Published in final edited form as:

Neuroscience. 2010 February 17; 165(4): 1490. doi:10.1016/j.neuroscience.2009.11.070.

Suppression of spontaneous firing in inferior colliculus neurons during sound processing

S.V. Voytenko¹ and A.V. Galazyuk²

¹ Department of Neuronal Networks Physiology, Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine

² Department of Anatomy and Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio, USA

Abstract

Spontaneous activity is a well-known neural phenomenon that occurs throughout the brain and is essential for normal development of auditory circuits and for processing of sounds. Spontaneous activity could interfere with sound processing by reducing the signal-to-noise ratio. Multiple studies have reported that spontaneous activity in auditory neurons can be suppressed by sound stimuli. The goal of this study was to determine the stimulus conditions that cause this suppression and to identify possible underlying mechanisms. Experiments were conducted in the inferior colliculus (IC) of awake little brown bats using extracellular and intracellular recording techniques. The majority of IC neurons (82%) fired spontaneously, with a median spontaneous firing rate of 6 spikes/sec. After offset of a 4 ms sound, more than half of these neurons exhibited suppression of spontaneous firing that lasted hundreds of milliseconds. The duration of suppression increased with sound level. Intracellular recordings showed that a short (<50ms) membrane hyperpolarization was often present during the beginning of suppression, but it was never observed during the remainder of the suppression. Beyond the initial 50 ms period, the absence of significant changes in input resistance during suppression suggests that suppression is presynaptic in origin. Namely, it may occur on presynaptic terminals and/or elsewhere on presynaptic neurons. Suppression of spontaneous firing may serve as a mechanism for enhancing signal-to-noise ratios during signal processing.

Keywords

IPSP; intracellular recording; signal-to-noise ratio; awake animal; bat; frequency modulated sweep

INTRODUCTION

Neurons throughout various brain structures, including those of sensory systems, have been shown to fire in the absence of overt external stimuli. Spontaneous firing is an important and necessary contributor to normal brain function. Research in this area has grown (Walsh and

Corresponding author: Alexander Galazyuk Ph.D., Assistant Professor, Northeastern Ohio Universities, College of Medicine, Department of Anatomy and Neurobiology, 4209 State Route 44, Rootstown, OH 44272, Phone: (330)325-6640, Fax: (330)325-5916, agalaz@neucom.edu.

Section Editor: Dr. Richard Weinberg.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

McGee, 1987; Gummer and Mark, 1994; Hermann et al., 2007; Jones et al., 2001, 2007); however, the understanding of its biological significance is still nascent.

Spontaneous activity in sensory neurons has been shown to serve several functions. Neurons of the lateral geniculate nucleus that synapse in the visual cortex are highly spontaneously active. Spontaneous activity keeps these synapses in a state of saturated synaptic depression (Boudreau and Ferster, 2005). As a consequence, geniculocortical synapses show no further depression in response to long and temporally complex visual stimuli. Neurons of the pretectal nuclear complex generate spontaneous firing intrinsically (Prochnow and Schmidt, 2004). Spontaneous activity in these neurons maintains normal function of oculomotor reflexes. In the somatosensory system, spontaneously active neurons of the zona incerta modulate responses of neurons in the posterior medial thalamus to sensory inputs (Trageser et al., 2006). Spontaneous activity of neurons in the somatosensory system is necessary for mediation of synaptic pruning (O'Leary et al., 1994).

Normal performance of the auditory system also depends upon spontaneous activity. Neurons from the medial nucleus of the trapezoid body enhance transmission across Calyx of Held synapses by introduction of spontaneous activity (Hermann et al., 2007). Analogous to the visual system, spontaneous activity improves the ability of the postsynaptic neurons to follow trains of high-frequency sound pulses and decreases recovery time following synaptic depression. Spontaneous activity is also critical for neuronal survival and for the refinement and maintenance of tonotopic maps before the onset of hearing (Walsh and McGee, 1987; Gummer and Mark, 1994; Jones et al., 2001, 2007). Although spontaneous activity is important for normal brain function, it is also important that a signal of interest is not swamped by spontaneous activity during signal processing.

Sound-evoked suppression of spontaneous activity has frequently been observed in neurons of the auditory system (Smith 1977; Harris and Dallos 1979; Relkin and Turner 1988; Ebert and Ostwald 1995; Galazyuk et al., 2005; Wehr and Zador 2005; Portfors and Roberts 2007; Nelson et al. 2009). Such suppression, lasting hundreds of milliseconds, has been observed in fibers of the auditory nerve (Smith 1977; Harris and Dallos 1979; Relkin and Turner 1988), cochlear nucleus (Ebert and Ostwald 1995; Portfors and Roberts 2007), IC (Galazyuk et al. 2005; Nelson et al. 2009) and auditory cortex (Wehr and Zador 2005). Despite the common occurrence of this phenomenon, it has not been studied systematically.

In vivo intracellular recordings in the IC have revealed hyperpolarizations that exceed the duration of the sound stimulus in many neurons (Nelson and Erulkar 1963; Torterolo et al. 1995; Covey et al. 1996; Kuwada et al. 1997; Pedemonte et al. 1997, Voytenko and Galazyuk 2007; 2008; Peterson et al, 2008). However, these potentials lasted less than 100 ms after the end of a stimulus, even in barbiturate anesthetized animals (Covey et al. 1996; Kuwada et al. 1997; Pedemonte et al. 1997). It is unclear whether long-lasting hyperpolarizations are responsible for prolonged suppressions of spontaneous firing.

The aim of this research was to determine which characteristics of a sound reduce spontaneous firing of auditory neurons and to verify the contributions of synaptic inhibition to stimulus suppression. We addressed this aim with both extracellular and intracellular recordings from IC neurons in awake bats, which served as a mammalian model. A majority of IC neurons showed suppression of spontaneous firing for hundreds of milliseconds following a short sound stimulus. An increase in stimulus sound level prolonged the duration of suppression. Intracellular recordings suggest that membrane hyperpolarization does not contribute to suppression beyond a few tens of milliseconds after stimulus offset. These data suggest that suppression is expressed at presynaptic terminals and/or elsewhere in presynaptic neurons.

EXPERIMENTAL PROCEDURES

Experimental preparation

All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee at the Northeastern Ohio Universities College of Medicine. Experimental subjects comprised 34 little brown bats, *Myotis lucifugus*. Each bat was anesthetized using isoflurane inhalation (1.5 – 2.0%, isoflurane administered by a precision vaporizer) prior to surgery. A midline incision of the skin over the cranium was made. The tissue overlying the skull then was removed and a small metal rod was glued to the skull using glass ionomer cement (3M ESPE, Germany). Following surgery, animals were allowed to recover for 2–3 days in individual holding cages.

