Physiological Response Properties of Neurons in the Superior Paraolivary Nucleus of the Rat

Randy J. Kulesza Jr.,1,2,4 George A. Spirou,1,3,4 and Albert S. Berrebi1,2,4

Departments of 1Otolaryngology–Head and Neck Surgery, 2Neurobiology and Anatomy, 3Physiology and Pharmacology, and 4The Sensory Neuroscience Research Center, West Virginia University School of Medicine, Morgantown, West Virginia 26506

Submitted 11 July 2002; accepted in final form 18 December 2002

Kulesza, Randy J. Jr., George A. Spirou, and Albert S. Berrebi. Physiological response properties of neurons in the superior paraolivary nucleus of the rat. J Neurophysiol 89: 2299–2312, 2003. First published December 27, 2002; 10.1152/jn.00547.2002. The superior paraolivary nucleus (SPON) is a prominent nucleus of the superior olivary complex. In rats, this nucleus is composed of a morphologically homogeneous population of GABAergic neurons that receive excitatory input from the contralateral cochlear nucleus and inhibitory input from the ipsilateral medial nucleus of the trapezoid body. SPON neurons provide a dense projection to the ipsilateral inferior colliculus and are thereby capable of exerting profound modulatory influence on collicular neurons. Despite recent interest in the structural and functional features of SPON, little is presently known concerning the physiological response properties of this cell group or its functional role in auditory processing. We utilized extracellular, in vivo recording methods to study responses of SPON neurons to broad band noise, pure tone, and amplitude-modulated pure tone stimuli. Localization of recording sites within the SPON provides evidence for a medial (high frequency) to lateral (low frequency) tonotopic representation of frequencies within the nucleus. Best frequencies of SPON neurons spanned the audible range of the rat and receptive fields were narrow with V-shaped regions near threshold. Nearly all SPON neurons responded at the offset of broad band noise and pure tone stimuli. The vast majority of SPON neurons displayed very low rates of spontaneous activity, and both sustained and phasic discharges (Behrend et al. 2000; Kulesza and Berrebi 2000; Kulesza et al. 2002), the nucleus is poised to exert a profound inhibitory influence on neurons in the IC.

In contrast to our understanding of SPON anatomy, the physiological response properties of its neurons are not well studied. Previous recordings from the SPON in gerbils indicates a heterogeneous population of units with mixed binaural and monaural responses, wide-ranging rates of spontaneous activity, and both sustained and phasic discharges (Behrend et al. 2002; Dehmel et al. 2002; Spitzer and Semple 1995). The few published recordings from the SPON of cat (Guinan et al. 1972) and rat (Finlayson and Adams 1997) further support the notion of heterogeneous responses to sounds. Thus we undertook a systematic study of auditory evoked responses of SPON neurons in the rat to shed light on the functional role of this nucleus in auditory processing. Our choice of this species was based, in large part, on the previously demonstrated homogeneity of neuronal morphologies, neurochemical phenotypes, and efferent projections of SPON neurons in rats (Kulesza and Berrebi 2000; Kulesza et al. 2002; Saldana and Berrebi 2000).

METHODS

Stereoaxial surgery

This study employed 40 female albino rats (Sprague-Dawley strain) and 5 female hooded rats (Long Evans strain) weighing between 250 and 320 g. Animals were anesthetized by intramuscular injection of a mixture of xylazine and ketamine (8.6 and 57 mg/kg body wt, respectively). Once determined to be areflexic, the rats were placed into a stereotaxic frame, their heads secured by a bite bar, and hollow brass earbars inserted into the cartilaginous external auditory meatus. A

Address for reprint requests: A. S. Berrebi, Sensory Neuroscience Research Center, P.O. Box 9303 Health Sciences Ctr., West Virginia University School of Medicine, Morgantown, WV 26506-9303 (E-mail: aberrebi@hsc.wvu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
midline incision was then made in the scalp, a small bone flap overlying the cerebellum was removed, and the dura mater was incised to permit penetration of the recording electrode. The anesthetic state of each animal was monitored throughout the experiment and supplemental doses of the same anesthetics were given, as needed, at 2/5 the original dose.

Sound stimuli and delivery

Acoustic stimuli were delivered via Stax speakers contained within custom built housings (Sokolich 1977) attached to the hollow ear bars. To permit calibration of the sound delivery system, the hollow ear bars were machined with a small tube joining the sound delivery tube at a 45° angle. Prior to or immediately following each recording session, with the rat in situ, a B&K microphone was placed into this tube and the sound-delivery system calibrated for broadband noise (BBN) and pure tones between 1 and 40 kHz. Stimulus intensities were converted to dB SPL off-line to correct for microphone attenuation.

Sound stimuli were created digitally with SigGen or RP Visual Design Studio software (Tucker-Davis Technologies, Gainesville, FL), and had 5 ms cos² ramps. Single-unit data were collected using Brainware software (Tucker-Davis Technologies) and analyzed using Microsoft Excel and custom written Matlab scripts.

