Published in final edited form as:

Neuroscience. 2012 January 10; 201: 307–319. doi:10.1016/j.neuroscience.2011.11.027.

EFFECTS OF KETAMINE ON RESPONSE PROPERTIES OF NEURONS IN THE SUPERIOR PARAOLIVARY NUCLEUS OF THE MOUSE

Richard A. Felix II, Alexander Kadner, and Albert S. Berrebi

Departments of Otolaryngology–Head and Neck Surgery, Neurobiology and Anatomy, and the Sensory Neuroscience Research Center, PO Box 9303 Health Sciences Center, West Virginia University School of Medicine, Morgantown, West Virginia 26506 USA

Abstract

The superior paraolivary nucleus (SPON; alternative abbreviation: *SPN* for the same nucleus in certain species) is a prominent brainstem structure that provides strong inhibitory input to the auditory midbrain. Previous studies established that SPON neurons encode temporal sound features with high precision. These earlier characterizations of SPON responses were recorded under the influence of ketamine, a dissociative anesthetic agent and known antagonist of NMDA receptors. Because NMDA alters neural responses from the auditory brainstem, single unit extracellular recordings of SPON neurons were performed in the presence and absence of ketamine. In doing so, this study represents the first *in-vivo* examination of the SPON of the mouse. Herein, independent data sets of SPON neurons are characterized that did or did not receive ketamine, as well as neurons that were recorded both prior to and following ketamine administration. In all conditions, SPON neurons exhibited contralaterally-driven spikes triggered by the offset of pure tone stimuli. Ketamine lowered both evoked and spontaneous spiking, decreased the sharpness of frequency tuning and increased auditory thresholds and first-spike latencies. In addition, ketamine limited the range of modulation frequencies to which neurons phase-locked to sinusoidally amplitude-modulated tones.

Keywords

anesthesia; superior olivary complex; inhibition; amplitude modulation

INTRODUCTION

The ability to precisely encode temporal sound features is a critical requirement of the central auditory system, and the superior paraolivary nucleus (SPON) appears well-suited to carry out temporal processing tasks. This nucleus has been most extensively studied in rats, where SPON neurons respond sensitively to gaps in pure tones and to modulations of sinusoidally amplitude-modulated (SAM) tones (Kadner and Berrebi, 2008). These

© 2011 IBRO. Published by Elsevier Ltd. All rights reserved.

Corresponding Author Albert S Berrebi, Ph.D., Sensory Neuroscience Research Center, PO Box 9303 – Health Sciences Center, West Virginia University School of Medicine, Morgantown, WV 26506-9303, aberrebi@hsc.wvu.edu, Phone: 304-293-2357, Fax: 304-293-7182.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

properties are consistent with the cell types from which the SPON receives its synaptic innervation (discussed in Behrend et al., 2002; Kulesza Jr. et al., 2007). Specifically, the SPON is the target of excitatory inputs from octopus and multipolar cells of the cochlear nucleus (Friauf and Ostwald, 1988; Thompson and Thompson, 1991; Schofield, 1995; Saldaña et al., 2009), which fire precisely-timed action potentials that exhibit a high degree of entrainment in response to SAM stimuli (Rhode et al., 1983; Rhode and Greenberg, 1994; Golding et al., 1995; 1999; Ferragamo et al., 1998; Oertel, 1999; Trussell, 1999). Moreover, the medial nucleus of the trapezoid body (MNTB), whose glycinergic neurons produce primary-like responses to pure tones and also phase-lock with high precision to SAM tones, provides a strong inhibitory input to the SPON (Morest, 1968; Helfert et al., 1989; Bledsoe et al., 1990; Banks and Smith, 1992; Forsythe and Barnes-Davies, 1993; Sommer et al., 1993; Smith et al., 1998; Kadner and Berrebi, 2008).

Anesthetic agents used during experimentation are known to substantially alter the behavior of auditory neurons (Kuwada et al., 1989; Schumacher et al., 2011). Previous *in-vivo* studies of SPON physiology were conducted under ketamine anesthesia (Behrend et al., 2002; Dehmel et al., 2002; Kulesza Jr. et al., 2003; 2007; Kadner et al., 2006; Kadner and Berrebi, 2008), which is known to alter brainstem auditory evoked potentials, resulting in higher peak latencies (Church and Gritzke, 1987; Smith and Mills, 1989; 1991) and elevated thresholds (van Looij et al., 2004). Both of these effects are thought to be mediated by antagonism of the N-methyl-D-aspartate glutamate receptor (NMDAR; Anis et al., 1983; Harrison and Simmonds, 1985). NMDARs are widely distributed in auditory brainstem structures, including the SPON (Petralia et al., 1994a, b; Sato et al., 1999), cochlear nucleus (Bilak et al., 1996; Sato et al., 1998; Petralia et al., 1994a, b; 2000) and MNTB (Petralia et al., 1994a, b; Sato et al., 1999; Nakagawa et al., 2000). Moreover, *in-vivo* and *in-vitro* physiological studies of the auditory brainstem and midbrain have shown that pharmacologic blockade of NMDARs reduces excitability in many cell types (*cochlear nucleus*: Martin, 1985; Isaacson and Walmsley, 1995; Ferragamo et al., 1998; *lateral superior olive*: Caspary and Faingold, 1989; Wu and Kelly, 1992; *medial superior olive*: Smith et al., 2000; *MNTB*: Forsythe and Barnes-Davies, 1993; Hamann et al., 2003; *auditory midbrain*: Faingold et al., 1989; Feldman and Knudsen, 1994; Zhang and Kelly, 2001; Wu et al., 2002; Sanchez et al., 2007). Thus, given the known effects of ketamine, it is plausible that *in-vivo* responses of SPON neurons reported previously may not accurately represent the activity of this nucleus in the unanesthetized state.

Single units were recorded in the mouse SPON, both in the absence and presence of ketamine, and the characteristic frequency, threshold, response map, sharpness of frequency tuning, and accuracy with which they phase-locked to SAM stimuli was determined. Quantitative analyses were performed to determine whether administration of ketamine anesthesia was accompanied by changes in these response properties.

This study represents the first *in-vivo* characterization of the mouse SPON. The mouse is becoming an increasingly common experimental animal for auditory studies because it affords two unique opportunities: Unlike the rat, where removing the cerebellum – which requires anesthesia – is necessary to make the brainstem practically accessible to micropipettes, the small head size of the mouse is compatible with microelectrode penetrations through the intact brain, so that recordings from the auditory brainstem are possible without anesthesia. Moreover, mice adapt well to the restraint required for an awake recording preparation (Portfors and Felix, 2005; Felix and Portfors, 2007; Bryant et al., 2009). Beyond addressing anesthesia-related concerns, establishing the mouse as an experimental model for brainstem recording studies opens future avenues for investigating the consequences of targeted genetic alterations.

EXPERIMENTAL PROCEDURES

Sound-evoked responses of single neurons in the SPON of 29 female CBA/CaJ mice (Jackson, Bar Harbor, ME, USA) were recorded. This strain has been established as a normal-hearing control in presbycusis studies, and its audiogram has been well studied (Willott, 1983). All mice were housed in the vivarium at the West Virginia University Health Sciences Center and were eight to twelve weeks of age at the time of testing. Experimental procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of West Virginia University.

Surgical procedure

Mice were initially anesthetized with isoflurane gas (1.5%; IsoFlo, Abbott, North Chicago, IL, USA) and placed in a stereotaxic apparatus using blunt ear bars to prevent damage to the tympanic membrane. Once the animal was determined to be areflexic, the scalp was incised and reflected laterally. A fine tungsten grounding pin was then inserted through the skull overlying the cortex contralateral to the recording site and cemented into place. To immobilize the head during recordings, a lightweight hollow metal head-pin was glued to the exposed skull using dental cement cured with ultraviolet light (Charisma, Heraeus Kulzer, Armonk, NY, USA). A small (1–2 mm diameter) craniotomy was then opened over the right hemisphere of the cerebellum without penetrating the dura mater; this procedure was performed while the mice were still under isoflurane anesthesia to eliminate pain associated with the craniotomy. Local anesthetic (5% Lidocaine gel, Teva, Sellersville, PA, USA) was applied topically to the margins of the open wound at this time and reapplied every two hours thereafter. The animal was returned to its cage and given one to two hours to recover from the isoflurane. During this period the mouse regained normal activity and resumed exploratory behavior within the cage.

