Neurons in the Medial Nucleus of the Trapezoid Body and Superior Paraolivary Nuclei of the Rat May Play a Role in Sound Duration Coding

Alexander Kadner, Randy J. Kulesza Jr, and Albert S. Berrebi
Departments of Otolaryngology–Head and Neck Surgery, Neurobiology and Anatomy and The Sensory Neuroscience Research Center, West Virginia University School of Medicine, Morgantown, West Virginia

Submitted 30 August 2005; accepted in final form 29 November 2005

Kadner, Alexander, Randy J. Kulesza Jr, and Albert S. Berrebi.
Neurons in the medial nucleus of the trapezoid body and superior paraolivary nucleus of the rat may play a role in sound duration coding. J Neurophysiol 95: 1499–1508, 2006. First published November 30 2005; doi:10.1152/jn.00902.2005. We describe neurons in two nuclei of the superior olivary complex that display differential sensitivities to sound duration. Single units in the medial nucleus of the trapezoid body (MNTB) and superior paraolivary nucleus (SPON) of anesthetized rats were studied. MNTB neurons produced primary-like responses to pure tones and displayed a period of suppressed spontaneous activity after stimulus offset. In contrast, neurons of the SPON, which receive a strong glycineergic input from MNTB, showed very little or no spontaneous activity and responded with short bursts of action potentials after the stimulus offset. Because SPON spikes were restricted to the same time window during which suppressed spontaneous activity occurs in the MNTB, we presume that SPON offset activity represents a form of postinhibitory rebound. Using characteristic frequency tones of 2–1,000-ms duration presented 20 dB above threshold, we show that the profundity and duration of the suppression of spontaneous activity in MNTB as well as the magnitude and first spike latency of the SPON offset response depend on stimulus duration as well as on stimulus intensity, showing a tradeoff between intensity and duration. Pairwise comparisons of the responses to stimuli of various durations revealed that the duration sensitivity in both nuclei is sharpest for stimuli <50 ms.

INTRODUCTION

Duration is an important temporal feature of acoustic stimuli. It is not surprising, therefore, that certain neurons in the central auditory system respond preferentially to sounds within a limited range of durations. Duration tuned cells have been most extensively studied in a few species whose behavioral ecology imposes a special need for calibrating signal duration. For example, sound duration is critical to eliciting appropriate behavioral responses to mating calls of the Puerto Rican tree frog (Narins and Capranica 1980) and duration tuned neurons are found in the auditory midbrain of several species of frogs (Feng et al. 1990; Gooler and Feng 1992; Narins and Capranica 1980; Potter 1965). Additionally, certain species of bats emit echolocation calls of different lengths in the various phases of the pursuit of prey, confronting the animals with the problem of avoiding a temporal overlap of the outgoing echolocation call with an incoming echo (reviewed by Schnitzler and Kalko 2001). Measurement of sound duration presumably plays a role in this process, and duration tuned neurons are found in the bat inferior colliculus (Casseday et al. 1994, 2000; Ehrlich et al. 1997; Faure et al. 2003) and auditory cortex (Galazyuk and Feng 1997). However, neurons tuned to sound duration have also been found in the auditory systems of nonecholocating mammals, including cats and rodents, whose behavioral ecology doesn’t present an obvious need for them. Specifically, neurons that respond preferentially to stimuli of specific durations have been demonstrated in the inferior colliculus of chinchillas (Chen 1998), rats (Perez-Gonzalez et al. 2005), and mice (Brand et al. 2000) as well as the medial geniculate body of guinea pigs (He 2002) and the auditory cortex of cats (He et al. 1997). It is interesting that psychophysical data show that humans can discriminate sounds differing in duration by only 30 ms (Creelman 1962), suggesting that this feature may be important for speech processing. Evidence of duration processing has been found in the form of mismatch negativity in a study of human auditory evoked potentials (Kaukoranta et al. 1989), and lack of duration mismatch negativity is associated with schizophrenia (Michie et al. 2000). It is notable, however, that duration sensitive neurons have been found in the auditory midbrain, thalamus and cortex, but not at lower levels of the auditory pathway.

The most detailed studies of duration tuning have been performed in the inferior colliculus (IC) of the big brown bat, Eptesicus fuscus, (Casseday et al. 1994, 2000; Ehrlich et al. 1997; Faure et al. 2003). The results of these studies have led to a model proposing that duration tuned neurons preferentially respond when a delayed excitatory input coincides with the rebound from a short latency inhibitory input (for a mechanistic description of the model see Fig. 1 in Faure et al. 2003). Moreover, iontophoresis studies showed that the GABA_A receptor blocker bicuculline abolishes this response selectivity in the IC (Casseday et al. 2000). Although focused on duration tuning mechanisms in the midbrain, the model of Casseday et al. (1994) acknowledges that the synaptic inputs required to create duration-tuned IC neurons could originate from lower levels of the auditory pathway.