Physiological recordings

Both tungsten and glass micropipette electrodes (8–20 MΩ, filled with 3 M KCl and 2.5% biocytin) were used to record from 116 units in the SPON. Guided by stereotaxic coordinates obtained from a rat brain atlas (Paxinos and Watson 1986), electrodes were advanced into the nucleus from a dorsal approach with a Burleigh Inchworm (Burleigh Instruments, Victor, NY). The data obtained with tungsten and glass electrodes are identical and will therefore be considered together. Although not included in this report, units recorded with glass electrodes filled with 0.45% NaCl also yielded similar results. Recording sites were marked with electrolytic lesions when using tungsten electrodes (8 μA for 10 s) or iontophoretic deposits of biocytin (Sigma Chemical, St. Louis, MO) when using glass micropipettes (200 nA for 5 min, 50% duty cycle).

Binaural BBN (50 ms in duration, 20-dB attenuation) was used as a search stimulus. Responses were determined to be from a single unit if they had biphasic waveforms and constant amplitude peaks. On isolation of a single unit, its aurality was determined by examining responses to 20 repetitions each of binaural, ipsilateral, and contralateral BBN. Neurons were considered monaural if their firing rate in response to unilateral stimulation did not differ significantly from their response to binaural stimulation or considered to be binaurally facilitated if they displayed significantly more spikes in response to the binaural presentation.

Response maps were generated from unit responses to presentations of numerous frequency-intensity combinations of pure tones (each 50 ms in duration). Best frequency (BF), the frequency that elicited a response from the unit at the lowest sound intensity, was determined from the response map. Rate-level curves to BF tones were collected at a single repetition per decibel, typically covering a 50- to 60-dB range, and smoothed by using a 1–2–1 triangular smoothing algorithm. Threshold was defined as the lowest sound intensity that resulted in any spike activity, and the dynamic range was calculated by subtracting the unit’s response threshold from the stimulus intensity corresponding to the first plateau on the rate-level curve. Some cells showed another increase in rate at higher intensities, but this feature was not considered in our measurement if it did not occur within 15 dB of the initial plateau. We interpreted such a broad intensity range without a rate increase as indicative of a lack of sensitivity to sound level. Peristimulus time histograms (PSTHs) were generated from responses to 50 presentations of 50-ms BF tones at 20 dB above threshold. Spontaneous activity was monitored in a 10-ms time window before the stimulus presentation during each of 50 sweeps for total time of 5 s.

A subset of SPON units (n = 30) was presented with sinusoidally amplitude-modulated (SAM) pure tones (100% modulation). Each stimulus was presented 20 times, and the carrier frequency was a 500-ms BF tone (20 dB above threshold) coupled with modulation frequencies of 25, 50, 100, 200, 300, or 400 Hz. Fidelity of phase locking was determined by calculating vectors strengths (VS) (Goldberg and Brown 1969) at each modulation frequency. Responses at the termination of the 500-ms SAM tone were not considered in the calculation of vector strengths.

Localization of recording sites

On completion of the recording session, each animal was given a supplemental dose of xylazine and ketamine and perfused through the ascending aorta with a vascular rinse of normal saline followed by a fixative composed of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.12 M sodium phosphate buffer, pH 7.2. The brain was then dissected from the cranium and cryoprotected overnight in 30% sucrose in the same buffer. Brain stems were coronally sectioned on a freezing microtome at a thickness of 60 μm. If recording sites in that animal that were marked by electrolytic lesions, the tissue sections were dry mounted onto glass slides from gelatin-alcohol and stained for Nissl substance with cresyl violet using standardized protocols. Sections from animals that received biocytin injections were processed, free-floating, according to the ABC method (Vector Laboratories) using 0.05% diaminobenzidine, 0.01% hydrogen peroxide, 0.025% cobalt chloride, and 0.02% nickel ammonium sulfate as the chromogen. The sections processed in this manner were then mounted onto glass slides and counterstained with neutral red. The boundaries of the SPON were determined relative to other SOC nuclei and prominent fiber bundles that demarcate its borders. The characteristic morphology of labeled SPON neurons was also used to confirm the location of recording sites. If we had difficulty discerning the boundaries of the SPON at any particular level, we referred to previously published borders of SOC nuclei in the rat derived from various sources (Kulesza and Berrebi 2000; Kulesza et al. 2002; Saldaña and Berrebi 2000).

Camera lucida drawings were made of tissue sections containing biocytin deposits, electrolytic lesions, or evidence of electrode passage. The distance between any two landmarks (lesions or deposits of biocytin) along a recording track was measured and used to calculate tissue shrinkage. Using depth measurements taken directly from the Burleigh microdrive readout and adjusting for tissue shrinkage, recording site locations along the electrode track were plotted by superimposing the camera lucida drawing onto a standardized template of the rat SOC (Paxinos and Watson 1986) at the appropriate rostrocaudal level and aligning it to achieve the best possible fit.

