Distinct Roles for Glycine and GABA in Shaping the Response Properties of Neurons in the Superior Paraolivary Nucleus of the Rat

Randy J. Kulesza, Jr,[1,2] Alexander Kadner,[1] and Albert S. Berrebi[1]

[1]*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; and* [2]*Auditory Research Center, Lake Erie College of Osteopathic Medicine, Erie, Pennsylvania*

Kulesza RJ Jr, Kadner A, Berrebi AS. Distinct roles for glycine and GABA in shaping the response properties of neurons in the superior paraolivary nucleus of the rat. *J Neurophysiol* 97: 1610–1620, 2007. First published November 22, 2006; doi:10.1152/jn.00613.2006. The superior paraolivary nucleus (SPON) is a prominent periolivary cell group of the superior olivary complex. SPON neurons use γ-aminobutyric acid (GABA) as their neurotransmitter and are contacted by large numbers of glycinergic and GABAergic punctate profiles, representing a dense inhibitory innervation from the medial nucleus of the trapezoid body (MNTB) and from collaterals of SPON axons, respectively. SPON neurons have low rates of spontaneous activity, respond preferentially to the offset of pure tones, and phase-lock to amplitude-modulated tones. To determine the roles of glycine and GABA in shaping SPON responses, we recorded from single units in the SPON of anesthetized rats before, during, and after application of the glycine receptor antagonist strychnine, the GABA$_A$ receptor antagonist bicuculline, or both drugs applied simultaneously. Strychnine caused a major increase in spike counts during the stimulus presentation, followed by the disappearance of offset spikes. In half of the recorded units, bicuculline caused moderately increased firing during the stimulus. However, in 86% of units bicuculline also caused a large increase in the magnitude of the offset response. Application of the drug cocktail caused increased spontaneous activity, dramatically increased spike counts during the stimulus presentation, and eliminated the offset response in most units. We conclude that glycinergic inhibition from the MNTB suppresses SPON spiking during sound stimulation and is essential in generating offset responses. GABAergic inhibition, presumably from intrinsic SPON collaterals, plays a subtler role, contributing in some cells to suppression of firing during the stimulus and in most cells to restrict firing after stimulus offset.

INTRODUCTION

The superior paraolivary nucleus (SPON) is a prominent GABAergic cell group of the superior olivary complex (SOC), a constellation of brain stem nuclei involved in auditory processing. In rats, the SPON contains a morphologically homogeneous population of roughly 2,400 large multipolar neurons that project to the ipsilateral inferior colliculus (Kelly et al. 1998; Kulesza Jr and Berrebi 2000; Saldaña and Berrebi 2000). Ascending inputs to the SPON arise from the contralateral cochlear nucleus, specifically from octopus and multipolar/stellate cells (Banks and Smith 1992; Friauf and Ostwald 1988; Kuwabara and Zook 1991; Schofield and Cant 1995; Thompson and Thompson 1991).

Neurochemical assays, tract-tracing studies, and immuno-histological experiments demonstrated that SPON neurons receive abundant inhibitory inputs. In fact, measurements of amino acid concentrations in the SPON of rats indicate that this nucleus contains higher levels of γ-aminobutyric acid (GABA) and glycine than any other SOC cell group (Godfrey et al. 2000). Moreover, light microscopy studies in rats and guinea pigs reveal that SPON cell bodies and dendrites are densely innervated by glycine-immunoreactive punctate profiles (Kulesza Jr and Berrebi 2000) that presumably represent synaptic inputs originating from the ipsilateral medial nucleus of the trapezoid body (MNTB; Banks and Smith 1992; Schofield, 1994. Sommer et al. 1993) and by GABAergic boutons that derive, at least in part, from axonal collaterals of SPON neurons (Kulesza Jr and Berrebi 2000). Furthermore, preliminary electron microscopic observations of the rat SPON indicate that two thirds of inhibitory synapses are strategically located on the somata and proximal dendrites of SPON neurons, whereas boutons with excitatory morphology are mainly distributed on their distal dendritic branches (Holt and Berrebi 1999). Taken together, these studies suggest a profound modulation of SPON activity by both glycinergic and GABAergic synapses.

The response properties of SPON neurons were previously studied in gerbils (Behrend et al. 2002; Dehmel et al. 2002), cats (Guinan Jr et al. 1972b), and rats (Finlayson and Adam 1997; Kulesza Jr et al. 2003a). Whereas earlier studies reported binaural inputs to the nucleus and a variety of SPON response types, our recent findings in the rat present a different picture (Kulesza Jr et al. 2003a). Based on single-unit extracellular recordings, SPON neurons were shown to have very low rates of spontaneous activity and respond only to contralateral stimulation. Moreover, SPON cells fire action potentials (APs) at the offset of pure-tone and broadband noise stimuli, and phase-lock to sinusoidally amplitude modulated tones. These offset responses are usually transient, often with neurons firing a single AP per stimulus. Some SPON neurons, termed offset-choppers, display a brief train of two or more well-timed spikes, whereas yet another subpopulation of units display longer-lasting (>20 ms) spike bursts, termed offset-sustained responses. We also demonstrated that the timing of SPON APs coincides with the suppression of spontaneous activity in MNTB neurons and suggested that these two nuclei, working in concert, may form a brain stem circuit capable of encoding

the duration of a sound stimulus (Kadner et al. 2006). Based on these observations, we hypothesized that SPON offset spikes are triggered by a rebound from glycinergic inhibition originating in the MNTB and, furthermore, that GABA, released from the intrinsic collaterals of SPON axons, serves to modulate SPON responses primarily after the stimulus offset.

To test these hypotheses, we recorded pure-tone responses of rat SPON neurons before, during, and after iontophoretic application of the glycine receptor antagonist strychnine (Curtis et al. 1971a), the GABA$_A$ receptor antagonist bicuculline (Curtis et al. 1971b), or the two drugs in combination. Portions of these results were previously reported in abstract form (Kulesza Jr et al. 2003b).

METHODS

Animals and surgery

Thirty-five female Sprague–Dawley albino rats (Hilltop Lab Animals, Scottsdale, PA) weighing between 230 and 300 g were used for this study. Animals were housed in the vivarium at the West Virginia University Health Sciences Center, an AAALAC-approved animal facility. All procedures were approved 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.

Animals were anesthetized by intramuscular injection of a mixture of xylazine and ketamine (8.6 and 57 mg/kg body weight, respectively). Once determined to be completely areflexic, the rats' pinnae were removed bilaterally. Animals were then mounted in a stereotaxic instrument and their heads secured with custom-made hollow brass earbars inserted into the external auditory meatus. A midline incision was made in the scalp to expose the skull and a craniotomy (about 4 mm rostrocaudal × 4 mm mediolateral) performed such that the rostral edge of the bone defect extended to the posterior aspect of the transverse sinus. The meninges were then incised and the underlying cerebellum aspirated to expose the floor of the fourth ventricle, whose midline was used as a landmark for electrode penetrations. The anesthetic state of the animal was monitored throughout the experiment and supplemental injections of the same anesthetics were given at two fifths the original dose, as needed.

Microelectrodes

All recordings were made with "piggyback" electrode configurations (Havey and Caspary 1980). The recording electrodes were pulled from single-barrel glass micropipettes with tips broken back to an outside diameter of 2.5 μm (10–20 MΩ). Five-barrel pipettes [World Precision Instruments (WPI), Sarasota, FL] were pulled and broken back to a total outside tip diameter of 10–20 μm. Recording electrodes were glued onto the five-barrel pipettes so that they extended about 10 μm past the tip of the five-barrel pipettes. Recording pipettes were filled with a solution of 3 M KCl with 2.5% biocytin (Sigma Chemical, St. Louis, MO). We used strychnine (Sigma), an antagonist of the glycine receptor, to block glycinergic synapses and bicuculline (Sigma) to block GABAergic transmission through the GABA$_A$ receptor. Both drugs were delivered at a concentration of 10 mM and dissolved in 0.165 M NaCl (pH 3), using a constant current source (Microiontophoresis Dual Current Generator Model 260; WPI). A retaining current of −15 nA was applied to prevent leakage of drug from the electrode. Strychnine was delivered with positive currents ranging from 20 to 36 nA, whereas bicuculline was delivered with positive currents ranging from 15 to 25 nA. To block glycine and GABA$_A$ receptors simultaneously, strychnine and bicuculline were combined (at 10 mM each) into a drug "cocktail" and delivered with positive currents ranging from 30 to 40 nA. Another barrel of the five-barrel pipette was filled with 0.9% NaCl and used as a ground channel.