Our laboratory has previously examined the structure and function of one possible source of such precisely timed GABAergic input to the IC, namely the superior paraolivary nucleus (SPON). In rats, this nucleus is composed of a homogeneous population of GABAergic neurons that send a massive topographic projection to the ipsilateral IC (Kulesza and Berrebi 2000; Saldaña and Berrebi 2000). In vivo extracellular single unit recordings show that the vast majority of SPON
neurons display little or no spontaneous activity and fail to discharge during characteristic frequency tone stimulation. Instead, SPON neurons fire transiently at the stimulus offset (Kulesza et al. 2003a). A postinhibitory rebound mechanism was suggested to account for these offset responses, and preliminary iontophoresis studies indicate that offset spikes in the SPON are mediated by the neurotransmitter glycine (Kulesza et al. 2003b). Glycinergic inhibition to SPON neurons likely originates in the nearby MNTB, whose neurons display high rates of spontaneous activity and primary-like responses to characteristic frequency tones (cats: Guinan et al. 1972a,b; Smith et al. 1998; gerbils: Kopp-Scheinflug et al. 2003a,b; rats: Kulesza et al. 2003a; Sommer et al. 1993). This study was designed to examine the effect of stimulus duration on the response properties of MNTB and SPON neurons. We also assessed whether response components occurring after the stimulus offset in both nuclei encode information about stimulus duration that could be relayed to the IC. Because psychophysical observations in humans (McFadden 1975) and behavioral experiments in budgerigars (Dooling et al. 1987) show that increases in stimulus duration and intensity can lead to indistinguishable perceptions, the responses to tones of varying intensity were also studied.

METHODS

Animals and surgery

The animals used in this study were female Sprague-Dawley rats (Hilltop Lab Animals, Scottsdale, PA) weighing 210–290 g. Animals were housed in the vivarium at the West Virginia University Health Sciences Center, an AAALAC-approved animal facility. All procedures were reviewed by the Institutional Animal Care and Use Committee at West Virginia University and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Rats were anesthetized by intramuscular injection of a mixture of ketamine (70 mg/kg) and xylazine (5 mg/kg). Supplementary injections of this mixture (one-third of the original dose) were administered during the recording sessions as needed. After the rat was determined to be areflexic, the head was shaved, and the animal mounted in a stereotaxic frame using blunt hollow earbars to avoid injury to the tympanic membrane. A scalp incision was made to expose the skull and the connective tissue covering the bone removed. A custom-fabricated head post was attached to the skull at bregma by drilling into the skull and securing it in place with a screw.

To gain access to both sides of the brain stem, a craniotomy (~3 mm rostro-caudal × 7 mm medio-lateral) was performed with the rostral edge of the bone defect extending to the posterior aspect of the transverse sinus. The dura mater was opened and the underlying cerebellum aspirated to expose the floor of the fourth ventricle, whose midline was used as a landmark for electrode penetrations. At this point the rats were moved to the recording booth and placed on a heating blanket to maintain body temperature. When deemed necessary, subcutaneous injections of 1 ml physiological saline were given at hourly intervals to compensate for loss of body fluids.

Acoustic stimuli and sound delivery

The acoustic stimuli were created as digital waveforms by the BATLAB control software (Dr. Donald P. Gans, Northeastern Ohio Universities College of Medicine, Rootstown, OH). The digital signals were converted to analog by a Microstar DAP5216a data acquisition processor and passed through an anti-aliasing filter [FT6-2, Tucker Davis Technologies (TDT), Alachua, FL]. Attenuation was controlled by PA-5 programmable attenuators (TDT). The signal was routed to a TDT ED1 speaker driver and presented through TDT ES1 free field speakers that were mounted in the stereotaxic frame ~5 mm from the opening of the external ear canal. To avoid spectral contamination of the stimuli by on and offset clicks, the broadband noise search stimuli were phased in and out using cos2 ramps. The duration of the ramps used to present pure tone stimuli were determined by the stimulus frequency such that they contained at least two full periods of the stimulus. The speakers were calibrated off-line using a Bruel and Kjaer Type 4939 microphone connected to a type 2610 measuring amplifier.

Electrophysiological recordings

The responses of single units were recorded using either glass pipette electrodes filled with 2.5% biocytin or neurobiotin dissolved in physiological saline, tip diameter 2–3 μm, or elgiloy electrodes with a tip impedance of 7 MΩ (World Precision Instruments). The extracellular signal was amplified and band-pass filtered between 200 and 3,000 Hz with a Krohn-Hite Model 3364 Filter. The overall signal gain was set so that the amplitude of an action potential at the end of the amplifier chain was between 2 and 3 V. The waveforms were digitized at a rate of 42 kHz using a Microstar DAP5216a data acquisition processor. A unit was considered well isolated if the auditory evoked spike waveforms appeared homogeneous and could be separated from background noise by a trigger window. The isolation of each unit was later verified off-line by examining a digital record of the spike waveforms.