RESULTS

Localization of units and tonotopic mapping of BFs

To elucidate the auditory evoked responses of SPON neurons, we recorded from a total of 116 well-isolated single units from 45 rats. Each electrode track was reconstructed, and the location of each unit within the SPON confirmed by biocytin deposition or lesion demarcation (Fig. 1).

The BF of each unit was then determined to reveal the representation of frequency in the SPON, and a tonotopic map of the nucleus was constructed. In our recordings, BFs ranged from 1.1 to 40 kHz, which covers the most sensitive range of hearing in rats (Kelly and Masterton 1977). Moreover, we found that the BFs of SPON units were distributed in a sys-
more spikes than the contralateral stimulus alone ($P \leq 0.047$, paired $t$-test). Moreover, virtually all SPON units (96%) responded only at the termination of the BBN (Fig. 3). A single neuron responded only at the onset of the BBN, and one neuron responded at the onset and again at the offset of the BBN. Spontaneous rates of activity in SPON neurons were typically very low (see following text), but in the few units with measurable spontaneous activity, we noted a depression in the spike rate during the contralaterally derived inhibitory input. Moreover, because most neurons displayed virtually no spontaneous activity, we cannot rule out with certainty the possibility of an ipsilaterally driven inhibitory input.

Response types

We then attempted to characterize the fundamental response profiles of SPON cells. First, PSTHs were constructed for all 116 SPON units’ responses to BF pure tones at 20 dB above threshold (Fig. 4). More than 95% of the units displayed spike activity primarily at the end or “offset” of the stimulus. Distinguishing features of these offset responses, however, enabled us to further subdivide our sample into five response classes including: 1) neurons that responded only transiently at the stimulus offset with single spikes [average of 1.13 ± 0.55 (SD) spikes/stimulus], termed offset-transient responders (Fig. 4A); 2) units that responded only at the stimulus offset with two or more regularly spaced spikes (average of 1.98 ± 0.82 spikes per stimulus), termed offset-choppers (Fig. 4B); 3) units that responded only at the stimulus offset with spike activity that was sustained for more than 20 ms (average of 2.01 ± 1.07 spikes per stimulus), termed offset-sustained responders (Fig. 4C); 4) units that responded with a few spikes during the stimulus as well as offset spikes, termed on-offset responders (Fig. 4D); and 5) neurons that displayed a mixture of onset and sustained responses during the stimulus but no offset spikes, termed on-sustained responders (Fig. 4E). Interestingly, members of this last response class, that accounted for only 5% of all units, were all localized near the dorsolateral border of the SPON and all but one had BFS less than 4 kHz. For the remaining response classes, BFS ranged from 1.2 to 40 kHz for offset-transient neurons, from 2.7 to 20.2 kHz for offset-choppers, from 2.8 to 11.7 kHz for offset-sustained responders, and from 3.5 to 26.9 kHz for the on-sustained units.

For 74 units, PSTHs in response to contralateral BBN were compared with the PSTHs derived in response to their BF pure tone stimuli. The majority of units (64 of 74, 86%) were classified similarly for both stimulus types, and 9 of the 10 neurons whose classification differed remained within offset classes I-III.

If the offset responses observed in response to 50-ms stimuli were the result of a late-arriving excitatory input, we would expect SPON neurons to fire during stimuli of durations longer than the latency of their excitatory input. To be certain that the offset responses were not generated in response to a long-latency excitatory input, we tested a small population of neurons with stimuli of various durations (20–500 ms) and, regardless of the stimulus duration, we observed offset responses (Fig. 5). During the course of our experiments, we also recorded from neurons whose locations were confirmed by lesions or biocytin deposits to the MNTB. An example of a
PSTH from one such MNTB neurons is illustrated in Fig. 4F. MNTB units have high rates of spontaneous activity and exhibit a dramatic increase in firing rate during the stimulus. At stimulus offset, MNTB units cease to fire for a brief period that appears to coincide with the time window during which SPON neurons respond with offset spikes.

**Response latencies**

The average first spike latency in response to 50-ms BF tones (20 dB above threshold) for SPON neurons in response classes I–III (n = 78) was determined to be 7.04 ± 3.56 ms from the stimulus offset. Latencies of offset-transient units (n = 35; 6.05 ± 2.33 ms) and offset-chopper cells (n = 25; 6.62 ± 2.21 ms) were statistically equivalent, (P = 0.47, unpaired t-test), but both classes had significantly shorter first spike latencies than offset-sustained units (n = 18; 9.67 ± 5.28 ms; P < 0.02; unpaired t-test). Furthermore, the first-spike latency within response classes I–III did not correlate with the unit’s BF (r = −0.127). However, in 12 of 26 units so tested, response latency decreased with increasing tone duration (10, 25, 50, 75, 100, 200, and 500 ms). In two units the latency increased with increasing tone duration, and in the remaining 12 units, latency was unaffected by stimulus duration.