Experimental procedures and data analysis

All recordings were performed within a sound-attenuated booth. Recording electrodes were advanced remotely with a Burleigh Inchworm (Burleigh Instruments, Victor, NY). The electrode signal was amplified, passed through a spike conditioner, digitized, and sent to an RP2 real-time processing unit [Tucker-Davis Technologies (TDT), Alachua, FL].

Stimuli were created digitally using a TDT System 3 Virtual Design Studio and converted to analog signals with an RP2 real-time processor (TDT), sent to a programmable switch attenuator, and then fed into a weighted summer. Sound intensities were controlled by separate left- and right-channel attenuators. Stimuli were then amplified and presented through custom-built Stax speakers mounted onto the hollow brass earbars (Sokolich 1977). The earbars were machined with a small calibration tube that joined the sound delivery tube at a 45° angle. Before or after each experiment, a Brüel & Kjær microphone (Brüel & Kjær North America, Norcross, GA) was placed into this tube and the sound delivery system calibrated for pure tones between 1 and 40 kHz. Stimulus intensities were converted to dB SPL off-line.

Broadband noise bursts, 50 ms in duration and containing frequencies from 20 Hz to 61 kHz, were used to search for single units. When an isolated single unit was found, pure tones were presented. The total duration of the tone stimuli was 55 ms, including 5-ms cosine^2 ramps; the repetition rate was four per second. Each recording trace was 120 ms long, with stimulus presentation commencing after a 10-ms delay. Each unit's characteristic frequency (CF), defined as the frequency to which the unit responded at the lowest sound intensity, and threshold at CF (with 1-dB precision) were determined.

For all subsequent recordings 100 repetitions of a CF tone, 20 dB above threshold, were presented to establish a baseline response before drug application. Drugs were then delivered by iontophoresis and responses to 100 pure-tone presentations were observed every 2–5 min until the spike counts remained unchanged for at least three trials, signaling the maximal drug effect. Once a unit's response reached this plateau, 100 stimulus presentations were used to characterize its response properties in the presence of strychnine, bicuculline, or the drug cocktail. The drug-retaining current was then switched on again and the neuron was allowed to recover. If a unit was not held until recovery was complete, we waited ≥30 min before searching for another cell to avoid recording baseline data from a neuron influenced by carryover drug effects.

Spike counts from recordings in the baseline, drug, and recovery conditions were used to construct peristimulus time histograms (PSTHs). For quantitative analysis of spike data we segregated the 120-ms recording traces into three time windows. The first time window preceded the stimulus presentation, spanning from 0 to 10 ms after the start of the recording, and was used to assess spontaneous activity. The second time window, hereafter the "stimulus window," coincided with the stimulus presentation (from 10 to 65 ms after the start of the recording). In baseline conditions the earliest spikes that could be attributed to the offset response occurred 72 ms after the start of the recording. Spikes occurring in the interval 65–72 ms after the start of the recording could neither be attributed with certainty to any excitatory response component uncovered in the drug conditions nor to the offset response and were therefore not included in the quantitative analyses. To include the entire baseline offset response across the population of recorded neurons, the third "poststimulus window" began at 72 ms and continued for the remainder of the recording. For quantitative analyses, spike counts from the spontaneous activity (0–10 ms), stimulus (10–65 ms), and poststimulus (72–120 ms) time windows were calculated, as were median first-spike latencies in the stimulus and poststimulus windows. Latencies in the stimulus window were measured from stimulus onset; latencies in the poststimulus

window were measured from the stimulus offset. Examination of data from our entire population of units revealed that the distributions of spike count values and latencies were asymmetric and that variances were unequal between the baseline and drug conditions. Because nonparametric tests do not assume normal distribution or equal variances, we used the Wilcoxon matched-pairs signed-ranks test to compare, on a cell-by-cell basis, baseline spike counts with spike counts occurring during drug application. Paired values were not always available for the comparison of spike times and thus the Mann–Whitney U test was used for statistical analyses of latency data.

Recording sites were marked with small deposits of biocytin, animals were perfused, and brain stem tissue sections were prepared as previously described (Kadner et al. 2006; Kulesza Jr et al. 2003a).

RESULTS

Localization of recording sites and controls

Biocytin deposits were used to confirm that all neurons presented here were located within the borders of the SPON, as defined in previous reports (Kulesza Jr and Berrebi 2000; Kulesza Jr et al. 2002; Saldaña and Berrebi 2000). To ensure that observed drug effects were not confounded by injection of current or pH changes in the vicinity of the unit, we recorded from neurons while injecting a 40-nA current through electrode barrels containing either physiological saline or the drug vehicle solution (0.165 M NaCl, pH 3). Each of these control experiments was performed on a small number of cells from separate animals; these units were not included in the drug experiments described below. Neither treatment had an effect on the number of spikes observed or the timing of their occurrence (Fig. 1).

Strychnine experiments

We recorded from 12 SPON neurons before, during, and after application of the glycine receptor antagonist strychnine (STRYCH). CFs of these units ranged from 2.4 to 23.5 kHz (Fig. 2) and baseline PSTHs showed that this sample contained all the major SPON offset response subtypes previously described (Kulesza Jr et al. 2003a). On average, the full effect of STRYCH was observed after 23 min of current application. Complete recovery data were collected for nine of the 12 units in this sample. Where observed, recovery from STRYCH application took an average of 53 min. Units in this sample were held for an average of 76 min.

Strychnine-induced changes in SPON response patterns, apparent from the PSTHs in Fig. 2, were assessed quantitatively by comparing spike counts in each recording window between the baseline and drug conditions. STRYCH application had no effect on spontaneous activity ($P = 0.953$, Wilcoxon signed-ranks test; Fig. 3, A and B). However, STRYCH caused a dramatic and statistically significant increase in spikes occurring within the stimulus window, from an average of 19.33 ± 3.48 baseline spikes to 124.00 ± 22.97 spikes during STRYCH application ($P < 0.01$, Wilcoxon signed-ranks test; Fig. 3, C and D). Moreover, spike counts in the poststimulus window fell significantly from an average of 127.33 ± 27.34 spikes in the baseline condition to 10.58 ± 2.42 spikes during STRYCH application ($P < 0.01$, Wilcoxon signed-ranks test; Fig. 3, E and F). In baseline conditions there was very little spiking activity during the stimulus presentation; therefore first-spike latencies were not calculated. However, a robust response in the stimulus window was present during strychnine application; here the median first spike latency averaged 14.6 ± 2.1 ms.

To gain a general impression of the responses of our entire sample of neurons, average PSTHs were constructed (Fig. 4). It was evident from these histograms that STRYCH transformed offset responders into units that responded during the stimulus presentation. Furthermore, the few action potentials occurring in the poststimulus window during STRYCH application represented a continuation of the response that started in the stimulus window, rather than an offset response triggered by the end of the stimulus (see also Fig. 2). Where recovery data were collected they invariably showed that the neurons returned to their baseline response patterns; two examples are shown in Fig. 5.

Bicuculline experiments

We recorded from 12 SPON neurons before, during, and after focal application of the GABA$_A$ receptor antagonist bicuculline (BIC). Baseline PSTHs showed that this sample also contained offset-transient, offset-chopper, and offset-sustained neurons, indicating that all the major offset response subtypes were represented in our data set. CFs of these units ranged from 2.4 to 40.0 kHz (Fig. 6). On average, the full effect of BIC was observed after 11 min of current application. Complete recovery data were obtained for eight of the 12 units in this sample. Where observed, recovery from BIC application
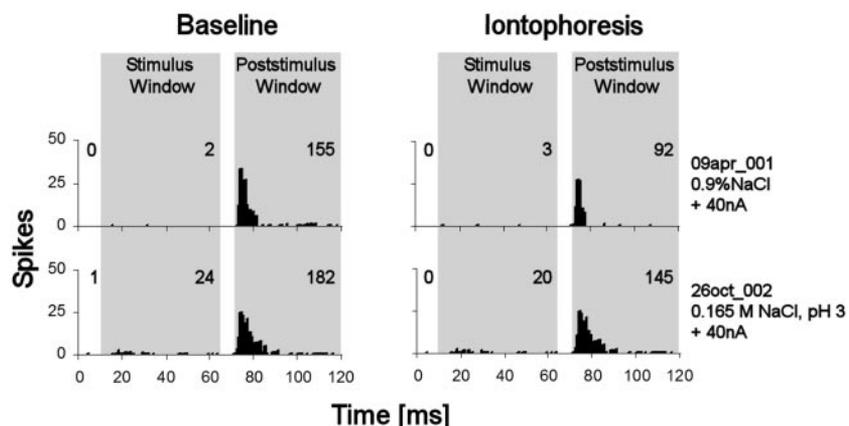

