 ms delay case or for the first FM subcomponent presented alone (compare Figs. 9B, E and I). Thus, for these three IC neurons an early inhibition in response to the second FM subcomponent played a critical role in determining their response sensitivity to the rate of FM sweep.

The remaining 5 of 8 neurons were affected differently as the delay between FM subcomponents was increased. For three, the response to the entire FM sweep was mainly determined by the response to the first FM subcomponent. In other words, they showed near identical responses to the
entire FM sweep and to the first FM subcomponent. Therefore, increasing the delay between FM subcomponents did not alter their response patterns.

The other two of five neurons fired spikes to the entire FM sweep, but displayed subthreshold PSPs in response to individual FM subcomponents presented alone. Increasing the inter-subcomponent delay increased the interval between these PSPs without their modification. It suggests that firing of these neurons was a result of temporal summation across wide range of sound frequencies.

Discussion:
The primary findings of this study were that the majority of IC neurons show postsynaptic responses to a wide range of sound frequencies and the most common response to FM sweep was IPSP-EPSP(spike)-IPSP. One third of IC neurons performed nearly linear temporal summation across wide range of sound frequencies whereas two thirds of IC neurons exhibited nonlinear temporal summation with different degrees of nonlinearity. Some IC neurons showed different latencies of postsynaptic potentials in response to different FM subcomponents. Often responses to the later FM subcomponent occurred before responses to the earlier ones (Fig.9).

Methodological issues
Intracellular recording from awake animals is a technically challenging procedure that results in very short recording time and therefore small quantities of data for individual neurons. During our experiments we usually could not hold IC neurons longer than 2 – 3 minutes. However, in spite of short recording time, we do believe that the quality of our intracellular recordings is comparable with previous intracellular studies. The range of resting membrane potentials and spike amplitudes presented in this study are similar to those reported by others (Nelson and Erulkar 1963; Volkov and Galazyuk 1991, 1992; Kuwada et al 1997; Pedemonte et al 1997; Nayagam et al 2005, 2006). All but two neurons that we report here displayed both synaptic and action potentials. Therefore we feel confident that our intracellular data are valid to determine postsynaptic mechanisms underlying FM sound processing in the inferior colliculus of awake bats.

Temporal summation of postsynaptic events

Previous studies suggests that integration of information across multiple frequency bands play a crucial role in processing of complex sounds in echolocating bats (Fuzessery and Hall, 1996; Portfors and Wenstrup 2001; Wenstrup and Leroy, 2001; O'Neill and Brimijoin, 2002; Fuzessery et al, 2006) as well as in nonecholocating animals (Phillips and Irvine, 1981; Sutter and Schreiner, 1991; Linden et al 2003; Portfors and Felix 2005). Linear summation of inhibitory and excitatory events has been shown to play a major role in cellular mechanisms responsible for response selectivity to the direction of visual motion.
in neurons of the cat primary visual cortex (Jagadeesh et al., 1993; Priebe and Ferster 2005). Our study supports these conclusions. We found that one third of IC neurons can integrate information using nearly linear summation.

In this study we demonstrated that the majority of IC neurons (39 out of 41) receive inputs over a wide range of sound frequencies at sound levels close to unit’s threshold for spikes because they show postsynaptic responses to two or three FM subcomponents. Summation of these inputs determines neural responses to the entire FM sweep. IC neurons performed either near linear (30% of units) or nonlinear summation (70% of units) of these inputs over time. The result of this summation facilitated or suppressed responses of IC neurons.