Preparation for recordings

The mouse was then moved to a sound-attenuated chamber and placed in a molded foam “sandwich” to support its trunk while leaving the limbs unencumbered, and the previously attached head-pin was mounted onto a custom-designed stereotaxic attachment to maintain a fixed head position during the recording session (Portfors and Felix, 2005; Felix and Portfors, 2007; Felix et al., 2011). To minimize the potential for distress, animals assigned to recording in the absence of ketamine were acclimated by placement in the foam support for increasing durations over a period of several days prior to the recording session. In order to minimize stress related to head restraint, animals assigned for recording in the awake state were habituated by first attaching the head-pin briefly (5 minutes), and gradually building up to successively longer intervals of head restraint on the day of the experiment.

Manipulation of anesthetic state

In order to ascertain the influence of ketamine on SPON response properties, recordings were conducted in either the unanesthetized state (i.e., in the absence of ketamine) or under the influence of ketamine anesthesia. Typically, recordings began in the unanesthetized state. After performing the recording protocol for several unanesthetized units, the animals (while remaining in the head restraint) were administered ketamine (70 mg/kg, i.p.). Approximately 15 minutes later, a foot pinch was given to confirm the absence of a nociceptive reflex. If no reflex was elicited, we considered the animal to be in the anesthetized state and recordings commenced. Using this approach, data were obtained from independent groups of unanesthetized and ketamine-anesthetized units.

In a second set of experiments, the recording protocol was refined to include paired recordings conducted in both unanesthetized and anesthetized states in the same units. To control for sequence effects, it would have been desirable to alternate or randomize the sequence of anesthetic states such that some units were recorded prior to and then again following administration of ketamine, whereas other units were recorded first under ketamine anesthesia and then again after complete recovery. Unfortunately, the recovery period from the systemic delivery of ketamine was too long to permit recordings before and after recovery. As a consequence, all units were recorded first in the unanesthetized state.

For paired recordings, each unit was presented with a CF pure tone (20 dB above threshold) following completion of the recording protocol in the unanesthetized state, and then immediately following the ketamine injection and during the subsequent transition to the anesthetized state. The unit's response was continually monitored to be sure it was not altered by movement of the animal. Once the animal ceased to respond to the foot pinch, the complete recording protocol was repeated on the same unit in the anesthetized state.

Sedation

To facilitate placement in the restraining device some animals were lightly sedated with the phenothiazine sedative acepromazine maleate (1 mg/kg, i.p.; Henry Schein, Melville, NY, USA) (Portfors and Felix, 2005). Acepromazine inhibits dopaminergic receptors (Feinberg and Snyder, 1975; Seeman and Lee 1975), and is recommended at a dosage of 1.0–5.0 mg/kg body weight for laboratory mice (Hawk et al., 2005). Although dopamine receptors have not been localized to the mouse brainstem (Camps et al., 1990), there is evidence that tyrosine hydroxylase, an enzyme necessary for dopamine synthesis, is present in the superior olivary complex (Darrow et al., 2006). Thus, the potential exists for acepromazine to alter SPON responses through modulatory inputs originating from neurons that are innervated by dopaminergic centers but are not dopaminergic themselves. With this possibility in mind, quantitative analyses were performed to determine whether acepromazine affected SPON responses, either alone or in combination with ketamine anesthesia.

Animal Monitoring During Recordings

In the case of anesthetized animals, the depth of anesthesia was assessed by attending to the level of reflex activity and supplemental doses of ketamine were administered as needed. In animals that were either sedated or awake during recordings, stress and discomfort were closely monitored for ethical reasons and also because any body or limb movements would interrupt the neural recordings; i.e., physical movements or vocalizations would cause large shifts in the electrical signal of the recordings. Even when such baseline modifications were not detected in the recording traces, each time the experimenter entered the booth to reposition the recording electrode (maximally at 15–20 minute intervals), the mouse was examined directly for signs of distress. Our experience with this restraint procedure is that with habituation performed as described, the mice remained essentially motionless and without apparent signs of distress for periods of up to 2 hours. If signs of distress were noted, at any time, either ketamine was administered and data were obtained in the anesthetized condition, acepromazine was administered and recording continued in the sedated condition, or the experiment was terminated and the animal was euthanized. Mice were offered water with a small dropper after each electrode penetration.

Acoustic stimulation and data acquisition

Acoustic stimulus generation and data acquisition were controlled outside the recording chamber by a PC running custom-written software (BATLAB, Donald Gans, Northeast Ohio Universities College of Medicine, Rootstown, OH). To avoid unwanted onset and offset clicks, stimuli were generated with 2 ms $\cos^2$ ramps. The digital waveforms were output

through a 16-bit digital-to-analog converter (Microstar Laboratories, Bellevue, WA, USA; model DAP521a, 400,000 samples/second). Analog signals were fed to an anti-aliasing filter (Tucker Davis Technologies (TDT), Alachua, FL, USA, model FT6-2), and attenuation of acoustic signals was controlled by a programmable attenuator (TDT, model PA-5). Signals were then routed to a speaker driver (TDT, model ED1) and presented to the animal through free field speakers (TDT, model ES1) placed approximately 5 mm from the opening of the external auditory meatus. Speaker output was calibrated offline using a ¼ inch condenser microphone (Brüel & Kjær model 4939, Norcross, GA), connected to a measuring amplifier (Brüel & Kjær model 2610). To determine whether a unit was contralaterally, ipsilaterally or binaurally driven, acoustic crosstalk between the ears was estimated by presenting pure tones (0.5–60 kHz) from one speaker and comparing recordings of the tones obtained with a condenser microphone located in the positions of the ipsilateral and contralateral ears. The sound intensity measured at the position of the contralateral ear was consistently 35–40 dB lower than at the ipsilateral ear. Thus, sounds up to 35 dB above threshold were known to stimulate only the ear closest to the speaker.

Data collection and analysis procedures

The dura overlying the exposed brain surface was opened in preparation for recordings. Electrode penetrations through the SPON were guided by coordinates provided in the atlas of the mouse brain (Paxinos and Franklin, 2001). Responses from single neurons were recorded using micropipette electrodes filled with 1.5% biocytin dissolved in 0.5 M NaCl (tip diameter 2–4 µm, 10–30 MΩ resistance), and advanced through the brain using a hydraulic micropositioner (David Kopf Instruments, Tujunga, CA, USA, model 650). Action potentials were amplified (Dagan Corporation, Minneapolis, MN, USA, model 2400), and passed through a band-pass-filter (Krohn-Hite, Brockton, MA, USA, model 3364) and then to a spike enhancer (Fredrick Haer, Bowdoinham, ME, USA). The output was digitized by a 16-bit high-speed analog-to-digital converter at a rate of 42 kHz (Microstar Laboratories, model DAP5216a). Individual neural waveforms, raster plots, peristimulus time histograms (PSTHs) and statistics were displayed by BATLAB software during data acquisition, and raw waveforms were stored for offline analysis. Homogeneity of sound-evoked spike waveforms and the separation of action potential waveforms from background noise by a trigger window were required for classification as a single unit recording.

