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Forward Masking in the Superior Paraolivary Nucleus of the Rat

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Abstract

In natural acoustic environments, perception of acoustic stimuli depends on the recent contextual history. Forward masking describes a phenomenon whereby the detection threshold of a probe stimulus is markedly increased when it is preceded by a masking stimulus. The aim of this study was to characterize the offset response of single units in the superior paraolivary nucleus (SPON) to a forward masking paradigm. We observed two distinct response types to forward masked stimuli, namely inhibited and facilitated responses. In the presence of a default masking stimulus, inhibited responses to probe stimuli were characterized by elevated thresholds and/or diminished spike counts, whereas facilitated responses were characterized by reduced thresholds and increased spike counts. In units with inhibited responses to the probe stimuli, probe thresholds increased and spike counts decreased as masker intensity was raised or the masker-to-probe delay was shortened. Conversely, in units with facilitated responses to the probe stimuli, probe thresholds decreased and spike counts increased as masker intensity was raised or the masker-to-probe delay was shortened. Neither inhibited nor facilitated responses to the forward masking paradigm were significantly dependent on masker frequency. These findings suggest that SPON responses are not themselves consistently subject to the same forward masking properties observed in other nuclei along the ascending auditory pathway. The potential neural mechanisms of the forward masking responses observed in the SPON are discussed.

Keywords

SPON; Auditory scene analysis; Context dependence; Forward suppression; Forward facilitation

Introduction

The mammalian auditory system is a highly complex neural network, which consists of interconnected subcortical nuclei and cortical areas that extract, encode, and interpret sounds arising from a dynamic auditory environment. Briefly, acoustic stimuli received by the

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cochlea are transmitted by auditory nerve fibers to the auditory brainstem, where the auditory information is split into several parallel auditory processing pathways traversing the superior olivary complex. These parallel pathways converge in the inferior colliculus (IC), at the level of auditory midbrain. Finally, the integrated auditory information from the inferior colliculus is sent to the medial geniculate body of the thalamus and passed on to the auditory cortex for the advanced processing underlying perception; for a review see (Malmierca and Hackett 2010).

In natural environments multiple sound sources tend to be active at the same time, creating a challenging situation where sounds occur at various temporal relationships to each other. Forward masking is thought to help in the processing of stimuli occurring in such a complex acoustic environment. Psychophysical studies have demonstrated that the perception threshold of an acoustic stimulus, hereafter called the *probe*, can be impaired by a preceding sound, hereafter called the *masker* (Jesteadt et al. 1982; Plack and Oxenham 1998). This forward masking or forward suppression phenomenon is thought to play multiple roles in the analysis of complex acoustic environments, including speech processing (Brosch and Schreiner 1997), echo suppression (Kaltenbach et al. 1993) and auditory stream segregation (Fishman et al. 2004).

The first stage of the neural computations underlying forward masking occurs at the level of the auditory nerve (Harris and Dallos 1979; Relkin and Turner 1988). Harris and Dallos (1979) found that probe-evoked responses of auditory nerve fibers were reduced by the addition of a masking stimulus, and that the forward suppression was dependent on the masker-to-probe delay, but independent of masker level and masker frequency. Relkin and Turner (1988) reexamined forward masking in the auditory nerve and showed that response threshold for the probe stimulus could be raised by up to 21 dB when a masker was presented. In our own previous study of forward masking in the medial nucleus of the trapezoid body (MNTB) of the rat, we found a maximum threshold shift of 54 dB, with 26% of the units in our sample exhibiting threshold shifts greater than 21 dB (Gao and Berrebi 2015). We also demonstrated that the masker-induced threshold shift in MNTB neurons depended on both masker level and masker-to-probe delay, with louder maskers and shorter masker-to-probe delays producing greater threshold shifts. The masker-induced threshold shift was also dependent on masker frequency, with maskers presented at the neurons' characteristic frequency (CF) producing greater threshold shifts than off-CF maskers. Compared with the MNTB, where we found an average threshold shift of 9.95 dB (Gao and Berrebi 2015), the inferior colliculus produced a more pronounced forward suppression, with an average shift of 18.8 dB in response to a forward masking paradigm (Nelson et al. 2009). The large threshold shifts found in the IC were very similar to those measured in psychophysical studies (Plack and Oxenham 1998). Nelson et al. (2009) postulated that the forward masking features in the IC could be explained by an inhibitory input that is time locked to the offset of the masker, and specifically proposed the superior paraolivary nucleus (SPON) as a potential source of this input.

The mammalian SPON is a prominent GABAergic cell group located in the superior olivary complex of the brainstem (Kulesza and Berrebi 2000; Saldaña and Berrebi 2000; Tollin 2003; Grothe et al. 2010). In addition to an excitatory input that originates mainly from the

posteroventral cochlear nucleus (Felix et al. 2013), the SPON receives strong, tonic glycinergic inhibitory input from the MNTB (Kulesza et al. 2003; Kadner et al. 2006; Kulesza et al. 2007) and GABAergic inhibitory input from the tectal longitudinal column (TLC) (Viñuela et al. 2011). The SPON provides a major source of GABAergic inhibition to neurons in the inferior colliculus (Kelly et al. 1998; Kulesza and Berrebi 2000). Ninety-five percent of SPON neurons produce offset spikes following their release from tonic glycinergic inhibition originating in the MNTB (Kulesza et al. 2003; Kadner et al. 2006; Kulesza et al. 2007; Kadner and Berrebi 2008, Felix et al. 2011). Thus, the MNTB and the SPON form a neural circuit that transforms an excitatory input from globular bushy cells into a transient GABAergic signal that occurs in response to stimulus offsets and discontinuities (Kadner and Berrebi 2008; Felix et al. 2011). By reversibly inactivating the SPON, we recently demonstrated that SPON-derived inhibition serves to induce temporal segmentation in responses of IC neurons (Felix et al. 2014). Taken together, these findings suggest that the SPON offset response may play a critical role in mediating forward masking in the IC. We explored the potential contribution of the SPON to forward masking in the IC, by assessing the offset responses of single units in the rat SPON to a forward masking paradigm. Parts of this study have been reported in abstract form (Gao and Berrebi 2014).