It is of interest that the majority of IC neurons (about 70%) show nonlinear integration. There are a few potential intrinsic cellular mechanisms that could be involved in such integration. For instance, the hyperpolarization-activated current such as Ih current in auditory brain stem neurons can contribute to the precise analysis of temporal information (Koch and Grothe 2003). This current can either reduce or improve temporal summation of excitatory and inhibitory potentials in auditory neurons (Oertel 1999; Bal and Oertel 2000; Koch and Grothe 2003). Additional evidence of hyperpolarization-activated intrinsic mechanisms comes from both in-vitro study of rat brain slices and in-vivo recording in the inferior colliculus of unanesthetized rabbits (Sivaramakrishnan et al. 2004). Since majority of IC neurons in our study showed an initial hyperpolarization in their responses these hyperpolarization-activated intrinsic mechanisms might play a role in
creation of complex responses to FM sweeps. Depolarization-activated low threshold potassium current has been recorded in the auditory brain stem neurons (Adam et al. 2001). Low and high threshold Ca2+ channels which can be activated by depolarization have been found in the inferior colliculus neurons of adult rats (N’Gouemo and Morad, 2003). Thus, in addition to patterns of synaptic input, response properties of the inferior colliculus neurons can be shaped by the intrinsic properties of the postsynaptic neuron. Future study of the intrinsic properties of the IC neurons may clarify the role of these mechanisms in temporal integration.

Excitation surrounded by inhibition

Our data suggest that the vast majority of IC neurons responds to the entire FM sweep with an IPSP-EPSP(spike)-IPSP response function. Independent of sound level these neurons usually show one or sometimes two action potentials in their responses. Early inhibition in auditory neuron responses to sounds has been seen by others (Nelson and Erulkar, 1963; Carney and Yin, 1989; Park and Pollak, 1993; Faure et al., 2003). Both early and late inhibitory components were observed during in-vivo patch-clamp recording from the inferior colliculus neurons in big brown bats (Covey et al 1996). It is unclear whether the early and late inhibitions are two parts of a long-lasting inhibition triggered by FM stimulus onset and interrupted by a strong excitation arriving a few milliseconds later than inhibition. Our data can not provide a definitive answer to this question. In response to individual FM subcomponents presented alone some neurons showed (IPSP-EPSP(spike)-IPSP response pattern which was similar to that of
the entire FM sweep (Fig.3,4). Therefore for these neurons it is difficult to speculate about the origin of the early and late inhibitions for their responses to FM sweeps. Other neurons showed short latency IPSP in response to one FM subcomponent while exhibiting longer latency EPSP(spike) or EPSP(spike)-IPSP responses to another (Fig.9). It suggests that these neurons receive early and late inhibitory inputs from different frequency ranges. Future studies that utilize intracellular recordings from individual IC neurons in response to both FM sweeps and pure tones presented at different frequencies might clarify this point.

Whether the late IPSP is caused by activation of intrinsic conductances after action potential(s) or after EPSP is unclear. Our data suggest that intrinsic mechanism is unlikely to be responsible for the late IPSPs. First of all, because in many of our neurons we did not observe spikes while they showed clear IPSP-EPSP-IPSP response patterns for some stimulus conditions. Second, we have also often seen spontaneous spikes which were not followed by an IPSP. Nevertheless, we cannot rule out the possibility for an intrinsic source of the late inhibitory response component. Future experiments that utilize blocking of different intrinsic conductances may clarify this point.

The fact that inhibition flanks excitation in many IC neurons restricting the time available to response might have an important behavioral relevance for bats. When a bat pursues a flying insect, its sonar emission rate is increased from 5-20 pulses per second during the search phase and to 100-200 pulses per second just prior to prey capture (Griffin 1986; Schnitzler et al. 1987; Kalko 1995). It is important for bats to analyze all returning echoes separately from
each other when the time interval between them can be as small as a few milliseconds. Therefore, it would be very important for these animals to restrict the time of neural response in order to avoid response overlap. At the same time this mechanism should be level independent, because of the dramatic difference between sound level of emitted pulses and returning echoes. Thus, a narrow response window for IC neurons should be critical for successful behavioral performance of bats.