For recordings using broadband noise and pure tone stimuli, analysis windows for determining evoked spiking rates began at the stimulus onset and ended 50 ms after the stimulus offset. For the majority of units, this time window contained no spontaneous spiking. Bursts of broadband noise (50 ms duration, ~80 dB SPL, 4 presentations/second) were used as search stimuli. Once a single unit was isolated, its characteristic frequency (CF), defined as the frequency requiring the lowest intensity to elicit stimulus-evoked spikes to at least 50% of the presentations, and its minimum threshold, defined as the lowest intensity that elicited a consistent spiking response, were determined and the neuron's frequency response area was mapped. Excitatory frequency ranges were first determined by presenting pure tones covering the most sensitive region of the mouse's hearing range (1–60 kHz at 20 dB attenuation, corresponding to 70–85 dB SPL, depending on the frequency presented). A series of automated tests were then performed in which the predetermined excitatory frequency range of tone responses was presented in increments of 1 or 2 kHz, beginning at 10 dB below threshold and extending to 50 dB above threshold intensity in steps of 10 dB. From these tests Q values were determined for responses observed at 10 and 30 dB above threshold as a measure of the sharpness of frequency tuning. First spike latencies were determined from responses to CF tones presented at 20 dB above threshold. The aurality of each unit was determined by presenting 20 repetitions of broadband noise bursts at 5 dB above threshold, first monaurally to each ear, then binaurally. Sinusoidally

amplitude-modulated (SAM) tones of 500 ms duration and 100% modulation depth were presented using the unit's CF as the carrier frequency at 20 dB above threshold (1 presentation/second). The analysis window for SAM tests began at the stimulus onset and ended at the offset. Each SAM stimulus was presented eight times, and modulation frequencies (MFs) were varied from 40 to 600 Hz in 40 Hz increments. The fidelity of phase-locking at each MF was quantified by calculating the vector strength (VS):

$$VS = \frac{1}{n} \sqrt{\left[\sum \sin(a_i) \right]^2 + \left[\sum \cos(a_i) \right]^2}$$

where a_i is the phase angle of spike i relative to the modulation cycle of the stimulus, and n is the total number of spikes in the analysis window (Goldberg and Brown, 1969). A VS of 1 indicates perfect synchronization between the neuronal response and the modulation phase of the stimulus, whereas a VS of 0 indicates no correlation. In cases where responses contained two or fewer spikes per stimulus presentation, VS was set to 0 to reduce the influence of isolated spikes that were time-locked to the stimulus onset and therefore occurred at a constant phase angle (Kadner and Berrebi, 2008). The Rayleigh test (Batschelet, 1981) was used to determine whether spike distributions relative to phase angles of stimuli differed significantly from randomness. As with VS, responses that had ≤ 2 spikes per stimulus were considered unsynchronized and excluded from Rayleigh test analyses.

For all other statistical analyses, unless otherwise noted, the Mann Whitney U test was applied for comparing group means in unpaired recordings and the Wilcoxon matched-pairs test was used for comparing pre- and post-ketamine responses in paired unit recordings.

Histological localization of recording sites

To confirm that single unit recordings were located in the SPON, the neural tracer biocytin (Sigma Chemical, St. Louis, MO) was deposited during an electrode penetration in each animal. Confined tracer deposits were achieved using a current generator to deliver 1 μ A of positive ejection current (Stoelting Co., Wood Dale, IL, USA; model 51413; 5 minute duration, 50% duty cycle). Detailed descriptions of histological processing techniques and reconstruction of electrode tracts were previously described for SPON recordings in the rat (Kulesza Jr. et al., 2003; Kadner and Berrebi, 2008) and were readily applied to the mouse. Briefly, at the conclusion of each recording session, mice were perfused through the ascending aorta with phosphate-buffered 4% paraformaldehyde. Brainstems were removed from the calvaria, cryoprotected overnight in 30% sucrose, and sectioned transversely at 40 μ m using a freezing microtome. Alternating sections were either mounted and counterstained for Nissl substance to reveal the boundaries of superior olivary nuclei, or processed to reveal biocytin deposits following the Avidin:Biotinylated enzyme complex procedure (Vectastain ABC, Vector Laboratories, Burlingame, CA, USA) using diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA) as chromogen. Photographs of adjacent tissue sections stained for Nissl substance and biocytin, respectively, were overlaid, using tissue landmarks to guide alignment, and the locations of tracer deposits within the superior olivary complex were determined.

RESULTS

A total of 83 single units were recorded from the mouse SPON. Of these, 70 units were recorded in either an unanesthetized ($n=36$) or ketamine-anesthetized state ($n=34$), and are referred to as *unpaired units*. The remaining 13 units were recorded both before and after ketamine administration and are therefore referred to as *paired units*. An accurate localization of one recording site per animal was achieved by ejecting biocytin from the

electrode tip immediately following completion of the recording protocol for a single unit response (Fig. 1A). The remaining recording sites were inferred by calculating their distances from tracer deposits.

Effect of acepromazine sedation

To determine if acepromazine maleate sedation altered neuronal response properties, a factorial ANOVA was employed with sedative state and anesthetic state as grouping factors. We compared spontaneous spiking rates, evoked spiking to CF tones, CF tone thresholds, first spike latencies and Q10 values between neurons recorded with or without acepromazine in unanesthetized ($n = 28$ and 8 units, respectively) and ketamine-anesthetized ($n = 12$ and 22 units, respectively) conditions. None of the comparisons revealed a significant main effect of acepromazine (spontaneous spiking, $p = 0.83$; evoked spiking, $p = 0.17$; threshold, $p = 0.48$; latency, $p = 0.14$; Q10, $p = 0.56$) nor a significant interaction effect between acepromazine and ketamine (spontaneous spiking, $p = 0.51$; evoked spiking, $p = 0.41$; threshold, $p = 0.26$; latency, $p = 0.82$; Q10, $p = 0.09$). Because each of the response properties recorded in the presence of acepromazine was statistically equivalent to that obtained in the absence of acepromazine, the effects of sedation will not be considered further.

Responses to pure tones

Characteristic frequencies (CFs) of SPON units were distributed from 2 to 41 kHz, covering the most sensitive area of the mouse's audible range (Fig. 1B). CFs increased when the electrode was positioned medially and decreased as the electrode was shifted laterally, consistent with the known topographic arrangement of the SPON in the rat, where high CF neurons are located medially and progressively lower CF neurons are positioned laterally (Kulesza Jr. et al., 2003). Evoked spiking thresholds for responses to CF tones were significantly elevated in the presence of ketamine compared to responses recorded in the unanesthetized state for both unpaired and paired data sets (Table I; Fig. 1B).

All units exhibited offset responses with little or no spiking during the presentation of tone stimuli (Fig. 2). The three prominent offset response sub-types previously described in the rat SPON (Kulesza Jr. et al., 2003) were also identified in the mouse (Fig. 2A). *Offset-transient* responses consisting of one or two well-timed action potentials were observed in 28% of unanesthetized and 34% of anesthetized units. Units that responded with bursts of regularly spaced offset spikes were termed *offset-choppers*. The regularity of spiking for these units was assessed by analysis of first-order interspike intervals (Fig. 2A, insets). To be classified as an offset chopper, the interspike interval histogram for a response had to show a well-defined peak; 33% of unanesthetized and 45% of anesthetized units met this criterion. The third response type, termed *offset-sustained*, was characterized by a brief burst of prominent offset spiking followed by at least 20 milliseconds of weaker evoked discharges, and accounted for 39% and 21% of unanesthetized and anesthetized responses, respectively. In paired unit recordings, we noted that two neurons displaying offset-sustained spiking patterns changed to offset-choppers following the delivery of ketamine (Fig. 2B). Likewise, one offset-chopper response was transformed into an offset-transient after ketamine was applied.

In addition to elevating spiking thresholds, ketamine significantly lowered evoked and spontaneous spiking rates. Moreover, anesthetized units had increased first spike latencies compared to responses recorded without anesthesia (Table I).

Responses to broadband noise and aurality

Two distinct spiking patterns were observed in response to broadband noise stimuli (Fig. 3). One type was characterized by an offset response with very little or no spiking during the stimulus, similar to the typical response to a pure tone. The second response type, observed in 69% of unanesthetized and 53% of anesthetized units, respectively, was marked by substantial spiking during the stimulus, in addition to offset spiking. For both types of noise responses, spiking rates reached their peaks shortly after the stimulus offset. It is noteworthy that for units with this second response type, spiking during the stimulus was observed only in response to noise stimuli; when CF pure tones were presented to the same units, only offset spikes occurred (Fig. 3A).