Materials and methods

Animal preparation

Twenty-seven female Sprague-Dawley albino rats (Harlan, Indianapolis, IN, USA) weighing 190–240 g, were used in this study. Animals were housed in the West Virginia University Health Sciences Center vivarium. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committee of West Virginia University.

Anesthesia was administered by intramuscular injection of an initial dose of 70 mg/kg ketamine and 5 mg/kg xylazine. Animals were then placed in a stereotaxic frame using blunt hollow ear bars to avoid injury to the tympanic membrane. After exposing the skull, a custom-fabricated head post was bonded to the skull rostral to bregma by applying screws and dental cement. A craniotomy (approximately 3×7 mm) was then made over the cerebellum, and the dura mater was opened. The exposed cerebellum was removed to uncover the floor of the fourth ventricle, whose midline was employed as a landmark for electrode penetrations. The animals were transferred to a sound-attenuated recording booth and the head post used to secure the animal in a stereotaxic frame. A thermostatically controlled heating blanket was used to maintain body temperature at 37 °C. When deemed necessary, supplementary injections of one-third of the original dose of ketamine were given as needed during surgery and physiological recording sessions. Lidocaine was applied to the margins of the incision every two hours.

Acoustic stimuli

Acoustic stimuli were generated as digital waveforms and converted to analog signals using SciWorks (DataWave Technologies Corporation, Loveland, CO). Analog signals were passed through an antialiasing filter (FT6-2; Tucker-Davis Technologies (TDT), Alachua,

FL) and sent to PA-5 programmable attenuators (TDT). Signals were then routed to a TDT speaker driver and presented through TDT free-field speakers mounted in the stereotaxic frame approximately 5 mm from the opening of each ear. The output of each speaker was calibrated over a frequency range from 1 to 65 kHz using a B&K Type 4939 microphone connected to a type 2610 measuring amplifier (Brüel and Kjaer North America, Norcross, GA, USA) and converted to dB SPL (sound pressure level, re. 20 μ P) offline.

Single-unit recording and data collection

Single units in the SPON were recorded through glass recording pipettes (tip diameter 2–3 μ m, tip impedance 15–30 M Ω) filled with 2.5 % Biocytin (Sigma Chemical, St. Louis, MO) dissolved in 0.9 % NaCl, or 10 % biotinylated dextran amine (BDA) dissolved in 0.1 M sodium phosphate buffer. Electrode signals were amplified using a Dagan 240 extracellular preamplifier (Dagan Corporation, Minneapolis, MN), and band-pass filtered from 0.3 to 3.0 KHz with a Krohn-Hite 3364 Filter (Krohn-Hite, Brockton, MA). The processed signals were then fed into the SciWorks software for digitization, display and storage.

Noise bursts of 50 ms duration with 2 ms rise/fall times, presented at 80 – 90 dB SPL, were used as search stimuli. Units were considered well isolated if the auditory-evoked spike waveforms were homogeneous and could be reliably separated from the background noise by a trigger window. Once a single unit was identified, its response threshold for noise bursts was estimated. To do so, the intensity of the search stimulus was reduced until no further spikes were evoked. The lowest intensity that could still evoke spikes was considered the threshold for the noise stimulus. Binaural interaction was then assessed by presenting 50 ms noise bursts at 5 dB above the unit's noise threshold to the ipsilateral and then to the contralateral ear, as well as to both ears simultaneously. Units were considered to be driven by the contralateral ear if they responded to stimulation of the contralateral but not the ipsilateral ear, and the responses to stimulation of the contralateral ear was similar to stimulation of both ears. Only units that responded to the offset of the search stimulus and were driven by the contralateral ear were studied further. Because only offset responses were assessed in this study, in the following the term “SPON responses” refers to offset responses exclusively.

Tone bursts were used to determine the basic auditory response properties and forward masking properties of SPON units. The frequency at which the unit produced evoked responses to the lowest sound level was considered the unit's CF, and the lowest sound level at CF that evoked spikes was considered the unit's threshold for pure tones. Peristimulus time histograms (PSTHs) were constructed from responses to 100 consecutive presentations of CF tones of 50 ms duration, at 20 dB above the unit's threshold. Response maps (RMs) were generated by presenting a matrix of tone stimuli in which the stimulus frequency varied with linear spacing from 1/2 CF to 3/2 CF in 1/20 CF steps, and stimulus intensity varied from 20 dB below to 60 dB above the unit's threshold in 20 dB steps. All tones used for recording the response maps were of 200 ms duration with 10 ms rise/fall times. If the threshold intensity was not clear after recording the RM, a rate-level function (RLF) was recorded using 200 ms CF tones. Stimulus intensities ranged from 10 dB below the initially determined thresholds to ~40 dB above the unit's threshold, or to the maximal intensity our

sound system was capable of producing, in 1 dB steps. All RLFs in this study were smoothed using a 1–2–1 triangular smoothing algorithm, as described previously (Kulesza et al. 2003). The sequence of stimulus presentations for the RM and RLF experiments was randomized, and responses to five presentations of each stimulus were recorded.