Two days after surgery each bat was trained to stay inside a small plastic tube. This tube was used as animal holding device during recording sessions. We directed the head of the bat into the opening of the tube, and the animal crawled inside and stayed there for several hours without movement. Recordings were made from awake bats within a single walled sound attenuating chamber (Industrial Acoustics Company, Inc). The metal rod on the head of the bat was secured to a small holder designed to restrain the head of the animal without causing distress, while the ears were unobstructed for free-field acoustic stimulation. A small hole (~ 50 μ m) penetrating the dura was drilled in the skull overlying the IC, through which a recording electrode was inserted into the IC. Throughout the recording session, the animal was offered water periodically and monitored for signs of discomfort. After a recording session of 4–6 hours, the exposed skull was covered with sterile bone wax, and the animal was returned to its holding cage. Experiments were conducted every 2–3 days for a maximum of 2 weeks. No sedative drugs were used during recording sessions. If the animal showed any signs of discomfort, the recording session was terminated and the bat was returned to its cage.

Acoustic Stimulation

We used linear downward frequency modulated (FM) sweeps that mimicked echolocation signals as our acoustic stimuli. Sounds 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 FM sweeps fell from 80 kHz to 20 kHz over 4 ms, with a 0.25 ms rise/fall time. The majority of IC neurons are tuned to the sound frequencies within this frequency range and respond well to downward FM sweeps (Galazyuk et al., 2005; Voytenko and Galazyuk, 2007). FM sweeps were presented once every two seconds at sound levels ranging from 0 to 80 dB SPL in 4 dB increments.

Neurons in the inferior colliculus of the little brown bat respond well to FM stimuli (Wang et al., 2007; Voytenko and Galazyuk, 2007). Pure tones were omitted as a stimulus in order to enable comparison between extracellular and intracellular recordings in awake animals. Pure tones can not be used for intracellular recordings because determining characteristic frequency of the recorded neuron is time-consuming whereas intracellular recordings from awake animals are typically short (3 – 4 min). The outputs of the loudspeaker were measured with a 1/4-inch microphone (Brüel and Kjaer 4135) and found to be ± 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 a sampling rate of 200 kHz.

Recording procedure

Extracellular recordings—Extracellular single-unit recordings were made with borosilicate glass micropipettes (10–20 M Ω impedance, 2–3 μ m tip) filled with 0.5 M sodium acetate. The electrode was positioned above the IC by means of a precision (1 μ m) digital micromanipulator and lowered to the dorsal surface of the brain. The relative position of each

electrode was monitored from the readouts of digital micrometers using a common reference point on the skull. Vertical advancement of the electrode was made by a precision piezoelectric microdrive from outside the sound attenuating chamber. Recorded action potentials were amplified (Dagan 2400A preamplifier), monitored audiovisually on a digital oscilloscope (DL1640, YOKOGAWA), digitized and then stored on a computer hard drive using EPC-10 digital interface and PULSE software from HEKA at a bandwidth of 100 kHz.

Intracellular recordings—Intracellular recordings were made using microelectrodes made from 1.0 mm diameter quartz pipettes (Sutter Instruments, Novato, CA) filled with 1 M potassium acetate. Micropipettes, with impedance between 70–160 M Ω , were pulled on a micropipette puller (P2000, Sutter Instruments). The electrode was positioned above the IC by a digital micromanipulator (MP-285, Sutter Instruments) and lowered to the dorsal surface of the brain. Vertical advancement of the electrode was made by a precision microdrive (KOPF, Model 660) in 2 – 3 μ m steps. The electrode was placed on the surface of the IC using a surgical microscope (Leica MZ9.5).

Intracellular responses of IC neurons were amplified through a single channel amplifier (CYGNUS Technology Inc., model IR183A) and monitored on a digital oscilloscope (DL1640, YOKOGAWA). Intracellular waveforms from the IR183A and sound stimuli from the Tucker Davis system were digitized and then stored on a computer hard drive using EPC-10 digital interface and PULSE software from HEKA at a bandwidth of 100 kHz. While searching for a cell, small (5–100 nA) current pulses of 100 ms duration were delivered through the microelectrode. Measuring the amplitude of these pulses allowed us to monitor impedance changes in the recording electrode. Such changes were used to determine whether the recording electrode was approaching an IC neuron. 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. A prolonged (> 3 min), stable drop (>40 mV) in the DC level was an indicator of a stable impalement of an IC neuron. Intracellular recordings typically lasted 3 – 4 min (maximum 50 min). During intracellular recording cell membrane resting potentials fluctuated by less than 4 mV. Successful intracellular recording was always accompanied by postsynaptic potentials and large action potentials (> 30 mV). A sudden decrease in membrane resting potential was typical just before a recorded neuron was lost. Intracellular recordings were performed for IC neurons located in the central nucleus of the IC at depths between 200 μ m and 1400 μ m. Different angles of electrode penetration were used for multiple recordings from the same animal to avoid local damage to the IC.

Data analysis

During extracellular recordings, dot-raster histograms were used to depict the temporal discharge patterns of a unit for each sound level. Each dot in a raster histogram indicates a spike at time relative to the beginning of the recording. Response latency was defined as the elapsed time from stimulus onset to the beginning of a response and was quantified by peristimulus time histograms (PSTHs) constructed with 1 ms time bins. Responses were determined within a “response window” in which the firing rate was 25% greater than (or less than, in the case of inhibitory responses) the background rate of spontaneous firing over 10–20 repetitions of the same stimulus. Response threshold was defined as the minimum stimulus sound level required to evoke a response in 50% of presentations. To determine the duration of suppression of spontaneous firing elicited by sound stimuli, spontaneous firing rates were measured for 200 ms before and after the stimulus and were compared over 21 different stimulus levels (from 0 to 80 dB SPL in 4 dB increments). The majority of IC neurons had low spontaneous firing rates; thus, we typically repeated every stimulus 50 times to obtain a sufficient number of data points (Fig. 1A, B). A total of 1050 stimuli (50 repetitions of 21 different sound levels) were typically presented. Changes in firing rates were difficult to assess

from PSTHs, despite multiple repetitions of a single stimulus; thus we measured these changes using a 100 ms sliding window. The window of analysis was initially aligned with 0 ms on the time axis of the PSTH and it was shifted in 10 ms increments until the end of the recording trace (usually 2 s). Each window of analysis overlapped with the previous window by 90 ms. This approach dampened small fluctuations in the PSTH, providing a clearer view of changes in spontaneous firing rates (Fig. 1C). Each point on the curve in Fig. 1C corresponds to the start time of the analysis window. Data for 200 ms preceding a stimulus was used to compute the mean spontaneous firing rate (Fig. 1C). Spontaneous firing was defined as suppressed for time intervals after a stimulus in which the spike rate was continuously less than two standard deviations below the spontaneous rate (95% confidence limits) (Fig. 1C). This protocol was repeated for all 21 sound levels used in the recording session, thereby allowing measurement of suppression for a wide range of sound levels (Fig. 3B). A population of IC neurons (24%) fired spontaneously with relatively higher rates (>20 spikes/sec). For these neurons we limited the number of stimulus repetitions to 10 because there were no significant effects from greater numbers of repetitions on the PSTH.

