Duration selective neurons in the inferior colliculus of the rat: topographic distribution and relation of duration sensitivity to other response properties.

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ABSTRACT

Many animals use duration to help them identify the source and meaning of a sound. Duration sensitive neurons have been found in the auditory midbrain of mammals and amphibians, where their selectivity appears to correspond to the lengths of species-specific vocalizations. In this study, single neurons in the rat inferior colliculus (IC) were tested for sensitivity to sound duration. About half (54%) of the units sampled showed some form of duration selectivity. The majority of these (76%) were long pass neurons that responded to sounds exceeding some duration threshold (range: 5 to 60 ms). Band pass neurons, which only responded to a restricted range of durations, made up 13% of duration sensitive neurons (best durations: 15 to 120 ms). Other units displayed short pass (2%), or mixed (9%) response patterns. The majority of duration sensitive neurons were localized outside the central nucleus of the IC, especially in the dorsal cortex, where over half of the neurons sampled had long pass selectivity for duration. Band pass duration tuned neurons were only found outside the central nucleus. Characteristics of duration sensitive neurons in the rat support the idea that this filtering arises through an interaction of excitatory and inhibitory inputs that converge in the IC. Band pass neurons typically responded at sound offset, suggesting that their tuning is created through the same mechanisms that have been described in echolocating bats. The finding that the first spike latencies of all long pass neurons were longer than the shortest duration to which they responded supports the idea that they receive transient inhibition prior to, or simultaneously with a sustained excitatory input. The ranges of selectivity in rat IC neurons are within the range of durations of rat vocalizations. These data suggest that a population of neurons in the rat IC have evolved to transmit

information about behaviorally relevant sound durations using mechanisms that are common to all mammals, with an emphasis on long pass tuning characteristics.

INTRODUCTION

Neurons that are selective for the duration of a sound have been described in the central auditory system of several vertebrates including frogs (Potter 1965; Narins and Capranica 1980), bats (Pinheiro et al. 1991; Casseday et al. 1994, 2000; Ehrlich et al. 1997; Fuzessery 1994; Mora and Kössl 2004), cats (He et al. 1997), chinchillas (Chen 1998) and mice (Brand et al. 2000). Duration selective neurons have only been found at or above the level of the inferior colliculus (IC) or its homolog in any of the species studied, so this form of neural filtering appears to be an emergent property that results from circuitry operating within the midbrain. The IC is innervated by multiple lower brainstem structures with excitatory or inhibitory inputs that vary in latency, temporal pattern, and sensitivity to multiple sound parameters including frequency and amplitude (e.g., Casseday et al. 2002). Previous studies have suggested that duration selectivity is generated through the interaction of such inputs, in particular, the combination of convergent excitatory and inhibitory inputs with specific temporal relationships (Ehrlich et al. 1997; Casseday et al. 1994, 2002; Brand et al. 2000; Faure et al. 2003).

In certain species with specialized auditory behavior, the ranges of sound durations to which neurons are tuned correspond closely to the range of durations found in behaviorally important sounds. This matching of neural duration sensitivity to the duration of vocalizations has been described in frogs, where neurons' duration tuning matches the durations of communication calls (Narins and Capranica 1980) and in bats, where neurons' duration tuning matches the durations of echolocation signals (Pinheiro et al. 1991; Ehrlich et al. 1997; Faure et

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al. 2003). More recently, duration sensitive neurons have been found in animals that lack obvious auditory specializations. Although the advantages of duration sensitivity in more generalized species have not been thoroughly investigated, it potentially serves to identify specific sounds. For example, the IC of mice contains a high proportion of long pass neurons selective for sounds longer than several tens of ms. The few observed band pass neurons typically have best durations on the order of tens of ms (Brand et al. 2000). Both of these filtering properties are congruent with the duration of adult and pup ultrasound vocalizations (Liu et al. 2003).

Many calls in rats have highly stereotyped temporal patterns (Kaltwasser 1990) in which call duration could convey important information such as individual identity, social status, or contextual situations. For example, rat ultrasonic vocalizations include long (>300 ms) calls that are emitted during a negative affective state when animals are anticipating punishment or engaging in avoidance behaviors and short (<300 ms) calls that are produced during a positive affective state when they are anticipating a reward or displaying approach behaviors (Knutson et al. 2002). Isolation calls of rat pups range from about 80 to 140 ms, decreasing in duration as a function of the pup's age. They also differ in length between individuals and show greater similarity within than across litters (Brudzynski et al. 1999). Thus, duration could be used by receivers to recognize kin and offspring (Brudzynski et al. 1993). Rats have a rich repertoire of sounds that are produced during exploration, agonistic encounters, sexual behavior, and other social interactions for which duration could convey meaning to conspecifics. To date, there have been no studies to determine whether the rat central auditory system contains duration sensitive neurons and, if so, how these relate to durations of vocal communication signals. The aim of this study was to determine whether there are duration selective neurons in the midbrain of the rat,

one of the most commonly used laboratory animals and, if so, how the range of duration selectivity corresponds to the duration of their known repertoire of vocalizations. Preliminary reports have been presented in abstract form elsewhere (Pérez-González et al. 2004a,b).

METHODS

All experimental procedures were approved by the University of Salamanca Animal Care and Use Committee, and conformed to the guidelines of the U.S. National Institutes of Health. Sixty-one adult male rats (*Rattus norvegicus*; 47 Long Evans pigmented, 14 Wistar albino strain), weighing between 166 and 365 grams, were used in this study. Anesthesia was induced with an intraperitoneal injection of urethane (1.5 g/kg) and maintained with supplementary doses (0.5 g/kg) as needed. Urethane is a standard anesthetic used in the rat, and was chosen because it is known to affect inhibitory processes less than barbiturate anesthetics (e.g., Hara and Harris 2002). A tracheotomy was performed in order to assure adequate ventilation, and atropine sulfate was administered subcutaneously (0.05 mg/kg) to reduce bronchial secretions. Body temperature was maintained at 38 ± 1 ºC with a thermostatically controlled electric blanket (Malmierca et al. 2003, Hernández et al. 2005). The rat's head was immobilized by placing it in a stereotaxic frame in which the ear bars had been replaced by hollow specula that accommodated a sound delivery system (Rees et al. 1997, Hernández et al. 2005). A craniotomy was performed to expose the tissue over the recording site, usually over the right IC, and the dura was reflected to allow entry of the electrode. The surface of the brain was irrigated regularly with saline to prevent desiccation.

Acoustic stimulation and electrophysiological recording was performed inside a sound attenuated booth. A tungsten electrode (Merrill and Ainsworth 1972) was placed over the

exposed cortex and moved along the dorsoventral axis using a piezoelectric microdrive (Burleigh 6000, EXFO Burleigh, Quebec, Canada) that was advanced by remote control from outside the booth. Once the IC was reached, single neurons were isolated, using white noise and pure tones as search stimuli, and sound-evoked action potentials were recorded extracellularly.