Inhibition preceding excitation has also been reported for nonecholocating animals (Nelson and Erulkar 1963). It may play an important role for central auditory processing to control a balance between excitation and inhibition. This balance can vary systematically as a function of stimulus parameters. For example, balance between inhibition and excitation can be altered as a function of sound level, causing response latency to increase. Neurons exhibiting so-called “paradoxical latency shift” have been reported for echolocating bats (Sullivan 1982; Galazyuk and Feng 2001; Galazyuk et al 2005) as well as for nonecholocating animals including insects (cats, Rose et al., 1963; gerbils, Klug et al., 2000; insects Krahe et al., 2002; frogs Galazyuk et al 2005; ).

*Response latency and FM response selectivity*

Our data demonstrate that some of IC neurons showed different PSP latencies in response to different FM subcomponents. Furthermore, the latencies to different FM subcomponents often did not correlate with the sequential order
of these subcomponents within the entire FM sweep. Response latencies to the second FM (60 kHz-40 kHz) subcomponent (inhibitory input) were a few milliseconds shorter than response latencies to the first subcomponent (80 kHz-60 kHz) (excitatory input). Suppression of spikes when excitation to the initial part of the FM sweep was preceded by the inhibition to later part of the FM sweep is equivalent to backward masking (Zwicker and Fastl, 1990). It is unclear what mechanism is responsible for the long latency excitation or short latency inhibition. One plausible scenario is that a sustained excitation has the same latency as the initial inhibition but the strength of inhibition is greater. Therefore, excitation is delayed by this stronger inhibition. This explanation is consistent with extracellular recording studies that show that some IC neuron discharge latency can be shortened by GABA or glycine receptor antagonist application (Johnson 1993; Park and Pollak 1993, Casseday and Covey 1996). In the IC, inhibition may extend the range of latencies which are larger than would be expected from synaptic delays and axon length (Haplea et al, 1994; Casseday and Covey 1996). Another piece of the evidence comes from study of auditory neurons exhibiting a “paradoxical latency shift” (Sullivan 1982; Galazyuk and Feng 2001; Galazyuk et al 2005). These neurons show longer response latencies at high sound levels. Application of GABAa blocker to these IC neurons revealed shorter-latency firing in their discharge pattern. In contrast to these findings several studies demonstrated that in the inferior colliculus inhibition has little if any contribution to the lengthening of response latencies (Le Beau et al 1996; Fuzessery et al 2003). Further studies are necessary to clarify this point.
Fast inhibitory inputs which were described in our study might be responsible for the FM sweep rate selectivity. Such neurons would not respond to FM sweeps with long durations (low sweep rate) because a fast inhibition in response to the middle part of the entire FM sweep might then coincide with a late excitation in response to the beginning of the entire FM sweep. This is exactly what happened when we increased inter-subcomponent delay in a train of FM subcomponents from 2 – 4 ms. Thus, for such neurons fast inhibitory input plays a crucial role in creation of FM sweep response selectivity. A similar mechanism has been reported for duration selective neurons in the inferior colliculus of big brown bats (Faure et al, 2003). The authors presented evidence that inhibition can play a significant role in temporal masking phenomena, including forward, simultaneous, and backward masking.

The importance of GABAergic inhibition in FM sweep direction and rate response selectivities has been shown for IC neurons (Fuzessery and Hall 1996; Fuzessery et al, 2006). Using a two-tone inhibition paradigm these authors described a few inhibitory mechanisms that can shape selectivity for FM sweep direction and rate. It turns out that two different inhibitory mechanisms can create similar tuning to rate of FM sweeps. The first mechanism suggests that duration tuning determines rate tuning. In the IC neurons that are not duration tuned another mechanism plays a role for the rate tuning. It is delayed high-frequency inhibition which at slow FM rates overlaps excitation. The authors suggested that it is possible for both mechanisms to work in concert. The cellular mechanism underlying FM rate selectivity which was discovered in our study is different from
those two described above. Our data suggests that delayed high frequency excitation and short latency low frequency inhibition might be responsible for both FM sweep rate and direction selectivity. Thus, it further supports a hypothesis that there is more than a single mechanism which can be used by the auditory system in order to achieve the same endpoint (Fuzessery et al, 2006). A similar conclusion has been made after multiple attempts to find a single cellular mechanism which might be responsible for sound duration tuning in the auditory system of different animals (Casseday et al, 1994; Fuzessery and Hall 1999).