**Spontaneous activity**

For the entire population of SPON units examined, spontaneous rates of activity averaged 2.65 ± 10.42 spikes/s. However, more than half of the units (71 of 116; 62%) displayed no spontaneous activity at all, and only six cells (5%) had spontaneous firing rates more than 6 spikes/s (Fig. 6). For comparison, the spontaneous firing rates we recorded from 16 MNTB units averaged 46.06 ± 27.37 spikes/s.

**Receptive fields**

Response maps were constructed for 54 SPON units with BFs ranging from 2.3 to 40 kHz (Fig. 7). These response maps typically had narrow V-shaped peaks near BF and low-frequency tails. Generally, SPON units exhibited only offset spikes throughout their response maps. Only on rare occasion were spikes observed during the stimulus presentation, and these were exclusively elicited by high-intensity, low-frequency tones (Fig. 8).

**FIG. 2.** Tonotopic arrangement of best frequencies (BFs) in the SPON. Single-unit recording sites are plotted according to BF on a series of standardized templates spanning the caudal (top left) to rostral (bottom right) extent of the SPON. The rostrocaudal spacing is indicated relative to the caudal end of the SOC. BFs of SPON units ranged from 1.2 to 40 kHz, spanning the rat’s audible range. Neurons with low BFs (up to 10 kHz) were located laterally, those with high BFs (20–40 kHz) are located medially, and neurons with intermediate BFs were interposed centrally within the nucleus, LNTB, lateral nucleus of the trapezoid body; M, medial.
Sharpness of tuning of SPON units was determined by calculating $Q_{10}$ and $Q_{30}$ values, which averaged 6.77 ± 3.30 and 1.64 ± 0.99, respectively (Fig. 9A). The $Q$ values were not statistically different across PSTH categories ($P > 0.52$, unpaired $t$-test). Although there was a general trend of $Q$ values increasing with BF, the statistical correlation was not significant ($Q_{10}$ $r = 0.307$ and $Q_{30}$ $r = 0.308$). We noted that the $Q_{10}$ values obtained for SPON units were similar to those reported for MNTB neurons in cat ($Q_{10}$ = 7.02, Fig. 9B) (Guinan 1968) and DNLL neurons in rat ($Q_{10}$ = 7.9) (Kelly et al. 1998a).

Thresholds for SPON neurons at their BF averaged 25.4 ± 11.53 dB SPL and ranged from 5 to 48 dB SPL (Fig. 10). Furthermore, their thresholds were not significantly different across the PSTH classes ($P > 0.25$, unpaired $t$-test).

Dynamic range

To determine the responsiveness of SPON neurons to sound intensity, rate level curves were collected for a subset of units in each response class. These were classified as having dynamic ranges that were narrow (less than or equal to 20 dB) or wide (>20 dB) or unsaturated at the highest intensity (Fig. 11). Because most neurons had spontaneous activity (Fig. 6), dynamic range was measured between the lowest sound intensity that generated a spike and the first plateau in that unit’s response profile. The majority of offset-transient units (12 of 14, 86%), cells that typically fire only one or two spikes per stimulus, had narrow dynamic ranges, as did the majority of offset-choppers (4 of 7 cells). However, the offset-sustained units tended to display wider dynamic ranges, with only 4 of 10 cells showing dynamic ranges less than or equal to 20 dB. Because offset-transient and offset-chopper units account for two-thirds of the population, we surmised that most SPON neurons have short dynamic ranges.

Responses to AM

To examine the responses of SPON units to more complex sounds, SAM pure tones at BF were presented to 30 units. The majority of these units (25 of 30, 83%) responded to the envelope of the SAM stimulus as if each period of modulation was detected as a separate stimulus and discharged to each modulation with single offset spikes (Fig. 12). Fourteen of the units responsive to SAM stimuli were offset-transients, 7 displayed an offset-sustained response, 3 were offset-choppers and 1 was an on-offset unit. Regardless of the modulation frequency, all units invariably displayed an offset response at the end of each presentation of the 500-ms SAM stimulus. BFs for the units responding to SAM stimuli ranged from 1.2 to 35 kHz, and all 25 cells demonstrated high-fidelity phase-locking to modulations up to 100 Hz (average vector strength = 0.81 ± 0.02). At the lower modulation frequencies tested (25–100 Hz), SPON units followed the stimulus envelope faithfully as indicated by vector strengths more than 0.75 (Figs. 12 and 13). At the higher modulation frequencies (200–400 Hz), vector strengths decreased as discharge rates declined sharply. However, as shown in the modulation transfer function, even at modulation rates of 200 and 400 Hz, the few elicited spikes remained reasonably well timed to the SAM stimulus envelope. Neurons that did not respond to SAM stimuli had BFs that ranged from 6.6 to 17.1 kHz and were distributed among the first four PSTH response classes (offset-transient, offset-chopper, offset-sustained, and on-offset).