Stimuli were synthesized by a TDT System II workstation (Tucker-Davis Technologies, Alachua, FL, USA) using custom software, and delivered through a closed field delivery system (Rees 1990) via two electrostatic speakers (TDT EC1) controlled by an electrostatic speaker driver (TDT ED1). The output of the system at each ear was calibrated in situ using a condenser microphone (Brüel and Kjær 4134, Nærum, Denmark) and a DI-2200 spectrum analyser (Diagnostic Instruments Ltd., Livingston, Scotland, UK). The maximum output of the system was flat from 0.3-5 kHz ($\approx$100 $\pm$ 7 dB SPL), from 5-40 kHz (90 $\pm$ 5 dB SPL) with a notch at about 22-23 kHz with a slope of approximately 15 dB/octave. Second and third harmonic components in the signal were 45 dB or more below the level of the fundamental at the highest output level. Because of the nonlinearities of the loudspeaker output, all values are expressed as dB SPL. The highest frequency produced by our system was limited to 40 kHz. Action potentials were amplified (x10000) with a Bioamp amplifier (TDT) and filtered (0.5 – 3 kHz, TDT DB4) before being processed in a spike discriminator (TDT SD1). The spike times were then stored on a computer.

Once a single unit was isolated, its characteristic frequency (CF) and threshold were determined by an automated procedure that consisted of the randomized presentation of pure tones across a matrix of frequency and intensity values that extended beyond the visually estimated response area. The standard duration of these stimuli was 75 ms. For duration sensitive neurons, it was set at the neuron's estimated best duration. For the experiments in which duration

was varied, stimuli were pure tones at the neuron's CF, typically 10 to 20 dB above threshold, and a rise-fall time of 1 or 2 ms. Stimuli were presented to the ear contralateral to the IC from which recordings were obtained. Stimulus duration was varied randomly among 10 or 20 stepwise values starting at 2-5 ms and extending up to 100-200 ms. To avoid confounds associated with spectral artifacts, we did not test durations below 2 ms. Each duration was presented 10 times. The data obtained were analyzed and plotted using commercial software (Microsoft Excel, Sigmaplot, and SPSS).

To determine whether a neuron was sensitive to sound duration, we plotted response probability as a function of duration. Response probability (also referred to as spike probability) was defined as the number of presentations (or trials) of a given stimulus for which there was at least one stimulus-evoked action potential, divided by the total number of presentations for that stimulus. For neurons with spontaneous activity, response probability was calculated after subtracting the spontaneous rate. This measure was used instead of spike counts in order to distinguish long pass duration sensitivity from simple systematic increases in spike count with increased duration in units with sustained discharge patterns. A neuron was considered to be duration sensitive if, at any point, the response probability function dropped below 50% of the maximum. For long pass or short pass neurons the cutoff duration was defined as the point at which the probability function dropped below 50% of the maximum; if this point was between two tested durations, it was calculated by linear interpolation. For band pass neurons, which had cutoffs at both long and short durations, the best duration was defined as that at which spike count was maximal. Because we were unable to test durations shorter than 2 ms, it is possible that those neurons that we classified as short pass were actually band pass, or that they might have had band pass characteristics at some sound levels (Fremouw et al. 2005).

Selected recording sites and other landmarks were marked with electrolytic lesions. These marks were used to reconstruct electrode tracks and to confirm the locations of the recorded units. For histological examination of the brain, the animal was administered a lethal dose of sodium pentobarbital (Nembutal; 60 mg/kg in saline, intraperitoneal), and perfused transcardially with Ringer's solution followed by fixative (1% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer, pH: 7.4). The brain was immersed in a 30% sucrose solution for 2-3 days before being cut into 40 μm thick transverse sections using a freezing microtome. Sections were stained with cresyl violet. Neurons were assigned to the IC subdivisions according to the parcellation scheme of Malmierca et al. (1993).

RESULTS

We recorded responses of 160 neurons to sounds of different durations. Of these, 5 neurons showed some level of habituation to repeated stimuli or responded weakly to all stimuli (Covey et al. 2003; Malmierca et al. 2004) and were consequently excluded from all analyses. Of the remaining 155 neurons, 84 (54.2%) showed some form of duration sensitivity. By far the most common form of duration sensitivity was long pass filtering. Long pass neurons were defined as those with a 50% cutoff on the short duration side, but a higher probability of firing at all longer durations tested. Neurons with long pass sensitivity comprised 41.3% of all IC neurons from which we recorded and 76.2% of duration sensitive neurons. Long pass neurons' cutoff durations ranged from 5 ms to 60 ms. An example of a long pass neuron is shown in Figure 1A and B. This neuron had no spontaneous activity. It was completely unresponsive to durations less than about 35 ms. From this point, the response probability gradually increased until reaching the 50% cutoff at about 60 ms and a plateau at about 80 ms. This neuron's response took the form of a sparse sustained discharge. Figure 1B shows that the response probability and sound duration

were positively correlated until the neuron reached its maximum probability of firing. First spike latency was extremely variable across trials, but was always more than 35 ms. Figure 1C shows the response from another neuron with a sustained discharge pattern, but this neuron was not duration sensitive (all pass). Although the number of spikes per trial was positively correlated with sound duration, as would be the case if recording from an auditory nerve fiber, there was no duration tested at which the neuron failed to respond reliably. The spike probability function of this neuron (Fig. 1D) was high (>70%) and essentially flat across all of the durations tested.

Figure 2A and B shows another example of a long pass duration sensitive neuron, in this case one with a robust transient response of no more than 2 spikes per trial. This neuron was completely unresponsive to 5 ms stimuli, but responded reliably to durations greater than or equal to about 15 ms. Once the duration exceeded the cutoff, the number of spikes per stimulus remained constant and response probability remained flat with further increases in duration. Response latency was consistently about 15 ms, about the same as the cutoff duration. For comparison, Figure 2C and D show the response of an onset responder that was not duration sensitive (all pass). This neuron responded with one spike on every trial, even at the shortest duration tested. The spike probability was 1.0 for all durations.

Because there appeared to be a correlation between the cutoff duration and response latency for long pass neurons, we compared the first spike latencies of long pass neurons to those of non-duration sensitive neurons at 20 dB above threshold (Fig. 3). The average latency of long pass neurons with sustained discharge patterns (30.5 ms, SD 15.1) was significantly longer than that of the other classes of neurons (two-tailed t-test, $P < 0.001$). Non-duration sensitive neurons with sustained responses had a mean latency of just 14.0 ms (SD 5.0). Long pass neurons with transient responses had a mean latency of 16.8 ms (SD 3.9) compared with non-duration

sensitive neurons with transient responses (12.9 ms, SD 3.8). The difference between the two groups of transient responders, although relatively small, was statistically significant (P = 0.001).