In conclusion, the main point we wish to emphasize is that the majority of IC neurons integrate information across a wide range of sound frequencies. Some of these neurons perform nearly linear summation whereas the other exhibit nonlinear summation with different degrees of nonlinearity. This is an important finding because it may shed light on mechanisms by which individual auditory neurons can analyze complex sounds.

Acknowledgments:

We thank Jeffrey Wenstrup, David Gooler, Nick Fuzessery for their valuable comments on earlier versions of this manuscript. This work was supported by a grant R01DC005377-01 from the National Institute on Deafness and Other Communication Disorders of the NIH.
References:


Figure 1. Statistical evaluation of postsynaptic responses using multiple stimulus presentations. Upper panel shows individual traces of postsynaptic responses of an IC neuron to the same downward FM sweep 80-60 kHz presented five times at sound level of 20 dB SPL. Bottom panel shows an algebraic average for all five traces shown in the upper panel. A horizontal solid line represents mean value of the baseline (see Method section for further details). Horizontal dotted lines represent values of two standard deviations (95% confidence interval) from the baseline. $V_{rest} = -45$ mV, threshold for PSP = 0 dB SPL, threshold for spike = 40 dB SPL.
Figure 2. Representative response patterns from two populations of IC neurons in response to a 80 kHz to 20 kHz FM sweep. Left column, Intracellular responses of an IC neuron representing a population with the most common response pattern (IPSP-EPSP(spike)-IPSP) to FM sweeps presented at different sound levels as indicated. Right column, Intracellular responses of an IC neuron representing a population with a less typical response pattern. Sound stimuli are shown by black horizontal bars below (same time scale as intracellular traces). Time and amplitude scales are shown in the bottom of each column. Horizontal dotted lines indicate the resting membrane potential. For cell shown in the left column: Vrest = - 43 mV, threshold for PSP = 0 dB SPL, threshold for spike = 40 dB SPL, depth of recording = 500 µm. For cell shown in the right column: Vrest = - 52 mV, threshold for spike = 0 dB SPL, depth of recording = 200 µm.
Figure 3. Intracellular responses of an IC neuron to the entire FM sweep and to its three subcomponents. Individual response traces to (A) the entire 80-20 kHz FM sweep and to each of three FM subcomponents: (B) 80-60 kHz, (C) 60-40 kHz, and (D) 40-20 kHz (shown in black). Red curves represent an algebraic average of postsynaptic responses to the same stimulus presented four times. Sound stimuli are shown by black horizontal bars below each response trace (same time scale as intracellular traces). The frequency range of each FM sweep is shown to the left of each sound stimulus. Time and amplitude scales are shown at the bottom right. Horizontal lines indicate the resting membrane potential. A horizontal solid line represents mean value of the baseline. Horizontal dotted lines represent values of two standard deviations (95% confidence interval) from the baseline. All sound stimuli were presented at 10 dB above the neuron’s threshold for spikes. Vrest = - 43 mV, threshold for PSP = 0 dB SPL, threshold for spike = 40 dB SPL.
depth of recording = 550 μm.
Figure 4. Temporal summation of postsynaptic events on an IC neuron. (A) Postsynaptic response to the entire FM sweep (shown in black). (B, C, D) Postsynaptic potentials in response to three different FM subcomponents arranged in time as they occur within the entire FM sweep (shown by four vertical dotted lines between stimulus envelopes). Red curves represent an algebraic average of postsynaptic responses to the same stimulus presented four times. Vertical dashed line indicates postsynaptic events in response to three different FM subcomponents which might coincide post-synaptically on this neuron in response to the entire FM sweep to give a rise of an action potential. (B+C+D) algebraic sum of postsynaptic responses to all three FM subcomponents (shown in green) superimposed with the response to the entire FM sweep (shown in black). Sound stimuli are shown by black horizontal bars below each of the response traces. Frequency range of each FM sweep is shown to the left of each sound stimulus. Time and amplitude scales...