Once the basic response properties of an SPON unit were known, responses to the forward masking paradigm were recorded. The masker (200 ms in duration with 10-ms rise/fall times) was varied in intensity, frequency and masker-to-probe delay, whereas the probe (20 ms in duration with 10 ms rise/fall times) only varied in intensity (Fig. 3; Figs 5 and 6, panels a, d and g respectively). The first step in observing a unit's forward masking properties was recording an RLF in response to the 20 ms probe (five stimulus presentations for each intensity tested) in the absence of a masker, followed by recording of a RLF under a so-called default masker condition. The default masker was a 200 ms CF tone, presented at either ~20 dB or ~40 dB above the unit's threshold, with a 10 ms masker-to-probe delay (Fig. 1a). The default masker was presented at 20 dB above threshold if the unit's threshold was higher than 40 dB attenuation relative to the maximum output of our sound system. Subsequently, the unit's RLF was recorded when the masker was varied in level, frequency or masker-to-probe delay. To determine the effects of the masker parameters on SPON responses to the probe, only one masker parameter was varied at a time while holding the remaining two parameters constant. In experiments assessing the dependence of forward masking on masker level, low, medium and high intensity maskers were presented. In units where the default masker was 40 dB above threshold, the low, medium and high intensity maskers were set at 20, 40 and 60 dB above threshold, respectively. In units where the default masker was 20 dB above threshold, the low, medium and high intensity maskers were set at 10, 20 and 30 dB above threshold, respectively. Three masker frequencies (CF, above- and below-CF) were all chosen within the RM of the recorded SPON unit to determine the effect of masker frequency. The masker-to-probe delays were set to 0, 10, 30, 70, 150 or 310 ms. In the case of the 0 ms masker delay, no true silent period existed between the masker and probe stimuli. All stimuli were presented at 1 second intervals, regardless of whether they were used to assess the unit's baseline response properties or to assess the effects of masker parameters. Recordings continued until the unit was lost. When well-isolated units were recorded, biocytin or BDA was deposited at the recording site by applying an anodic current of 1.0 μ A for 5 min. Recording sites were subsequently verified histologically as described previously (Kulesza et al., 2003).

Data analysis

SPON offset responses to the probe were calculated from a time window that started immediately after the offset of the probe and lasted for 40 ms (Fig. 1b). Masker-evoked offset responses were calculated from an analysis window that started at the offset of the masker and lasted 20 ms (Fig. 1b). The analysis windows for the offset responses to the masker and probe were immediately adjacent in the 0 ms delay condition, but no overlap occurred, so that the analysis windows were separate for all masker conditions. In addition, we confirmed by online inspection of the raster plots and offline inspection of individual PSTHs that the offset responses to masker and probe were separate for all masker and probe combinations tested. Probe thresholds for each unit was determined from its RLF in both

unmasked and forward-masked conditions. The probe threshold was defined as the lowest probe tone level that evoked a reliable increase in spike count of 20% or more of average spontaneous activity. For neurons that had responses at the lowest probe intensity tested, we estimated the probe threshold from the regression lines of their RLFs. Spontaneous rates for estimating probe threshold were determined from the last 200 ms of each recording trace for the masker alone. The probe threshold shift was defined as the difference between the probe thresholds determined under the unmasked and masked conditions. The total number of spikes in the unit's probe RLF was counted between the unit's probe threshold and the maximum probe intensity tested under unmasked and masked conditions. The relative change of response magnitude was calculated as: $(1 - \text{total spikes under masked condition} / \text{total spikes under unmasked condition}) \times 100\%$. We assessed the dependence of the probe threshold shift and response change magnitude on the unit's CF, probe threshold and first-spike latency. A unit's responses were only analyzed if their thresholds could be estimated under at least two frequency or masker-to-probe delay conditions.

Data were analyzed using DataWave SciWorks software (DataWave Technologies Corporation, Loveland, CO), or our own Python and Visual Basic routines. Statistical analyses were performed using SPSS 13.0, and between-group differences were considered significant if $p < 0.05$.

Results

Fifty-seven well-isolated single SPON units (CFs: 1 – 31 kHz) from 27 female Sprague Dawley rats were recorded. The anatomical location of each recorded unit was verified by recovering biocytin or BDA deposits (Fig. 2).

Inhibited and facilitated SPON responses to the forward masking paradigm

In response to the forward masking stimulus paradigm, SPON neurons produced distinct and clearly separate responses to both the masker and probe stimuli under all stimulus conditions assessed. This separation of responses to the masker and probe stimuli was necessary to perform all subsequent analyses of the forward masking properties of SPON responses. Two basic types of SPON response to the forward masking paradigm were found under default masker conditions, hereafter called inhibited and facilitated responses. In inhibited responses, the presence of the default masker caused an elevation of the probe threshold and/or a decrease of the response magnitude, whereas in facilitated responses the presence of the default masker caused a reduction of the probe threshold and an increase of the response magnitude. An example of an inhibited response is shown in Figure 3a–c, and an example of a facilitated response in Figure 3d–f. It is notable the inhibited response in Figure 3a and b contained an onset response component when the probe was presented alone, but not when a masker was presented. For the inhibited unit the probe threshold was elevated by 10 dB, and the total spike count over the full range of probe intensities tested was reduced by 180 (77%) when the default masker was presented (Fig. 3a–c). Conversely, for the facilitated unit illustrated, the probe threshold was reduced by 15 dB and the total spiking over the full range of probe intensities tested was increased by 406 (64%) when the masker was presented (Fig. 3d–f).

The threshold shifts and relative response changes were determined under the default masker condition for a sample of 57 SPON units. Within this sample, 33 (58%) units showed inhibited responses, and 24 (42%) units showed facilitated responses in the presence of the default masker. Under the default masker condition, the average threshold shifts for inhibited and facilitated SPON responses were 12.5 ± 1.7 dB and -12.6 ± 2.9 dB, and the average relative response changes for units producing inhibited and facilitated responses were $-40.2 \pm 4.5\%$ and $278.1 \pm 157.6\%$, respectively. To determine whether threshold shifts and relative response changes were correlated to the neuron's CF and baseline unmasked threshold, we constructed scatter plots of threshold shift and relative response change over CF (Fig. 4a, b) and probe threshold (Fig. 4c, d). Across the entire sample of 57 SPON units, neither response threshold shift nor relative response change was significantly correlated with CF (Pearson correlation; for threshold shift, $p = 0.061$; for response change, $p = 0.073$). The probe threshold shift was negatively correlated with the unmasked probe threshold (Pearson correlation, $r = -0.274$, $p = 0.039$). Conversely, the relative response change had no significant correlation with the unmasked probe threshold (Pearson correlation, $p = 0.143$). The first-spike latencies of SPON responses to probes in unmasked and default masker conditions were determined from probe RLF tests and compared between the two conditions. For the inhibited neurons the default masker delayed the SPON response latency by 0.38 ± 0.43 ms, and for the facilitated neurons the default masker shortened the response latency by 2.08 ± 0.81 ms. Scatter plots of threshold shift and relative response change over the latency change of SPON responses are shown in Figure 4e and f, respectively. Response threshold shift was significantly positively correlated with latency change (Pearson correlation, $r = 0.525$, $p = 0.00009$), and relative response change was significantly negatively correlated with latency change (Pearson correlation, $r = -0.478$, $p = 0.00044$).