DISCUSSION

This study represents the first systematic characterization of the auditory evoked physiological responses of SPON neurons in the rat. Our sample of 116 single units were nearly exclu-
sively monaurally activated and always by contralaterally presented stimuli. Most SPON units responded at the offset of pure tone stimuli with a first spike latency on the order of 6–10 ms. Furthermore, many SPON units displayed impressive phase-locking to SAM tones. The homogeneity of responses observed in this study is consistent with the morphological, connectional, and neurochemical homogeneity previously reported for SPON neurons in the rat (Kulesza and Berrebi 2000; Saldanha and Berrebi 2000). In light of reports indicating considerable variability and interspecies diversity in the anatomical and physiological features of SPON neurons in other species (Adams 1983; Behrend et al. 2002; Covey et al. 1984; Dehmel et al. 2002; Guinan et al. 1972; Kulesza and Berrebi 2000; Moore and Goldberg 1966; Osen et al. 1984; Saint Marie and Baker 1990; Saldana and Berrebi 2000; Schofield 1991; Spangler and Warr 1991; Spitzer and Semple 1995; Strutz and Spatz 1980; Thompson and Thompson 1991), we suggest that future attempts to understand the functional role of SPON-derived inhibition to the IC may be most efficiently undertaken in rats.

**Previous studies of SPON physiology**

Kuwada and Batra (1999) described a population of neurons in the SOC of the unanesthetized rabbit that exhibited offset responses and phase-locked to SAM stimuli. Although the nature of their chronic recording preparation precluded precise histological localization of recording sites, these units were located medial to the MSO and likely represent neurons of the SPON or its homologue in the rabbit. In the mustached bat, the MSO contains a population of GABAergic neurons (Winer et al. 1995) as well as units that demonstrate offset responses to contralateral stimulation but do not respond to ipsilateral stimulation and have very low rates of spontaneous activity and low-pass filter characteristics for amplitude-modulated tones (Grothe 1994). These findings have led to the speculation that neurons that constitute the SPON in rodents may have merged into the MSO in the mustached bat (Grothe et al. 1992, Vater 1995).

Finlayson and Adams (1997) recorded from auditory brain stem neurons of Long-Evans hooded rats anesthetized with a mixture of pentobarbital, xylazine, and ketamine. They reported that the vast majority of SPON units were binaurally excited (“EE”) and received matching BF inputs from the two ears. In the course of the present study, we recorded from more than 250 neurons in the SOC or reticular formation that were clearly outside the borders of the SPON. Only 18 units from within this sample had “EE” properties, and 13 of these were localized to the reticular formation dorsal to the SOC. It is not

**FIG. 4.** Classification of SPON response types. The vast majority of SPON neurons (100 of 116, 86%) responded only at the offset of BF tone stimuli (presented at 20 db above threshold). Based on the timing of the spikes observed over 500 stimulus presentations for the entire population of cells, peristimulus time histograms (PSTHs) were subdivided into 5 unit response classes as follows: offset-transient units (A; 41% of units) typically responded with 1 or 2 spikes at the stimulus offset; offset-choppers (B; 23% of units) fired 2 or more regularly spaced spikes, however their responses were also transient, lasting less than 20 ms; offset-sustained units (C; 22% of units) also fired only at the stimulus offset, but their spike activity lasted more than 20 ms; on-offset cells (D; 9% of units) responded with a few spikes during the stimulus as well as offset spikes; and on-sustained cells (E; 5% of units) displayed onset or sustained responses during the tone stimulus. F: a PSTH constructed from an MNTB neuron demonstrates its typical “primary-like” responses, high rate of spontaneous activity, the dramatic increase in firing during the stimulus presentation, and the brief cessation in discharges on termination of the stimulus. Note that the latency of the offset response in SPON units is on the order of 6–10 ms, coinciding with the brief period of quiescence in MNTB neurons (i.e., compare C and F).
possible to fully reconcile this discrepancy, although differences in anesthetic agents employed is one obvious factor that may have contributed to the disparate results. We cannot exclude the possibility that there are substantial numbers of “EE” neurons in the rat SPON that we failed to isolate. However, it is more likely, in our opinion, that at least some of the binaurally excited neurons described by Finlayson and Adams may have been encountered outside the SPON within the dorsal ribbon of the SOC (Feliciano et al. 1995) or the reticular formation.