It might be argued that long pass neurons were not sensitive to duration *per se*, but rather that they integrated energy over the initial part of the stimulus, responding only when the total energy surpassed some threshold. If this were the case, we would expect these neurons' cutoff thresholds to shift to shorter durations as sound amplitude increased. When tested with different amplitudes, neurons typically followed one of two patterns, as shown in Figures 4 and 5. For 8 neurons (62% of long pass neurons tested at different sound levels), cutoffs did shift to shorter durations. For example, the neuron in Figure 4 had a cutoff duration of about 28 ms at 5 dB above threshold, changing to about 5 ms or less at 25 dB above threshold. Over this range, latency also changed from about 35 ms at 5 dB above threshold to about 12 ms at 25 dB above threshold. The level-dependent changes shown by this neuron were typical of those whose cutoff durations shifted to shorter values. For the neurons in this category, the mean decrease in cutoff duration was 35 ms (range 3-92 ms). The mean decrease in latency was 18 ms (range 3.6-28.3 ms). Two of these neurons lost their duration sensitivity at higher sound levels.

Only 2/13 (15%) long pass neurons that were tested at different sound levels showed a shift of cutoff durations to longer values when sound amplitude was increased (Fig. 5). For the neuron in Figure 5, the cutoff duration shifted from about 10 ms at 20 dB above threshold to about 30 ms at 40 dB above threshold. In addition, latency lengthened from about 25 ms at 20 dB above threshold to 45 ms at 40 dB above threshold. The other neuron (not shown) displayed a smaller change in cutoff duration (42 to 47 ms) but a similar increase in latency (45 to 58 ms). The cutoff durations of the remaining 3 neurons did not change with sound level.

Figure 6 shows a scatter plot of cutoff durations versus first spike latency for 48 long pass duration sensitive neurons at 20 dB above threshold. All but one of these neurons had first spike latencies that were longer than their cutoff durations as would be expected if latencies depended on energy integration or the same inhibitory input that set the cutoff duration. Sustained responders had a large range of latencies (10-68 ms) and cutoff durations (4.5-60.5 ms) with a strong positive correlation between latency and cutoff duration ($R^2 = 0.88$). Transient responders, on the other hand, all had relatively short latencies (12-24 ms) and cutoff durations (4.5-15 ms). When calculated in a case by case basis, the latency for transient responders was 7.4 ms longer than the cutoff duration on average, while for sustained neurons it was 8.3 ms longer.

Neurons that responded to a restricted range of durations and were unresponsive to longer and shorter durations were classified as band pass. Units with this response characteristic comprised 7.1% of all IC neurons in our sample and 13.1% of duration sensitive neurons. The best durations of band pass neurons ranged from 4 ms to 128 ms. Figure 7A and B shows an example of a band pass duration tuned neuron that responded to sounds with durations between about 25 and 160 ms, with the most reliable response at 55 ms. This neuron was typical of band pass units in that it responded at the offset of the sound, so latency relative to sound onset varied as a function of sound duration.

Some neurons responded to the shortest stimuli that were presented, but failed to respond to longer sounds. Figure 7C and D shows an example of a short pass neuron. This unit responded robustly to 5 ms stimuli and moderately to 15 ms stimuli, but was mostly unresponsive to longer sounds. Short pass neurons made up 1.3% of all IC neurons from which we recorded and 2.4% of duration sensitive units. Cutoff durations of short pass neurons were all less than 20 ms.

Seven neurons in our sample (4.5% of all IC neurons, 8.4% of duration sensitive neurons) had responses that we classified as 'mixed'. Three of these had "band-reject" responses to stimulus duration, responding most reliably to both short and long durations, but poorly to sounds of intermediate durations. An example of such a neuron is shown in Figure 8A and B. This neuron had a sparse response to durations of 80 ms and longer and a robust response to durations shorter than 10 ms, but little or no response to durations in between. Band-reject duration tuning of this sort has been described in the chinchilla (Chen 1998), where it was suggested that the decreased response to intermediate durations was due to neural inhibition.

Four neurons had complex multipeaked (or multiple band-reject) duration-response functions, a typical example of which is shown in Figure 8C and D. For this neuron, the spike probability peaked at three different durations separated by two minima at about 35 and 80 ms where spike probability dropped to zero. Even at the maxima, however, the spike probability for this neuron was low. Maximal spike probability for other neurons with multipeaked duration sensitivity varied considerably but was typically less than 1.0 (range = 0.4-0.8).

Figure 9 summarizes the distribution of neuron classes based on their patterns of duration selectivity. Long pass duration sensitivity was by far the most common type observed, followed by band pass, while only a few of the other types were found. Figure 10 shows the relation between neurons' discharge patterns and their sensitivity to sound duration. Neurons that were insensitive to duration responded with a variety of temporal discharge patterns. The majority of neurons with transient and pauser discharge patterns were insensitive to duration, whereas the majority of units with sustained responses were long pass. Offset responses were typical of band pass units, but were also occasionally seen in long pass and short pass units. Interestingly, all of the units that responded at sound offset exhibited some form of duration sensitivity.

Ten additional units were tested with durations of up to 400 ms. These ten neurons were not included in the original sample of 160 since the range of durations tested was different. Of these, 7 were duration sensitive. Five of the 7 showed long pass characteristics out to a duration of 400 ms (Fig. 11C,D). One neuron was band pass (Fig. 11A,B) with a best duration around 250 ms. Interestingly, this unit was an offset responder. Thus, we cannot entirely rule out the possibility that some off responders that were classified as long pass using stimuli up to 200 ms could actually be band pass with best durations longer than 200 ms. One neuron (not shown) had a multipeaked duration sensitivity function that included peaks at durations between 200-400ms. The remaining 3 units were not duration sensitive and their filtering properties did not change at durations longer than 100 ms (Fig. 11E,F).

The CFs of the duration sensitive neurons ranged from 0.5 to 37 kHz, covering almost the entire range of frequencies tested. The CFs were evenly distributed for band pass and long pass neurons; but the short pass neurons, probably due to the small number found, seemed to be clustered at low frequencies around 2-3 kHz. At every CF for which data were obtained there was a broad range of best durations (band pass neurons) and cutoff durations (short pass and long pass neurons). There was no significant difference in the distribution of CFs or thresholds for the population of duration sensitive neurons and those that were not sensitive to duration, nor did there appear to be any frequency-specific bias for a particular range of durations.

To determine whether duration sensitive neurons are confined to a particular region within the IC, we plotted the locations of 56 neurons at marked sites within the subdivisions of the IC. The results are shown in Figure 12. Eighty percent of neurons in the central nucleus of the inferior colliculus (CNIC) were insensitive to the duration of the stimulus. However, the CNIC was also the only region in which we found short pass neurons. In contrast, more than half

of all neurons in the dorsal cortex of the IC (DCIC) and external cortex of the IC (ECIC) were duration sensitive. In the DCIC more than half of all neurons were long pass. In the ECIC, there was a relatively high percentage of band pass neurons. Band pass neurons were found only in the DCIC or the ECIC, and multipeaked neurons only in the ECIC.