FIG. 1. Offset responses in superior paraolivary nucleus (SPON) neurons are unaffected by extracellular current injection or vehicle pH. To demonstrate a specific action of pharmacological manipulations, we recorded from SPON neurons while iontophoretically delivering physiological saline (*top*) or drug vehicle solution (*bottom*) with currents greater than those used for drug delivery. Neither current injection nor local pH change affected neuronal discharges. Numbers within each peristimulus time histogram (PSTH) indicate the spike count in the spontaneous, stimulus, and poststimulus analysis windows; the latter two windows are highlighted in gray shading. Presentation of the 55-ms pure-tone stimulus coincided with the stimulus window. Each unit's ID number and the injection parameters are given in the *right* margin.
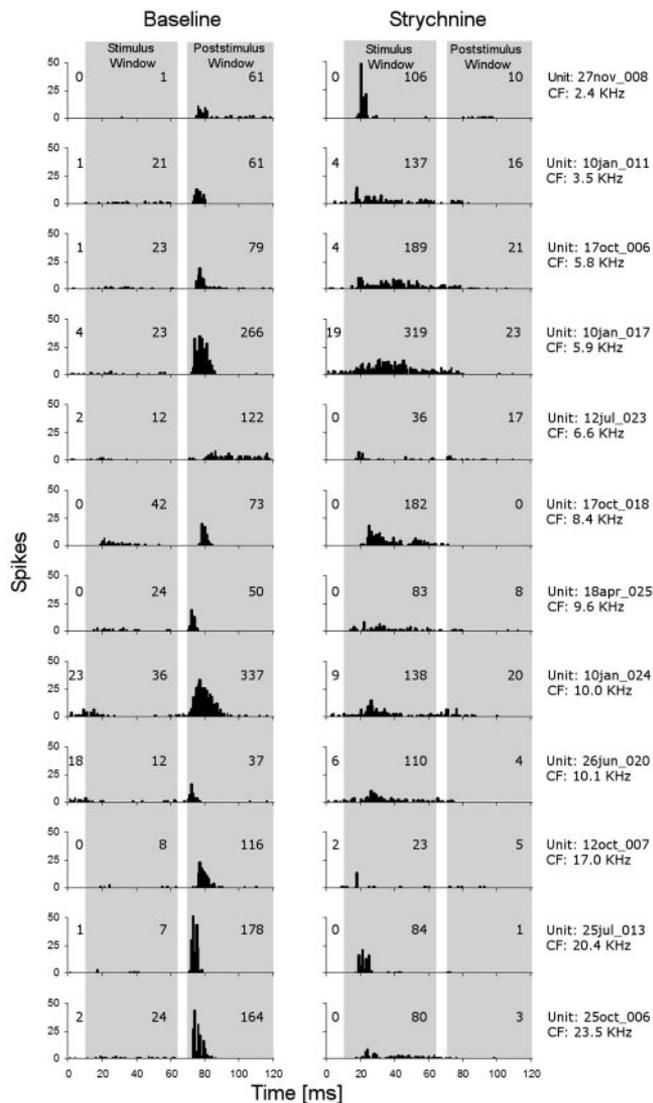
FIG. 2. Responses of strychnine-treated SPON units, sorted by characteristic frequency (CF). PSTHs recorded in the baseline condition are displayed on the *left*, whereas those recorded under strychnine treatment are presented on the *right* for each neuron. Numbers within each PSTH indicate the spike count in the spontaneous, stimulus, or poststimulus analysis windows; the latter 2 windows are highlighted in gray shading. Presentation of the 55-ms pure-tone stimulus coincided with the stimulus window. Each unit's ID number and CF are given in the *right* margin. Strychnine administration essentially abolished the offset response in all cells and, in some units, caused a transient response to appear during the stimulus presentation. In most cells, strychnine caused a sustained response during the stimulus presentation that continued after the stimulus offset. As discussed in the text, these late-occurring spikes were not considered offset responses.

took an average of 51 min. Units in this sample were held for an average of 65 min.

As in the STRYCH experiments, application of BIC had no effect on spontaneous activity ($P = 0.891$, Wilcoxon signed-ranks test; Fig. 3, *G* and *H*) but resulted in increased spike counts in the stimulus window, from an average of 10.0 ± 30.6 spikes in the baseline condition to 53.3 ± 25.9 spikes during BIC application ($P < 0.01$, Wilcoxon signed-ranks test; Fig. 3, *I* and *J*). Despite this statistically significant increase, close examination of the PSTHs revealed that for half the units spike counts in the stimulus window remained near zero in both conditions. BIC also caused an average 36% increase in post-

stimulus spikes, from a mean of 140.2 ± 19.0 spikes in the baseline condition to 191.3 ± 30.6 spikes during drug application (Fig. 3, *K* and *L*), although this difference did not quite reach statistical significance ($P = 0.06$, Wilcoxon signed-ranks test). There was very little spiking activity during the stimulus presentation in both baseline and bicuculline conditions and thus first-spike latencies are not reported. Within the post-stimulus window, the median first-spike latencies were 10.2 ± 0.6 ms after the stimulus offset in the baseline condition and 10.0 ± 0.7 ms after the stimulus offset in the BIC condition; this difference was not significant ($P = 0.887$, Mann–Whitney
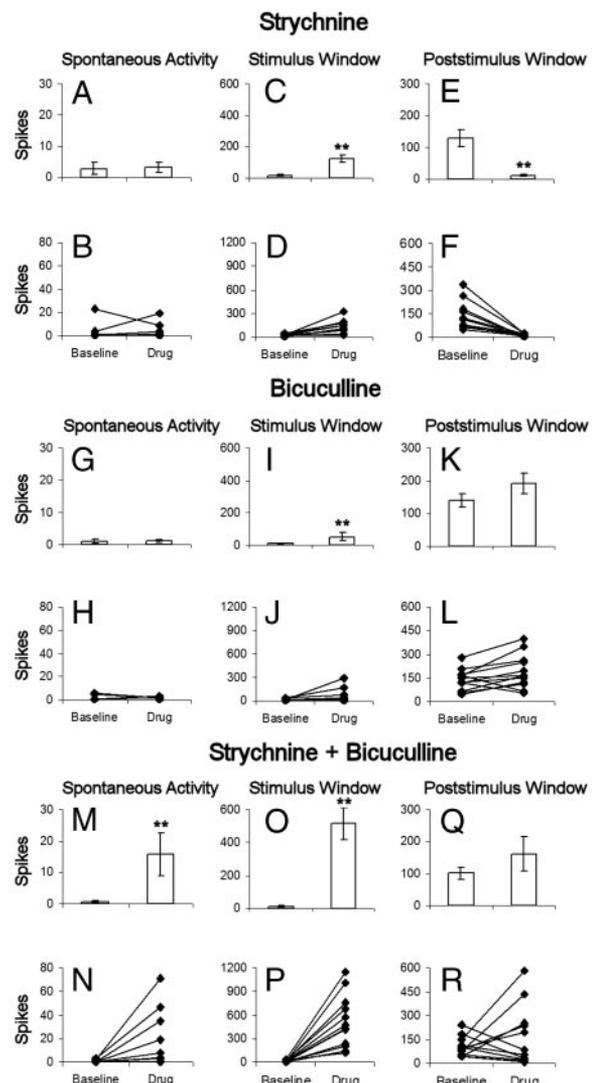