The distribution of inhibited and facilitated responses to the forward masking paradigm across our sample largely coincided with the distribution of on-offset responses and offset responses to pure tones (Kulesza et al., 2003). Specifically, 20/23 (87%) of on-offset SPON units displayed inhibited responses to the forward masking paradigm, whereas 21/34 (61.8%) of offset SPON units showed facilitated responses. Rate-level functions of both neuron groups fell broadly into 3 classes, namely monotonic, saturating, and non-monotonic. All RLFs in our sample contained small fluctuations, so that plotting spike count over stimulus intensity resulted in functions containing a series of peaks and troughs. To account for these fluctuations, we classified RLFs as monotonic if they consisted of a series of peaks of increasing height over the entire range of intensities recorded (Fig. 6g). RLFs were classified as saturating (Fig. 5a and Fig. 6a) if the highest peak was followed by further peaks and troughs with spike counts not exceeding the value at the highest peak, and not falling more than 20% below the highest peak value. RLFs were classified as non-monotonic if the highest peak was followed by peaks and troughs with spike counts decreasing more than 20% below the highest peak value (Fig. 3c and f; Fig. 5d, g and Fig. 6d). Of the neurons displaying inhibited responses to the forward masking paradigm, 52% (17/33) showed monotonic RLFs, 24% (8/33) showed saturating RLFs, and 24% (8/33) showed nonmonotonic RLFs. Of the neurons displaying facilitated responses, 58% (14/24) showed monotonic RLFs, 29% (7/24) showed saturating RLFs, and 13% (3/24) showed non-monotonic RLFs.

Table 1 shows a summary of basic response properties for SPON neurons displaying inhibited or facilitated responses, including CF, unit's threshold, probe threshold, first spike latency, spontaneous rates, spike count per stimulus and Q_{20} values. The first spike latency and spike rates shown here were estimated from the SPON neurons' PSTHs. Comparison of these response properties using the Mann-Whitney Rank Sum test revealed no significant differences in any of these response properties between the two unit populations, although the comparison of Q_{20} approached significance (Mann-Whitney Rank Sum test, $p = 0.052$), with inhibited neurons displaying sharper frequency tuning than facilitated units. Below we first describe the inhibited responses and then the facilitated responses to the forward masking paradigm.

Properties of inhibited SPON responses to the probe in the forward masking paradigm

The effects of masker level on inhibited responses to the forward masking paradigm were studied in a sample of 33 SPON units. The probe RLFs of a representative SPON unit determined at low, medium and high masker levels are shown in Figure 5a. When masker level was set to 20 dB above threshold of the unit (in this case 36 dB SPL), the probe threshold was increased by 10 dB and its response magnitude was diminished by 32% (not shown). As the masker levels increased to 40 and 60 dB above the threshold (in this case 56 and 76 dB SPL), the probe threshold was increased by 13 dB and 15 dB, respectively. Similarly, at masker intensities of 40 and 60 dB above threshold, the response magnitude was diminished by 34% and 42% respectively, compared to the unmasked response. The probe threshold shift (Fig. 5b) and relative response change (Fig. 5c) at low, medium and high masker level conditions were determined for each unit in the sample. The average threshold shifts and relative response changes depended significantly on the masker level (threshold shift: Fig. 5b; repeated measures ANOVA, $df = 2$, $F = 6.076$, $p = 0.013$; relative response change: Fig. 5c; repeated measures ANOVA, $df = 2$, $F = 5.013$, $p = 0.023$). Threshold shifts and relative response changes at medium and high masker levels were significantly greater than at low masker levels (post-hoc LSD pairwise comparisons; for threshold shift: low vs medium masker level, $p = 0.010$; low vs high masker level: $p = 0.018$; for response change: low vs medium masker level, $p = 0.026$; low vs high masker level: $p = 0.041$), but no significant increase in threshold shift or relative response change occurred when the masker level was raised from medium to high (post-hoc LSD pairwise comparisons; for threshold shift: $p = 0.251$ and for response change: $p = 0.415$).

The frequency dependence of inhibited responses to the forward masking paradigm was studied in 16 SPON units. Probe RLFs of a typical unit are shown in Figure 5d. The probe threshold of this example unit was raised by 10 dB when the masker frequency was set at the unit's CF (26 kHz). We selected masker frequencies below and above the CF within the unit's RM, in this case 24 kHz and 28 kHz (See the inset in Fig. 5d). When the masker was set to these frequencies the probe threshold of this unit was raised by 10 dB and 8 dB, respectively. Similarly, a 77% reduction in the relative response change was seen when the masker was presented at the unit's CF, and 70% and 74 % reductions were observed when the masker were set at below and above the CF, respectively. Figure 5, panels e and f show the probe threshold shift and relative response change determined in various masker frequency conditions for each SPON unit studied. For most units ($n = 12$) in our sample, the

CF and the off-CF maskers showed similar effects on the probe threshold and the SPON responses to the probe at various intensities. For some units ($n = 4$), the CF masker caused a stronger or weaker forward masking effect on the SPON responses than the off-CF masker did.