During intracellular recordings, we often observed slight variability in the postsynaptic responses of individual neurons to a given stimulus presented multiple times. Therefore, responses were analyzed based on averages of 3 to 4 repetitions of recordings for a given stimulus. A baseline value for resting membrane potential was first calculated by determining the mean value for all samples of each waveform over a time window of at least 200 ms duration (sampling rate 10 μ s). Spikes and/or large de- or hyperpolarizations altered the mean value; thus we eliminated all values that exceeded one standard deviation and a new baseline mean value was calculated. For this purpose, large depolarizations or hyperpolarizations were defined as fluctuations of membrane potential away from the baseline by two standard deviations (95% confidence limits) occurring after the onset of the stimulus. Off-line data and statistical analyses were performed using custom software.

RESULTS

Spontaneous firing in the IC

Responses were obtained extracellularly from 67 neurons and intracellularly from 94 neurons. The majority of neurons recorded extracellularly (82%, 55/67) and intracellularly (83%, 88/94) fired spontaneously. Ranges of spontaneous firing rates and their distributions were remarkably similar over the two populations of neurons recorded with different techniques (Fig. 2). Firing rates ranged from 0.5 to 60.4 spikes per second (sp/s) for extracellular recordings, and 0.3 to 72 sp/s for intracellular recordings (Fig. 2A and B, respectively). The mean firing rates were 15.2 sp/s for extracellular and 12.3 sp/s for intracellular recordings. Mean values were skewed by very high spontaneous rates in a few neurons. Median spontaneous firing rates of 6 sp/s for extracellular recordings and 5.8 sp/s for intracellular recordings better reflect the population.

I. Extracellular study

Effect of a short FM sweep on spontaneous firing: Spontaneous activity of a majority of IC neurons (56%, 31/55) was suppressed after stimulus offset. Two principal characteristics of this suppression are illustrated by the neuron in Fig. 3A: firstly, the suppression extended well beyond the stimulus duration, and second, the duration of suppression increased with sound level. Presented at 16 dB SPL, FM sweeps evoked a suppression of spontaneous firing that lasted about 62 ms. This period almost doubled (122 ms) at 44 dB SPL. The duration of the suppression progressively increased to 395 ms at 80 dB SPL. Thus, the longest period of suppression was evoked by the highest sound level. This neuron, as was typical for the population, had quite a low (2.9 sp/s) spontaneous firing rate. All 31 spontaneously active

neurons showed level-dependent increases in duration of suppression (Fig. 3B). Threshold of suppression ranged from 12 to 72 dB SPL (mean 36 dB SPL, standard deviation 18.4).

Relationship between suppression and sound-evoked firing: We investigated whether the suppression of spontaneous firing in IC neurons depended on the presence of sound-evoked firing. Response patterns of IC neurons varied over a wide range of sound levels (Fig. 4). A large population of neurons that exhibited sound-evoked suppression of spontaneous firing (68%, 21/31) also showed monotonic increases in firing rate with stimulus level, or plateaus in firing rates. A representative neuron from this population shown in Fig 4A, fired in response to FM sounds presented from 36 to 72 dB SPL. The response spike count showed few changes within this range of sound levels. Suppression of spontaneous firing in this neuron occurred over the range of sound levels from 24 to 72 dB SPL. Thus, the threshold of the suppression (24 dB, SPL) was lower than the threshold of sound evoked firing (36 dB, SPL). A relatively small population of IC neurons (26%, 8/31) responded non-monotonically: their firing increased to a maximum and decreased thereafter with further increases in sound level (Fig. 4B). Two of the 31 neurons (6%) did not fire in response to FM sounds presented at any sound level (Fig. 4C). All 31 neurons showed suppression of spontaneous firing that was independent of response pattern. The duration of suppression, as was typical for IC neurons, increased with sound level. In the majority of IC neurons (90%, or 28/31), the threshold of the suppression was 4 to 16 dB lower than the threshold of sound-evoked firing (Fig. 4A, B).

Neurons with low spontaneous firing rates showed longer suppression: Neurons of the IC showed a range of spontaneous rates that varied from 0.3 to 72 sp/s (Fig. 2). At the highest sound levels, the duration of suppression ranged from 62 to 809 ms (Fig. 3B). Spontaneous firing rates in IC neurons were negatively correlated with the maximal durations of suppression ($r = -0.73$, $p < 0.001$). Neurons that exhibited lower rates of spontaneous firing showed longer durations of maximal suppression (Fig. 5).

Suppression of spontaneous firing in response to a train of FM sweeps: An individual FM sweep stimulus can suppress spontaneous firing in IC neurons for hundreds of milliseconds. We tested whether long trains of FM sweeps with inter-sweep intervals that were less than the duration of the suppression would completely suppress spontaneous firing for a duration equivalent to the train. We monitored responses of seven IC neurons exhibiting suppression of spontaneous firing to a train of five FM sweeps with a 100 ms inter-sweep interval and a total duration of 420 ms. This train duration and interval pattern approximates the echolocation signal that bats emit when in search of prey (Moss et al., 1997). Responses of a representative neuron are shown in Fig. 6. The neuron showed suppression of spontaneous firing that lasted about 120 ms in response to a single FM sweep presented at 60 dB SPL (Fig. 6A). When the train was presented this neuron fired to every FM sweep of the train, but spontaneous activity was suppressed in the inter-sweep intervals (Fig. 6B). In this case, suppression occurred beyond the train duration. Approximately 300 ms after the train, the rate of spontaneous activity was restored to the level observed before the train was introduced. Suppression of spontaneous firing within the train was 100% in 3 neurons and 92–95% in 4 neurons.

II. Intracellular study—Although intracellular recordings from awake animals provide valuable data, they have the major limitation of short recording time (typically 3–4 min). Therefore we could not repeat the same stimulus as many times as in extracellular recordings. For these reasons, the intracellular study was primarily focused on qualitative rather than quantitative features of long-term suppression.

Intracellular recordings were collected from 94 neurons in the IC of awake little brown bats. Neurons were included in the study only for recordings that met the following criteria: 1) resting membrane potential remained below -40 mV throughout the recording period, 2) resting

membrane potential varied by less than 4 mV throughout the recording period, and 3) spike height exceeded 30 mV. Intracellularly recorded response patterns of IC neurons were consistent with discharge patterns reported using extracellular recording techniques.

As stated previously, the vast majority of IC neurons recorded intracellularly (88%, 83/94) fired spontaneously, consistent with our observations from extracellular recordings (Fig. 2). Resting membrane potentials ranged from -40 to -75 mV (mean -49.3 mV, standard deviation 10.3). No significant correlation was observed between resting membrane potentials and rates of spontaneous firing in IC neurons ($r=0.04$).