Recording sites were approached using stereotaxic coordinates provided in an atlas of the rat brain (Paxinos and Watson 1986). Location of recorded units was confirmed as follows: cells in both the MNTB and SPON were presumed to be contralaterally driven (Kulesza et al. 2003a; Smith et al. 1998), and this was verified for all units included in this study. Whenever well-isolated units were recorded, electrode tracks were marked. When using glass electrodes biocytin or neurobiotin was deposited by applying an anodic current of 500 μA for 10 min. With metal electrodes, an iron deposit was made by applying an anodic current of 2 μA for 180 s. Actual recording sites were subsequently verified histologically, taking into account tissue shrinkage and using adjacent Nissl-counterstained sections to elucidate the borders of superior olivary nuclei (Kulesza et al. 2002, 2003a).

Experimental paradigm

Single units in the MNTB and SPON were found using repetitive 50-ms broadband noise bursts. Search stimuli were presented at 80–100 dB SPL and a repetition rate of 4/s. Characteristic frequency (CF) and threshold were determined for all recorded units. Contralateral drive and the absence of binaural facilitation were verified by presenting 50-ms noise bursts at 10 dB above threshold monaurally to each ear and binaurally. The spontaneous activity of each unit was calculated from recordings of twenty 200-ms control traces during which no stimulus was presented. Tuning curves were recorded using 50-ms tone bursts, presented at 10 dB below the unit’s threshold, at threshold intensity, and at 10, 20, and 30 dB above the threshold. Finally, the responses to various stimulus durations were assessed by presenting CF tones at 20 dB above threshold. Tones of 2- to 10-ms durations were presented in 1-ms steps; tones of 20- to 100-ms duration were presented in 10-ms steps, and tones of 200- to 1,000-ms duration were presented in 100-ms steps. All stimuli ≤100 ms in duration were presented at a rate of 4/s. Tones of 200- to 1,000-ms duration were presented at a rate of 0.5/s. Because completion of the recording procedure required 60–90 min for each unit, during which the stimuli were presented almost continuously, the potential existed for chronic rise of the animals’ hearing thresholds. However, we observed no systematic trend toward increasing thresholds during the recording sessions.
TABLE 1. Characteristic frequencies of units recorded in the MNTB and SPON

<table>
<thead>
<tr>
<th>Unit ID</th>
<th>Characteristic Frequency</th>
<th>MNTB</th>
<th>SPON</th>
<th>Unit ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>04-0129-01</td>
<td>1.5</td>
<td>0.8</td>
<td></td>
<td>04-0159-04</td>
</tr>
<tr>
<td>04-0102-03</td>
<td>1.8</td>
<td>0.9</td>
<td></td>
<td>04-0159-02</td>
</tr>
<tr>
<td>04-0110-01</td>
<td>2.1</td>
<td>1.1</td>
<td></td>
<td>04-0159-05</td>
</tr>
<tr>
<td>05-0012-03</td>
<td>2.3</td>
<td>1.2</td>
<td></td>
<td>04-0144-02</td>
</tr>
<tr>
<td>04-0089-02</td>
<td>2.5</td>
<td>1.2</td>
<td></td>
<td>04-0159-03</td>
</tr>
<tr>
<td>04-0108-02</td>
<td>2.6</td>
<td>5.3</td>
<td></td>
<td>04-0104-05</td>
</tr>
<tr>
<td>04-0118-01</td>
<td>4.2</td>
<td>8.0</td>
<td></td>
<td>04-0102-04</td>
</tr>
<tr>
<td>04-0118-02</td>
<td>4.7</td>
<td>8.2</td>
<td></td>
<td>04-0104-02</td>
</tr>
<tr>
<td>04-0104-04</td>
<td>5.0</td>
<td>8.2</td>
<td></td>
<td>04-0140-02</td>
</tr>
<tr>
<td>04-0114-01</td>
<td>5.0</td>
<td>8.4</td>
<td></td>
<td>04-0104-03</td>
</tr>
<tr>
<td>04-0113-03</td>
<td>5.2</td>
<td>8.5</td>
<td></td>
<td>04-0154-01</td>
</tr>
<tr>
<td>04-0140-01</td>
<td>5.2</td>
<td>8.8</td>
<td></td>
<td>04-0144-01</td>
</tr>
<tr>
<td>05-0012-02</td>
<td>7.0</td>
<td>9.0</td>
<td></td>
<td>04-0146-01</td>
</tr>
<tr>
<td>04-0122-01</td>
<td>7.7</td>
<td>10.3</td>
<td></td>
<td>04-0122-03</td>
</tr>
<tr>
<td>04-0102-02</td>
<td>9.0</td>
<td>12.0</td>
<td></td>
<td>04-0159-01</td>
</tr>
<tr>
<td>04-0113-01</td>
<td>11.9</td>
<td>13.0</td>
<td></td>
<td>04-0146-06</td>
</tr>
<tr>
<td>04-0105-02</td>
<td>17.0</td>
<td>18.0</td>
<td></td>
<td>05-0015-04</td>
</tr>
<tr>
<td>04-0155-01</td>
<td>17.0</td>
<td>23.0</td>
<td></td>
<td>04-0140-04</td>
</tr>
<tr>
<td>04-0155-03</td>
<td>20.0</td>
<td>33.0</td>
<td></td>
<td>04-0110-02</td>
</tr>
<tr>
<td>05-0008-01</td>
<td>22.0</td>
<td>34.0</td>
<td></td>
<td>04-0098-01</td>
</tr>
<tr>
<td>04-0106-01</td>
<td>24.0</td>
<td>44.0</td>
<td></td>
<td>04-0144-02</td>
</tr>
<tr>
<td>04-0105-04</td>
<td>34.0</td>
<td>50.0</td>
<td></td>
<td>04-0110-04</td>
</tr>
<tr>
<td>04-0103-02</td>
<td>36.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04-0138-02</td>
<td>41.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-0004-01</td>
<td>43.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04-0099-02</td>
<td>51.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04-0105-01</td>
<td>55.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>05-0005-01</td>
<td>56.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MNTB, medial nucleus of the trapezoid body; SPON, superior paraolivary nucleus.