FIG. 3. Quantitative analyses of SPON spike counts before and during drug application. *Top*: strychnine results. *Middle*: bicuculline results. *Bottom*: strychnine + bicuculline results. Individual panels present the average spike counts recorded in the spontaneous (*left*), stimulus (*middle*), and poststimulus (*right*) time windows before and during strychnine treatment (*A*, *C*, *E*), bicuculline treatment (*G*, *I*, *K*), or treatment with the drug cocktail combining strychnine and bicuculline (*M*, *O*, *Q*). Corresponding spike counts for each unit in the strychnine experiments (*B*, *D*, *F*), bicuculline experiments (*H*, *J*, *L*), or the drug cocktail experiments (*N*, *P*, *R*) are also provided. Error bars represent standard error. Significance symbols: **$P < 0.01$, Wilcoxon signed-ranks test for paired samples. For *K*, which compares average spike counts in the poststimulus time window between baseline and bicuculline conditions, $P = 0.06$.
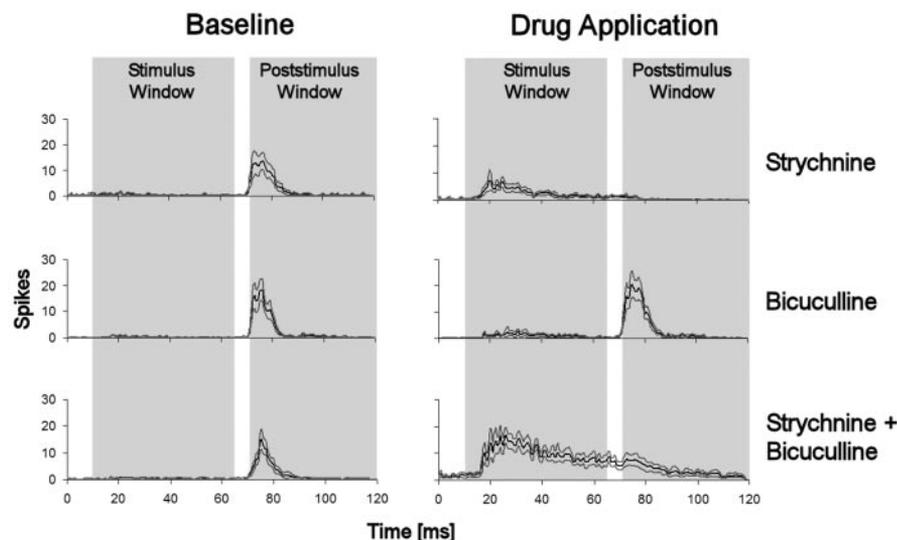
FIG. 4. Average PSTHs for the sample of recorded SPON units. Average PSTHs constructed for the sample of strychnine-treated units (*top*), bicuculline-treated units (*middle*), and units treated with the strychnine + bicuculline cocktail (*bottom*). Responses recorded in the baseline condition are displayed on the *left*, whereas those recorded during drug treatment are presented on the *right*. Black lines represent the mean cumulative spike counts; gray lines represent the mean cumulative spike counts ± SEs.

U test). When BIC administration ceased, the neurons returned to their baseline response patterns (Fig. 7).

To summarize, in half of the units sampled, BIC caused an increase in spiking activity during the stimulus presentation, and in 10 of 12 neurons (83%) BIC application resulted in a larger offset response (Figs. 3, *K* and *L* and 6). Interestingly, the BIC-induced increase in spiking during the stimulus presentation was most pronounced in those neurons that showed activity during the stimulus presentation under baseline conditions. It is also notable that two neurons (units 22jan_007 and 15jan_006 in Fig. 6) reacted to BIC in a manner remarkably different from that of the remaining cells in our sample, that is, by dramatically increasing their spike counts during the stimulus presentation followed by reducing their poststimulus spiking. Thus in these two cells, the response to BIC resembled the response of most SPON neurons to STRYCH. The remaining ten units in the sample showed a BIC-induced increase in poststimulus spiking that ranged from 12 to 160% (mean increase of 70%). Average PSTHs constructed for this sample of units showed, in both the baseline and BIC conditions, that

action potentials occurring in the poststimulus window clearly represented offset responses (Fig. 4).

Drug cocktail experiments

We recorded from 12 SPON neurons before, during, and after application of a drug "cocktail" composed of a mixture of STRYCH and BIC. CFs of these 12 units ranged from 3.8 to 22.9 kHz (Fig. 8). Baseline PSTHs showed that this sample contained all the major SPON offset response subtypes previously described (Kulesza Jr et al. 2003a), except offset-sustained responses (i.e., offset-transient, offset-chopper). On average, the full drug effect was observed after 24 min of current application. We held five of the 12 units in this sample long enough to observe complete recovery from the effects of the drug cocktail. When observed, recovery from the drug cocktail took an average of 43 min. Units in this sample were held for an average of 66 min.

Drug-cocktail–induced changes in SPON response patterns, apparent from the PSTHs in Fig. 8, were assessed quantitatively by comparing spike counts in each recording window for the baseline and drug conditions. Unlike the effects of either STRYCH or BIC applied in isolation, application of the drug cocktail caused a significant increase in the spontaneous activity of SPON units, from an average of 0.5 ± 0.3 spikes in the baseline condition to an average of 15.8 ± 6.7 spikes during application of drugs ($P < 0.01$, Wilcoxon signed-ranks test; Fig. 3, *M* and *N*). The drug cocktail also caused a significant, 50-fold increase in the spike counts within the stimulus window, from an average of 11.5 ± 3.3 baseline spikes to 516.6 ± 96.1 spikes during cocktail application ($P < 0.01$, Wilcoxon signed-ranks test; Fig. 3, *O* and *P*).

As in the STRYCH and BIC experiments, there was very little spiking activity during the stimulus presentation in baseline conditions and thus first-spike latency is not reported. However, a robust response in the stimulus window was present during application of the drug cocktail; here the first-spike latency was 13.6 ± 1.9 ms.

Interestingly, spike counts in the poststimulus window were not significantly altered in the drug condition, averaging 101 ± 18.1 spikes in the baseline condition compared with 161.2 ± 54.4 spikes during drug cocktail application ($P = 0.53$, Wil-
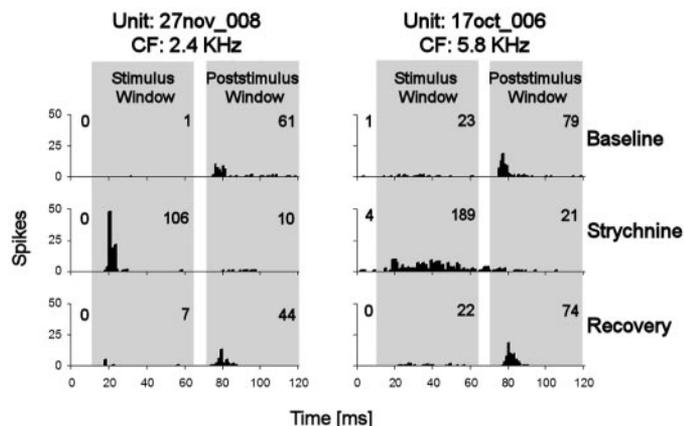

FIG. 5. Recovery of SPON responses after cessation of strychnine application. Responses are shown of 2 representative neurons for which full recovery data were obtained. Baseline, strychnine, and recovery PSTHs are displayed in the *top*, *middle*, and *bottom traces*, respectively. In all cases the response observed after recovery from strychnine was essentially similar to the baseline response.
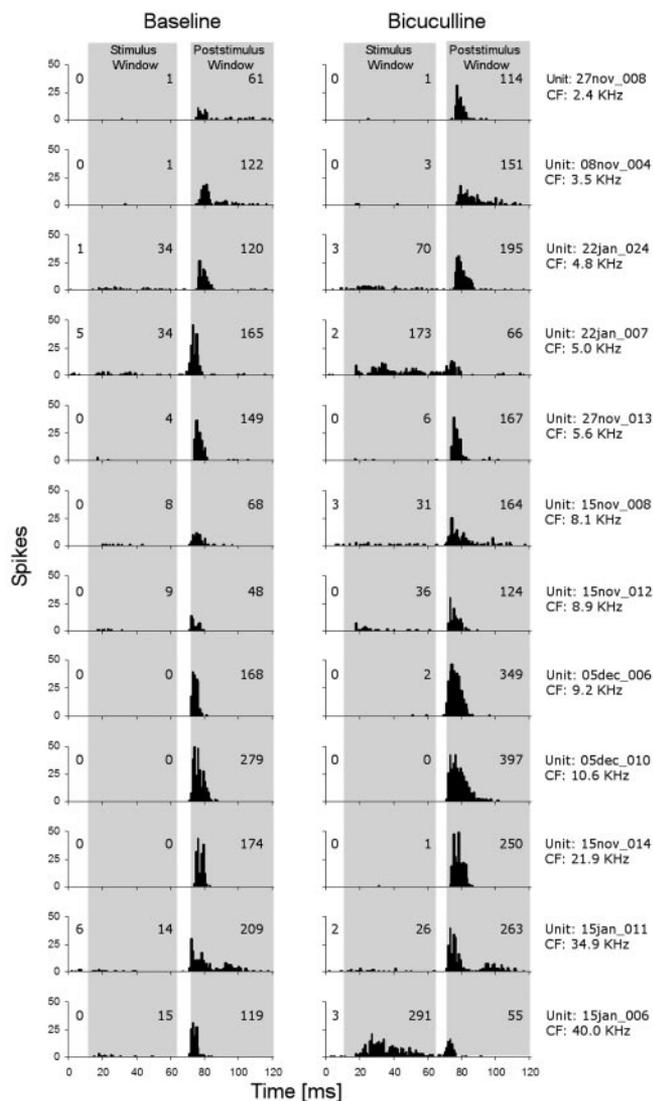
FIG. 6. Responses of bicuculline-treated SPON units, sorted by CF. PSTHs recorded in the baseline condition are displayed on the *left*, whereas those recorded under bicuculline treatment are presented on the *right* for each neuron. Numbers within each PSTH indicate the spike count in the spontaneous, stimulus, or poststimulus analysis windows; the latter 2 windows are highlighted in gray shading. Presentation of the 55-ms pure-tone stimulus coincided with the stimulus window. Each unit's ID number and CF are given in the *right* margin. In most SPON neurons, spiking activity was strongly inhibited during the stimulus presentation and bicuculline administration caused an enlarged offset response. Some SPON neurons displayed spikes during the stimulus presentation in the baseline condition and in these units bicuculline also caused increased spiking during the stimulus presentation.