Changes in membrane potential during suppression: Inhibition is a possible cause of the observed suppression of spontaneous firing. We tested this hypothesis by recording postsynaptic responses to single FM sweeps in 83 spontaneously active IC neurons. Consistent with results of the extracellular study, the majority of IC neurons (59%, 49/83) exhibited long-term suppression of spontaneous firing in response to a single FM sweep. Duration of suppression also increased as sound level increased. The maximal duration of suppression ranged from 70 to 560 ms (mean 264 ms, standard deviation 134 ms). A representative neuron (Fig. 7) exhibited suppression of spontaneous firing that lasted about 100 ms in response to an FM sweep presented at 20 dB SPL. Duration of suppression increased with sound level and lasted longer than 300ms at 80 dB SPL (Fig. 7). Contrary to our expectations, intracellular recordings showed that a short (<50 ms) membrane hyperpolarization was often (86%, 42/49) present at the beginning of the suppression but never lasted for the entire suppression.

Sound-evoked transient potentials during suppression

Previous studies have shown that shunting inhibition contributes to suppression of neuronal firing (Chance et al, 2002; Ulrich, 2003; Ayaz and Chance, 2009). During shunting inhibition, the chloride equilibrium potential approximates the resting potential of a neuron, and openings of chloride channels associated with GABA_a or glycine receptors cause little or no change in membrane potential. We hypothesized that shunting inhibition could explain the absence of hyperpolarizing potentials during the entire duration of suppression. We tested six IC neurons for the presence of shunting conductances by hyperpolarizing their membranes during the suppression. Membrane hyperpolarization was accomplished by applying a long (350 ms) hyperpolarizing current pulse, injected through the recording microelectrode, 50 ms after stimulus onset. Injection of hyperpolarizing currents into all six IC neurons tested elicited no evidence of shunting inhibition during the suppression. Postsynaptic responses of a representative IC neuron before, during, and after current injection are shown in Fig. 8. We observed neither a response to the stimulus, nor suppression of spontaneous firing, in response to an FM sweep presented at 10 dB SPL (Fig. 8A). When the sound level was raised to 80 dB SPL, this neuron showed a short and hyperpolarizing postsynaptic response to the stimulus, followed by a 200 ms suppression of spontaneous firing (Fig. 8B). If shunting inhibition were responsible for suppression, we would expect to see less hyperpolarization than in the control (10 dB SPL) condition. Instead, current injection produced a waveform similar to the response observed in the absence of the suppression when a low sound level was presented (Fig. 8C and D). These data suggest that shunting inhibition does not play a role in suppressing spontaneous firing in IC neurons.

An interesting phenomenon we consistently observed during hyperpolarization of IC neurons was a reduction of postsynaptic membrane activity during the period of suppression. This phenomenon was readily observed during hyperpolarization because postsynaptic activity during this period was not masked by action potentials. The neuron shown in Fig. 8 exhibited postsynaptic potentials with peak amplitudes of about 8 mV after its response to a 10 dB SPL FM sweep, when suppression of spontaneous activity was not present (Fig. 8C). However, this

synaptic excitation was reduced during the period of suppression when the FM sweep level was increased to 80 dB SPL (Fig. 8D). A suppression of excitation during suppression of spontaneous firing may reflect a reduction of neurotransmitter release from terminals presynaptic to the recorded IC neuron and/or a reduction of presynaptic firing.

Input resistance is unchanged during suppression

We further tested whether active ionic conductances play a role in postsynaptic responses of IC neurons during suppression of spontaneous firing. These changes were assessed by measuring input resistance in IC neurons before, during, and after suppression. This method can detect whether suppression of spontaneous firing is accompanied by openings of ion channels, which would significantly reduce neuronal input resistance (Volkov and Galazyuk, 1992). We injected a train of short (10 ms) hyperpolarizing current pulses, with a 10 ms interpulse interval, through an intracellular recording electrode and measured the amplitudes of neuronal responses. The beginning of a current pulse injection was synchronized with the beginning of each recording trace; therefore current pulses occurred at the same time over multiple recording trials. Two hyperpolarizing current pulses that were injected before each sound stimulus were used as reference points for comparison with similar measurements obtained after sound presentation. We averaged measurements for at least three trials to reduce the impact of possible variations in input resistance. We did not observe a reduction in input resistance during the suppression. However, we did observe a significant reduction, during the initial postsynaptic response, to an FM sweep in the five IC neurons studied. A representative neuron is shown in Fig. 9. This neuron exhibited no suppression of spontaneous firing in response to an FM sweep presented at 10 dB SPL (Fig. 9A). We did not detect significant changes in neuron input resistance (Fig. 9A, C, E). When the sound level was increased to 80 dB SPL, this neuron showed typical response patterns for IC neurons: IPSP-EPSP-spike-IPSP (Voytenko and Galazyuk, 2007, 2008; Peterson et al, 2008) followed by a suppression of spontaneous firing that lasted about 100 ms (Fig. 9B). We observed no significant changes in input resistance during suppression, except for a period lasting 50 ms which was started 10 ms after stimulus onset (Fig. 9B, D, F). During this short time period, a postsynaptic response was evident, and the input resistance was greatly reduced (Fig. 9D, F). The absence of change in input resistance during the entire period of suppression suggests that suppression occurs presynaptically, on presynaptic terminals and/or on presynaptic neurons, rather than postsynaptically.

DISCUSSION

Our research produced two significant observations. Firstly, the majority of IC neurons greatly suppressed their spontaneous firing when sound stimuli were presented. This suppression persisted long after the end of the sound stimulus and increased in duration with sound level. Secondly, neither membrane hyperpolarization nor shunting inhibition contributed to suppression, except for a few tens of milliseconds after the stimulus.

A possible origin of spontaneous firing in IC neurons

Spontaneous firing in the IC can be generated intrinsically and/or may be a reflection of synaptic inputs. Data from multiple *in vitro* studies suggest that an intrinsic source is unlikely. A number of studies have reported that spontaneous firing is rare in IC brain slices (Wu et al., 2004; Sun et al., 2006; Sun and Wu, 2008); however, robust spontaneous firing in the IC has been shown by one *in vitro* study (Basta and Ernest, 2004). The rarity of spontaneous firing *in vitro* further suggests that local IC circuits are not likely to contribute to spontaneous firing. The most probable scenario is that synaptic inputs to IC neurons trigger spontaneous firing in the IC. These inputs may arise from multiple sources (ascending and/or descending) provided by auditory as well as non-auditory brain structures (Irvine, 1986; Jain and Shore, 2006; Zhou

and Shore, 2006; Dehmel et al., 2008). The ventral cochlear nucleus is a primary excitatory projection to the IC (Helfert et al., 1991; Ramachandran et al., 1999; Covey and Casseday, 1995). Multipolar or stellate cells in both anterior and posterior ventral cochlear nucleus project directly to the contralateral IC (Cant, 1982; Schofield and Cant, 1996). A majority of these neurons exhibit robust spontaneous firing as a consequence of spontaneous firing in auditory nerve fibers (Geisler et al., 1985; Liberman, 1978; Tsuji and Liberman, 1997).