refer to stimuli and intracellular traces. All sound stimuli were presented at 20 dB above neuron threshold for spikes. \( V_{rest} = -43 \text{ mV} \), threshold for PSP = 10 dB SPL, threshold for spike = 30 dB SPL, depth of recording = 500 \( \mu \text{m} \). See legend of Figure 3 for other details.
Figure 5. Similarity of postsynaptic responses to the entire FM sweep with the algebraically summed postsynaptic potentials to the three FM subcomponents. (A) An algebraic average of postsynaptic responses to the entire FM sweep presented three times. (B, C, D) averaged postsynaptic responses of the same neuron to different FM subcomponents presented three times. (B+C+D) algebraic sum of wave forms shown in B, C, and D. (E) Comparison of averaged wave form shown in A with the summed potential (shown in B+C+D). (F, G, H) comparison of averaged postsynaptic response shown in A with each of averaged postsynaptic responses shown in B, C, and D, respectively. All sound stimuli were presented at 20 dB above neuron threshold for PSP. Threshold for PSP = 0 dB SPL, Vrest = -66 mV, depth of recording = 550 μm. See legend of Figure 3 for other details.
Figure 6. Comparison of postsynaptic responses to the entire FM sweep with the summated responses to three FM subcomponents at different sound levels. (A) Mean of correlation coefficient values between 6 individual traces to the entire FM sweep and 6 summated PSPs to three FM subcomponents (15 measurements, filled circles). Correlation coefficients are calculated for 5 different sound levels (0, 20, 40, 60, and 80 dB SPL). Vertical bars represent standard deviation values. (B-F) Representative examples of postsynaptic responses to the entire FM sweep shown in black traces superimposed with the summated PSP in response to three FM subcomponents (shown in gray) presented at different sound levels (indicated by arrows). Vrest = -42 mV, threshold for PSP = 20 dB SPL, threshold for spike = 40 dB SPL, depth of recording = 500 μm.
Figure 7. Effect of sound level on correlation coefficient values between postsynaptic responses to the entire FM sweep and the summated responses to three FM subcomponents for 14 IC neurons. See legend of Figure 6 for other details.
Figure 8. Postsynaptic responses of three IC neurons in response to the entire FM sweep (left column) and to a train of three FM subcomponents with 0 ms inter-subcomponent interval (right column). Sound stimuli are shown by black horizontal bars below each of the response traces (same time scale as intracellular traces). Frequency range of each FM sweep is shown below the stimuli at the bottom. Time and amplitude scales are shown at the bottom. Horizontal dotted lines indicate the resting membrane potential. Unit#1: Vrest = -40 mV, threshold for PSP = 10 dB SPL, threshold for spike = 30 dB SPL, depth of recording = 400 μm. Unit#2: Vrest = -41 mV, threshold for PSP = 20 dB SPL, threshold for spike = 40 dB SPL, depth of recording = 390 μm. Unit#3: Vrest = -52 mV, threshold for PSP = 10 dB SPL, threshold for spike = 40 dB SPL, depth of recording = 780 μm. All sound stimuli were presented at the level of each neuron's threshold for spikes.
Figure 9. Later segment of the entire FM sweep modulates the response of an IC neuron to the initial part of the FM sweep. (A) Intracellular response of an IC neuron to the entire FM sweep. (B-D) Intracellular responses of the same neuron in response to three different FM subcomponents presented alone. (E-I) Intracellular responses of the same unit in response to a train of three FM subcomponents presented at different inter-component time intervals (0, 2, 4, 8, 16 ms, respectively). Red curve represents an algebraic average of postsynaptic responses to the same stimulus presented five times. See legend of Figure 3 for other details. Vrest = -42 mV, threshold for PSP = 0 dB SPL, threshold for spike = 20 dB SPL. All sound stimuli were presented at 40 dB SPL, depth of recording = 410 μm. Action potentials were truncated in order to better see postsynaptic potentials (indicated by stars).