Because of the nature of the responses of MNTB and SPON neurons, which are described in detail below, we will hereafter use the term “offset response” to denote all deviations from a neuron’s spontaneous activity that occurred after the stimulus offset. In the case of MNTB neurons, the offset response took the form of suppression of spontaneous activity, whereas in the case of SPON neurons, the offset response took the form of suppression of spontaneous activity that occurred after the stimulus offset. In the case of MNTB neurons, the term “offset response” to denote all deviations from a neuron’s spontaneous activity rate is 14 spikes/s. Note suppression of spontaneous activity that occurred after the stimulus offset, albeit differently. Whereas the offset response of MNTB cells took the form of a transient suppression of spontaneous activity, 22 of 24 (92%) SPON neurons in our sample responded to the stimulus offset by generating action potentials (for examples, see Fig. 1).

Results

Basic cellular responses

A total of 30 MNTB neurons were recorded, ranging in CF from 1.5 to 56 KHz (Table 1). Of these, 28 (93%) displayed spontaneous activity, with the sample averaging 14.04 spikes/s. All response types found in earlier observations of neurons in the rat MNTB (Kulesza et al. 2003a; Sommer et al. 1993). A total of 24 SPON neurons were recorded, ranging in CF from 0.8 to 50 KHz (Table 1). These neurons typically displayed little or no spontaneous activity, with the sample averaging 0.44 spikes/s. All response types found in earlier recordings of the rat SPON (Kulesza et al. 2003a) were encountered, including offset-transient, offset-chopper, offset-sustained, on-offset, and onset-sustained responses. Examples of an offset-transient response and an offset-sustained response are shown in Figs. 1B and 4, A–F, respectively.

Thus both MNTB and SPON neurons responded to the stimulus offset, albeit differently. Whereas the offset response of MNTB cells took the form of a transient suppression of spontaneous activity, 22 of 24 (92%) SPON neurons in our sample responded to the stimulus offset by generating action potentials (for examples, see Fig. 1).

Stimulus intensity

To determine whether the magnitude of these offset responses is dependent on stimulus intensity, we recorded from MNTB and SPON units presented with stimuli of various intensities. Specifically, 50-ms CF tone bursts were presented with a 10-ms delay relative to the start of the recording, the overall duration of which was 100 ms. The defined analysis window began 5 ms after the stimulus offset to avoid registering spikes belonging to the sustained portion of the MNTB response and continued until the end of the recording, 40 ms after the stimulus offset. This analysis window was sufficient to capture the entire offset response of all units in both nuclei. In this time window, we measured the mean number of spikes over six stimulus presentations. The Friedman test was used for all within-subjects comparisons because the data often showed unequal variance across durations and intensities (e.g., Fig. 5C) that precluded the use of within-subjects ANOVA.

The intensity dependence of the offset response was assessed in all MNTB neurons that displayed spontaneous activity (n = 28). Within this sample, increasing stimulus intensity caused a significant decrease (Friedman test, P < 0.001) in the number of spikes per presentation, corresponding to a more sustained, on-offset, and onset-sustained responses.
profound and/or longer lasting suppression of spontaneous activity (Fig. 2A). In SPON, we assessed the intensity dependence of the offset response in all neurons that produced spikes at the stimulus offset \((n = 22)\). In SPON neurons, increasing the stimulus intensity caused a significant increase (Friedman test, \(P < 0.001\)) in the number of spikes per presentation (Fig. 2B). Note that while increasing stimulus intensity led to fewer spikes over the entire range of intensities tested in the MNTB (Fig. 2A), the SPON offset response increased with stimulus intensity only over part of the range of intensities tested, reaching a plateau at 20 dB above threshold (Fig. 2B).

**Stimulus duration**

The duration dependence of the offset responses was assessed by presenting CF tone bursts of various durations at 20 dB above threshold, as described above (e.g., Figs. 3 and 4). As before, a time window was defined to begin 5 ms after the stimulus offset to avoid registering spikes belonging to the sustained portion of the response of MNTB neurons. However, because long duration stimuli can cause suppression periods of several tens of milliseconds (e.g., Fig. 3F), the analysis window extended to 80 ms after stimulus offset to make sure that some unsuppressed spontaneous activity would be registered. Within this window, the number of spikes per presentation and median first spike latency relative to the stimulus offset were measured. MNTB neurons without spontaneous activity were excluded from this analysis. We assessed duration dependence in all remaining neurons that displayed any offset response and for which the stability of our recording platform was sufficient to collect a complete set of duration data (MNTB: \(n = 24\); SPON: \(n = 18\)).