DISCUSSION

Neurons that respond selectively to sounds of certain durations have been described in the IC of several different mammalian species. This is the first study to show that the rat IC also contains a large population of neurons that are sensitive to sound duration. Our finding that the different classes of duration sensitive neurons present in the rat are qualitatively similar to the band pass, short pass and long pass neurons that have been described in other species provides strong evidence that the same integrative mechanisms operate in all species to produce each form of duration sensitivity, and that duration sensitivity is a fundamental filtering process that contributes to the analysis of complex sounds for a variety of purposes. Despite the similarity of response types, there are some major quantitative differences between the rat and other species, especially echolocating bats, in terms of the relative proportions of the different classes of duration sensitive units, the ranges of durations to which they respond, and their location within the IC. We cannot completely rule out the possibility that some of the differences among species could be due to the preparation in which duration sensitivity was studied, particularly the use of anesthesia in some studies and awake animals in others. However, the qualitative similarities among species make it highly probable that whatever differences are observed are truly species differences related to the temporal characteristics of behaviorally important sounds as well as

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differences in the relative strengths and filter characteristics of the various inputs to IC neurons in different species.

The population of duration sensitive neurons found in the IC of the rat closely resembles that of the mouse (Brand et al. 2000). Both species have a high proportion of long pass neurons, accounting for 40% of all units sampled in the rat and 38% in the mouse (Brand et al. 2000). Moreover, band pass and short pass neurons comprised less than 10% of all IC neurons in rats, and 17% in mice (Brand et al. 2000). Bats have nearly opposite ratios of these response types. In the bat species that have been studied, band pass and short pass neurons comprised approximately one-third to two-thirds of all IC neurons and long pass neurons were in the minority (Ehrlich et al. 1997; Galazyuk and Feng 1997; Fuzessery and Hall 1999; Faure et al. 2003; Mora and Kössl 2004; Fremouw et al. 2005).

The durations to which most neurons in the rat and mouse are selective are considerably longer than those in bats. Figure 13 compares the range of cutoff durations and best durations of IC neurons in the rat and the big brown bat, *Eptesicus fuscus*, a species typical of the bats that have been studied. In the rat, the range of durations to which band pass neurons are tuned extends to over 120 ms. In contrast, best durations of band pass neurons in the bat IC typically range from about 1 ms to 25 ms, with the majority under 10 ms (Ehrlich et al. 1997; Galazyuk and Feng 1997; Fuzessery and Hall 1999; Mora and Kössl 2004). Bats' echolocation calls typically range from about 20 ms to < 1 ms (Simmons 1989). Although echolocation calls are short, communication calls in *Eptesicus* may be longer in duration, ranging up to 100 ms (Gould 1971). Although not as common as in the rat or mouse, long pass duration sensitive neurons are also found in the bat, where they respond to sounds longer than those used for echolocation (Faure et al. 2003). The close match between band pass duration tuning and the durations of bats'

echolocation calls suggests that the large number of band pass neurons in the bat represents a specialization for echolocation, whereas long pass neurons may be used for processing communication sounds, and therefore be the more primitive characteristic.

In rats, the cutoff durations of short pass neurons and the best durations of band pass neurons are consistent with the longer durations of rats' vocalizations, and fall mainly in the duration range of audible calls emitted during exploration, agonistic behavior, and sexual behavior. Some are sensitive to durations corresponding to the range of pup calls (Brudzynski et al. 1999; Kaltwasser 1990). Long pass neurons' cutoff durations were distributed over a fairly wide range, from about 5-60 ms, indicating that a subset of these neurons would be unresponsive to short duration calls such as the "broadband" signals emitted during exploration or the "short" and "wave-like modulated" calls emitted during sexual behavior. These cells would presumably be responsive to infant calls as well as the "screams" and "frequency step" calls emitted during agonistic behavior (Kaltwasser 1990).

Our finding that most band pass and long pass neurons were located outside the central nucleus of the IC also seems different from the situation in the bat, where most duration sensitive neurons, at least band pass ones, are found in the caudal half of the central nucleus (Ehrlich et al. 1997). The finding that the rat has a high proportion of long pass neurons in the dorsal part of the IC is consistent with the idea that neurons in this region have longer integration times due to the influence of cascaded intrinsic projections within the IC itself (Miller et al. 2005) and/or descending input from the cortex (Caicedo and Herbert 1993; Saldaña et al. 1996; reviewed in Malmierca 2003).

Previous studies in which inhibition was blocked (Casseday et al. 1994, 2000; Fuzessery and Hall 1999) provide convincing evidence that duration sensitivity of all types can be created

through the interaction of excitation and inhibition within the IC. However, there is also evidence that inhibition may not be directly responsible for the duration tuning of all IC neurons (Fuzessery and Hall 1999). The fact that latencies of long pass neurons were always longer than their cutoff duration, and the average latency of long pass neurons was longer than the average latency of those that were not sensitive to duration, is consistent with the idea that the duration of a single onset-evoked inhibitory input determines both the cutoff duration and response latency of long pass neurons (Fig. 14 A,B) (Brand et al. 2000; Faure et al. 2003). For neurons with sustained responses and paradoxical latency shift (Sullivan 1982), whose cutoff durations shifted to longer values with increasing sound level, this clearly seems to be the case. For these neurons, the relative strength and duration of a transient, onset-evoked inhibitory input apparently grew at a faster rate than that of a sustained, onset-evoked excitatory input (Fig. 14C). Previous studies have shown that in many IC neurons, inhibition precedes excitation (e.g., Covey et al. 1996; Kuwada et al. 1997), and that such inhibition can cause paradoxical latency shift (Covey et al. 1996).