The dependence of inhibited SPON responses on the masker-to-probe delay was assessed in 15 units. Masker-to-probe delays ranged from 0 to 310 ms; an example of probe RLFs obtained at all masker-to-probe delays is shown in Figure 5g. At 0 ms masker-to-probe delay, the probe could only evoke spikes at high intensities; the response magnitude was reduced by 52% and the threshold increased by 19 dB. As the masker-to-probe delay increased, more spikes were evoked across the range of probe intensities, and probe thresholds gradually approached the unmasked threshold as the masker-to-probe delays were lengthened. At the longest 310 ms masker-to-probe delay, the probe-evoked responses almost recovered to those observed in the unmasked condition, with a 23% reduction in response magnitude and a 7 dB threshold increase remaining. Figure 5h and i show the probe threshold shift and relative response change of each unit in our sample as well as the sample average plotted over the masker-to-probe delays tested. For most units, probe threshold shift and response change functions, as well as their relative average functions, recover approximately linearly in log time, i.e. with an exponential decay as the masker-to-probe interval is lengthened (Fig. 5h and i, inset). We fitted exponential decay functions to the probe threshold and response change data and found R^2 -values of 0.9375 and 0.8579, respectively, indicating that both recovery kinetics were well described by exponential decay functions. For most SPON neurons, recovery to baseline levels occurred between the 150 ms and 310 ms masker-to-probe delay conditions. A small subset of units ($n = 5$) recovered to baseline between the 70 ms and 150 ms masker-to-probe delay conditions. The average threshold shift and relative response change showed a similar trend with nearly complete recovery to baseline values at the 310 ms masker-to-probe delay.

Properties of facilitated SPON responses to the probe in the forward masking paradigm

The effect of masker level on facilitated SPON responses were assessed in 24 units. Probe RLFs of a representative unit in this sample, determined at three masker levels, are shown in Figure 6a. The presence of the masker decreased the probe threshold and increased the response magnitude of this unit in response to the probe tones over the full range of probe intensities tested. When the masker level was set at 20 dB above the threshold (in this case 34.5 dB SPL), the unit's probe threshold was reduced by 8 dB and its response magnitude was increased by 98% (not shown). As the masker levels were increased to 40 dB and 60 dB above the threshold (in this case 54.5 and 74.5 dB SPL), the probe threshold of the unit declined by 21 dB and 29 dB, and the response magnitude increased by 190% and 765%, respectively. Probe threshold shifts and relative response changes under low, medium and high level masker conditions were determined for each unit in our sample and are illustrated in Figure 6b and c. Threshold shifts depended significantly on masker intensity (Fig. 6b; repeated measures ANOVA, $df = 2$, $F = 8.146$, $p = 0.002$). Moreover, threshold shifts determined at low masker levels were significantly smaller than those determined at medium (Fig. 6b; LSD Pairwise comparisons, $p = 0.002$) and high masker levels (Fig. 6b; LSD Pairwise comparisons, $p = 0.023$), but increasing masker intensities from medium to high

levels caused no further increase of threshold shift (Fig. 6b; post-hoc LSD pairwise comparisons, $p = 0.741$). Conversely, we found no significant difference of probe-evoked response changes across masker intensities (Fig. 6c; repeated measures ANOVA, $df = 2$, $F = 2.153$, $p = 0.134$).

The effect of masker frequency on the facilitated SPON responses was assessed in 14 units. Probe RLFs of a representative unit determined at three masker frequencies are shown in Figure 6d. This unit showed a reduced threshold and an increased response magnitude to the probe across all probe intensities tested. The probe threshold of this unit was reduced by 8 dB when the masker frequency was set at the unit's CF, 10 kHz. The same threshold shifts were also seen when the masker frequencies were set at below and above the CF of the unit, in this case 9 kHz and 11 kHz (See the inset in Fig. 6d). Varying the masker frequency had a similar effect on the response magnitude. The relative response change was 61% when the masker was presented at 10 kHz, and 57% and 59% when the masker was set at 9 kHz and 11 kHz, respectively. Figure 6, panels e and f illustrate the probe threshold shift and the relative response change determined in various masker frequency conditions for each SPON unit studied. For some units in our sample, the CF masker caused slightly stronger effects on the probe threshold ($n = 10$) and SPON responses ($n = 8$) than the off-CF maskers did. Conversely, for the other units, the off-CF maskers caused slightly stronger effects on the probe threshold ($n = 4$) and the SPON responses ($n = 6$) than the CF masker did.

The effect of masker-to-probe delay on the facilitated responses to the forward masking paradigm was assessed in 15 units. Probe RLFs of a representative unit determined at various masker-to-probe delays are shown in Figure 6g. In the unmasked condition, this unit displayed an RLF with a comparatively high threshold, as well as a comparatively low spike rate. When the masker was presented with a 310 ms masker-to-probe delay, the threshold was reduced by 11 dB and the response magnitude increased by 24% across all probe intensities tested. As the masker-to-probe delay shortened, probe thresholds gradually decreased and response magnitude gradually increased. With a 0 ms masker-to-probe delay, the threshold was decreased by 22 dB and response magnitude increased by 276%. The probe threshold shift and relative response change as a function of masker-to-probe delay were plotted for all units studied, and are shown in Figure 6h and i. While the threshold shifts of most individual units show a linear recovery with log time towards the baseline, some units show a different recovery kinetic that was also reflected in the average (Fig. 6h). With the changes of response magnitude, a linear in log time recovery occurred only in a subset of individual units and was not reflected in the average response magnitude function. Instead, the remainder of the units showed a minimal change in response magnitude at masker-to-probe delays up to 150 ms (Fig. 6g). We fitted exponential decay functions to our threshold shift and response change data and found R^2 -values of 0.5758 and 0.1964, respectively, indicating that in units with facilitated responses the recovery from forward masking as the masker-to-probe interval was lengthened was not well described by exponential decay functions.