coxon signed-ranks test; Fig. 3, *Q* and *R*). To gain a general impression of the responses of this sample of neurons, average PSTHs were constructed (Fig. 4). From these PSTHs, as well as from the individual PSTHs in Fig. 8, it is evident that (with the exception of unit 27nov_013), the action potentials occurring in the poststimulus window during cocktail application represented a continuation of the response that started during the stimulus presentation, rather than an offset response triggered by the end of the stimulus. Where recovery data were obtained they demonstrated that the neurons returned to their baseline response patterns; two examples are shown in Fig. 9.

DISCUSSION

The present study focuses on the respective roles of glycine and GABA in shaping neuronal response properties in the SPON. Fortuitously, the main inhibitory inputs to SPON neurons use separate neurotransmitter systems, rendering it possible to study the effects of each input in isolation by applying selective antagonists of the glycine and/or GABA receptors. Thus ours joins several previous studies of the auditory brain stem and midbrain showing differential effects of GABA and glycine on neuronal response properties (Davis and Young 2000; Klug et al. 1995; Nataraj and Wenstrup 2005; Vater et al. 1992; Yang and Pollak 1994a,b).

In the following sections, the effects of strychnine, bicuculline, and the drug cocktail are discussed first. We then consider the relative contributions of glycine and GABA to the spontaneous activity of SPON neurons, as well as to SPON response components occurring during and after the stimulus presentation. For the purpose of this discussion an offset response is defined as spiking activity that begins in the poststimulus time window, as opposed to spikes in the poststimulus window that represent a continuation of a response starting earlier. To aid in relating the findings of this study to the known synaptic inputs to SPON neurons, a schematic diagram is presented in Fig. 10.

Effects of strychnine

On the basis of the known response properties of SPON neurons and their synaptic inputs, we hypothesized that glycinergic inhibition originating in the MNTB is essential to the formation of the SPON offset response (Kulesza Jr et al. 2003a).

The present data support this hypothesis, insofar as SPON offset responses disappeared during application of STRYCH. Interestingly, the disappearance of the offset responses was accompanied by the emergence of a rather long latency response during the stimulus that sometimes extended into the poststimulus window. Examination of the PSTHs revealed that poststimulus spikes in the absence of glycinergic inhibition were not offset responses, but rather represented a continuation of the response that was initiated
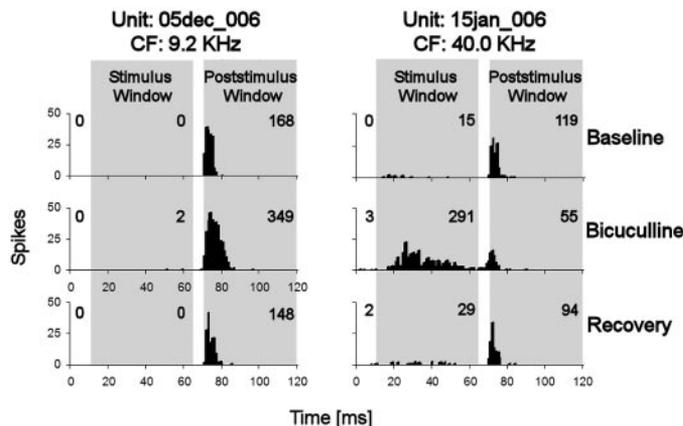

FIG. 7. Recovery of SPON responses after cessation of bicuculline application. Responses are shown of 2 representative neurons for which full recovery data were obtained. Baseline, bicuculline, and recovery PSTHs are displayed in the *top*, *middle*, and *bottom* traces, respectively. In all cases the response observed after recovery from bicuculline was essentially similar to the baseline response.
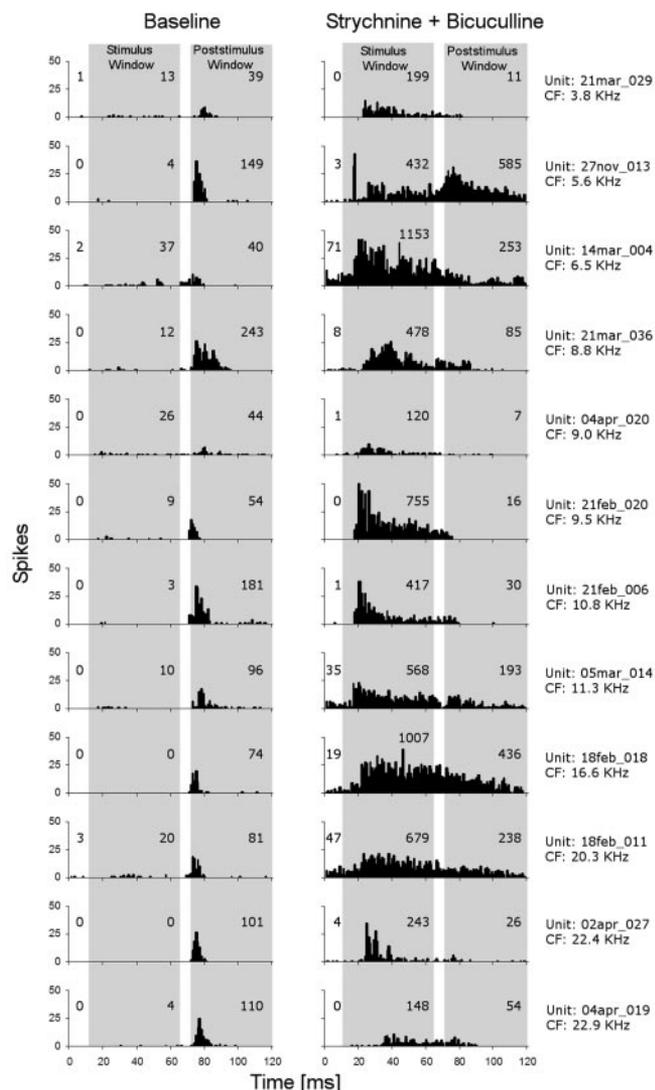
FIG. 8. Responses of drug-cocktail–treated SPON units, sorted by CF. PSTHs recorded in the baseline condition are displayed on the *left*, whereas those recorded under STRYCH + BIC treatment are presented on the *right* for each neuron. Numbers within each PSTH indicate the spike count in the spontaneous, stimulus, or poststimulus analysis windows; the latter 2 windows are highlighted in gray shading. Presentation of the 55-ms pure-tone stimulus coincided with the stimulus window. Each unit's ID number and CF are given in the *right* margin. In about 50% of SPON units we recorded from in the absence of inhibition, spontaneous activity did not change, spiking activity initiated during the stimulus presentation was primarily confined to the stimulus window, and offset responses were abolished. In other SPON cells, although a clear offset response was not apparent, spike bursts beginning in the stimulus interval continued into the poststimulus window; this latter group of neurons also showed increased spontaneous activity. Only one unit displayed an unequivocal offset response in the presence of the drug cocktail (unit 27nov_013) and we suggest that this isolated result represents an incomplete drug effect.

during the stimulus presentation. This dramatic shift in response pattern suggests that spikes occurring during the stimulus presentation (in the presence of strychnine) were generated by an entirely different mechanism than the offset spikes; we presume that they are driven by an excitatory input arriving during the stimulus presentation. This excitatory input, which presumably originates from cochlear nucleus octopus neurons, and perhaps multipolar/stellate cells, is discussed in detail below.

Effects of bicuculline

Our hypothesis concerning the role of GABA in the SPON was based on three separate observations: that SPON neurons are GABAergic (Kulesza Jr and Berrebi 2000), give rise to axonal collaterals that branch within the nucleus (Kulesza Jr et al. 2000; Saldaña and Berrebi 2000), and fire action potentials after the stimulus offset (Kulesza Jr et al. 2003a). Therefore we expected the primary effect of BIC treatment would be an augmentation of the offset response.