For those neurons whose cutoffs shifted to shorter durations with increases in amplitude, this shift could be explained if the situation were reversed so that the strength and duration of inhibition grew at a slower rate than that of excitation (Fig. 14D). Consistent with this idea is the finding that inhibition can have a lower threshold than excitation (Covey et al 1996; Kuwada et al. 1997) and that blocking inhibition causes a lowering of threshold in some IC neurons (Vater et al. 1992, LeBeau et al. 2001). An equally plausible alternative explanation would be that these neurons were integrating subthreshold excitatory inputs over a period as long as 90 ms, possibly through projections from a cascaded system of delay lines within the IC (Miller et al. 2005) or convergence of ascending inputs with descending excitatory projections from outside the IC. In

this case, increases in sound amplitude would result in larger, longer lasting EPSPs, allowing temporal summation to occur. The finding that blocking inhibition in some IC neurons in the pallid bat did not abolish their duration tuning is consistent with this hypothesis, and suggests that duration sensitivity may arise through multiple mechanisms. Moreover, the relative prevalence of each mechanism may vary across species. For long pass neurons with transient responses, a transient onset-evoked inhibition could not easily create a long pass response, because it would presumably have the same effect regardless of stimulus duration. However, if transient inhibition were evoked by the offset of the stimulus, it could eliminate responses to sounds with durations shorter than the latency of the excitatory input, while permitting responses to sounds at all longer durations. The latency of an offset response (relative to the onset of the stimulus) is equal to the duration of the stimulus plus the latency of the inhibition relative to sound offset. Based on the data in Fig. 6, the average value for the theoretical 50% cutoff duration at which the latency of the offset inhibition becomes long enough to permit a response to onset-evoked transient excitation is 7.3 ms (SD 3.1), a perfectly plausible value for latency in the rat IC.

The observation that band pass neurons were typically offset responders is consistent with previous studies showing that transient onset-evoked excitation and sustained inhibition followed by an excitatory rebound at sound offset is responsible for this response pattern (Casseday et al. 1994, 2000; Faure et al. 2003). To further support this view, all of the offset responders in this study showed some kind of duration sensitivity. Although the number of offset neurons in our sample is relatively small (7), the same finding has been published in other species, including the mouse (Brand et al. 2000) and chinchilla (Chen 1998). In the bat, up to 80% of duration sensitive neurons are offset responders (Fremouw et al. 2005), a finding that is

consistent with the observation that the majority of duration sensitive neurons in the bat have band pass tuning (Ehrlich et al. 1994). It seems this relationship also holds in regions outside the IC. For example, in the dorsal zone of the auditory cortex of the cat, 24/28 offset neurons were described as duration sensitive (He et al. 1997). Therefore, it seems to be a highly consistent finding, both across species and across sound levels, that a majority of offset responders are sensitive to stimulus duration. The conclusion to be drawn from considering all of these data together is that duration sensitivity in the IC takes many forms, and may arise through diverse mechanisms, but that these mechanisms are consistent across species.

Although it is tempting to think of duration sensitivity as an independent filtering mechanism that creates "feature detectors" selective for biologically relevant sound durations, we must also consider the possibility that it is but one component of a larger scale mechanism underlying the filtering and analysis of complex temporal sequences of natural sounds. The fact that duration tuned neurons in the bat experience suppression analogous to forward and backward masking when presented with sounds of different durations (Faure et al. 2003) suggests that their responses are also determined by the temporal context in which a given sound occurs.

In the rat, we found that most duration sensitive neurons were located in the cortical areas of the IC. This finding was surprising because in the bat they have been reported only in the CNIC, mainly in the caudal portion (Ehrlich et al. 1997). In the other studies, either the recordings were performed only in the CNIC (Ehrlich et al. 1997; Faure et al. 2003; Fremouw et al. 2005) or there was no precise indication of the location (Pinheiro et al. 1991; Chen 1998; Fuzessery and Hall, 1999; Brand et al. 2000; Mora and Kössl 2004), so the opportunities for comparison are limited. There are some anatomical differences that may explain the different

distribution of the duration sensitive neurons in the rat and bat. In *Eptesicus fuscus*, the bat in which duration tuned neurons were localized, the "cortical" areas surrounding the CNIC are not nearly as prominent as they are in rats. In *Eptesicus* the "pericentral area" is quite small (less than 200 μm thickness) and includes regions that would presumably correspond to both the ECIC and DCIC in rats (Casseday and Covey 1992; Covey and Carr 2005). Even though we cannot rule out a sampling bias due to the relatively larger size of the cortical areas in the rat, it is still significant that only 20% of the CNIC units in our sample had any kind of duration sensitivity while the percentage was much higher in the cortical areas. This finding suggests that duration sensitive neurons in the rat may be important for orienting to sounds of specific durations.

The finding that all of the different types of duration sensitive neurons that have been described in other species are present in the rat IC reinforces the idea that duration tuning is an emergent property in the midbrain of all vertebrate species, and that it arises through multiple mechanisms and takes multiple forms. The selectivity of rat IC neurons for relatively long durations that correspond to those within the rat's vocalization repertoire suggests that there are species-specific adjustments in latencies, discharge patterns, and synaptic strengths that optimize the range of sound duration sensitivity in each species to produce filtering properties that are important for behaviors that depend on analyzing the temporal pattern of sound.

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REFERENCES

Brand A, Urban A, and Grothe B. Duration tuning in the mouse auditory midbrain. *J Neurophysiol* 84: 1790-1799, 2000.

Brudzynski SM, Bihari F, Ociepa D and Fu XW. Analysis of 22 kHz ultrasonic vocalization in laboratory rats: long and short calls. *Physiol Behav* 54: 215-221, 1993.

Brudzynski SM, Kehoe P and Callahan M. Sonographic structure of isolation-induced ultrasonic calls of rat pups. *Dev Psychobiol* 34: 195-204, 1999.

Caicedo A and Herbert H. Topography of descending projections from the inferior colliculus to auditory brainstem nuclei in the rat. *J Comp Neurol* 328: 377-392, 1993.

Casseday JH and Covey E. Frequency tuning properties of neurons in the inferior colliculus of an FM bat. *J Comp Neurol* 319: 34-50, 1992.

Casseday JH, Ehrlich D and Covey E. Neural tuning for sound duration: Role of inhibitory mechanisms in the inferior colliculus. *Science* 264: 847-850, 1994.

Casseday JH, Ehrlich D and Covey E. Neural measurement of sound duration: Control by excitatory-inhibitory interactions in the inferior colliculus. *J Neurophysiol* 84: 1475-1487, 2000.

Casseday JH, Fremouw T and Covey E. The inferior colliculus: Hub of the auditory system. In: *Integrative Functions in the Mammalian Auditory Pathway*, edited by D. Oertel, A.N. Popper and R.R. Fay. New York: Springer-Verlag, 2002, pp 238-318.

Chen GD. Effects of stimulus duration on responses of neurons in the chinchilla inferior colliculus. *Hear Res* 122: 142-150, 1998.

Covey E and Carr CE. The inferior colliculus in bats and birds. In: *The Inferior Colliculus*, edited by J.A. Winer and C.E. Schreiner. New York: Springer-Verlag, 2005, pp 493-536.

Covey E, Kauer JA and Casseday JH. Whole-cell patch-clamp recording reveals subthreshold sound-evoked postsynaptic currents in the inferior colliculus of awake bats. *J Neurosci* 16(9): 3009-18, 1996.

Covey E, Pérez-González D, Hernández O and Malmierca MS. Novelty units in the inferior colliculus of the rat. *Neurosci Abstr* 29, 2003.

Ehrlich D, Casseday JH and Covey E. Neural tuning to sound duration in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. *J Neurophysiol* 77: 2360-2372, 1997.