Discussion

SPON responses in the forward masking paradigm: summary of results

To better understand the function of the MNTB/SPON circuit in generating forward masking in the inferior colliculus, we have undertaken a series of studies to observe the responses of MNTB and SPON neurons to a forward masking paradigm. The present study is, to our knowledge, the first to examine offset response properties of SPON units in this context. We found that SPON neurons produce clearly separate offset responses to both masker and probe stimuli. Presumably, this separation of responses to masker and probe is induced by a glycinergic inhibitory input from the MNTB in response to the probe stimulus. SPON responses to the forward masking paradigm fell into two distinct groups, namely inhibited and facilitated responses. Inhibited responses were characterized by elevated response thresholds and/or decreased response magnitudes when a default forward masker was presented. Conversely, facilitated responses were characterized by decreased response thresholds and increased response magnitudes when a default masker was presented. Across the population of SPON neurons from the two groups, we found that probe threshold shifts under default masker conditions were significantly correlated with the units' probe thresholds (Fig. 4c), and both the probe threshold shifts (Fig. 4e) and relative response changes (Fig. 4f) were significantly correlated with the change of the SPON response latency to the probe under default masker conditions.

We assessed the dependence of both response types on masker level, masker frequency and masker-to-probe delay. Inhibited responses showed a significant dependence on masker level, with medium and high intensity maskers producing greater threshold elevations and reductions of response magnitude than low intensity maskers (Fig. 5b, c). Increasing the masker level from medium to high intensity induced no further threshold shifts or response magnitude changes. The shortest masker-to-probe delays produced the greatest elevation of probe threshold as well as the greatest reduction of response magnitude (Fig. 5h, i). Both probe threshold and response magnitude recovered to near baseline levels in a linear fashion with log time, evidenced by a good fit to an exponential decay function, as masker-to-probe intervals were lengthened (insets, Fig. 5h, i). Facilitated responses were also significantly dependent on masker level, with medium and high intensity maskers resulting in greater threshold decreases and greater increases of response magnitude than low intensity maskers, and high intensity maskers producing no further threshold shifts or response magnitude changes than medium intensity maskers (Fig. 6b, c). For facilitated responses to the forward masking paradigm, 0 ms masker-to-probe delays produced the greatest threshold decrease and the greatest increase of response magnitude. Both measures recovered to near baseline levels as masker-to-probe intervals were lengthened. However, we observed a linear recovery with log time only in the probe threshold (Fig. 6h), whereas the average response magnitude changes showed a minimum at 150 ms masker-to-probe intervals (Fig. 6i). Fitting our probe threshold and response change values to an exponential decay function yielded substantially lower R^2 -values than those seen in the case of inhibited units, indicating that with facilitated responses the recovery to baseline conditions, as the masker-to-probe interval was lengthened, did not follow an exponential decay function. In summary,

manipulation of masker level and masker-to-probe delay had opposite effects on inhibited and facilitated responses.

As demonstrated in previous studies (auditory cortex: Brosch and Schreiner 1997; inferior colliculus: Nelson et al. 2009; MNTB: Gao and Berrebi 2015), masker frequency may play an important role in shaping auditory responses to probe stimuli in a forward masking paradigm. In these previous studies, CF-maskers usually caused the strongest inhibition on auditory responses and this inhibition declined as the masker's frequency was shifted away from the unit's CF. In this study, however, we found the effects of the masker frequency on SPON responses were complex, and did not follow the same pattern as in other auditory nuclei.

Potential neural mechanism of forward inhibition and facilitation

SPON offset responses are attributed to a post-inhibitory rebound mechanism (Kulesza and Berrebi 2003, Kulesza et al. 2007, Kadner et al. 2008; Felix et al. 2011; Kopp-Scheinflug et al. 2011). This rebound mechanism is driven by the MNTB offset response, defined as a period of suppressed neural activity following the stimulus offset. The MNTB offset response presumably occurs simultaneously in large populations of MNTB neurons and has the effect of releasing their synaptic targets in the SPON from glycinergic inhibition, which in turn triggers the SPON offset response (Kadner and Berrebi 2008). Our previous study of MNTB responses to the forward masking paradigm demonstrated that the magnitude of the onset and sustained components of the MNTB response are diminished in the presence of a forward masker (Gao and Berrebi 2015). Therefore, the release from inhibition triggering the SPON offset response will also be diminished, leading to a smaller offset response and to an increased probe threshold. This mechanism, whereby forward masking is passed on from the MNTB to the SPON, may partially explain the inhibited, but not the facilitated responses to the forward masking paradigm observed in the present study.

One putative mechanism explaining the opposite behavior of SPON units with inhibited and facilitated responses to the forward masking paradigm lies in the interaction of glycinergic and GABAergic inhibitory inputs. SPON neurons receive glycinergic inhibitory inputs from the MNTB and their own neurotransmitter is GABA. In addition, SPON neurons receive GABAergic inhibitory inputs from the tectal longitudinal column (TLC) (Viñuela et al. 2011) and give rise to extensive axonal collaterals that project to targets within the SPON (Kulesza and Berrebi 2000; Saldaña and Berrebi 2000). Moreover, MNTB-derived glycinergic inhibition and TLC- or SPON-derived GABA-ergic inhibition within the SPON act on different timescales. TLC-derived GABAergic inhibition arrives at a much longer latency than glycinergic inhibition from the MNTB (Marshall et al. 2008), and while inhibitory postsynaptic potentials mediated by glycinergic receptors have decay times of 4.5–7.5 ms (Singer et al. 1998; Russier et al. 2002), GABA_A receptors produce inhibitory postsynaptic potentials with decay times between 15.8 and 54.8 ms, depending on the receptor's subunit composition (Nusser et al. 1999; Russier et al. 2002; Bacci et al. 2003). Due to the short decay times of glycinergic inhibitory inputs, SPON neurons can produce offset responses that closely follow the release from glycinergic inhibition. Conversely, the longer-acting GABA-ergic inhibition is a candidate mechanism for mediating the both

inhibited and facilitated responses to the forward masking paradigm. Inhibited responses to the forward masking paradigm may be mediated by GABA-ergic inhibition occurring after the offset of the probe stimulus. Such persisting GABA-ergic inhibition would then mask the release from glycinergic inhibition which occurs at the stimulus offset, so that the SPON neuron would remain inhibited and unable to produce spikes. This would result in an elevation of probe thresholds and a reduction of response magnitudes. In contrast, facilitated responses may be mediated by a release from GABA-ergic inhibition occurring during the period of the probe stimulus that may coincide with the release from glycinergic inhibition at the end of the probe stimulus. This release from combined inhibition may produce a stronger effect than the release from glycinergic inhibition alone, and as a consequence the response magnitude may be increased above baseline levels and the probe threshold lowered.