Recently, two reports of SPON physiology in the gerbil have appeared in the literature and the findings of these studies are somewhat contradictory (Behrend et al. 2002; Dehmel et al., 2002). For example, while Dehmel and colleagues indicate that nearly 65% of SPON neurons responded to the stimulus offset, Behrend and co-workers report that only 6% of SPON units displayed off responses. Dehmel and co-workers did not include responses to SAM stimuli to their report, but Behrend et al. indicate that the small population of off responders in the SPON were not capable of synchronizing to SAM tones. This finding is particularly difficult to reconcile with our demonstration that offset neurons in the rat SPON phase-lock quite well to modulation frequencies up to 200 Hz. One significant difference between the SPON of gerbils and rats is the existence in the former of a population of neurons with descending projections to the cochlear nuclei (Faye-Lund 1986; Helfert et al. 1988; unpublished observations). Therefore we can only speculate that perhaps the majority population of sustained discharging neurons recorded in the gerbil SPON by Behrend et al. (2002) may serve a role in the descending auditory pathway that is simply not performed by the SPON of the rat. More difficult to comprehend is the fact that despite using similar procedures in the same species, Behrend et al. (2002) observed so few offset responders in comparison to Dehmel and colleagues (2002).

**Tonotopy in the SPON**

We identified a tonotopic organization within the rat SPON, with high BFs represented medially and low BFs laterally. The appearance of a slight dorsomedial-to-ventrolateral tilt to the isofrequency contours in the nucleus is consistent with a similar observation previously made on the basis of retrograde tracing studies (Saldaña and Berrebi 2000). Overall, the tonotopic mapping we report is compatible with the topographic arrangement of afferent inputs to SPON from the ipsilateral MNTB (Banks and Smith 1992; Sommer et al. 1993) and the efferent projections of SPON to the ipsilateral IC (Kelly et al. 1998b; Saldaña and Berrebi 2000). Thus the SPON joins the principal nuclei of the SOC in having a defined tonotopic axis.

**How is the offset response formed?**

Neurons responding at the offset of a stimulus are not a rare finding in the mammalian nervous system. OFF neurons have been described in the retina and lateral geniculate nucleus (Hubel and Wiesel 1961; Schiller and Malepli 1978) and at several levels of the auditory pathway including the MNTB and dorsomedial periolivary nucleus (Guinan et al. 1972), MSO (Grothe 1994), ventral nucleus of the lateral lemniscus (VNLL) (Buta and Fitzpatrick 1999; Guinan et al. 1972), dorsal nucleus of the lateral lemniscus (DNLL) (Bajo et al. 1998), IC (Faingold et al. 1986), medial geniculate nucleus (He 2001), and auditory cortex (He 1997). Moreover, inhibition timed to the offset of acoustic stimuli has been reported in DNLL and IC and is at least partially mediated by GABA (Bajo et al. 1989; Bauer et al. 2000; Faingold et al. 1986). Thus it appears that offset synaptic activity may play a fundamental role in central processing of sensory information.

There are several possible mechanisms to account for the offset responses of SPON units. The offset response may result from a long-latency excitatory input to SPON, or one that arrives coincident with the stimulus offset. A descending projection from the tectal commissural column (TCC) of the midbrain has recently been described (Vũnhuela and Saldaña 2001) that could represent a long-latency input to the SPON. However, preliminary observations suggest that this projection is probably inhibitory (unpublished observations). Furthermore, when presented with long tone stimuli (up to 1 s in duration) SPON neurons maintained offset activity, suggesting that a long-latency excitatory input is probably not a sufficient explanation for the offset responses observed. Transient responses timed to the stimulus onset or offset can also be generated through a complex interaction of sustained excitatory and inhibitory inputs (Grothe 1994; Yang and Pollak 1998b; Saldanha and Berrebi 2000). Overall, the tonotopic mapping we report is compatible with the topographic arrangement of afferent inputs to SPON from the ipsilateral MNTB (Banks and Smith 1992; Sommer et al. 1993) and the efferent projections of SPON to the ipsilateral IC (Kelly et al. 1998b; Saldaña and Berrebi 2000). Thus the SPON joins the principal nuclei of the SOC in having a defined tonotopic axis.
1997). For example, if an inhibitory input reaches the neuron first and is outlasted by an excitatory input, the neuron would be expected to respond to the tail end of the excitatory input, resulting in an offset firing pattern. Alternatively, the offset response of SPON neurons may represent a rebound from strong inhibition during the stimulus. Consistent with this notion are immunocytochemical data showing that, in rats, SPON neurons receive dense axo-somatic and axo-dendritic

FIG. 6. SPON units have very low rates of spontaneous activity. The overwhelming majority of SPON units displayed spontaneous firing rates less than 6 spikes/s. The spontaneous rates of activity of 16 MNTB units are included for comparison.