In MNTB neurons, increasing the stimulus duration led to a significant decrease in the number of spikes per presentation and to a significant increase of the median first spike latency, i.e., a longer duration of the suppression (Friedman test, \(P < 0.001\) in each case; Fig. 5, A and B). Responses from five (21%) MNTB neurons showed a nearly monotonic decrease in the number of spikes, corresponding to successively longer suppression of spontaneous activity, for stimulus durations \(\leq 1,000\) ms. In the remaining 19 (79%) cells, the suppression of spontaneous activity saturated at an intermediate stimulus duration.

To obtain more detailed information about the significant outcome of the Friedman test, pairwise comparisons of the number of spikes and the first spike latencies were made using Wilcoxon signed rank tests for our entire sample of neurons from each nucleus. Each stimulus duration was used as a standard and the difference between that duration and the first longer and shorter stimuli that evoked a significantly different response were calculated and termed the upward and downward just significant difference (JSD), respectively. To visualize the duration sensitivity of the sample, the JSDs were plotted over standard duration (Fig. 6, A and B). Pairwise comparisons showed that in both the MNTB and SPON the sensitivity to stimulus duration changed as a function of the standard duration. Moreover, in both nuclei the number of spikes per presentation contained more information about stimulus duration than did the first spike latency, because in many cases, JSDs based on first spike latency were not found in the range of durations tested. Where latency-based JSDs were found, they were usually longer than the corresponding JSDs based on spike count. In MNTB, the highest sensitivity was found at a standard duration of 7 ms, where upward and downward JSDs of 3 ms were found. The duration sensitivity of MNTB neurons dropped sharply when the standard durations exceeded 50 ms. Because individual neurons may differ in the range over which they are duration sensitive, i.e., the range over which the spike count and latency vary as a monotonic function of stimulus duration, it is also worthwhile to assess the duration sensitivity of each individual neuron. This was achieved by using the spike counts from individual stimulus presentations to calculate the parameter \(d'\) from signal detection theory according to the formula

\[
d' = (\mu_{\text{hit}} - \mu_{\text{false}})/\sqrt{(\sigma_{\text{hit}}^2 + \sigma_{\text{false}}^2)}
\]

(Brown et al. 2004; Macmillan and Creelman 1991). The parameter \(d'\) measures the ability to discriminate between two stimuli. Discriminability thresholds are usually defined as 75% correct responses, corresponding to a \(d'\) of 1.35 (Green and Swets 1966). In analogy to the JSDs described above, each stimulus duration was used as a standard, the first longer and shorter durations yielding a \(d'\) of 1.35 or greater were identified, and the difference between the durations calculated to give the upward or downward just noticeable differences (JNDs), respectively. Like the JSDs, the JNDs were plotted over standard duration (Fig. 7A). As with the JSDs, JNDs
based on the comparison of responses from individual neurons varied as a function of standard duration. Seven of the 24 neurons in the sample (29%) failed to produce any JND within the range of stimulus durations tested, and in the remaining neurons, the JNDs for a given standard duration varied widely. However, as with the JSDs, the shortest JNDs were found for stimulus durations below 50 ms. Calculating JNDs in this manner also afforded the possibility of comparing the discrimination performance of individual neurons to behavioral duration discrimination data from the literature. Church et al. (1976) determined difference limens for the comparison of a 0.5-, 1-, 2-, 4-, and 8-s standard stimulus to a longer duration test stimulus using a 75% correct criterion. A comparison of our electrophysiological data and the behavioral data are only possible for the 0.5-s standard duration. The mean of the minimum difference limens for the 0.5-s standard obtained by Church et al. (1976) (calculated from their Table 1) is 627 ± 148 ms. Figure 7A shows that our sample of MNTB neurons contains only one cell with JNDs shorter than the difference limens at the 500-ms standard duration and whose responses were therefore consistent with the discrimination behavior observed by Church et al.

In SPON neurons (n = 18), increasing the stimulus duration led to a significant increase in the number of spikes per presentation within the analysis window as well as to a significant decrease of the median first spike latency (Friedman test, P < 0.001 in each case; Fig. 5, C and D). Responses from 4 (22%) of the 18 SPON neurons in our sample showed a nearly monotonic increase in the number of spikes for stimulus durations ≥1,000 ms, whereas in the remaining 14 (78%) cases, the magnitude of the offset response saturated at an intermediate stimulus duration. As with the MNTB neurons, the duration sensitivity of the SPON neurons was assessed in more detail using pairwise comparisons with the Wilcoxon signed rank test to calculate JSDs and the parameter d' from signal detection theory to calculate JNDs. These analyses revealed that, as in MNTB neurons, the spike count contains more information about the stimulus duration (i.e., shorter JSDs) than the first spike latency (Fig. 6, C and D). The highest duration sensitivity in SPON was found at a standard duration of 9 ms, with an upward JSD of 11 ms and a downward JSD of 7 ms, whereas at stimulus durations ≥10 ms the sensitivity fell off noticeably. In contrast to the MNTB, however, JSDs at longer standard durations were often relatively short, affording a degree of duration sensitivity even at high standard durations. Four of 18 SPON neurons (22%) failed to produce any JND within the range of durations tested (Fig. 7B). Similar to the findings in MNTB neurons, JNDs for any given reference duration varied widely but showed a trend toward shorter JNDs for shorter standard durations. The comparison with the previously reported behavioral data (Church et al. 1976) showed that the SPON sample contained two neurons that displayed an
upward JND shorter than the behavioral difference limens at the 500-ms standard duration and were thus capable of driving the behavioral performance observed. Taken together, the statistically significant duration dependence of the offset responses in each nucleus, coupled with the JSD and JND analyses, show that the MNTB-SPON circuit produces offset responses capable of representing stimulus duration.