SPON neurons may receive GABA-ergic collateral inhibitory inputs from multiple sources within the SPON. All these GABAergic inputs are triggered by the offset of the masker stimulus, but their arrival times may vary. As a consequence, the inhibited or facilitated responses of an SPON unit in response to the probe in the presence of a masker stimulus may occur over a range of masker-to-probe delays, and recover to baseline gradually as the masker-to-probe delay is lengthened. It is likely that most GABAergic inhibitory inputs arrive shortly after the cessation of the masker. Thus, the strongest forward suppression and facilitation should be observed at the 0 ms masker-to-probe delay for both inhibited and facilitated responses in the SPON.

The balance of excitatory and inhibitory inputs to the SPON provides another putative mechanism for inhibited and facilitated responses. The SPON receives excitatory input from the contralateral ventral cochlear nucleus, mainly from octopus and multipolar cells (Warr 1966; Friauf and Ostwald 1988; Saldaña et al. 1994; Schofield 1995). The timing of this input makes it a candidate mechanism for driving the onset response components in on-offset SPON units (Felix et al. 2013). We found that on-offset units most often produce inhibited responses to the forward masking paradigm, whereas facilitated responses predominate in offset units. In on-offset units excitatory inputs may counterbalance the GABAergic inputs generated by the masker offset, and diminish the post-inhibitory rebound occurring at the onset of the probe stimulus. It is also notable that in on-offset units the onset response component was abolished at probe intensities that could still evoke the offset response, indicating that forward masking acts differently on the on- and offset response components. Conversely, offset responding units may not receive similarly strong excitation, so that the sum of MNTB-derived glycinergic and SPON-derived GABA-ergic inhibition outweighs the excitatory input and combines to produce a stronger post-inhibitory rebound than observed in baseline conditions.

In summary, SPON responses to the forward masking paradigm appear to be determined by the balance of excitatory and inhibitory inputs, their relative timing, as well as intrinsic tuning properties of SPON neurons. These factors combine to produce either inhibited or facilitated responses to the forward masking paradigm, and lead to a situation where the average forward masking of SPON responses is less pronounced than previously found in the MNTB (Gao and Berrebi 2015).

The role of the SPON in forward masking

Forward masking has been extensively studied in many parts of the ascending auditory pathway, using a wide variety of animal preparations including the auditory nerve of the anesthetized chinchilla (Relkin and Turner 1988), the MNTB of the anesthetized rat (Gao and Berrebi 2015), the IC of awake marmosets (Nelson et al. 2009) and the auditory cortex of the anesthetized cat (Reale and Brugge 2000; Zhang et al. 2005, 2009; Nakamoto et al. 2006). As discussed in detail in our previous report of forward masking properties of the MNTB (Gao and Berrebi, 2015), forward masking, as quantified by elevation of probe thresholds, increases along the ascending auditory pathway until threshold shifts mirroring those found in psychophysical experiments are reached in the IC. In the awake marmoset preparation, Nelson et al. (2009) proposed that SPON-derived offset inhibition may play a critical role in mediating forward masking in the IC. A recent study in our laboratory (Felix et al. 2014) explored how the SPON contributes to gap detection and the processing of sinusoidally amplitude modulated (SAM) stimuli in the IC, and found that inactivation of the SPON significantly increased gap detection thresholds and decreased synchronization to SAM stimuli in IC units producing sustained responses. Thus, the main function of SPON-derived inhibition in the IC appears to be inducing a temporal segmentation of IC responses. In this context it is interesting that the present study found responses from the SPON are not themselves consistently subject to forward masking and that forward facilitation occurs in a large subset of SPON units.

The existence of inhibited and facilitated SPON responses to the forward masking paradigm may impact auditory processing in a number of different ways. Facilitated responses may ensure that even a suppressed response to a probe can be clearly segregated from a subsequent stimulus, whereas inhibited responses contribute only a weak inhibitory input and may enable the processing of stimuli with presently unknown characteristics. Alternatively, inhibited and facilitated SPON responses may not have separate functions, and neurons producing either type of response may project to the same synaptic targets. If so, the facilitated and inhibited responses may balance and produce an average SPON output that is relatively unaffected by forward masking, and can ensure temporal segregation of masked from subsequent, unmasked responses. Forward masking information extracted by the MNTB/SPON circuit may also reach the auditory forebrain, as evidenced by a direct projection from SPON to the medial geniculate body of the thalamus (Sloan et al. 2011; Berrebi et al. 2012).

Conclusion

In the present study, we reveal the SPON responses to a forward masking paradigm that is commonly used to simulate complex acoustic environments containing multiple sounds. The neural computation of forward masking in the SPON doesn't follow the features observed in previously studied auditory nuclei, including the auditory nerve, MNTB and IC. Instead, SPON neurons show either forward inhibition or forward facilitation. The neural mechanisms underlying both response types may involve the relative strength and timing of glycinergic and GABA-ergic inhibition of SPON units, as well their excitatory inputs. The functional relevance of producing forward facilitated responses may lie in ensuring that IC

neurons can temporally segregate even masked responses from subsequent stronger responses.

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Fig. 1.