Spiking rates to broadband noise decreased significantly in the presence of ketamine. In paired recordings, five of the ten units that initially exhibited spiking during the broadband noise stimulus displayed only offset spiking following ketamine administration (Fig. 3B).

Analysis of spike waveforms confirmed that broadband noise responses were indeed produced by the unit under study, rather than by a neighboring unit or a fiber of passage. To assess whether units that displayed peristimulus spiking to broadband noise represented a subtype of SPON unit separate from those that responded strictly to the stimulus offset, their pure tone thresholds, evoked and spontaneous spiking rates, sharpness of frequency tuning and vector strengths in response to SAM stimuli were compared for unpaired recordings. No significant differences were observed between any of these measures ($p > 0.05$ in all cases).

All SPON units responded with evoked spiking to contralateral broadband noise stimulation alone, but rarely to ipsilateral stimulation alone (Fig. 3C). Aurality tests in both unpaired and paired datasets revealed significantly different spiking rates in response to contralateral, ipsilateral, bilateral and no stimulus sound conditions ($F(3,144) = 31.93$; unanesthetized units and $F(3,136) = 20.47$ for anesthetized units; one-way ANOVAs; $F(3,52) = 19.4$, paired recordings; repeated measures ANOVA, all p values < 0.001). In each case, spiking in response to contralateral noise was significantly higher than to ipsilateral noise (Fig. 3C; Tukey post hoc test, $p < 0.001$ for both group comparisons), but statistically equivalent to bilateral stimulation (Tukey, $p > 0.99$ for both groups). Furthermore, there was no significant difference in response magnitude between the no sound and ipsilateral noise conditions (Tukey, $p > 0.99$ for both groups). Thus, the mouse SPON is monaurally driven by input to the contralateral ear.

Ketamine administration resulted in significant decreases in spiking in response to contralateral and bilateral noise compared to the unanesthetized state (unpaired: $p < 0.01$ for both cases; paired: $p = 0.02$ and 0.03 , respectively) (Fig. 3C). However, ketamine had no impact on spiking to no sound and to ipsilateral noise presentation (unpaired: $p = 0.13$ and 0.17 , respectively; paired: $p = 0.09$ and 0.13). Overall, the aurality of all responses remained unchanged regardless of anesthetic state, i.e., all responses were monaural and contralaterally driven.

Frequency response maps

Complete frequency response maps were obtained for 44 unanesthetized and 46 anesthetized units. For unanesthetized recordings, response maps were consistently V-shaped with low-frequency tails (Fig. 4A). Q values were calculated to assess the sharpness of tuning for each unit and to compare between anesthetic states. Overall, ketamine significantly broadened frequency tuning at 10 dB (Table I) and 30 dB above threshold (not shown; $p < 0.03$ for both unpaired and paired unit recordings) (Fig. 4B). For all units, offset responses were consistently observed throughout the response maps. Despite the fact that threshold shifts

were evident following ketamine treatment, tuning curves generally retained their characteristic broad shapes.

Responses to sinusoidally amplitude modulated tones

Responses to the full sequence of SAM tones, ranging from 40–600 Hz modulation frequency (MF), were recorded from 39 unanesthetized and 43 ketamine-anesthetized SPON neurons, representing the full range of CFs and spiking patterns in the data sets. Responses to SAM stimuli clearly displayed periodic spiking, indicating evoked spiking to individual modulation cycles, particularly at lower MFs (Fig. 5). Analysis of first-order interspike intervals for phase-locked responses revealed that at low MFs, a large proportion of spikes were separated by the duration of one modulation cycle. Moreover, interspike intervals representing multiples of the cycle duration also occurred, indicating that responses often skipped modulation cycles (Fig. 5A). As MFs increased, fewer spikes were elicited, and as a result, the evoked response skipped increasingly more modulation cycles. However, in some cases entrainment to SAM stimuli, as measured by vector strengths, remained high, even when spiking rates were low.

When presented with SAM tones, SPON units exhibited pre-ketamine responses that were phase-locked over a range of 40 to 440 Hz MF ($p < 0.05$ in all cases, Rayleigh test); after ketamine treatment, this range was reduced to 40–120 Hz MF (Figs. 5B and 6). Moreover, ketamine delivery caused a reduction in vector strengths for nearly all MFs tested ($p < 0.05$, Mann Whitney U and Wilcoxon signed-ranks test; Fig. 6). Spiking rates were also generally lower after ketamine administration, but this difference was only significant for MFs below 240 Hz ($p < 0.05$ for both unpaired and paired data sets).

Summary of Results

The effects of ketamine on SPON response properties for units recorded in only one anesthetic state (i.e., unpaired recordings) were consistent with those observed in units for which paired recordings were made (Fig. 7, Table I). Thresholds, spiking rates and first-spike latencies in response to pure tone stimuli were altered significantly by ketamine administration (Fig. 7A–C). In addition, spiking to broadband noise significantly decreased in the presence of ketamine (Fig. 7D). Furthermore, the sharpness of frequency tuning was lower in the anesthetized state (Fig. 7E), and vector strengths in response to SAM tones presented at each unit's best modulation frequency (BMF) decreased significantly following ketamine application (Fig. 7F).

DISCUSSION

Mouse SPON response properties

This study represents the first systematic investigation of the physiological characteristics of mouse SPON neurons *in vivo*. These neurons responded to the offset of pure tones with transient, chopper-like or sustained spiking. Responses to noise stimuli often contained additional spikes that occurred during the stimulus presentation, in addition to the characteristic offset response. All units responded to monaural stimuli presented to the contralateral ear and frequency response maps were generally V-shaped with low frequency tails. At low MFs, SPON responses to SAM stimuli were characterized by spiking that was entrained to fluctuations of the stimulus envelope. At higher MFs, responses to SAM tones contained fewer spikes, and as a result, had lower vector strengths. Moreover, as the MFs were systematically increased, SPON responses progressively skipped more modulation cycles until eventually the response ceased entirely. To explain similar phenomena, it has been proposed that increasing MFs narrows the time windows during which SPON neurons are released from MNTB-derived inhibition, thereby resulting in fewer elicited rebound

spikes, and in turn, lower vector strengths (Grothe, 1994; Kadner and Berrebi, 2008; discussed in detail below).

Effect of ketamine on SPON responses

Ketamine acts as an NMDA receptor (NMDAR) antagonist (Anis et al., 1983; Harrison and Simmonds, 1985) and also blocks GABA_A receptors (Kurt et al., 2008) as well as HCN1 cation channels (Chen et al., 2009). Relevant to the present results, HCN1 channels in conjunction with T-type Ca²⁺ channels were recently shown by us and others to be the primary mediators of SPON offset spiking (Felix et al., 2011; Kopp-Scheinpflug et al., 2011), raising the possibility that HCN1 channel blockade may account for some or all of the ketamine effects observed in the present study. However, in our recent *in-vivo* recordings from ketamine anesthetized mice, application of ZD7288, a blocker of HCN1 channels, significantly decreased SPON offset spiking and increased spiking that occurred during the stimulus presentation (Felix et al., 2011). Thus, at the dosage administered systemically (sufficient to induce general anesthesia) ketamine was not very effective at blocking Ih/HCN1 channels. Moreover, upon concurrent CNQX and AP5 blockade of NMDA and AMPA receptors *in-vitro*, although Kopp-Scheinpflug and colleagues (2011) observed well-timed offset action potentials in SPON neurons, the magnitude of the offset response appeared diminished (see their Fig. 1H). Consistent with this, our own unpublished *in-vivo* recordings confirm that blockade of NMDA neurotransmission with CPP reduces SPON offset spiking by ~22%, without changing its temporal characteristics (Felix, 2011). Therefore, while we cannot exclude the possibility that ketamine's effects on SPON neurons may have been mediated, in part, by blockade of HCN1 channels, for the reasons enumerated above we conclude that the primary mechanism of action of ketamine in the present study was through NMDAR blockade.