Faure PA, Fremouw T, Casseday JH and Covey E. Temporal masking reveals properties of sound-evoked inhibition in duration-tuned neurons of the inferior colliculus. *J Neurosci* 23: 3052-3065, 2003.

Fremouw T, Faure P, Casseday JH and Covey E. Duration selectivity of neurons in the inferior colliculus of the big brown bat: tolerance to changes in sound level. *J Neurophysiol* 94: 1869-1878, 2005.

Fuzessery ZM. Response selectivity for multiple dimensions of frequency sweeps in the pallid bat inferior colliculus. *J Neurophysiol* 72: 1061-1079, 1994.

Fuzessery ZM and Hall JC. Sound duration selectivity in the pallid bat inferior colliculus. *Hear Res* 137: 137-154, 1999.

Galazyuk AV and Feng AS. Encoding of sound duration by neurons in the auditory cortex of the little brown bat, *Myotis lucifugus*. *J Comp Physiol [A]* 180: 301-311, 1997.

Gould E. Studies of maternal-infant communication and development of vocalizations in the bats *Myotis* and *Eptesicus*. *Comm Behav Biol* 5: 263-313, 1971.

Hara K, Harris RA. The anesthetic mechanism of urethane: the effects on neurotransmitter-gated ion channels. *Anesth Analg* 94: 313-318, 2002.

He J, Hashikawa T, Ojima H and Kinouchi Y. Temporal integration and duration tuning in the dorsal zone of cat auditory cortex. *J Neurosci* 17: 2615-2625, 1997.

Hernández O, Espinosa N, Pérez-González D and Malmierca MS. The inferior colliculus of the rat: a quantitative analysis of monaural frequency response areas. *Neuroscience* 132: 203-217, 2005.

Kaltwasser MT. Acoustic signaling in the black rat (*Rattus rattus*). *J Comp Psychol* 104: 227-232, 1990.

Knutson B, Burgdorf J and Panksepp J. Ultrasonic vocalizations as indices of affective states in rats. *Psychol Bull* 128: 961-977, 2002.

Kuwada S, Batra R, Yin TC, Oliver DL, Haberly LB and Stanford TR. Intracellular recordings in response to monaural and binaural stimulation of neurons in the inferior colliculus of the cat. *J Neurosci* 17: 7565-7581, 1997.

LeBeau FE, Malmierca MS and Rees A. Iontophoresis in vivo demonstrates a key role for GABA(A) and glycinergic inhibition in shaping frequency response areas in the inferior colliculus of guinea pig. *J Neurosci* 21: 7303-7312, 2001.

Liu RC, Miller KD, Merzenich MM and Schreiner CE. Acoustic variability and distinguishability among mouse ultrasound vocalizations. *J Acoust Soc Am* 114: 3412-3422, 2003.

Malmierca MS. The structure and physiology of the rat auditory system: an overview. *Int Rev Neurobiol* 56: 147-211, 2003.

Malmierca MS, Blackstad TW, Osen KK, Karagulle T and Molowny RL. The central
nucleus of the inferior colliculus in rat: a Golgi and computer reconstruction study of
neuronal and laminar structure. *J Comp Neurol* 333: 1-27, 1993.

Malmierca MS, Hernández O, Falconi A, Lopez-Poveda EA, Merchán M and Rees A. The
commissure of the inferior colliculus shapes frequency response areas in rat: an in vivo
study using reversible blockade with microinjection of kynurenic acid. *Exp Brain Res*
153: 522-529, 2003.

Malmierca MS, Pérez-González D, Hernández O and Covey E. Novelty responses in single
neurons of the auditory midbrain are generated by rapid stimulus-specific habituation.
FENS Abst 2A15721*, 2004.*

Merrill EG and Ainsworth A. Glass-coated platinum-plated tungsten microelectrodes. *Med
Biol Eng* 10: 662-672, 1972.

Miller KE, Casseday JH and Covey E. Relation between intrinsic connections and
isofrequency contours in the inferior colliculus of the big brown bat, *Eptesicus fuscus*.
Neuroscience, In press, 2005.

Mora EC and Kössl M. Ambiguities in sound-duration selectivity by neurons in the inferior
colliculus of the bat *Molossus molossus* from Cuba. *J Neurophysiol* 91: 2115-2126, 2004.

Narins PM and Capranica RR. Neural adaptations for processing the two-note call of the
Puerto Rican treefrog, *Eleutherodactylus coqui*. *Brain Behav Evol* 17: 48-66, 1980.

Pérez-González D, Malmierca MS, Moore J and Covey E. Duration sensitive neurons in the
inferior colliculus of the rat. *Assoc Res Otolaryngol Abs:* 232, 2004a.

Pérez-González D, Malmierca MS, Moore J and Covey E. Sound duration selectivity in the
rat auditory midbrain. *FENS Abstr* vol 2, A226.6, 2004b.

Pinheiro AD, Wu M and Jen PHS. Encoding repetition rate and duration in the inferior

    colliculus of the big brown bat, *Eptesicus fuscus*. *J Comp Physiol [A]* 169: 69-85, 1991.

Potter HD. Patterns of acoustically evoked discharges in the mesencephalon of the bullfrog. *J*

    Neurophysiol 28: 1155-1184, 1965.

Rees A. A closed field sound system for auditory neurophysiology. *J Physiol Lond* 430: 6, 1990.

Rees A, Sarbaz A, Malmierca MS and LeBeau FEN. Regularity of firing of neurons in the

    inferior colliculus. *J Neurophysiol* 77: 2945-2965, 1997.

Saldaña E, Feliciano M and Mugnaini E. Distribution of descending projections from primary

    auditory neocortex to inferior colliculus mimics the topography of intracollicular

    projections. *J Comp Neurol* 371: 15-40, 1996.

Simmons JA. A view of the world through the bat's ear: the formation of acoustic images in

    echolocation. *Cognition* 33: 155-99, 1989.

Sullivan WE 3rd. Possible neural mechanisms of target distance coding in auditory system of

    the echolocating bat Myotis lucifugus. *J Neurophysiol* 48: 1033-1047, 1982.

Vater M, **Habbicht H**, **Kossl M and Grothe B**. The functional role of GABA and glycine in

    monaural and binaural processing in the inferior colliculus of horseshoe bats. *J Comp*

    Physiol [A] 171: 541-553, 1992.