Our experiments showed that blocking GABAergic inhibition caused not only an increase in the number of spikes in the stimulus window, but also an augmented offset response. Thus GABAergic inhibition must act on some SPON neurons during pure-tone stimulation and also plays a role in limiting the magnitude of the offset response.

However, the effects of bicuculline treatment must be interpreted with some caution. A recent comparison of the effects of bicuculline and the more specific GABA$_A$ receptor antagonist gabazine in the primary auditory cortex of the gerbil showed that bicuculline treatment induces non-GABAergic side effects, specifically by acting on calcium-dependent potassium channels (Kurt et al. 2006). In gerbil auditory cortex the most prominent side effect of bicuculline was the broadening of tuning curves. This side effect was dose dependent and observed mainly with 40-nA injection currents, whereas for 15-nA injection currents the effects of bicuculline and gabazine were similar. Because the injection currents in the present study were between 15 and 25 nA for BIC, we assume that the non-GABAergic effects of the drug did not contribute much to the observed alteration of SPON response patterns.

At present the intrinsic collaterals of SPON neurons themselves constitute the only *known* source of GABAergic input to the SPON (Kulesza Jr et al. 2000; Saldaña and Berrebi 2000). If this intrinsic projection is the *main* source of GABA to the nucleus, then the offset response may supply sufficient inhibition to limit further offset spikes in SPON cells. Response components occurring in the stimulus window may similarly limit their own magnitude by means of this intrinsic GABAergic inhibition. These speculations are consistent with the observed increases in spike counts in the stimulus and poststimu-
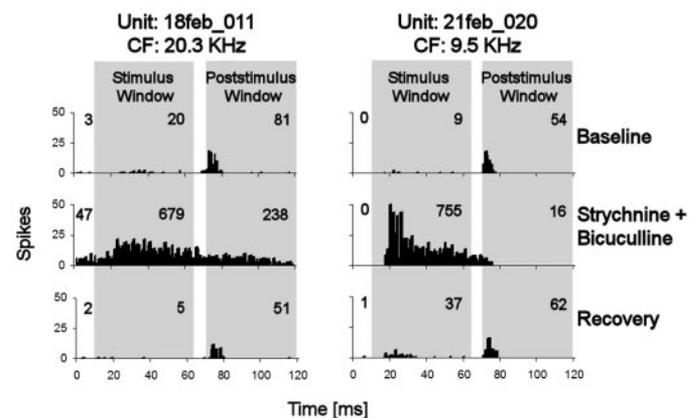

FIG. 9. Recovery of SPON responses after cessation of drug cocktail application. Responses are shown of 2 representative neurons for which full recovery data were obtained. Baseline, drug cocktail, and recovery PSTHs are displayed in the *top*, *middle*, and *bottom traces*, respectively. In all cases the response observed after recovery from strychnine + bicuculline was essentially similar to the baseline response.
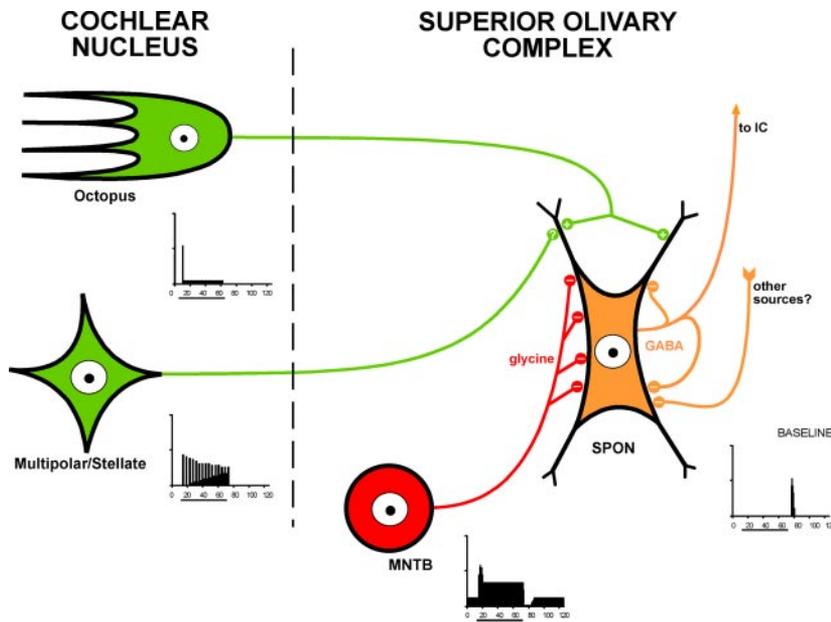
FIG. 10. Schematic depiction of the known synaptic inputs to SPON neurons. Ascending inputs to the SPON arise primarily from octopus neurons and perhaps also from multipolar/stellate cells of the contralateral cochlear nucleus. Projections of octopus neurons are presumed to be excitatory (green+), although multipolar/stellate cells are heterogeneous with respect to their neurochemical phenotypes (green ?). Both of these inputs are distributed distally, in line with preliminary electron microscopic observations, whereas glycine-immunoreactive and γ-aminobutyric acid (GABA)–immunoreactive boutons are densely distributed along the somata and proximal dendrites of SPON neurons. Glycinergic projection to SPON originates primarily from the ipsilateral medial nucleus of the trapezoid body (MNTB, red), whereas the GABAergic input presumably arises from intrinsic collaterals of SPON neurons (orange) whose axons innervate the ipsilateral inferior colliculus; other, as yet unidentified, sources of GABA may also be present. Boxed areas contain a schematic representation, based on the literature, of a typical PSTH for each cell type. Horizontal bar depicts the pure-tone stimulus. Vertical dashed line denotes the brain stem midline.

lus windows when this self- and/or collateral inhibition was removed by BIC administration.

Several potential extrinsic sources of GABA to the SPON, including the ventral nucleus of the trapezoid body (VNTB) and both ventral and dorsal nuclei of the lateral lemniscus (VNLL and DNLL, respectively), were previously suggested but not demonstrated in the rat; nonetheless these cannot be excluded on the basis of the present pharmacological or earlier anatomical data (discussed in Kulesza Jr and Berrebi 2000). Another recently identified candidate source of a late-arriving inhibitory input to SPON is the tectal longitudinal column, which contains GABAergic neurons and projects to the SPON (Saldaña et al. 2002; Viñuela et al. 2002). Systematic cell counts and/or double-labeling experiments will be needed, however, to determine whether the SPON-projecting neurons in this midbrain structure use GABA.

Effects of the drug cocktail

Application of the drug cocktail yielded results similar to those obtained with strychnine treatment alone, to the extent that in both cases SPON offset responses disappeared. However, when GABAergic and glycinergic inhibition were blocked together, the response component occurring during the stimulus presentation was much larger than that during application of strychnine alone. One interpretation of this response is that it represents a summation of the strychnine-induced shift of response pattern and the bicuculline-induced increase of the response magnitude.

Examination of the individual unit PSTHs revealed two different ways that SPON neurons reacted to treatment with the drug cocktail. In about half the SPON neurons we recorded during application of STRYCH and BIC *1*) there was no substantial increase in spontaneous activity, *2*) the uncovered response during the stimulus remained largely confined to the stimulus interval, and *3*) the offset response was abolished. In other SPON cells, although the offset response was not discernible as a separate response component, spiking activity beginning in the stimulus interval extended well into the

poststimulus interval. These neurons also showed a substantial increase in spontaneous activity. Therefore the effects of the drug cocktail on poststimulus spike counts were mixed: seven neurons showed a reduction in spikes, whereas the remaining five displayed an appreciable increase in poststimulus spiking. Consequently, even though the poststimulus spiking activity of all neurons in this sample were altered by the drug treatment, the opposing direction of the changes in these two subgroups caused the statistical comparison to return a nonsignificant result. In one case (unit 27nov_013) a clear offset response was observed in the presence of the drug cocktail. We suggest that this isolated result represents an incomplete drug effect, perhaps arising from our failure to distribute the drug cocktail over the entire dendritic tree of the neuron.

Effects of inhibition on spontaneous activity

Neither strychnine nor bicuculline treatment led to an increase of spontaneous activity in SPON neurons. This suggests that either GABAergic or glycinergic inhibition by itself is sufficient to suppress spontaneous activity in SPON neurons. When both inhibitory transmitter systems were blocked together, spontaneous activity increased, but only in about half of the SPON cells we recorded. Therefore inhibition serves to suppress spontaneous activity in at least a subset of SPON neurons (see the earlier discussion of the drug cocktail experiments).