Spontaneous activity in the auditory nerve originates from two primary sources. First, postsynaptic potentials are generated by stochastic release of neurotransmitter from hair cells (Furukawa and Ishii, 1967; Ishii et al., 1971; Furukawa et al., 1972, 1978; Sewell, 1984). A second source is the responses of cochlear afferents (Tsuji and Liberman, 1997) to low-level background acoustic noise (Lima da Costa et al., 1997; Popelar et al., 1996). Guinan and Gifford (1988) reported a consistent, but incomplete, suppression of spontaneous firing in auditory nerve fibers in response to efferent stimulation in cats. Cook et al. (2002) also demonstrated a partial (approximately 40%) reduction in spontaneous activity of auditory nerve fibers in gerbils after acute conductive hearing loss. Conversely, bilateral cochlear removal dramatically, but not completely, suppresses spontaneous firing in neurons of brain stem auditory nuclei in chicks (Born et al., 1991) and cats (Koerber et al., 1966). Thus, ascending auditory inputs are likely to be the dominant source of spontaneous firing in IC neurons. However, descending auditory pathways (Aitkin et al., 1981; Huffman and Henson, 1990; Winer et al., 1998; Coomes et al., 2005), as well as inputs from non-auditory brain structures (Tokunaga et al., 1984) may also contribute to spontaneous firing in IC neurons.

Possible cellular mechanisms underlying suppression of spontaneous firing

Suppression of stimulus-driven or spontaneous firing in the auditory cortex has been attributed to synaptic inhibition (Brosch and Schreiner, 1997; Calford and Sample, 1995; Tan et al., 2004). This has been directly tested in a study utilizing both extracellular and intracellular recordings from the auditory cortex (Wehr and Zador, 2005). It has also been demonstrated that neurons exhibiting forward suppression that persists for hundreds of milliseconds do not show inhibitory conductances for longer than 50–100 ms. In our present and previous intracellular studies (Voytenko and Galazyuk 2007, 2008), we also rarely observed membrane hyperpolarizations in the IC lasting longer than 50 ms, even though suppression can last for hundreds of milliseconds. In an attempt to identify a possible cellular mechanism underlying this long-lasting suppression, we tested whether input resistance in IC neurons was altered during the suppression. A significant drop in input resistance would suggest that extra ion channels open that do not open under control conditions. We found that input resistance remained unchanged during suppression. By applying a long hyperpolarizing current pulse to IC neurons during suppression, we also showed that shunting inhibition does not play a role in this phenomenon. During these experiments it was evident that excitation was greatly reduced during suppression, suggesting that a presynaptic, rather than a postsynaptic, mechanism contributes to the phenomenon.

Collectively these different approaches provide strong evidence that long-term suppression of spontaneous firing occurs on presynaptic terminals and/or in presynaptic neurons elsewhere in the auditory system. However, we can not rule out the possibility that long lasting inhibition may occur postsynaptically on distal dendrites, making it undetectable by intracellular somatic recordings. A similar explanation has recently been proposed to explain an inhibition-based mechanism underlying combination-sensitive responses observed in IC neurons (Peterson et al, 2008).

A sound stimulus that lasts only four milliseconds can trigger a suppression of spontaneous firing that lasts up to hundreds of milliseconds. The prolonged suppression of spontaneous activity raises the question of the membrane receptors involved in this phenomenon. Ionotropic

receptors, upon activation, offer fast but short responses. However, the effects of metabotropic receptors, often triggered by the same neurotransmitters as ionotropic receptors, can persist for hundreds of milliseconds or even minutes. We speculate that metabotropic glutamate receptors (mGluRs) may contribute to the suppression of spontaneous firing (see review by Ferraguti and Shigemoto, 2006). The amount of glutamate released depends on the stimulus intensity (Nelson and Erulkar 1963; Erulkar 1983), thus if the duration of suppression is driven by mGluRs, then the duration would be expected to increase with sound level. All neurons in our study that exhibited suppression also showed increases in duration of suppression with increases in sound level.

Contradictory to our hypothesis a small population of neurons did not respond to the sound stimulus, but still showed suppression of spontaneous activity. There are two possible explanations for this discrepancy. One explanation is that these neurons do receive strong excitatory input, but this excitation is suppressed by a stronger inhibitory input that arrives slightly earlier. Such an inhibitory-excitatory interaction has been described for IC neurons (Voytenko and Galazyuk 2007; 2008). It is also possible that mGluRs are activated in neurons at lower levels of the auditory system, and these neurons in turn provide excitatory inputs to the IC.

The olivocochlear reflex might also contribute to suppression in the IC. Electrical stimulation of the olivocochlear bundle raises thresholds and suppresses responses in the auditory periphery (Galambos, 1956; Wiederhold and Kiang, 1970). Furthermore, the olivocochlear reflex in the natural sound environment enhances responses to transient stimuli by suppressing responses to steady background noise (Winslow and Sachs, 1987; Kawase et al., 1993). However, this reflex is unlikely to be fully responsible for the suppression in the IC, because it can not suppress non-auditory inputs (Irvine, 1986; Jain and Shore, 2006; Zhou and Shore, 2006; Dehmel et al., 2008).

Implications for signal processing

Real-world acoustic signals, including human speech, are composed of a sequence of sound elements. The present and other studies (Smith 1977; Harris and Dallos 1979; Delgutte 1980; Moore et al., 1988; Relkin and Turner, 1988; Nelson et al, 2009) have shown that a majority of auditory neurons exhibit sound-evoked suppression of both spontaneous firing and sound-evoked responses. Therefore, if a sound sequence occurs, the first sound of this sequence will be likely to trigger suppression, whereas subsequent sounds may maintain the suppression. Consequently, all but the first sound in a sequence will be processed by auditory neurons amongst little or no spontaneous activity (Fig. 6). When spontaneous firing is absent or greatly suppressed the presence of a spike in response to a sound is a more reliable indicator of the presence of a sound stimulus. Investigations still need to be made to ascertain the cellular mechanisms mediating the suppression of spontaneous firing along with its biological significance.

Acknowledgments

We thank Jeffrey Wenstrup, David Gooler, Jon Walro, Jasmine Grimsley, Marie Gadziola, and Calum Grimsley for their valuable comments on earlier versions of this manuscript. Thank is also due to Olga Galazyuk for developing software that allowed off-line data analysis and statistical evaluation. This work was supported by a grant R01DC005377 from the National Institute on Deafness and Other Communication Disorders of the NIH.