The mean duration at which the number of spikes per presentation in the analysis window saturated was 442.67 ± 84.97 (SE) ms in the MNTB and 270.43 ± 88.45 ms in the SPON (Mann-Whitney U test, P = 0.14). We attempted to correlate the durations at which the offset response saturated with the unit’s CF, as well as with the number of spikes per presentation contained in the offset response at the 50-ms stimulus duration. However, these correlation coefficients were not statistically significant in either nucleus.

**DISCUSSION**

Offset responses such as those shown here (the suppression of spontaneous activity in MNTB and offset spikes in SPON) may be particularly well suited to encode two auditory cues, namely that a stimulus has ended and its total duration. The offset suppression of spontaneous activity in MNTB is dependent on stimulus intensity and extends several milliseconds beyond the stimulus. By in vivo intracellular recording from

**FIG. 4.** Duration-dependent responses of a typical SPON neuron. A–F: PSTHs show responses of an SPON neuron to CF tone stimuli of durations ranging from 3 to 1,000 ms. Stimulus is represented by a black horizontal bar at the top of each panel, and the time window in which the offset response was analyzed is shaded gray. Twenty repetitions of the stimulus were presented; bin width is 1 ms. Mean spike counts and median latency of the 1st spike in the analysis window relative to the stimulus offset are plotted over stimulus duration in G and H, respectively.

**FIG. 5.** Summary of the duration dependence of MNTB and SPON offset responses. Number of spikes per stimulus and median 1st spike latency relative to the analysis window is plotted over stimulus duration for the sample of 24 MNTB neurons (A and B, respectively) and for our sample of 18 SPON neurons (C and D). Means and error bars are displayed in black, with individual units in gray. Error bars represent SE.
principal cells of the rat MNTB, Sommer et al. (1993) reported high rates of spontaneous activity and phasic-tonic responses to best frequency tones, followed by a period of suppression of spontaneous activity that coincided with a transient hyperpolarization of the membrane potential and was dependent on stimulus intensity. It was also noted that this hyperpolarization persisted for several milliseconds after the stimulus offset, i.e., during the period that spontaneous activity is suppressed. In this study, we confirm the general shape of the MNTB response and a period of suppressed spontaneous activity after the stimulus offset, the duration of which depends on stimulus intensity. This study also shows that the duration and/or strength of this suppression is not exclusively determined by stimulus intensity but also by stimulus duration and can extend up to 70 ms beyond the stimulus offset, depending on stimulus parameters.

SPON neurons responded to the stimulus offset with spikes at all durations tested in this study. The reliable temporal relationship of these spikes to the stimulus offset is consistent with their resulting through a postinhibitory rebound mechanism (Kulesza et al. 2003a). The major inhibitory input to the SPON is glycinergic and originates in the MNTB (Banks and Smith 1992; Kulesza and Berrebi 2000; Kulesza et al. 2003a,b; Kuwabara and Zook 1991; Sommer et al. 1993). Furthermore, this inhibition is presumably tonic because of the high spontaneous activity rates of MNTB neurons and is maximal during the onset and sustained components of the MNTB neuron’s response to CF tones. By the same reasoning, factors shaping the MNTB offset response will also determine the strength of the SPON offset response and similar intensity and duration dependencies should be expected from both nuclei. While this is generally the case, it is noteworthy that the magnitude of the SPON offset response reaches a plateau at intensities 20 dB above threshold, whereas the MNTB offset response does not.

Consideration of other potential roles of MNTB and SPON offset responses

Given our current understanding of the MNTB-SPON circuit described herein, it seems that only the offset component of the MNTB response is required to drive SPON spike activity. However, the innervation of SPON occurs through collateral branches of MNTB axons whose primary synaptic targets are in the lateral superior olive (LSO) (Banks and Smith 1992; Spangler et al. 1985). The computational function of LSO is most easily understood as the summation of a primary-like excitatory and a primary-like inhibitory input, which encodes the localization of high-frequency sounds based on interaural intensity differences (Park et al. 1997; Pollak et al. 2003). Based on our results, one might expect that LSO responses to contralateral tones would include a transient burst of activity at stimulus offset, similar to that observed in SPON. Indeed, examination of published peristimulus time histograms (PSTHs) from LSO suggests that this may be the case (see Fig. 3 in Caird and Klinke 1983; Tollin and Yin 2002), but to our knowledge, this particular feature of LSO physiology has not been systematically studied. This line of reasoning leads us to believe that the MNTB may have at least two functional roles: the first is its well known contribution to sound localization circuitry, whereas its offset response mediates spiking activity in the SPON and ultimately generates GABAergic inhibition in the inferior colliculus.