FIG. 7. SPON unit response maps. Representative response maps are shown for 6 SPON units ranging in BF from 3.4 to 26.9 kHz. Tuning curves of most units displayed narrow, V-shaped peaks near BF and low-frequency tails. For the 6 units depicted, thresholds (Th) ranged from 6 to 38 dB SPL. An index of the sharpness of tuning of each unit is indicated by $Q_{10}$ and $Q_{30}$ values.
glycinergic and GABAergic inhibition (Kulesza and Berrebi 2000). The precise role of each of these inhibitory neurotransmitter systems in the generation of the offset response is currently being investigated using selective pharmacologic blockade of their receptors using a multi-barrel recording electrode configuration. Preliminary data support the notion that SPON offset responses are produced by a postinhibitory rebound mechanism (Kulesza et al. 2003).

In the basal ganglia and thalamus, so-called OFF neurons reportedly generate action potentials after a hyperpolarization, and this phenomenon has been termed “postinhibitory rebound” (Bando et al. 1980; Grenier et al. 1998; Plenz and Kital 1999). Postinhibitory rebound has been attributed to the opening of low-threshold, voltage-gated Ca\(^{2+}\) channels or to \(I_{h}\), a hyperpolarization-activated cationic current (Aizenman and Linden 1999; Cooper and Stanford 2000). The Ca\(^{2+}\) channels are reportedly de-inactivated during hyperpolarization, such as that which would be provided by a strong glycinergic inhibition, and open on the return to more depolarized membrane potentials. This results in an influx of calcium and the generation of a Ca\(^{2+}\) spike that may be capped by Na\(^{+}\) spikes. The calcium channels may be required to generate the offset-chopper and offset-sustained responses in the SPON, while sodium spikes may be sufficient to account for responses of offset-transient units. Interestingly, in the subthalamic nucleus, rebound spike bursts have been elicited in vivo by application of GABA (Plenz and Kital 1999), indicating that a hyperpolarizing inhibitory input alone is sufficient to generate this response.

**Functional implications**

**DURATION TUNING.** Duration is a biologically important feature of natural sounds and neurons with selectivity for certain sound durations have been described in the IC of bats (Casseday et al. 1994), frogs (Feng et al. 1990), and mice (Brand et al. 2000). In their most recent model, Casseday and co-workers (2000) proposed an important role for late arriving or offset inhibition in duration tuning. Moreover, this response selectivity can be abolished by application of the GABA\(_A\) receptor antagonist bicuculline in the IC, indicating that GABAergic inhibition in particular is a crucial element in the synaptic mechanisms underlying duration tuning (Casseday et al. 2000).

In rats, SPON neurons have been shown to utilize GABA as their neurotransmitter and provide an impressive projection to the IC (Kulesza and Berrebi 2000; Saldaña and Berrebi 2000). In light of the present data, it is quite possible that GABAergic inhibition derived from the SPON may contribute to the duration tuning capability of IC neurons.

Many naturally occurring rat vocalizations, including sounds elicited in threatening situations or in the context of sexual behaviors, consist of a series of 2–20 constant frequency calls in the 18- to 32-kHz range and of varying duration (from 20 ms to nearly 4 s) (Brudzynski et al. 1993). The combination of response features that we have described in SPON suggests that this cell group is particularly well suited for a role in signaling the end (offset-transient) or duration (offset-choppers and offset-sustained) of species-specific vocalizations.
**Fig. 9.** Sharpness of tuning of SPON units. **A:** $Q_{10}$ values (●) and $Q_{30}$ values (○), reflective of tuning curve sharpness, are plotted for the population of SPON units according to their BFs. Average $Q_{10}$ and $Q_{30}$ values were calculated to be $6.77 \pm 3.30$ and $1.64 \pm 0.99$, respectively. **B:** $Q_{10}$ values for SPON units are superimposed on a plot of $Q_{10}$ values previously reported for MNTB neurons in the cat (●; average $Q_{10} = 7.02$) (taken from Guinan 1968). It is apparent from the trendlines for each data set that SPON neurons have slightly wider receptive fields than MNTB neurons at all BFs.

**Fig. 10.** SPON unit thresholds. Thresholds of 60 SPON units (●) for BF tones averaged $25.41 \pm 11.53$ dB SPL and ranged from 5 to 48 dB SPL. A behavioral audiogram for the albino rat, determined from the literature (○) (from Kelly and Masterton 1977), is included for comparison.
FIG. 11. Rate-level curves of SPON neurons. Most offset-transient units (top left) and offset-chopper units (lower right) display narrow dynamic ranges. A minority of offset-transient cells (top right) and most offset-sustained units (lower left) displayed dynamic ranges >20 dB. The threshold of each unit is depicted by the upward pointing triangle, and the first plateau, the intensity at which the unit response was considered saturated, is indicated by the downward pointing triangle.