Abbreviations

FM	frequency modulated
IC	inferior colliculus

mGluR	metabotropic glutamate receptor
PSTH	peristimulus time histogram

References

- Aitkin LM, Kenyon CE, Philpott P. The representation of the auditory and somatosensory systems in the external nucleus of the cat inferior colliculus. *J Comp Neurol* 1981;196:25–40. [PubMed: 7204665]
- Ayaz A, Chance FS. Gain Modulation of Neuronal Responses by Subtractive and Divisive Mechanisms of Inhibition. *J Neurophysiol* 2009;101:958–968. [PubMed: 19073814]
- Basta D, Ernest A. Noise-induced changes of neuronal spontaneous activity in mice inferior colliculus brain slices. *Neurosci Lett* 2004;368:297–302. [PubMed: 15364415]
- Born DE, Durham D, Rubel EW. Afferent influences on brainstem auditory nuclei of the chick: nucleus magnocellularis neuronal activity following cochlea removal. *Brain Res* 1991;557:37–47. [PubMed: 1747768]
- Boudreau CE, Ferster D. Short-term depression in thalamocortical synapses of cat primary visual cortex. *J Neurosci* 2005;25:7179–7190. [PubMed: 16079400]
- Brosch M, Schreiner CE. Time course of forward masking tuning curves in cat primary auditory cortex. *J Neurophysiol* 1997;77:923–943. [PubMed: 9065859]
- Calford MB, Semple MN. Monaural inhibition in cat auditory cortex. *J Neurophysiol* 1995;73:1876–1891. [PubMed: 7623087]
- Cant NB. Identification of cell types in the anteroventral cochlear nucleus that project to the inferior colliculus. *Neurosci Lett* 1982;32:241–246. [PubMed: 7177487]
- Chance FS, Abbott LF, Reyes AD. Gain modulation from background synaptic input. *Neuron* 2002;35:773–782. [PubMed: 12194875]
- Cook RD, Hung TY, Miller RL, Smith DW, Tucci DL. Effects of conductive hearing loss on auditory nerve activity in gerbil. *Hear Res* 2002;164:127–137. [PubMed: 11950532]
- Coomes DL, Schofield RM, Schofield BR. Unilateral and bilateral projections from cortical cells to the inferior colliculus in guinea pigs. *Brain Res* 2005;1042:62–72. [PubMed: 15823254]
- Covey E, Casseday JH. The lower brainstem and auditory pathways. In: Popper, AN.; Fay, RR., editors. *Hearing by Bats*. New York: Springer-Verlag; 1995. p. 235-295.
- Covey E, Kauer JA, Casseday JH. Whole-cell patch-clamp recording reveals subthreshold sound-evoked postsynaptic currents in the inferior colliculus of awake bats. *J Neurosci* 1996;16:3009–3018. [PubMed: 8622130]
- Dehmel S, Cui YL, Shore SE. Cross-modal interactions of auditory and somatic inputs in the brainstem and midbrain and their imbalance in tinnitus and deafness. *Am J Audiol* 2008;17:193–209.
- Delgutte B. Representation of speech-like sounds in the discharge patterns of auditory-nerve fibers. *J Acoust Soc Am* 1980;68:843–857. [PubMed: 7419820]
- Ebert U, Ostwald J. GABA can improve acoustic contrast in the rat ventral cochlear nucleus. *Exp Brain Res* 1995;104:310–322. [PubMed: 7672023]
- Erulkar SD. The modulation of neurotransmitter release at synaptic junctions. *Rev Physiol Biochem Pharmacol* 1983;98:63–175. [PubMed: 6140742]
- Ferraguti F, Shigemoto R. Metabotropic glutamate receptors. *Cell Tissue Res* 2006;326:483–504. [PubMed: 16847639]
- Furukawa T, Ishii Y. Neurophysiological studies of hearing in goldfish. *J Neurophysiol* 1967;30:1377–1403. [PubMed: 6066445]
- Furukawa T, Ishii Y, Matsuura S. Synaptic delay and time course of postsynaptic potentials at the junction between hair cells and eighth nerve fibers in the goldfish. *Jap J Physiol* 1972;22:617–635. [PubMed: 4347488]
- Furukawa T, Hayashida Y, Matsuura S. Quantal analysis of the size of excitatory post-synaptic potentials at synapses between hair cells and afferent nerve fibers in goldfish. *J Physiol* 1978;276:211–226. [PubMed: 206683]

- Galambos R. Suppression of auditory nerve activity by stimulation of efferent fibers to cochlea. *J Neurophysiol* 1956;19:424–437. [PubMed: 13367873]
- Galazyuk AV, Lin W, Llano D, Feng AS. Leading inhibition to neural oscillation is important for time-domain processing in the auditory midbrain. *J Neurophysiol* 2005;94:314–326. [PubMed: 15772243]
- Geisler CD, Deng L, Greenberg SR. Thresholds for primary auditory fibers using statistically defined criteria. *J Acoust Soc Am* 1985;77:1102–1109. [PubMed: 3980864]
- Guinan JJ Jr, Gifford ML. Effects of electrical stimulation of efferent olivocochlear neurons on cat auditory-nerve fibers. II. Spontaneous rate. *Hear Res* 1988;33:115–127. [PubMed: 3397322]
- Gummer AW, Mark RF. Patterned neural activity in brain stem auditory areas of a prehearing mammal, the tammar wallaby (*Macropus eugenii*). *Neuroreport* 1994;5:685–688. [PubMed: 8199338]
- Harris DM, Dallos P. Forward masking of auditory nerve fiber responses. *J Neurophysiol* 1979;42:1083–1107. [PubMed: 479921]
- Helfert, RH.; Snead, CR.; Altschuler, RA. The ascending auditory pathways. In: Altschuler, RA.; Bobbin, RP.; Clopton, BM.; Hoffman, DW., editors. *Neurobiology of Hearing: The Central Auditory System*. New York: Raven Press; 1991. p. 1-25.
- Hermann J, Pecka M, von Gersdorff H, Grothe B, Klug A. Synaptic transmission at the calyx of Held under in vivo like activity levels. *J Neurophysiol* 2007;98:807–820. [PubMed: 17507501]
- Huffman RF, Henson OW. The descending auditory pathway and acousticomotor systems: connections with the inferior colliculus. *Brain Research Review* 1990;15:295–323.
- Jain R, Shore S. External inferior colliculus integrates trigeminal and acoustic information: unit responses to trigeminal nucleus and acoustic stimulation in the guinea pig. *Neurosci Lett* 2006;395:71–75. [PubMed: 16298057]
- Jones TA, Jones SM, Paggett KC. Primordial rhythmic bursting in embryonic cochlear ganglion cells. *J Neurosci* 2001;21:8129–8135. [PubMed: 11588185]
- Jones TA, Leake PA, Snyder RL, Stakhovskaya O, Bonham B. Spontaneous discharge patterns in cochlear spiral ganglion cells before the onset of hearing in cats. *J Neurophysiol* 2007;98:1898–1908. [PubMed: 17686914]
- Irvine, DRF. The auditory brainstem. In: Ottoson, D., editor. *Progress in Sensory Physiology*. Vol. 7. Berlin: Springer-Verlag; 1986. p. 1-279.
- Ishii Y, Matsuura S, Furukawa T. Quantal nature of transmission at the synapse between the hair cells and eighth nerve fibers. *Jap J Physiol* 1971;21:91–98. [PubMed: 4340492]
- Kawase T, Delgutte B, Liberman MC. Antimasking effects of the olivocochlear reflex. II. Enhancement of auditory-nerve response to masked tones. *J Neurophysiol* 1993;70:2533–2549. [PubMed: 8120597]
- Koerber KC, Pfeiffer RR, Warr WB, Kiang NY. Spontaneous spike discharges from single units in the cochlear nucleus after destruction of the cochlea. *Exp Neurol* 1966;16:119–130. [PubMed: 5922930]
- Kuwada S, Batra R, Yin TC, Oliver DL, Haberly LB, Stanford TR. Intracellular recordings in response to monaural and binaural stimulation of neurons in the inferior colliculus of the cat. *J Neurosci* 1997;17:7565–7581. [PubMed: 9295401]
- Liberman MC. Auditory-nerve response from cats raised in a low-noise chamber. *J Acoust Soc Am* 1978;63:442–455. [PubMed: 670542]
- Lima da Costa D, Erre JP, Charlet de Sauvage R, Popelar J, Aran JM. Bioelectrical cochlear noise and its contralateral suppression: relation to background activity of the eighth nerve and effects of sedation and anesthesia. *Exp Brain Res* 1997;116:259–269. [PubMed: 9348125]
- Moore BC, Glasberg BR, Plack CJ, Biswas AK. The shape of the ear's temporal window. *J Acoust Soc Am* 1988;83:1102–1116. [PubMed: 3356815]
- Moss CF, Redish D, Gounden C, Kunz TH. Ontogeny of vocal signals in the little brown bat, *Myotis lucifugus*. *Anim Behav* 1997;54:131–141. [PubMed: 9268443]
- Nelson PG, Erulkar SD. Synaptic mechanisms of excitation and inhibition in the central auditory pathway. *J Neurophysiol* 1963;26:908–923. [PubMed: 14084166]
- Nelson PC, Smith ZM, Young ED. Wide-dynamic-range forward suppression in marmoset inferior colliculus neurons is generated centrally and accounts for perceptual masking. *J Neurosci* 2009;29:2553–2562. [PubMed: 19244530]