FIGURE CAPTIONS

Figure 1. Long pass duration sensitive neuron compared with a sustained responder that

was not sensitive to duration (all pass). *A:* Dot raster showing the response of the long pass

neuron to different durations. Sounds were pure tones presented at 10 dB above threshold. In this

and similar figures the duration of the sound is indicated by shading. *B:* Spike probability as a function of duration for the long pass neuron. For this and similar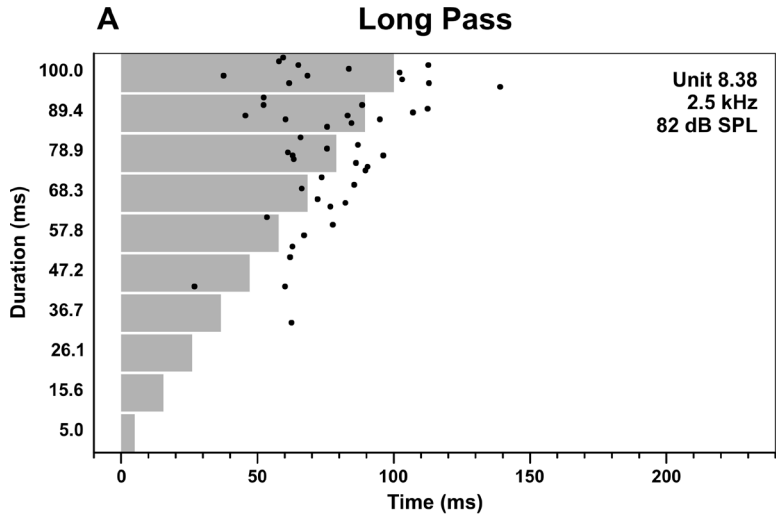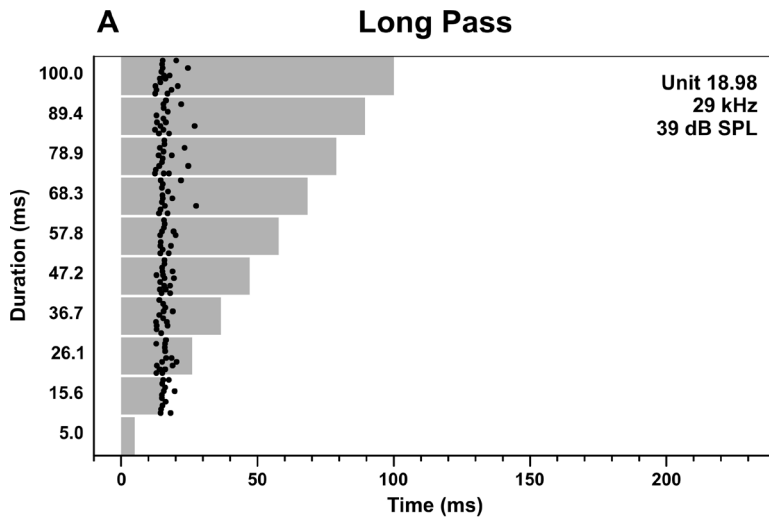 figures, the dotted line in the stimulus duration/spike probability plot indicates 50% of the maximum response probability. *C:* Dot raster illustrating the response of a neuron that was insensitive to duration. Stimuli were pure tones presented at 15 dB above threshold. *D:* Spike probability as a function of duration for the non-duration sensitive neuron.

Figure 2. Long pass duration sensitive neuron with a transient response (*A, B*), compared to a transient responder that was not sensitive to duration (all pass: *C, D*), recorded 10 dB above threshold. Stimuli in were pure tones presented at 15 dB above threshold. Format as in Figure 1.

Figure 3. Mean first spike latency of transient and sustained responders, for non-duration sensitive (NoDT) and long pass neurons (LP) calculated at 20 dB above threshold. The error bars indicate the standard deviation. Asterisks indicate that the means are statistically different (t-test, $P \leq 0.001$).

Figure 4. Responses of a long pass duration sensitive neuron to different sound levels of a pure tone (*A:* 5 dB above threshold; *C:* 25 dB above threshold). As sound level increased, this neuron's cutoff shifted to a shorter duration (*B*), and response latency decreased (*D*). The error bars indicate the standard deviation.

Figure 5. Responses of a long pass duration sensitive neuron to different sound levels of a pure tone (*A:* 20 dB above threshold; *C:* 40 dB above threshold). As sound level increased, this neuron's cutoff shifted to a longer duration (*B*), and response latency lengthened (*D*). The error bars indicate the standard deviation.

Figure 6. Scatter plot showing cutoff duration versus mean first spike latency for 48 long pass neurons calculated at 20 dB above threshold. Bold line is the diagonal (x = y); thin line is

the regression line for transient responders (filled circles); dotted line is the regression line for sustained responders (open circles).

Figure 7. Band pass and short pass duration sensitive neurons. *A:* Dot raster showing response of a band pass neuron at different durations. *B:* Spike probability of the band pass neuron as a function of duration. *C:* Dot raster showing response of a short pass duration sensitive neuron. *D:* Spike probability of the short pass neuron as a function of duration. For both neurons the stimulus was a pure tone at 10 dB above threshold.

Figure 8. Band-reject and multipeaked duration sensitive neurons. *A:* Dot raster showing the response of the band reject neuron. *B:* Spike probability of the band-reject neuron as a function of duration. *C:* Dot raster showing the response of a neuron with multiple peaks of duration sensitivity, or multiple durations at which its response was suppressed. *D:* Spike probability of the neuron with multipeaked duration sensitivity as a function of stimulus duration. For both neurons the stimulus was a pure tone at 10 dB above threshold.

Figure 9. Distribution of different types of duration sensitivity. No DT, not sensitive to duration (all pass); LP, long pass; BP, band pass; SP, short pass; BR, band-reject; MP, multipeaked.

Figure 10. Relationship between duration sensitivity and discharge pattern shown as the distribution of filter types for each type of discharge pattern. Abbreviations as in Figure 9.

Figure 11. Examples of 3 units tested with durations up to 400 ms. *A:* Dot raster showing the response of a band pass neuron with a best duration >200 ms. *B:* Spike probability as a function of duration for the band pass neuron. *C:* Dot raster illustrating the response of a long pass neuron. *D:* Spike probability as a function of duration for the long pass neuron. *E:* Spike

probability as a function of duration for a neuron that remained duration insensitive (all pass) at least up to 400 ms. *F:* Spike probability as a function of duration for the all pass neuron.

Figure 12. *A:* Distribution of different classes of duration sensitive neurons within the subdivisions of the IC, shown as percentages of the total neurons in each subdivision. *B:* Locations of duration sensitive neurons of each type within the IC, shown as number of neurons in each subdivision for each type. Inset shows subdivisions of the rat IC as seen in a frontal section. CNIC, central nucleus of the IC; DCIC dorsal cortex of the IC; ECIC, external cortex of the IC.

Figure 13. Comparison of duration sensitivity in rat and the big brown bat. The arrows indicate the range of cutoffs (for long pass and short pass neurons) or best durations (for band pass neurons). The thick vertical line indicates the mean value. The boxes show the approximate range of durations reported for different types of calls, and their frequencies. Bat data from Ehrlich et al. (1997); rat vocalization data from Kaltwasser (1990).