Effects of inhibition on response components occurring during the stimulus presentation

In a subset of SPON neurons, some spiking activity was present in the stimulus window under baseline conditions and this activity was enhanced by blockade of glycine and/or GABA receptors. In keeping with our hypothesis that SPON offset spikes are triggered by a rebound from glycinergic inhibition originating in the MNTB, these baseline responses may similarly result from a brief release of glycinergic inhibition after the pronounced onset component of the MNTB

response (for examples of in vivo responses of rat MNTB neurons, see Kadner et al. 2006; Kulesza Jr et al. 2003a). However, the enhancement of spiking during the stimulus in the absence of inhibition indicates an excitatory input is making a contribution to SPON activity. Tract-tracing studies report that the likeliest sources of this excitatory input are octopus cells, and perhaps multipolar/stellate cells, of the cochlear nucleus (Saldaña et al. 1994; Schofield 1995). Because the literature depicts multipolar/stellate cells as a rather heterogeneous population of neurons based on their neurochemistry and projection patterns and suggests that some multipolar/stellate cells are inhibitory (Doucet and Ryugo 2006; Needham and Paolini 2006; Schofield and Cant 1996), we focus next on the octopus cell input to SPON.

Octopus cells are characterized by pronounced and temporally precise onset responses followed by sustained discharges, whereas multipolar/stellate cells show a chopper-type response pattern (Rhode and Greenberg 1992; Rhode and Smith 1986). Interestingly, the SPON responses we observed during the stimulus window in the absence of inhibition do not appear to reflect either of these response types. One possible explanation for this inconsistency is that transporters for the excitatory neurotransmitter glutamate are not abundant in the rat SPON and occur mainly in the neuropil (Blaesse et al. 2005). If removal of glutamate from the synaptic cleft is indeed inefficient, one would predict glutamatergic activation of SPON neurons would continue for some time after presynaptic glutamate release, resulting in temporal imprecision of SPON excitatory responses. Moreover, preliminary electron microscopic studies indicate that excitatory synaptic boutons are preferentially distributed on the distal dendritic branches of SPON neurons (Holt and Berrebi 1999). Therefore the passive conduction of an excitatory presynaptic potential to the somata of SPON neurons should add a distance-dependent delay to this temporal imprecision. Results of our drug cocktail experiments suggest that GABAergic inhibition normally serves to suppress this excitatory response component because blocking glycinergic and GABAergic inhibition simultaneously results in dramatically larger excitatory responses than blocking glycinergic inhibition alone.

Effects of inhibition on poststimulus response components

Off responses are by no means a novel finding in the auditory system, having been described in the cochlear nucleus, SOC, nuclei of the lateral lemniscus, IC, medial geniculate body, and auditory cortex (Aitkin and Prain 1974; Bajo et al. 1998; Batra and Fitzpatrick 1999; Grinnell 1973; Grothe 1994; Guinan Jr et al. 1972a,b; He 2001; He et al. 1997; Kuwada and Batra 1999; Spitzer and Semple 1995). Our results show that SPON offset responses are a clearly discernible, discrete response component only in the presence of glycinergic inhibition. On the other hand, GABAergic inhibition does not play a critical role in generating the SPON offset response. Rather, GABA appears to suppress SPON spiking regardless of its timing relative to the stimulus. These findings are consistent with our hypothesis that glycine-mediated postinhibitory rebound (PIR) contributes to the formation of SPON offset responses.

The generation of offset responses by PIR is an interesting feature of the nervous system and provides a mechanism where one input can generate both inhibition and excitation. PIR is triggered by the opening of T-type, i.e., low-voltage–activated calcium channels that are deinactivated during hyperpolarization of the cell membrane and open on return to more depolarized membrane potentials, resulting in a large influx of calcium (Aizenman and Linden 1999). This calcium influx presumably leads to the depolarization that causes the opening of deinactivated sodium channels and results in sodium spikes. Another potential contributor to PIR is I_h, a mixed cation current that is activated during hyperpolarization and drives the membrane potential to more depolarized levels (Aizenman and Linden 1999; McCormick and Pape 1990). Intracellular recordings will be necessary to determine whether PIR contributes to offset responses in the SPON, as previously shown in the inferior colliculus (Koch and Grothe 2003; Sivaramakrishnan and Oliver 2001; Wu et al. 2002).

In contrast to our original hypothesis, the results of our drug cocktail experiments suggest that excitation may also play a role in the formation of SPON offset responses because some of the drug-cocktail–treated neurons displayed excitatory responses (normally suppressed by glycine) that extended far into the poststimulus interval. In these neurons it is conceivable that glycinergic inhibition simply serves to shunt excitation during the stimulus. At the end of the stimulus MNTB spiking ceases briefly, thereby interrupting glycinergic inhibition to the SPON. Because no SPON spikes have occurred yet, GABAergic self-inhibition is also low. At this point in time, excitatory input may drive SPON neurons for a short period, until spontaneous activity in the MNTB (and with it glycinergic inhibition of SPON neurons) resumes or SPON-derived GABAergic inhibition suppresses the excitatory response. Thus it appears that both PIR and excitation may contribute to the offset response with the relative contribution of each mechanism likely varying somewhat from neuron to neuron.

Other considerations

The interactions of inhibition and excitation in the rat SPON discussed above may not be shared among mammals or even rodents. Studies of the gerbil SPON described neurons excited by either ear and exhibiting ON–OFF, OFF, and sustained responses (Behrend et al. 2002; Dehmel et al. 2002). In particular, some neurons in the gerbil SPON produced a sustained response characterized by irregular spiking and an absence of phase locking to amplitude-modulated stimuli. These response properties are reminiscent of the response we observed in the presence of strychnine or the drug cocktail, leading us to speculate that inhibition in the gerbil SPON may not be as potent as that in the rat. Although an anatomical variation must underlie the bilateral drive found in gerbil SPON neurons, differences in the balance of inhibition and excitation may account for the disparity in response types between the two species.

Summary of results

In conclusion, our pharmacological data show that the coordinated activity of glycinergic and GABAergic inhibition plays an important role in shaping the responses of SPON neurons. Glycinergic inhibition is critical to the formation of SPON offset responses. In some SPON neurons, offset re-

sponses may be attributable to a rebound from glycinergic inhibition originating in the MNTB. In other SPON neurons, such inhibition may serve to suppress excitation during the stimulus presentation, with the interruption of glycinergic inhibition after the stimulus offset thereby allowing an excitatory offset response to be generated. GABAergic inhibition, presumably derived mainly from intrinsic collaterals of SPON cells, primarily serves to limit the response magnitude of SPON neurons.

REFERENCES

Aitkin LM, Prain SM. Medial geniculate body: unit responses in the awake cat. *J Neurophysiol* 37: 512–521, 1974.

Aizenman CD, Linden DJ. Regulation of the rebound depolarization and spontaneous firing patterns of deep nuclear neurons in slices of rat cerebellum. *J Neurophysiol* 82: 1697–1709, 1999.

Bajo VM, Villa AE, de Ribaupierre F, Rouiller EM. Discharge properties of single neurons in the dorsal nucleus of the lateral lemniscus of the rat. *Brain Res Bull* 47: 595–610, 1998.

Banks MI, Smith PH. Intracellular recordings from neurobiotin-labeled cells in brain slices of the rat medial nucleus of the trapezoid body. *J Neurosci* 12: 2819–2837, 1992.

Batra R, Fitzpatrick DC. Discharge patterns of neurons in the ventral nucleus of the lateral lemniscus of the unanesthetized rabbit. *J Neurophysiol* 82: 1097–1113, 1999.

Behrend O, Brand A, Kapfer C, Grothe B. Auditory response properties in the superior paraolivary nucleus of the gerbil. *J Neurophysiol* 87: 2915–2928, 2002.

Blaesse P, Ehrhardt S, Friauf E, Nothwang HG. Developmental pattern of three vesicular glutamate transporters in the rat superior olivary complex. *Cell Tissue Res* 320: 33–50, 2005.

Curtis DR, Duggan AW, Felix D, Johnston GA. Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. *Brain Res* 32: 69–96, 1971b.

Curtis DR, Duggan AW, Johnston GA. The specificity of strychnine as a glycine antagonist in the mammalian spinal cord. *Exp Brain Res* 12: 547–565, 1971a.

Davis KA, Young ED. Pharmacological evidence of inhibitory and disinhibitory neuronal circuits in dorsal cochlear nucleus. *J Neurophysiol* 83: 926–940, 2000.