- O'Leary DD, Ruff NL, Dyck RH. Development, critical period plasticity, and adult reorganizations of mammalian somatosensory systems. *Curr Opin Neurobiol* 1994;4:535–544. [PubMed: 7812142]
- Pedemonte M, Torterolo P, Velluti RA. In vivo intracellular characteristics of inferior colliculus neurons in guinea pigs. *Brain Res* 1997;759:24–31. [PubMed: 9219859]
- Peterson DC, Voytenko S, Gans D, Galazyuk A, Wenstrup JJ. Intracellular recordings from combination-sensitive neurons in the inferior colliculus. *J Neurophysiology* 2008;100:629–645.
- Popelar J, Lima da Costa D, Erre JP, Avan P, Aran JM. Contralateral suppression of the ensemble background activity of the auditory nerve in awake guinea pigs: effects of gentamicin. *Audit Neurosci* 1996;3:425–433.
- Portfors CV, Roberts PD. Temporal and frequency characteristics of cartwheel cells in the dorsal cochlear nucleus of the awake mouse. *J Neurophysiol* 2007;98:744–756. [PubMed: 17581852]
- Prochnow N, Schmidt M. Spontaneous activity of rat pretectal nuclear complex neurons in vitro. *BMC Neurosci* 2004;5:29. [PubMed: 15333139]
- Ramachandran R, Davis KA, May BJ. Single-unit responses in the inferior colliculus of decerebrate cats. I. Classification based on frequency response maps. *J Neurophysiol* 1999;82:152–163. [PubMed: 10400944]
- Relkin EM, Turner CW. A reexamination of forward masking in the auditory nerve. *J Acoust Soc Am* 1988;84:584–591. [PubMed: 3170950]
- Schofield BR, Cant NB. Projections from the ventral cochlear nucleus to the inferior colliculus and the contralateral cochlear nucleus in guinea pigs. *Hear Res* 1996;102:1–14. [PubMed: 8951445]
- Sewell WF. The relation between the endocochlear potential and spontaneous activity in auditory nerve fibers of the cat. *J Physiol* 1984;347:685–696. [PubMed: 6707972]
- Smith RL. Short-term adaptation in single auditory nerve fibers: some poststimulatory effects. *J Neurophysiol* 1977;40:1098–1111. [PubMed: 903799]
- Sun H, Ma CL, Kelly JB, Wu SH. GABA_B receptor-mediated presynaptic inhibition of glutamatergic transmission in the inferior colliculus. *Neurosci Lett* 2006;399:151–156. [PubMed: 16513264]
- Sun H, Wu SH. Physiological characteristics of postinhibitory rebound depolarization in neurons of the rat's dorsal cortex of the inferior colliculus studied in vitro. *Brain Res* 2008;1226:70–81. [PubMed: 18586018]
- Tan AY, Zhang LI, Merzenich MM, Schreiner CE. Tone-evoked excitatory and inhibitory synaptic conductances of primary auditory cortex neurons. *J Neurophysiol* 2004;92:630–643. [PubMed: 14999047]
- Tokunaga A, Sugita S, Otani K. Auditory and non-auditory subcortical afferents to the inferior colliculus in the rat. *J Hirnforsch* 1984;25:461–472. [PubMed: 6481158]
- Tortorolo P, Pedemonte M, Velluti RA. Intracellular in vivo recording of inferior colliculus auditory neurons from awake guinea-pigs. *Arch Ital Biol* 1995;134:57–64. [PubMed: 8919192]
- Trageser JC, Burke KA, Masri R, Li Y, Sellers L, Keller A. State-dependent gating of sensory inputs by zona incerta. *J Neurophysiol* 2006;96:1456–1463. [PubMed: 16775205]
- Tsuji J, Liberman MC. Intracellular labeling of auditory nerve fibers in guinea pig: central and peripheral projections. *J Comp Neurol* 1997;381:188–202. [PubMed: 9130668]
- Ulrich D. Differential arithmetic of shunting inhibition for voltage and spike rate in neocortical pyramidal cells. *Eur J Neurosci* 2003;18:2159–2165. [PubMed: 14622176]
- Volkov IO, Galazyuk AV. Peculiarities of inhibition in cat auditory cortex neurons evoked by tonal stimuli of various durations. *Exp Brain Res* 1992;91:115–120. [PubMed: 1301363]
- Voytenko SV, Galazyuk AV. Intracellular recording reveals temporal integration in inferior colliculus neurons of awake bats. *J Neurophysiol* 2007;97:1368–1378. [PubMed: 17135472]
- Voytenko SV, Galazyuk AV. Timing of sound-evoked potentials and spike responses in the inferior colliculus of awake bats. *Neuroscience* 2008;155:923–936. [PubMed: 18621102]
- Wang X, Galazyuk AV, Feng AS. FM signals produce robust paradoxical latency shifts in the bat's inferior colliculus. *J Comp Physiol A* 2007;193:13–20.
- Walsh EJ, McGee J. Postnatal development of auditory nerve and cochlear nucleus neuronal responses in kittens. *Hear Res* 1987;28:97–116. [PubMed: 3610862]