Blocking NMDARs may decrease the magnitude of MNTB neuronal responses, thereby weakening the post inhibitory rebound mechanism known to underlie the well-timed transient component of SPON offset spiking (Kulesza Jr. et al., 2007; Felix et al., 2011). Moreover, the shifts observed from sustained spiking patterns to more transient responses upon ketamine application may be caused by effectively removing sources of long lasting NMDAR-mediated excitation, which originates in large part from multipolar and globular bushy cells of the contralateral cochlear nucleus (Saldaña et al., 2009). Specifically, sustained offset responses could be created by a combination of MNTB-driven rebound spiking mediated through glycine receptors, and cochlear nucleus afferent inputs mediated through NMDARs, with the latter possibly outlasting the stimulus by tens of milliseconds in the absence of ketamine (Dale and Roberts, 1985; Forsythe and Westbrook 1988). Following ketamine application, these sustained responses would become more transient as NMDAR-mediated spiking decreases and glycine receptor-mediated transient spiking remains intact. In contrast, offset-chopper responses are likely governed primarily by inhibition, as similar responses have been observed in the mouse SPON *in vitro*, requiring only release from a sustained hyperpolarizing current stimulus (Felix et al., 2011). Apart from MNTB-derived glycinergic inhibition, GABAergic inputs may also contribute to forming offset-choppers *in vivo*. A role for GABA_A receptors underlying this response type may explain why ketamine changes some offset-chopper responses into offset-transients, presumably through its antagonism of the GABA_A receptors (Kurt et al., 2008). In line with this view, decreasing the strength of a hyperpolarizing current stimulus *in vitro* reduces the rebound response generated by SPON neurons, typically resulting in fewer evoked offset spikes SPON neu (Felix et al., 2011).

Over half of SPON neurons in the present study exhibited spiking during the presentation of a broadband noise stimulus, but not during pure tones. Moreover, ketamine abolished this peri-stimulus noise response in half of the paired unit recordings in which it was observed.

This observation suggests that the NMDAR mediates this response component, at least in part. Activation of NMDARs may depolarize neurons sufficiently, so that only a small contribution from a post-inhibitory rebound is needed to produce spikes. Such rebounds may be generated by fluctuations in the envelope of the noise stimulus, whereas pure tone stimuli would produce rebounds only at the offset. Because we used frozen noise, i.e. a waveform whose exact structure is repeated in each stimulus presentation, spiking tended to occur at the same time during each stimulus presentation, which may have given the response a structured appearance. Previous recordings in the rat SPON have always employed ketamine in the anesthetic regimen, but it is nonetheless difficult to reconcile why a peristimulus noise response was never observed in rats (Kulesza Jr. et al., 2003). One possible explanation for this discrepancy may be that NMDAR-mediated excitation of SPON neurons is more prominent in the mouse, whereas the rat SPON is driven mainly by the post-inhibitory rebound occurring at the end of a stimulus. Unfortunately, while the afferent and efferent connectivity of the rat SPON has been extensively investigated in tract-tracing studies (Saldaña and Berrebi, 2000; Saldaña et al., 2009; Viñuela et al., 2011), very little is currently known about the sources and relative abundance of synaptic inputs to this nucleus in the mouse, making direct comparisons between these species difficult.

In the absence of ketamine, mouse SPON neurons exhibited low-pass modulation transfer functions in response to SAM tones, and generally responded with entrainment to every MF to which there was sufficient spiking. Following ketamine application, the low-pass shape of SAM modulation transfer functions remained unchanged, with units responding with phase-locking to low MFs. However, the upper limit of MFs to which units were phase-locked was reduced after the introduction of ketamine, indicating that NMDAR-mediated excitation is also needed for SPON neurons to respond to higher MFs.

Comparison of mouse SPON physiology with other species

The majority of brainstem offset units have been reported in the SPON and its presumed homologue, the dorsal-medial paraolivary nucleus (DMPO; discussed in Saldaña and Berrebi, 2000). The bat medial superior olive (MSO) resembles the SPON/DMPO in several respects, including receiving its primary excitatory and inhibitory inputs from the contralateral cochlear nucleus and ipsilateral MNTB, respectively (Cant and Casseday, 1986; Covey et al., 1991), and the presence of offset responses that synchronize to SAM stimuli, particularly at low MFs (Grothe, 1994). Thus, the bat MSO is included in our discussion of SPON offset responses.

Many notable features of offset responses are shared across mammalian species. For instance, most studies in the superior olivary complex reported offset responses that were monaural (rabbit: Kuwada and Batra, 1999; gerbil: Dehmel et al., 2002; rat: Kulesza Jr. et al., 2003; 2007; Kadner and Berrebi, 2008). In addition, the rat SPON (Kulesza Jr. et al., 2003), cat DMPO (Guinan et al., 1972) and bat MSO (Grothe, 1994) exhibited low rates of spontaneous activity, similar to responses in the mouse SPON. All SPON response types seen in the mouse were also observed in similar proportions in the rat (Kulesza Jr. et al., 2003). Units in the cat DMPO also displayed offset-transient spiking patterns, while gerbil SPON units possessed patterns resembling offset-transient and offset-chopper types (Dehmel et al., 2002). Offset-sustained units were also reported in the DMPO of the rabbit (Kuwada and Batra, 1999), but had a much more robust sustained component compared to the mouse. Furthermore, responses of gerbil SPON units had V-shaped frequency tuning curves and elevated thresholds, relative to their behavioral audiogram (Dehmel et al., 2002). Despite the similarities of offset responses in these studies, several confounding differences exist (discussed in Behrend et al., 2002; Kulesza Jr. et al., 2003; Kadner and Berrebi, 2008; Pollak et al., 2011) and likely reflect evolutionary pressures that caused certain species to

develop specializations for hearing at high (e.g., mice, rats, bats) versus low (gerbils) frequencies (Rosowski et al., 1999).

Low-pass SAM modulation transfer functions similar to those observed herein were reported for the bat MSO (Grothe, 1994), and were explained by the timing of excitatory and inhibitory inputs relative to the occurrence of modulation cycles. In the rat, Kadner and Berrebi (2008) described a mechanism whereby cycles of SAM modulations elicit responses in the MNTB, which then cause spiking in the SPON via a post-inhibitory rebound mechanism. When the modulation frequency reaches an upper limit, overlapping inhibition from the previous cycle suppresses rebound spiking, and the SPON ceases to respond. The same type of low-pass SAM modulation transfer function seen in the bat MSO and rat SPON has also been described in the rabbit DMPO (Kuwada and Batra, 1999). Thus, offset responses to SAM stimuli in the mouse SPON are consistent with those from other species, suggesting a conserved role for this type of neuron in temporal processing.

We recorded from SPON neurons in mouse in the presence and absence of ketamine.
Ketamine lowered evoked and spontaneous spiking in SPON neurons.
Ketamine increased auditory thresholds and first spike latencies in SPON neurons.
Ketamine decreased spike count and response synchronization to SAM stimuli. SAM stimuli