Dehmel S, Kopp-Scheinpflug C, Dorrscheidt GJ, Rubsamen R. Electrophysiological characterization of the superior paraolivary nucleus in the Mongolian gerbil. *Hear Res* 172: 18–36, 2002.

Doucet JR, Ryugo DK. Structural and functional classes of multipolar cells in the ventral cochlear nucleus. *Anat Rec A Discov Mol Cell Evol Biol* 288: 331–344, 2006.

Finlayson PG, Adam TJ. Excitatory and inhibitory response adaptation in the superior olive complex affects binaural acoustic processing. *Hear Res* 103: 1–18, 1997.

Friauf E, Ostwald J. Divergent projections of physiologically characterized rat ventral cochlear nucleus neurons as shown by intra-axonal injection of horseradish peroxidase. *Exp Brain Res* 73: 263–284, 1988.

Godfrey DA, Farms WB, Godfrey TG, Mikesell NL, Liu J. Amino acid concentrations in rat cochlear nucleus and superior olive. *Hear Res* 150: 189–205, 2000.

Grinnell AD. Rebound excitation (off-responses) following nonneural suppression in the cochlear nucleus of echolocating bats. *J Comp Physiol* 82: 179–194, 1973.

Grothe B. Interaction of excitation and inhibition in processing of pure tone and amplitude-modulated stimuli in the medial superior olive of the mustached bat. *J Neurophysiol* 71: 706–721, 1994.

Guinan JJ Jr, Guinan SS, Norris BE. Single units in the superior olivary complex I: responses to sounds and classification based on physiological properties. *Int J Neurosci* 4: 101–120, 1972a.

Guinan JJ Jr, Norris BE, Guinan SS. Single units in the superior olivary complex II: localizations of unit categories and tonotopic organization. *Int J Neurosci* 4: 147–166, 1972b.

Havey DC, Caspary DM. A simple technique for constructing "piggy-back" multibarrel microelectrodes. *Electroencephalogr Clin Neurophysiol* 48: 249–251, 1980.

He J. On and off pathways segregated at the auditory thalamus of the guinea pig. *J Neurosci* 21: 8672–8679, 2001.

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

Holt GA, Berrebi AS. Ultrastructure of the superior paraolivary nucleus (SPON) of the rat. *Assoc Res Otolaryngol Abstr* 22: 277, 1999.

Kadner A, Kulesza RJ Jr, Berrebi AS. 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.

Kelly JB, Liscum A, van Adel B, Ito M. Projections from the superior olive and lateral lemniscus to tonotopic regions of the rat's inferior colliculus. *Hear Res* 116: 43–54, 1998.

Klug A, Park TJ, Pollak GD. Glycine and GABA influence binaural processing in the inferior colliculus of the mustache bat. *J Neurophysiol* 74: 1701–1713, 1995.

Koch U, Grothe B. Hyperpolarization-activated current (I_h) in the inferior colliculus: distribution and contribution to temporal processing. *J Neurophysiol* 90: 3679–3687, 2003.

Kulesza RJ Jr, Berrebi AS. Superior paraolivary nucleus of the rat is a GABAergic nucleus. *J Assoc Res Otolaryngol* 1: 255–269, 2000.

Kulesza RJ Jr, Holt GA, Spirou G, Berrebi AS. Intracellular labeling of axonal collaterals of SPON neurons. *Assoc Res Otolaryngol Abstr* 23: 5746, 2000.

Kulesza RJ Jr, Spirou GA, Berrebi AS. Physiological response properties of neurons in the superior paraolivary nucleus of the rat. *J Neurophysiol* 89: 2299–2312, 2003a.

Kulesza RJ Jr, Spirou GA, Berrebi AS. Selective effects of GABA and glycine on response properties of neurons in the superior paraolivary nucleus in the rat. *Assoc Res Otolaryngol Abstr* 26: 94, 2003b.

Kulesza RJ Jr, Viñuela A, Saldaña E, Berrebi AS. Unbiased stereological estimates of neuron number in subcortical auditory nuclei of the rat. *Hear Res* 168: 12–24, 2002.

Kurt S, Crook JM, Ohl FW, Scheich H, Schulze H. Differential effects of iontophoretic in vivo application of the GABA(A)-antagonists bicuculline and gabazine in sensory cortex. *Hear Res* 212: 224–235, 2006.

Kuwabara N, Zook JM. Classification of the principal cells of the medial nucleus of the trapezoid body. *J Comp Neurol* 314: 707–720, 1991.

Kuwada S, Batra R. Coding of sound envelopes by inhibitory rebound in neurons of the superior olivary complex in the unanesthetized rabbit. *J Neurosci* 19: 2273–2287, 1999.

McCormick DA, Pape HC. Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. *J Physiol* 431: 291–318, 1990.

Nataraj K, Wenstrup JJ. Roles of inhibition in creating complex auditory responses in the inferior colliculus: facilitated combination-sensitive neurons. *J Neurophysiol* 93: 3294–3312, 2005.

Needham K, Paolini AG. Neural timing, inhibition and the nature of stellate cell interaction in the ventral cochlear nucleus. *Hear Res* 216–217: 31–42, 2006.

Rhode WS, Greenberg S. Physiology of the cochlear nuclei. In: *Mammalian Auditory Pathway: Neurophysiology*, edited by Popper AN, Fay RR. New York: Springer-Verlag, 1992, p. 94–152.

Rhode WS, Smith PH. Encoding timing and intensity in the ventral cochlear nucleus of the cat. *J Neurophysiol* 56: 261–286, 1986.

Saldaña E, Berrebi AS. Anisotropic organization of the rat superior paraolivary nucleus. *Anat Embryol* (*Berl*) 202: 265–279, 2000.

Saldaña E, Lough D, Berrebi AS. Superior paraoliviary nucleus (SPON): connectivity revealed by transport of biotinylated dextran. *Soc Neurosci Abstr* 20: 975, 1994.

Saldaña E, Viñuela A, Aparicio MA, Castellanos O. A novel nucleus of the mammalian brain: the tectal commissural column. *Soc Neurosci Abstr* 28: 560.12, 2002.

Schofield BR. Projections to the cochlear nuclei from principal cells in the medial nucleus of the trapezoid body in guinea pigs. *J Comp Neurol* 344: 83–100, 1994.

Schofield BR. Projections from the cochlear nucleus to the superior paraolivary nucleus in guinea pigs. *J Comp Neurol* 360: 135–149, 1995.

Schofield BR, Cant NB. Origins and targets of commissural connections between the cochlear nuclei in guinea pigs. *J Comp Neurol* 375: 128–146, 1996.

Sivaramakrishnan S, Oliver DL. Distinct K currents result in physiologically distinct cell types in the inferior colliculus of the rat. *J Neurosci* 21: 2861–2877, 2001.

Sokolich W. Improved acoustic system for auditory research [Abstract]. *J Acoust Soc Am* 62: S12, 1977.

Sommer I, Lingenhohl K, Friauf E. Principal cells of the rat medial nucleus of the trapezoid body: an intracellular in vivo study of their physiology and morphology. *Exp Brain Res* 95: 223–239, 1993.

Spitzer MW, Semple MN. Neurons sensitive to interaural phase disparity in gerbil superior olive: diverse monaural and temporal response properties. *J Neurophysiol* 73: 1668–1690, 1995.

Thompson AM, Thompson GC. Projections from the posteroventral cochlear nucleus to the superior olivary complex in guinea pig: light and EM observations with the PHA-L method. *J Comp Neurol* 311: 495–508, 1991.

Vater M, Habbicht H, Kossl M, Grothe B. The functional role of GABA and glycine in monaural and binaural processing in the inferior colliculus of horseshoe bats. *J Comp Physiol A Sens Neural Behav Physiol* 171: 541–553, 1992.

Viñuela A, Aparicio MA, Saldaña E. Reciprocal connections between the superior olivary complex and the tectal commissural column. *Assoc Res Otolaryngol Abstr* 2002.

Wu SH, Ma CL, Sivaramakrishnan S, Oliver DL. Synaptic modification in neurons of the central nucleus of the inferior colliculus. *Hear Res* 168: 43–54, 2002.

Yang L, Pollak GD. The roles of GABAergic and glycinergic inhibition on binaural processing in the dorsal nucleus of the lateral lemniscus of the mustache bat. *J Neurophysiol* 71: 1999–2013, 1994a.

Yang L, Pollak GD. GABA and glycine have different effects on monaural response properties in the dorsal nucleus of the lateral lemniscus of the mustache bat. *J Neurophysiol* 71: 2014–2024, 1994b.