- Wiederhold ML, Kiang NY. Effects of electric stimulation of the crossed olivocochlear bundle on single auditory-nerve fibers in the cat. *J Acoust Soc Am* 1970;48:950–965. [PubMed: 5480390]
- Winer JA, Larue DT, Diehl JJ, Hefti BJ. Auditory cortical projections to the cat inferior colliculus. *J Comp Neurol* 1998;400:147–174. [PubMed: 9766397]
- Winslow RL, Sachs MB. Effect of electrical stimulation of the crossed olivocochlear bundle on auditory nerve response to tones in noise. *J Neurophysiol* 1987;57:1002–1021. [PubMed: 3585452]
- Wehr M, Zador AM. Synaptic mechanisms of forward suppression in rat auditory cortex. *Neuron* 2005;47:437–445. [PubMed: 16055066]
- Wu SH, Ma CL, Kelly JB. Contribution of AMPA, NMDA, and GABA(A) receptors to temporal pattern of postsynaptic responses in the inferior colliculus of the rat. *J Neurosci* 2004;24:4625–4634. [PubMed: 15140934]
- Zhou J, Shore S. Convergence of spinal trigeminal and cochlear nucleus projections in the inferior colliculus of the guinea pig. *J Comp Neurol* 2006;495:100–112. [PubMed: 16432905]

Figure 1.

Statistical evaluation of sound-evoked suppression of spontaneous firing using multiple stimulus presentations. *A*: Dot-raster time histogram of 50 individual traces recorded from an IC neuron exhibiting suppression of spontaneous firing in response to FM sweeps of 4 ms duration presented at 80 dB SPL and repeated 50 times. *B*: Peristimulus time histogram (PSTH) generated from data shown in *A*. Bin size is 10 ms. The time course of the FM sweep is shown at the bottom by a black vertical bar (histograms shown in *A* and *B* have the same time scale). *C*: A curve representing changes in spontaneous firing rates obtained by plotting spike count values within a 100-ms analyzing time window sliding along the PSTH shown in *B*, in 10 ms increments. Each point on the curve is identified by the start time of the window of analysis.

The dashed horizontal line on the graph indicates the spontaneous firing rate computed for 200 ms before a stimulus was presented. The horizontal solid lines indicate the 95% CLs of the spontaneous firing rate. Only values exceeding the 95% CLs are judged to be significant changes of spontaneous firing rate. A thick horizontal line indicates the time period when spontaneous firing was continually suppressed (below the 95% CL level).

Figure 2. Distribution of spontaneous firing rates for 59 neurons in the IC recorded extracellularly (A) and 83 neurons recorded intracellularly (B). Bin size is 2 ms.

Figure 3.

Suppression of spontaneous firing in IC neurons by sound stimuli. *A*: Cumulative dot-raster time histogram of a representative IC neuron exhibiting suppression of spontaneous firing in response to FM sweeps presented at different sound levels. Each of the 21 sound levels was repeated 50 times. Note that duration of the suppression increased with sound level. Spontaneous firing rate is indicated in the upper right. *B*: Sound level-dependent changes in the suppression duration in a population of 31 IC neurons.

Figure 4.

Suppression of spontaneous firing in IC neurons was largely independent of firing in response to sound stimuli. *A, B, C*: PSTHs of three IC neurons exhibiting the most typical firing patterns when sound level was increased from 0 to 72 dB SPL (monotonic, non-monotonic, and no firing, respectively). Independently of response pattern, these neurons showed suppression of the spontaneous firing. The time course of the FM sweep stimulus is represented below each column by a black vertical bar. Each stimulus was repeated 40 times. Bin size is 10 ms.

Figure 5.

Negative correlation ($r = -0.72$) between the spontaneous firing rate and the maximal duration of spontaneous firing suppression for 31 IC neurons.

Figure 6.

Spontaneous firing was greatly suppressed during responses to a long (400 ms) train of FM sweeps. *A*: typical suppression of spontaneous firing in an IC neuron in response to a single FM sweep. The duration of suppression increased with sound level. Each of the 21 sound levels was repeated 50 times. Spontaneous firing rate is indicated in the upper right. *B*: PSTH of the same neuron stimulated with a train of 5 FM sweeps with interstimulus intervals of 100 ms presented at 50 dB SPL. This stimulus was repeated 50 times. Bin size is 20 ms.

Figure 7.

Typical intracellular response traces of an IC neuron exhibiting suppression of spontaneous firing in response to a single FM sweep presented at different sound levels (shown on the left of each trace). The time course of an FM sweep is shown by a black vertical bar at the bottom (same time scale as intracellular traces). Horizontal dashed lines indicate the resting membrane potential (shown on the right of each trace). Black arrows indicate short membrane hyperpolarizations that typically occurred during the initial part of the suppression.

Figure 8. Long-lasting suppression of spontaneous firing is not explained by shunting inhibition. *A,B*: individual intracellular response traces of an IC neuron in response to a single FM sweep presented at 10 dB and 80 dB SPL, respectively. Note that in response to a loud sound this neuron showed suppression of spontaneous firing that lasted about 300 ms, whereas from stimulus onset it exhibited a short lasting (about 40 ms) membrane hyperpolarization. *C,D*: intracellular traces from the same neuron for hyperpolarizing current injections. Current injections hyperpolarized the neuron membrane by about 10 mV, but it did not show any potentially-inhibitory conductances. Arrows indicate large amplitude postsynaptic potentials.

Figure 9.

Input resistance in IC neurons was not altered during suppression of spontaneous firing. *A, B*: overlay of three individual intracellular response traces (shown in grey) and an averaged trace (shown in black) of an IC neuron in response to an FM sweep presented at 10 dB and 80 dB SPL, respectively. Note that at the initial part of response to an 80 dB SPL sweep this neuron showed a typical response pattern for the IC: hyperpolarization followed by depolarization followed by hyperpolarization [see inset in *B*] (Voytenko Galazyuk 2007; 2008). *C, D*: overlay of three individual intracellular traces from the same neuron when short (10 ms) hyperpolarizing current pulses were applied through the recording electrode to measure neuron's input resistance, while FM sweeps were presented at 10 dB and 80 dB SPL, respectively. *E, F*: mean and standard deviation of input resistance measured before, during, and after neuron's responses to 10 dB and 80 dB SPL FM sweeps, respectively. Note that the input resistance of this neuron did not change during the suppression, but was greatly reduced during postsynaptic response just before the suppression (outlined by two vertical dashed lines in *B, D*, and *F*).