![Diagram](http://jn.physiology.org/)

**Fig. 6.** Results of pairwise comparisons of responses to stimuli of different duration. Open symbols represent the upward just significant difference (JSD); filled symbols represent the downward JSD. Where no symbols are drawn, no JSD was found. Note that small absolute values of JSD, indicated by symbols near the horizontal axis, indicate high resolution of stimulus duration. A: JSD based on number of spikes per presentation in MNTB neurons. B: JSD based on 1st spike latency in MNTB neurons. C: JSD based on number of spikes per presentation in SPON neurons. D: JSD based on 1st spike latency in SPON neurons.
How then might SPON offset spikes contribute to response properties of IC neurons? In addition to the inhibition arising in SPON, the IC receives ascending GABAergic inputs from a number of other sources, most notably the dorsal and ventral nuclei of the lateral lemniscus (DNLL and VNLL, respectively; Gonzalez-Hernandez et al. 1996; Zhang et al. 1998). A detailed comparison of the pattern of axonal arborizations of each projection is not available, but based on unbiased stereological estimates of the number of neurons contained within each of these nuclei, the VNLL is presumably, by far, the largest source of GABAergic inputs to the IC, followed by the SPON (Kulesza et al. 2002).

The myriad effects of GABAergic inhibition on responses of IC neurons have been extensively studied (reviewed by Faingold 2002; Pollak et al. 2002, 2003). Based on our existing knowledge of the response properties and efferent projections of SPON neurons, we would postulate that the direct effects of this nucleus on IC physiology would be limited to those that are evoked by contralateral stimulation, mediated by GABA, and closely follow the stimulus offset. Particularly relevant to this discussion, therefore, is the iontophoresis study of Mexican free-tailed bats showing that cells in the central nucleus of the IC exhibit a monaural, contralaterally evoked excitation followed by a period of inhibition (Bauer et al. 2000). This inhibition outlasts the stimulus, increases at higher stimulus intensities and is sensitive to stimulus duration. Moreover, the inhibition is largely reduced or abolished by application of bicuculline, suggesting that it acts primarily through the GABA$_A$ receptor. Assuming that this inhibitory input to the IC is common to mammals, its properties are consistent with the properties of the MNTB-SPON circuit’s offset responses shown in our study.

Phenomena matching our criteria for SPON derived inhibition of IC neurons have also been described in the rat. In GABA-deficient rats prone to audiogenic seizures, IC neurons display an increased incidence of offset responses, which can also be induced in normal rats by iontophoretic application of the GABA$_A$ receptor antagonist bicuculline (Faingold et al. 1986). These findings indicate that GABA inhibits these IC neurons for a brief period after the stimulus offset. The SPON was suggested as the probable source of this inhibition in the rat IC (Faingold 2002). However, whether this offset inhibition in the IC is affected by changes in stimulus duration and intensity remains unknown.

**Neural mechanisms and models of duration sensitivity**

Our data show that together MNTB and SPON form a neural circuit capable of generating responses representing stimulus durations with increased durations resulting in larger offset responses. In MNTB neurons, the increased offset responses manifest as more profound and/or longer lasting suppression of spontaneous activity, whereas in SPON neurons more spikes occur. Longer stimulus durations saturate the offset response magnitude, so that all neurons in the circuit described here exhibit longpass filter characteristics with regard to duration. In the case of the MNTB, the number of spikes in the analysis window decreases less as stimulus duration is increased. In contrast, in the SPON average spike counts increase nearly monotonically with stimulus duration, with a corresponding increase in the SE. It seems therefore that at longer durations there is an increasing contrast between the response magnitudes of the neurons that saturate and those that can still respond to duration increases with an increased number of spikes. This representation of stimulus duration by the magnitude of an offset response is confounded, however, by the circuit’s reaction to stimulus intensity, which is largely identical to its representation of stimulus duration. It is interesting to note that a perceptual equivalence of stimulus duration and intensity has also been observed in behavioral experiments with budgerigars (Dooling et al. 1987) and psychophysical studies in humans (McFadden 1975).