REFERENCES

- Anis NA, Berry SC, Burton NR, Lodge D. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *Br J Pharmacol.* 1983; 79:565–575. [PubMed: 6317114]
- Banks MI, Smith PH. Intracellular recordings from neurobiotin-labeled cells in brain slices of the rat medial nucleus of the trapezoid body. *J Neurosci.* 1992; 12:2819–2837. [PubMed: 1351938]
- Batschelet, E. Circular statistics in biology. London: Academic Press; 1981.
- Behrend O, Brand A, Kapfer C, Grothe B. Auditory response properties in the superior paraolivary nucleus of the gerbil. *J Neurophysiol.* 2002; 87:2915–2928. [PubMed: 12037195]
- Bilak MM, Bilak SR, Morest DK. Differential expression of N-methyl-D-aspartate receptor in the cochlear nucleus of the mouse. *Neuroscience.* 1996; 75:1075–1097. [PubMed: 8938743]
- Bledsoe SC Jr, Snead CR, Helfert RH, Prasad V, Wenthold RJ, Altschuler RA. Immunocytochemical and lesion studies support the hypothesis that the projections from the medial nucleus of the trapezoid body to the lateral superior olive is glycinergic. *Brain Res.* 1990; 517:189–194. [PubMed: 2375987]
- Bryant JL, Roy S, Heck DH. A technique for stereotaxic recordings of neuronal activity in awake head-restrained mice. *J Neurosci Meth.* 2009; 178:75–79.
- Camps M, Kelly PH, Palacios JM. Autoradiographic localization of dopamine D1 and D2 receptors in the brain of several mammalian species. *J Neural Transm Gen Sect.* 1990; 80:105–127. [PubMed: 2138461]
- Cant NB, Casseday JH. Projections from the anteroventral cochlear nucleus to the lateral and medial superior olivary nuclei. *J Comp Neurol.* 1986; 247:457–476. [PubMed: 3722446]
- Caspary DM, Faingold CL. Non-N-methyl-D-aspartate receptors may mediate ipsilateral excitation at lateral superior olivary synapses. *Brain Res.* 1989; 503:83–90. [PubMed: 2558777]
- Chen X, Shu S, Bayliss DA. HCN1 channel subunits are a molecular substrate for hypnotic actions of ketamine. *J Neurosci.* 2009; 29:600–609. [PubMed: 19158287]
- Church MW, Gritzke R. Effects of ketamine anesthesia on the rat brain-stem auditory evoked potential as a function of dose and stimulus intensity. *Electroencephalogr Clin Neurophysiol.* 1987; 67:570–583. [PubMed: 2445550]

- Covey E, Vater M, Casseday JH. Binaural properties of single units in the superior olivary complex of the mustached bat. *J Neurophysiol.* 1991; 66:1080–1094. [PubMed: 1721651]
- Dale N, Roberts A. Dual-component amino-acid-mediated synaptic potentials: Excitatory drive for swimming in *xenopus* embryos. *J Physiol.* 1985; 363:35–59. [PubMed: 2862278]
- Darrow KN, Simons EJ, Dodds L, Liberman MC. Dopaminergic innervation of the mouse inner ear: evidence for a separate cytochemical group of cochlear efferent fibers. *J Comp Neurol.* 2006; 498:403–414. [PubMed: 16871528]
- Dehmel S, Kopp-Scheinflug C, Dorrscheidt GJ, Rubsamen R. Electrophysiological characterization of the superior paraolivary nucleus in the Mongolian gerbil. *Hear Res.* 2002; 172:18–36. [PubMed: 12361864]
- Ehret G. Development of absolute auditory thresholds in the house mouse (*Mus musculus*). *J Am Audiol Soc.* 1976; 1:179–184. [PubMed: 956003]
- Faingold CL, Hoffmann WE, Caspary DM. Effects of excitant amino acids on acoustic responses of inferior colliculus neurons. *Hear Res.* 1989; 40:127–136. [PubMed: 2570054]
- Feinberg AP, Snyder SH. Phenothiazine drugs: structure-activity relationships explained by a confirmation that mimics dopamine. *Proc Natl Acad Sci USA.* 1975; 72:1899–1903. [PubMed: 239403]
- Feldman DE, Knudsen EI. NMDA and non-NMDA glutamate receptors in auditory transmission in the barn owl inferior colliculus. *J Neurosci.* 1994; 14:5939–5958. [PubMed: 7931555]
- Felix, RA, II. Doctoral Thesis. West Virginia University; 2011. Encoding of temporal sound features in the rodent superior paraolivary nucleus. Online Abstract, <http://hdl.handle.net/10450/12467>
- Felix RA II, Portfors CV. Excitatory, inhibitory and facilitatory frequency response areas in the inferior colliculus of hearing impaired mice. *Hear Res.* 2007; 228:212–229. [PubMed: 17412539]
- Felix RA II, Fridberger A, Leijon S, Berrebi AS, Magnusson AK. Sound rhythms are encoded by postinhibitory rebound spiking in the superior paraolivary nucleus. *J Neurosci.* 2011; 31:12566–12578. [PubMed: 21880918]
- Ferragamo MJ, Golding NL, Oertel D. Synaptic inputs to stellate cells in the ventral cochlear nucleus. *J Neurophysiol.* 1998; 79:51–63. [PubMed: 9425176]
- Forsythe ID, Barnes-Davies M. The binaural auditory pathway: excitatory amino acid receptors mediate dual timecourse excitatory postsynaptic currents in the rat medial nucleus of the trapezoid body. *Proc Biol Sci.* 1993; 251:151–157. [PubMed: 8096081]
- Forsythe ID, Westbrook GL. Slow excitatory postsynaptic currents mediated by *N*-methyl-D-Aspartate receptors on cultured mouse central neurones. *J Physiol.* 1988; 396:515–533. [PubMed: 2900892]
- 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.* 1988; 73:263–284. [PubMed: 3215304]
- Goldberg JM, Brown PB. Response of binaural neurons of dog superior olivary complex to dichotic tonal stimuli: some physiological mechanisms of sound localization. *J Neurophysiol.* 1969; 32:613–636. [PubMed: 5810617]
- Golding NL, Ferragamo MJ, Oertel D. Role of intrinsic conductances underlying responses to transients in octopus cells of the cochlear nucleus. *J Neurosci.* 1999; 19:2897–2905. [PubMed: 10191307]
- Golding NL, Robertson D, Oertel D. Recordings from slices indicate that octopus cells of the cochlear nucleus detect coincidence firing of auditory nerve fibers with temporal precision. *J Neurosci.* 1995; 15:3138–3153. [PubMed: 7722652]
- 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.* 1994; 71:706–721. [PubMed: 8176433]
- Hamann M, Billups B, Forsythe ID. Non-calyceal excitatory inputs mediate low fidelity synaptic transmission in rat auditory brainstem slices. *Eur J Neurosci.* 2003; 18:2899–2902. [PubMed: 14656340]
- Harrison NL, Simmonds MA. Quantitative studies on some antagonists of *N*-methyl D-TL, aspartate in slices of rat cerebral cortex. *Br J Pharmacol.* 1985; 84:381–391. [PubMed: 2858237]
- Hawk, TC.; Leary, SL.; Morris, TH. Formulary for laboratory animals. Aimes: Blackwell; 2005.