(a) Forward masking paradigm and (b) Responses of a typical SPON unit (CF = 31 kHz; threshold = 45 dB SPL) to a default stimulus (5 repetitions). Horizontal black bars represent the masker (200 ms CF tone at 40 dB above threshold) and probe stimuli (20 ms CF tone at 86 dB SPL). Gray shaded areas indicate the analysis windows used to quantify responses to the stimuli, and to measure spontaneous activity.

Fig. 2.

Extracellular deposit of biotinylated dextran amine shows the localization of an SPON recording site. The recorded neuron was located around 10 mm caudal, 1.2mm lateral and 10.4 mm ventral of bregma and its CF was 4 kHz. D, dorsal; M, medial; LSO, lateral superior olive; MSO, medial superior olive; MNTB, medial nucleus of the trapezoid body.

Fig. 3.

a–c Example of inhibited SPON responses to the probe under the default masker condition (200 ms, 26 kHz pure tone at 20 dB above the unit's threshold, with a 10 ms masker-to-probe delay), shown as a spike time raster plot (**a**), a PSTH (**b**) and a rate-level function (**c**). **d–f** Example of facilitated SPON responses to the probe under the default masker condition (200 ms, 8.6 kHz pure tone at 40 dB above the unit's threshold, with a 10 ms masker-to-probe delay), shown as a spike time raster plot (**d**), a PSTH (**e**) and a rate-level function (**f**). Th: threshold of the studied unit.

Fig. 4.

Probe threshold shifts (**a, c, e**) and relative response change (**b, d, f**) under default masker conditions were plotted over CF (**a, b**), probe threshold (**c, d**) and the change of first-spike latency (**e, f**) for all 57 SPON neurons. Small/large symbols denote default masker intensities of ~20 dB/~40 dB above threshold, respectively. Positive threshold shifts and negative response changes (round symbols) indicate inhibited SPON responses; negative threshold shifts and positive response changes (square symbols) indicate facilitated SPON responses. Positive latency changes indicate that the latencies of probe responses were

lengthened by the default maskers, and negative latency changes indicate the latencies of probe responses were shortened. n: the number of SPON neurons with inhibited or facilitated responses.

Fig. 5.

Effects of masker level, masker frequency and masker-to-probe delay on units with inhibited responses to the forward masking paradigm. Left panels show examples of the effects of varying masker levels (**a**), masker frequencies (**d**) and masker-to-probe delays (**g**) on the responses of three typical SPON units. Center and right panels summarize the effects of masker level (**b**, **c**), masker frequency (**e**, **f**) and masker-to-probe delay (**h**, **i**) on probe threshold shift and the relative response change across our sample. Th: threshold of the studied unit. n: the number of neurons studied. *: repeated measures ANOVA, $p < 0.05$. Inset in panel **d** shows a partial RM, determined at 40 dB above threshold. oct: octaves. Inset in panels **h** and **i**: The recovery of the threshold (**h**) and total spikes (**i**) to the baseline value followed a linear decay in log time with increasing masker-to-probe delays. Center and right

panels: Open symbols represent individual units, closed symbols with error bars represent the group average $\pm$ SEM.

Fig. 6.

Effects of masker level, masker frequency and masker-to-probe delay on units with facilitated responses to the forward masking paradigm. Left panels show the examples of the effects of varying masker levels (**a**), masker frequencies (**d**) and masker-to-probe delays (**g**) on the responses of three typical SPON units with facilitated responses. Center and right panels summarize the effects of masker level (**b**, **c**), masker frequency (**e**, **f**) and masker-to-probe delay (**h**, **i**) on probe threshold shift and relative response change across our sample. Center and right panels: Open symbols represent individual units, closed symbols with error bars represent the group average $\pm$ SEM. Th: threshold of the studied unit. n: the number of neurons studied. Negative threshold shift indicates the probe threshold was decreased and positive response change indicates the response magnitude was increased. *: repeated

measures ANOVA, $p < 0.05$. Inset in panel **d** shows a partial RM, determined at 40 dB above threshold. oct: octaves. Inset in **h** and **i**: The recovery of the threshold to the baseline value followed a linear decay in log time with increasing masker-to-probe delays (**h**) and the recovery of the response magnitude followed a nonmonotonic kinetic with a minimum at 150 ms masker-to-probe delay (**i**).

Table 1

Summary of offset response properties for SPON neurons displaying inhibited or facilitated responses under default masker conditions.

Parameter	Neuron Groups	N	Mean±S.E.M.	Min	Max	Median	25%	75%	Sig.
CF (kHz)	Inhibited	33	15.3 ± 1.1	4.0	31.0	15.0	10.0	19.0	p=0.104
	Facilitated	24	12.5 ± 1.0	2.4	18.0	14.2	9.2	16.6	
Probe threshold (dB SPL)	Inhibited	33	49.7 ± 2.4	16.5	78.5	50.5	41.0	60.5	p=0.518
	Facilitated	24	52.3 ± 4.8	11.5	93.0	54.0	36.0	73.6	
1 st spike latency (ms)	Inhibited	33	6.5 ± 0.2	5.0	9.2	6.4	5.7	7.2	p=0.185
	Facilitated	24	7.1 ± 0.4	5.2	13.7	6.7	6.1	7.7	
Spont. Rates (sp/ms)	Inhibited	33	1.8 ± 0.5	0.0	13.2	0.8	0.2	2.8	p=0.404
	Facilitated	24	2.2 ± 0.6	0.0	12.0	1.5	0.3	3.1	
Spike count/Stim.	Inhibited	33	1.6 ± 0.2	0.3	6.5	1.4	1.0	2.1	p=0.865
	Facilitated	24	1.5 ± 0.2	0.6	4.4	1.2	1.0	2.1	
Q ₂₀	Inhibited	16	4.3 ± 0.78	1.4	12.7	6.0	3.8	8.6	p=0.052
	Facilitated	14	6.0 ± 0.82	3.2	9.5	3.2	2.0	4.7	

The Mann-Whitney Rank Sum test was used to compare the differences in response properties between inhibited and facilitated neurons. Differences were considered significant if $p < 0.05$.