Figure 14. Model of how different relative rates of growth of excitation and inhibition as a function of sound level could affect response latencies and cutoff durations in long pass duration sensitive neurons. All of the model neurons shown receive a transient inhibitory input with a fixed decay time (IPSP) and a sustained excitatory input (EPSP). *A:* At low sound levels all of the neurons respond in a similar fashion to a sound longer than the cutoff. *B:* At a higher sound level, if the growth of excitation is matched to that of inhibition, the response latency and cutoff duration will remain constant. *C:* If inhibition grows at a faster rate than excitation, latency will increase and cutoff duration will lengthen with increased sound level. At the sound level illustrated in the example, the spike count has decreased considerably; unless the IPSP were

to reach a plateau, further increases in sound level would eventually result in complete suppression of the response at this duration, and a longer duration sound would be required to drive the response. *D:* If excitation grows at a faster rate than inhibition, latency will shorten, and the cutoff duration will decrease with increased sound level as the effective period of the inhibition shortens.
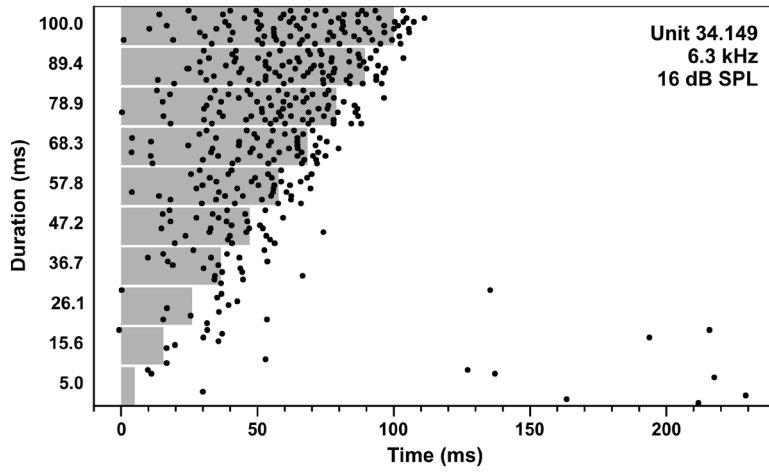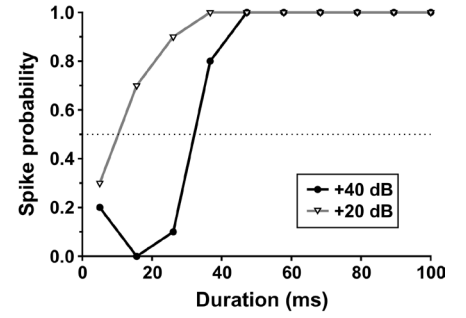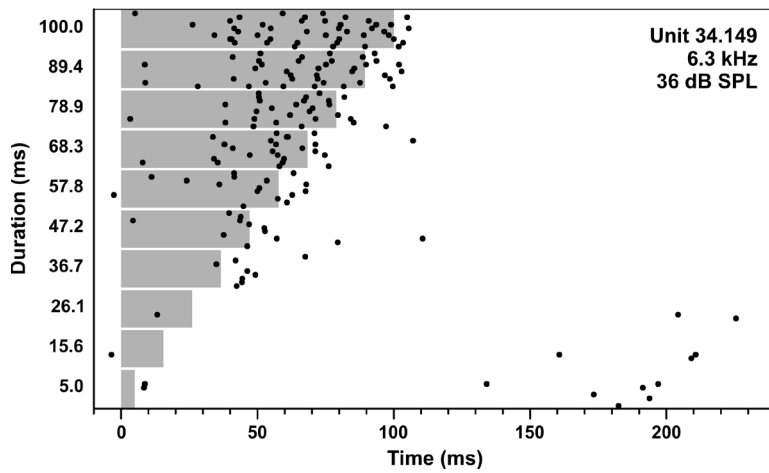
A Long Pass

Unit 8.38
2.5 kHz
82 dB SPL

B

C All Pass

Unit 3.7
1.3 kHz
71 dB SPL

D

30

A Long Pass

Unit 18.98
29 kHz
39 dB SPL

B

C All Pass

Unit 3.3
20 kHz
39 dB SPL

D

A 5 dB above threshold

Unit 40.169
38 kHz
9 dB SPL

B

C 25 dB above threshold

Unit 40.169
38 kHz
29 dB SPL

D

+25 dB
+5 dB

A **20 dB above threshold**

Unit 34.149
6.3 kHz
16 dB SPL

Duration (ms): 100.0, 89.4, 78.9, 68.3, 57.8, 47.2, 36.7, 26.1, 15.6, 5.0

Time (ms)

B

Spike probability vs Duration (ms)

+40 dB
+20 dB

C **40 dB above threshold**

Unit 34.149
6.3 kHz
36 dB SPL

Duration (ms): 100.0, 89.4, 78.9, 68.3, 57.8, 47.2, 36.7, 26.1, 15.6, 5.0

Time (ms)

D

First spike latency (ms) vs Duration (ms)

A **Band Pass**

Duration (ms): 200, 190, 180, 169, 159, 149, 138, 128, 118, 108, 97, 87, 77, 67, 56, 46, 36, 26, 15, 5

Time (ms)

Unit 39.164
7.5 kHz
65 dB SPL

B

Spike probability vs Duration (ms)

C **Short Pass**

Duration (ms): 100.0, 89.4, 78.9, 68.3, 57.8, 47.2, 36.7, 26.1, 15.6, 5.0

Time (ms)

Unit 9.39
2 kHz
77 dB SPL

D

Spike probability vs Duration (ms)

36

A   Band Reject

Unit 15.80
2.5 kHz
79 dB SPL

B

C   Multipeaked

Unit 13.71
1.7 kHz
81 dB SPL

D

37

A   **Band pass with a long BD**

Unit 271.3
35.6 kHz
62 dB SPL

B

C   **Long pass mantained at long durations**

Unit 278.2
8.7 kHz
47 dB SPL

D

E   **Duration insensitive at long durations**

Unit 278.3
1.8 kHz
65 dB SPL

F

41

RAT

LP

BP

SP

80 kHz

Modulated

60 kHz — Short pulse

Infant calls

50 kHz

40 kHz

Frequency step A

Tooth chatter

Frequency step B (up to 300 ms)

20 kHz

Broadband

Sniffing

Scream (up to 500 ms)

0 kHz

BAT

BP

Bat echolocation calls

0   20   40   60   80   100   120   140

Duration (ms)

A

Low Amplitude

B. Matched Growth of Excitation and Inhibition

no change

IPSP

EPSP

High Amplitude

C. Inhibition Grows More

IPSP

EPSP

High Amplitude

D. Excitation Grows More

IPSP

EPSP

High Amplitude