- Helfert RH, Bonneau JM, Wenthold RJ, Altschuler RA. GABA and glycine immunoreactivity in the guinea pig superior olivary complex. *Brain Res.* 1989; 501:269–286. [PubMed: 2819441]
- Isaacson JS, Walmsley B. Receptors underlying excitatory synaptic transmission in slices of the rat anteroventral cochlear nucleus. *J Neurophysiol.* 1995; 73:964–973. [PubMed: 7608781]
- Kadner A, Berrebi AS. Encoding of temporal features of auditory stimuli in the medial nucleus of the trapezoid body and superior paraolivary nucleus of the rat. *Neuroscience.* 2008; 151:868–887. [PubMed: 18155850]
- 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.* 2006; 95:1499–1508. [PubMed: 16319207]
- Kopp-Scheinpflug C, Tolnai S, Malmierca MS, Rubsamen R. The medial nucleus of the trapezoid body: comparative physiology. *Neuroscience.* 2008; 154:160–170. [PubMed: 18436383]
- Kopp-Scheinpflug C, Tozer AJ, Robinson SW, Tempel BL, Hennig MH, Forsythe ID. The sound of silence: ionic mechanisms encoding sound termination. *Neuron.* 2011; 71:911–925. [PubMed: 21903083]
- Kulesza RJ Jr, Berrebi AS. Superior paraolivary nucleus of the rat is a GABAergic nucleus. *J Assoc Res Otolaryngol.* 2000; 1:255–269. [PubMed: 11547806]
- Kulesza RJ Jr, Kadner A, Berrebi AS. Distinct roles for glycine and GABA in shaping response properties of neurons in the superior paraolivary nucleus of the rat. *J Neurophysiol.* 2007; 97:1610–1620. [PubMed: 17122321]
- Kulesza RJ Jr, Spirou GA, Berrebi AS. Physiological response properties of neurons in the superior paraolivary nucleus of the rat. *J Neurophysiol.* 2003; 89:2299–2312. [PubMed: 12612016]
- Kurt S, Moeller CK, Jeschke, Schulze H. Differential effects of iontophoretic application of the GABAA-antagonists bicuculline and gabazine on tone-evoked local field potentials in primary auditory cortex: interaction with ketamine anesthesia. *Brain Res.* 2008; 1220:58–69. [PubMed: 18022607]
- Kuwada S, Batra R. Coding of sound envelopes by inhibitory rebound in neurons of the superior olivary complex in the unanesthetized rabbit. *J Neurosci.* 1999; 19:2273–2287. [PubMed: 10066278]
- Kuwada S, Batra R, Stanford TR. Monaural and binaural response properties of neurons in the inferior colliculus of the rabbit: effects of sodium pentobarbital. *J Neurophysiol.* 1989; 61:269–82. [PubMed: 2918355]
- Martin MR. Evidence for an excitatory amino acid as the transmitter of the auditory nerve in the in vitro mouse cochlear nucleus. *Hear Res.* 1985; 20:215–220. [PubMed: 2867986]
- Morest DK. The collateral system of the medial nucleus of the trapezoid body of the cat, its neuronal architecture and relation to the olivo-cochlear bundle. *Brain Res.* 1968; 9:288–311. [PubMed: 5679830]
- Nakagawa H, Sato K, Shiraishi Y, Kuriyama H, Altschuler RA. NMDAR1 isoforms in the rat superior olivary complex and changes after unilateral cochlear ablation. *Brain Res Mol Brain Res.* 2000; 77:246–257. [PubMed: 10837919]
- Nelson PC, Smith ZM, Young ED. Wide-dynamic-range forward suppression in marmoset inferior colliculus neurons is generated centrally and accounts for perceptual masking. *J Neurosci.* 2009; 29:2553–2562. [PubMed: 19244530]
- Oertel D. The role of timing in the brain stem auditory nuclei of vertebrates. *Ann Rev Physiol.* 1999; 61:497–519. [PubMed: 10099699]
- Ollo C, Schwartz IR. The superior olivary complex in C57BL/6 mice. *Am J Anat.* 1979; 155:349–373. [PubMed: 474450]
- Paxinos, G.; Franklin, K. The mouse brain in stereotaxic coordinates. San Diego: Academic Press; 2001.
- Petralia RS, Rubio ME, Wang YX, Wenthold RJ. Differential distribution of glutamate receptors in the cochlear nuclei. *Hear Res.* 2000; 147:59–69. [PubMed: 10962173]
- Petralia RS, Wang YX, Wenthold RJ. The NMDA receptor subunits NR2A and NR2B show histological and ultrastructural localization patterns similar to those of NR1. *J Neurosci.* 1994a; 14:6102–6120. [PubMed: 7931566]

- Petralia RS, Yokotani N, Wenthold RJ. Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. *J Neurosci.* 1994b; 14:667–696. [PubMed: 8301357]
- Pollak GD, Gittelman JX, Li N, Xie R. Inhibitory projections from the ventral nucleus of the lateral lemniscus and superior paraolivary nucleus create directional selectivity of frequency modulations in the inferior colliculus: A comparison of bats with other mammals. *Hear Res.* 2011; 273:134–144. [PubMed: 20451594]
- Portfors CV, Felix RA II. Spectral integration in the inferior colliculus of the CBA/CaJ mouse. *Neuroscience.* 2005; 136:1159–1170. [PubMed: 16216422]
- Rhode WS, Greenberg S. Encoding of amplitude modulation in the cochlear nucleus of the cat. *J Neurophysiol.* 1994; 71:1797–1825. [PubMed: 8064349]
- Rhode WS, Oertel D, Smith PH. Physiological response properties of cells labeled intracellularly with horseradish peroxidase in cat ventral cochlear nucleus. *J Comp Neurol.* 1983; 213:448–463. [PubMed: 6300200]
- Rosowski JJ, Ravicz ME, Teoh SW, Flandermeyer D. Measurements of middle-ear function in the Mongolian gerbil, a specialized mammalian ear. *Audiol Neurotol.* 1999; 4:129–136. [PubMed: 10187920]
- Saldaña E, Berrebi AS. Anisotropic organization of the rat superior paraolivary nucleus. *Anat Embryol (Berl).* 2000; 202:265–279. [PubMed: 11000278]
- Saldaña E, Aparicio MA, Fuentes-Santamaria V, Berrebi AS. Connections of the superior paraolivary nucleus of the rat: projections to the inferior colliculus. *Neuroscience.* 2009; 163:372–387. [PubMed: 19539725]
- Sanchez JT, Gans D, Wenstrup JJ. Contribution of AMPA and NMDA receptors to temporal patterning of auditory responses in the inferior colliculus. *J Neurosci.* 2007; 27:1954–1963. [PubMed: 17314291]
- Sato K, Kuriyama H, Altschuler RA. Differential distribution of NMDA receptor subunit mRNA in the rat cochlear nucleus. *Microsc Res Tech.* 1998; 41:217–223. [PubMed: 9605339]
- Sato K, Nakagawa H, Kuriyama H, Altschuler RA. Differential distribution of N-methyl-D-aspartate receptor-2 subunit messenger RNA in the rat superior olivary complex. *Neuroscience.* 1999; 89:839–853. [PubMed: 10199617]
- Schofield BR. Projections from the cochlear nucleus to the superior paraolivary nucleus in guinea pigs. *J Comp Neurol.* 1995; 360:135–149. [PubMed: 7499559]
- Schumacher JW, Schneider DM, Woolley SMN. Anesthetic state modulates excitability but not spectral tuning or neural discrimination in single auditory midbrain neurons. *J. Neurophysiol.* 2011; 106:500–514.
- Seeman P, Lee T. Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. *Science.* 1975; 188:1217–1219. [PubMed: 1145194]
- Smith AJ, Owens S, Forsythe ID. Characterisation of inhibitory and excitatory postsynaptic currents of the rat medial superior olive. *J Physiol.* 2000; 529:681–698. [PubMed: 11118498]
- Smith DI, Mills JH. Anesthesia effects: auditory brain-stem response. *Electroencephalogr Clin Neurophysiol.* 1989; 72:422–428. [PubMed: 2469566]
- Smith DI, Mills JH. Low-frequency component of the gerbil brainstem response: response characteristics and anesthesia effects. *Hear Res.* 1991; 54:1–10. [PubMed: 1917708]
- Smith PH, Joris PX, Yin TCT. Anatomy and physiology of principal cells of the medial nucleus of the trapezoid body (MNTB) of the cat. *J Neurophysiol.* 1998; 79:3127–3142. [PubMed: 9636113]
- 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.* 1993; 95:223–239. [PubMed: 8224048]
- Sonntag M, Englitz B, Kopp-Scheinpflug C, Rübsamen R. Early postnatal development of spontaneous and acoustically evoked discharge activity of principal cells of the medial nucleus of the trapezoid body: an in vivo study in mice. *J Neurosci.* 2009; 29:9510–9520. [PubMed: 19641114]

- 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.* 1991; 311:495–508. [PubMed: 1757599]
- Trussell LO. Synaptic mechanisms for coding timing in auditory neurons. *Ann Rev Physiol.* 1999; 61:477–496. [PubMed: 10099698]
- van Looij MA, Liem SS, van der Burg H, van der Wees J, De Zeeuw CI, van Zanten BG. Impact of conventional anesthesia on auditory brainstem responses in mice. *Hear Res.* 2004; 193:75–82. [PubMed: 15219322]
- Viñuela A, Aparicio M-A, Berrebi AS, Saldaña E. Connections of the superior paraolivary nucleus of the rat: II. Reciprocal connections with the tectal longitudinal column. *Front Neuroanat.* 2011; 5:1–15. [PubMed: 21373368]
- Willott, JF. The auditory psychobiology of the mouse. Springfield: Charles C Thomas; 1983.
- Wu SH, Kelly JB. Synaptic pharmacology of the superior olivary complex studied in mouse brain slice. *J Neurosci.* 1992; 12:3084–3097. [PubMed: 1494947]
- Wu SH, Ma CL, Sivaramakrishnan S, Oliver DL. Synaptic modification of neurons of the central nucleus of the inferior colliculus. *Hear Res.* 2002; 168:43–54. [PubMed: 12117508]
- Zhang H, Kelly JB. AMPA and NMDA receptors regulate glutamate responses of neurons in the rat's inferior colliculus. *J Neurophysiol.* 2001; 86:871–880. [PubMed: 11495957]

FIG. 1.