FIG. 12. SPON unit responses to sinusoidally amplitude modulated tones. PSTHs were constructed from the responses of 2 typical SPON units to 20 presentations of their BF tones at 50-, 100-, 200-, or 400-Hz rates of AM. The entire sinusoidally amplitude-modulated (SAM) stimulus was 500 ms in duration. The modulation rates and calculated vector strengths (VS) are provided at the top of each PSTH. Both units demonstrate high-fidelity phase-locking to the stimulus envelope up to 100-Hz modulation, but vector strengths decline at higher modulation rates (200 Hz for the unit depicted in B). However, even at the 400-Hz modulation rate many units, including that depicted in A, had relatively high vector strengths because their spikes, although fewer, remained well-timed to the SAM stimulus. Note the prominent offset response at the termination of the 500-ms stimulus for all modulation rates. These offset spikes were not included in the calculation of vector strengths. The unit depicted in A was classified as offset-transient and that shown in B as offset-sustained.
SOUND ENVELOPE CODING. Another interesting feature of SPON physiology is that most of its constituent neurons display phase-locked responses to SAM stimuli. In fact, SPON neurons follow 25- to 200-Hz modulation frequencies quite precisely. However, at higher modulation rates SPON neurons, although still displaying relatively high vector strengths, fail to respond to every modulation cycle as the response rate during the stimulus falls off considerably. In fact, at the highest modulation frequency tested (400 Hz) many units responded only once at the termination of the SAM stimulus. The sensitivity of SPON neurons to amplitude fluctuations in the stimulus envelope supports a role for this nucleus in the processing of complex acoustic stimuli.

Reduced phase-locking at the higher modulation frequencies may be caused by the inhibition of SPON units originating from the ipsilateral MNTB. Indirect support for this notion comes from comparing SAM responses we recorded from MNTB cells with those of SPON neurons (Fig. 14). MNTB neurons fired robustly during the stimulus with their PSTH pattern apparently carved out by the modulation frequency. Only during the short period between each wave of modulation were the MNTB neurons quiescent for a few milliseconds. Interestingly, only during this same phase of the modulation cycle, corresponding to the trough of the SAM stimulus waveform, did SPON neurons discharge their action potentials. This apparent reciprocal temporal firing pattern suggests that SPON units are capable of firing only when released from their MNTB-derived inhibition. Along the same line of reasoning, it is plausible that during stimulation at high modulation rates SPON units may have insufficient time to recover from this inhibition and thus can respond only at the termination of the SAM stimulus.

Other known sources of input to the SPON, in particular cells located in the posteroventral cochlear nucleus with onset
PSTHs that presumably correspond to excitatory octopus neurons, reportedly have excellent phase-locking capabilities to SAM stimuli (Frisina et al. 1990). However, it is unclear at this point how input from octopus cells contributes to SPON neuron responses to SAM stimuli.

Effects of GABA on response properties of inferior colliculus neurons

The multitude of synaptic inputs arriving at the central nucleus of the IC are both excitatory and inhibitory, and it is well documented that GABAergic inhibition has a profound impact on response properties of IC neurons. For example, GABAergic inputs are required for interaural intensity disparity coding, shape PSTH response properties and tuning curves (Koch and Grothe 1998; Yang et al. 1992), and lengthen first-spike latencies of IC neurons (LeBeau et al. 1996; Park and Pollak 1993). GABA has also been shown to sharpen response maps and is essential for duration tuning in the IC (Casseday et al. 1994; Fuzessery and Hall 1996).

Our findings are consistent with previous reports of a contralaterally evoked offset inhibition to the rat IC that appears to be mediated by GABA. Specifically, when the GABA_α receptor was blocked by bicuculline application to IC units, their responses were transformed from ‘on’ neurons into ‘on-off’ neurons, suggesting that GABA normally suppresses their offset activity (Faingold 2002; Faingold et al. 1986). Interestingly, ‘on-off’ neurons are more common in the IC of the genetically epilepsy-prone rat (Faingold et al. 2002; Mugnaini and Oertel 1985) and the VNLL contains approximately 1,800 presumably GABAergic neurons (Kulesza et al. 2002).

The main sources of ascending inhibition reaching the IC are the SPON and the ventral and dorsal nuclei of the lateral lemniscus (VNLL and DNNL). Unbiased stereological estimates indicate that the rat SPON contains approximately 2,400 neurons, the overwhelming majority of which are GABAergic (Kulesza and Berrebi 2000; Kulesza et al. 2002). By comparison, the rat’s DNNL contains approximately 1,800 presumably GABAergic neurons (Kulesza et al. 2002; Mugnaini and Oertel 1985) and the VNLL contains about 14,000 neurons, two-thirds of which are reportedly inhibitory (Kulesza et al. 2002; Riquelme et al. 2001). Thus the SPON represents a significant source of GABAergic inhibition to the IC. Important new clues to the functions and mechanisms of SPON neurons will be provided by future investigations of the potentially profound and wide-ranging effects of SPON-derived inhibition to the IC.

The authors thank B. Pope and J. Thompson for technical assistance. We are also grateful for the presubmission critiques of the manuscript provided by Dr. C. Portfors and our colleagues in the West Virginia University School of Medicine, Department of Neurobiology and Anatomy.

REFERENCES