The mechanisms underlying duration sensitivity of neurons in the rat MNTB and SPON differ in several aspects from that proposed for duration tuned IC neurons in bats (Casseday et al. 1994, 2000; Ehrlich et al. 1997; Faure et al. 2003). Most obvious is the fact that the duration-sensitive neurons herein shown in rats reside in a lower part of the auditory pathway.
We acknowledge, however, that offset responses similar to those observed in the MNTB (suppression of spontaneous activity after stimulus offset) have also been reported at even earlier stations of the central auditory pathway, namely the auditory nerve (Kiang 1966) and cochlear nucleus (Palmer 1987; Pfeiffer 1966), although the duration dependence of these offset responses has not been studied. At present it is also unclear whether the MNTB offset response is inherited from inputs arising from the CN, generated by an inhibitory input or caused by some intrinsic property of MNTB neurons themselves. The model of Casseday et al. (1994) specifically allows for the possibility that some of the inputs necessary for duration tuning in IC originate from lower levels of the auditory brain stem, and it is therefore conceivable that the output of SPON neurons might represent a contributing input. However, in this model both excitatory and inhibitory inputs are triggered by the stimulus onset, whereas SPON cells provide inhibition triggered by the stimulus offset. We consider it unlikely therefore that the SPON contributes to duration tuning in the IC, at least as proposed by the model of Casseday et al. (1994).

Duration-tuned neurons have been described in the IC of the mouse (Brand et al. 2000) and most recently in the rat (Perez-Gonzalez et al. 2005). Duration tuned neurons in the mouse IC display either band-pass or longpass characteristics. Responses of the band-pass tuned neurons occurred after the stimulus offset and were compatible with the above-mentioned model of Casseday et al. However, the long-pass neurons only responded when stimuli exceeded a threshold duration and, in most cases, showed onset responses. Recently described duration sensitive neurons studied in the IC of the rat included long-pass neurons that fired only to stimuli exceeding some threshold duration, as well as band-pass neurons which most often responded to the stimulus offset (Perez-Gonzalez et al. 2005). These authors also report that all offset neurons in the IC display some sort of duration sensitivity, leading us to hypothesize that the role of SPON-derived inhibition may be to suppress offset responses in band-pass duration tuned neurons when stimuli fall outside their passband. In the case of stimulus durations within the passband, this inhibition is presumably overridden by excitation. Perez-Gonzalez et al. (2005) go on to speculate that transient inhibition synchronized to the stimulus offset, consistent with SPON response characteristics, might contribute to the generation of lowpass filter characteristics in IC neurons with transient responses.

Another hypothesis is that stimulus duration might be represented in the IC by GABA-mediated suppression of spontaneous activity after the offset, as observed by Bauer et al. (2000). As discussed previously, we consider it likely that GABAergic inhibition from the SPON causes the offset inhibition in the IC. Indeed, Bauer et al. (2000) indicate that offset inhibition in the IC is duration sensitive, a finding consistent with the notion that temporal information contained in SPON offset spikes is conserved in this response component of IC neurons.

Finally, He (2002) showed long-duration selective off responses in the medial geniculate body of the guinea pig. Our own preliminary tract-tracing results show a direct projection from SPON to MGB in the rat (Jin and Berrebi 2006), raising the possibility that the duration information observed in the present study is relayed to the level of the auditory thalamus.

Consideration of behavioral data

We attempted to align the duration sensitivity of neurons in the rat’s MNTB-SPON circuit to the discrimination ability observed in the behavioral experiments of Church et al. (1976) using signal detection theory. To drive the discrimination performance, the JNDs exhibited by MNTB and SPON neurons should be equal to or shorter than the corresponding behaviorally determined difference limens. Only three of the neurons tested in this study, one in the MNTB and two in the SPON, met this criterion. It is also notable that MNTB and SPON neurons show the highest duration sensitivity for durations shorter than ~50 ms. The only stimulus duration available for comparison between this study and that of Church et al. lies well outside this range. We can offer two possible conclusions from these findings. Either a very small subpopulation of MNTB and SPON neurons generates the duration measurements underlying the rat’s behavioral discrimination performance, or there exists a separate mechanism of duration measurement elsewhere in the brain that can more effectively discriminate between longer stimulus durations.

Behavioral studies of duration discrimination also give rise to the assumption that the neural mechanisms of duration perception are species specific. Duration discrimination has been studied in the Japanese macaque (Sinnott et al. 1987), bottlenose dolphin (Yunker and Herman 1974), parakeet (Dooling and Haskell 1978), European starling (Maier and Klump 1990), mouse (Klink and Klump 2004), and rat (Church et al. 1976). Killeen and Weiss (1987) differentiate two different models to account for the psychophysiological data gathered from these various species. In the so-called “clock” model of duration perception, a single measure of sound duration is taken, whereas in “clock-counter” models, impulses are generated throughout the ongoing sound and the measurement of duration relies on the ongoing count of these impulses. In each scenario, the error is dependent on the stimulus duration in a characteristic fashion, permitting the identification of the mechanism underlying duration perception in each species. For example, duration tuning in the mouse follows a clock-counter mechanism, whereas duration processing in the rat follows a clock mechanism. Given that behavioral studies suggest differences in duration processing even between relatively closely related species such as the mouse and rat, comparisons of electrophysiological data on duration processing between species have to be approached with caution.

Acknowledgments

We thank L. Bai, R. Fecek, and D. Cole for expert technical assistance. Drs. Enrique Saldaña, Ariel Agmon, George Spirou, Peter Mathers, Paul Brown, and Ronald Millecchia provided valuable comments on the manuscript.

Grants

This work was supported by National Institute for Deafness and Communication Disorders Grant RO1 DC-06626 to A. S. Berrebi.

References