A: SPON recording sites were marked by ejecting biocytin from recording pipettes during each experiment. Counterstaining with Neutral Red facilitated accurate delineation of the boundaries of nuclei within the superior olivary complex. Retrogradely labeled neuronal cell bodies were often seen in the ipsilateral MNTB. B: Distribution of thresholds and characteristic frequencies of unpaired and paired single unit recordings in the mouse SPON made either with ketamine (Ket +) or without ketamine anesthetic (Ket -). A behavioral audiogram of the mouse is outlined in each panel in B for comparison (from Ehret, 1976). Abbreviations: M: medial; D: dorsal; MNTB: medial nucleus of the trapezoid body; LSO: lateral superior olive.

FIG. 2.

Classification of the main response types observed in the mouse SPON. All units responded with offset spiking, with very little or no response during the presentation of CF tone stimuli. Insets in each panel show corresponding first order interspike interval histograms.

A: The most common response type was termed *offset-transient*, and consisted of one or two well-timed spikes triggered by the stimulus offset. The corresponding interspike interval histogram shows that when offset-transient units generate multiple spikes, the interspike interval is variable (top panel). The middle panel shows an example of a response consisting of a burst of offset spikes, with regularly timed spacing between spikes, as shown by the defined peak in the corresponding interspike interval plot. These units were classified as *offset-chopper*. A third type of discharge pattern was characterized by prominent transient offset spiking followed by a weaker sustained response that always exceeded 20 ms. These responses were classified as *offset-sustained*. Offset-sustained responders did not generate regularly spaced action potentials, as shown in the corresponding interspike interval plot (lower panel). **B:** Example of responses to CF pure tones obtained from a paired SPON unit recording directly prior to (Ket -) and following ketamine administration (Ket +).

Horizontal bars in each plot represent the time course of the pure tone stimulus.

Abbreviations: Thr, minimum threshold; Latency, first spike latency.

FIG. 3.

Comparison of responses to pure tones and broadband noise and aural localization of responses. *A*: Typical SPON unit responses to CF pure tone stimuli, marked by an absence of spiking during the stimulus and a transient offset response (*left panels*). The unit depicted in the top panels also responded to a broadband noise stimulus with a similar offset-transient response (*right panel*). However, the majority of SPON responses (~60%), regardless of anesthetic state, exhibited spiking during the presentation of broadband noise, in addition to a typical transient offset response, as shown in the lower panel. The response during the noise stimulus was characterized by a long latency relative to the stimulus onset and an intermittent spiking pattern. Note that when units that responded during noise stimulation

were presented with CF pure tones, spiking during the stimulus ceased and only the offset response was elicited. *B*: Responses from a paired unit recording to broadband noise before and after ketamine delivery. *C*: Mean spike rates shown for the four broadband noise stimulus conditions: no sound, stimuli presented to the contralateral ear alone, stimuli presented to the ipsilateral ear alone, and stimuli presented to both ears (bilateral). All units, regardless of anesthetic state, exhibited monaural, contralaterally-driven responses. Ketamine lowered spike rates in response to contralateral and bilateral stimulation. Error bars represent the standard errors of the means (* $p < 0.05$, Mann-Whitney U and Wilcoxon matched pairs test). Horizontal bars in *A*, *B* represent the time course of stimuli in the recording window. Thresholds for broadband noise stimuli are approximations based on the output characteristics of the speakers.

FIG. 4.

A: Typical frequency response maps of mouse SPON units. CFs for the examples shown ranged from 13–41 kHz, spanning most of the range of CFs recorded in the population of units. With few exceptions, response maps were broadly tuned and V-shaped near CF. Prominent low-frequency tails and sharp slopes at the high frequency ends of the response maps were also typical. *B*: Response maps for a paired single-unit recording before and after application of ketamine anesthesia.

FIG. 5.

SPON responses to sinusoidally amplitude-modulated (SAM) tones. *A*: Left panels show peristimulus time histograms (PSTHs) of responses to SAM tones at increasing modulation frequencies (MFs). Vector strengths (VSs) to all MFs were high in this example (p values < 0.05 , Rayleigh test (Batschelet, 1981); indicating significant phase locking), although spiking decreased in response to the highest MF SAM tone. First order interspike interval histograms are shown beside the corresponding PSTHs. *B*: The response to a SAM tone (120 Hz MF) in a paired recording decreased following ketamine treatment, accompanied by a substantial decrease in the vector strength. Horizontal waveforms indicate the time course of SAM stimuli within the recording window. Vertical dashed lines in *A* denote multiples of the duration of amplitude modulation cycles.

FIG. 6.

Mean vector strengths and spikes per stimulus presentation plotted as a function of modulation frequency for unpaired (left panels) and paired (right panels) recordings in unanesthetized (Ket $-$) and ketamine-anesthetized (Ket $+$) units. Vector strengths above 0.42 marked significant entrainment to stimuli, as determined by the Rayleigh test ($p < 0.05$). Error bars represent the standard errors of the means.

FIG. 7.

Population data for response properties of SPON units recorded in the absence (Ket −) or presence of ketamine anesthesia (Ket +). Data for characteristic frequency (CF) pure tone thresholds (A), spiking rates (B), and first spike latencies (C) are shown, as well as spiking rates to broadband noise (BBN) (D), sharpness of frequency tuning to pure tones represented by Q10 values (E), and vector strengths at each unit's best modulation frequency (BMF). In each plot, values for individual units are represented for both unpaired (open circles) and paired (filled circles) recordings. Group means are represented by solid and dashed horizontal lines for unpaired and paired groups, respectively. Values for units in the paired recording group are connected by solid lines.

Summary of response properties for unpaired and paired SPON recordings conducted with and without ketamine anesthesia.

TABLE 1

Anesthetic State	Threshold (dB SPL)	Tone Evoked Spikes (sp/stim)	First Spike Latency (ms)	Spontaneous Spikes (sp/sec)	BBN Evoked Spikes (sp stim)	Q10 Value	Vector Strength at BMF
Unpaired Recordings							
Ket-	37.1 ± 1.3**	2.2 ± 0.2**	6.8 ± 0.2**	3.0 ± 0.6**	3.1 ± 0.3*	3.5 ± 0.3**	0.85 ± 0.02**
Ket+	57.8 ± 2.0	1.2 ± 0.1	9.5 ± 0.3	1.1 ± 0.3	1.9 ± 0.3	2.6 ± 0.3	0.69 ± 0.03
Paired Recordings							
Ket-	40.7 ± 4.4**	2.0 ± 0.3**	7.1 ± 0.4**	4.2 ± 1.4	3.6 ± 0.7	3.2 ± 0.4	0.82 ± 0.03*
Ket+	58.7 ± 4.7	1.2 ± 0.2	9.2 ± 0.5	1.9 ± 0.8	1.8 ± 0.5	2.1 ± 0.3	0.68 ± 0.03

All differences between Ket - and Ket + conditions were significant (p < 0.05) as determined by the Mann Whitney U and Wilcoxon matched-pairs tests for unpaired and paired recordings, respectively.

* = p < 0.01;

** = p < 0.001.

BBN: broadband noise; BMF: best modulation frequency.