

Laura M. Hurley · George D. Pollak

Serotonin modulates responses to species-specific vocalizations in the inferior colliculus

Received: 14 July 2004 / Revised: 23 February 2005 / Accepted: 24 February 2005 / Published online: 14 April 2005
© Springer-Verlag 2005

Abstract Neuromodulators such as serotonin are capable of altering the neural processing of stimuli across many sensory modalities. In the inferior colliculus, a major midbrain auditory gateway, serotonin alters the way that individual neurons respond to simple tone bursts and linear frequency modulated sweeps. The effects of serotonin are complex, and vary among neurons. How serotonin transforms the responses to spectrotemporally complex sounds of the type normally heard in natural settings has been poorly examined. To explore this issue further, the effects of iontophoretically applied serotonin on the responses of individual inferior colliculus neurons to a variety of recorded species-specific vocalizations were examined. These experiments were performed in the Mexican free-tailed bat, a species that uses a rich repertoire of vocalizations for the purposes of communication as well as echolocation. Serotonin frequently changed the number of recorded calls that were capable of evoking a response from individual neurons, sometimes increasing (15% of serotonin-responsive neurons), but usually decreasing (62% of serotonin-responsive neurons), this number. A functional consequence of these serotonin-evoked changes would be to change the population response to species-specific vocalizations.

Keywords Serotonin · Inferior colliculus · Free-tailed bat · Vocalization · Neuromodulator

Abbreviations IC: Inferior colliculus

Introduction

Studies of how the nuclei of the auditory brainstem process acoustic information have been the subject of numerous reports for more than four decades. However, only a few studies have addressed how the processing in these nuclei is affected by neuromodulators, although neuromodulators are present in most auditory nuclei and have strong effects on auditory responses (for example, Olazábal and Moore 1989; Kössl and Vater 1989; Klepper and Herbert 1991; Henderson and Sheriff 1991; Ebert and Ostwald 1992; Thompson et al. 1995; Kaiser and Covey 1997; Wang and Robertson 1997; Manunta and Edeline 1999; Hurley and Pollak 1999, 2001; Hurley and Thompson 2001; Thompson and Hurley 2004). Serotonin, a major brain neuromodulator, is similarly present in most auditory nuclei (Steinbusch 1981; Klepper and Herbert 1991; Thompson et al. 1994; Kaiser and Covey 1997; Hurley and Thompson 2001). Similar to other neuromodulators, many of the effects of serotonin result from the activation of second messenger systems that lead to a strengthening or weakening of the actions of other neurochemicals (for example, Hoyer et al. 2002).

The serotonergic neurons that provide a large input to auditory nuclei are located in the dorsal raphe nucleus, and often fire in correlation with the level of arousal (Trulson and Jacobs 1979). Levels of serotonin in target regions of the brain are correspondingly higher during increased activity of raphe neurons, whether this activity is induced (for example, Bunin and Wightman 1998) or spontaneous (Trulson 1985). Serotonergic neurons may also transiently change their firing patterns in response to sensory stimuli (Trulson and Trulson 1982; Rasmussen et al. 1986). It is for these reasons that serotonergic innervation is thought to be one avenue by which behavioral state and neural activity in the auditory system are linked.

L. M. Hurley (✉)
Jordan Hall/ Biology Department,
Indiana University,
1001 East Third Street,
Bloomington, IN 47405, USA
E-mail: lhurley@bio.indiana.edu
Tel.: +1-812-8561991
Fax: +1-812-8556705

Present address: G. D. Pollak
University of Texas,
Austin, TX 78712, USA

Here we explore one aspect of this issue by evaluating the influence of serotonin on responses to species-specific calls in the inferior colliculus (IC) of Mexican free-tailed bats (*Tadarida brasiliensis mexicana*). The IC is a site for the convergence and integration of both excitatory and inhibitory inputs from the majority of lower auditory nuclei (for example, Beyerl 1978; Roth et al. 1978; Adams 1979; Brunso-Bechtold et al. 1981; Zook and Casseday 1982; Aitkin 1986; Ross et al. 1988; Oliver and Huerta 1992; Vater et al. 1992). The IC is also innervated by descending cortical projections (for example, Andersen et al. 1980; Luethke et al. 1989; Herbert et al. 1991; Winer et al. 1998), and by a dense network of serotonergic fibers (Steinbusch 1981; Klepper and Herbert 1991; Kaiser and Covey 1997; Hurley and Thompson 2001).

We chose Mexican free-tailed bats as experimental subjects for three major reasons. The first reason is that these bats use a rich repertoire of social communication calls for a variety of social interactions (Gelfand and McCracken 1986; Balcombe and McCracken 1992; Simmons et al. 1978, 1979; Schmidt and Thaller 1994; French and Lollar 1998, 2000). The second reason is that a great deal is known about the response features of neurons at various levels of their brainstem auditory system (Pollak et al. 1977; Bodenhamer et al. 1979; Grothe et al. 1994, 1997; Park et al. 1996, 1998). We know, for example, that IC neurons in Mexican free-tailed bats respond to species-specific communication calls with a high degree of selectivity (Klug et al. 2002). When 10–20 communication calls are played, IC neurons respond only to some calls but not to others, even though the calls that fail to evoke a response have suprathreshold energy that encroaches upon the excitatory tuning curves. This high degree of selectivity is a characteristic that is lacking in a nucleus that is only one synaptic relay prior to the IC, the dorsal nucleus of the lateral lemniscus (Bauer et al. 2002). The third reason for using free-tailed bats is that responses evoked in the IC by tones and by brief FM sweeps, signals that simulate echolocation calls, are often strongly modulated by serotonin (Hurley and Pollak 2001). The modulation in many neurons is complex, in that serotonin can facilitate or depress the responses to some frequencies in the cell's tuning curve while leaving the responses evoked by other frequencies unaffected.

Based on these previous findings, it seems likely that responses to species-specific communication calls are also modulated by serotonin. However, because the spectral structures of communication calls are substantially more complex than those of the stimuli used previously, and because species-specific vocalizations may elicit much stronger responses from auditory neurons than other types of sounds (Wang and Kadia 2001), it is not clear whether serotonin should similarly modulate responses to communication calls and other types of sounds.

To address this issue, we utilized the same suite of communication calls in this study that has been used in

previous studies, and evaluated the types of changes in response features that were caused by serotonin. We show that serotonin has substantial influence on responses to communication calls, and often changes response selectivity. The changes we observed in individual neurons would translate into changes in population response patterns to the communication calls.

Materials and methods

Electrodes

Recordings were made with “piggy back” multibarreled electrodes (Havey and Caspary 1980). A five-barrel blank was pulled and the tip blunted to 10–15 μm . Recordings were made with a single-barreled micropipette glued to the multibarreled array so that the tip of the recording electrode was 10–20 μm from the blunted end of the multibarreled pipette. The recording electrodes had resistances of 8–15 M Ω . The recording pipette and the central barrel of the multibarreled pipette were filled with 1M NaCl and 2% Fast Green (pH 7.4). The Fast Green allowed the pipette to be easily visualized during placement over the inferior colliculus. The remaining barrels were filled with serotonin creatinine sulfate, a compound that is relatively light-stable in comparison with other serotonin compounds (20 mM in 200 mM NaCl, pH 4) or with the carrier vehicle (200 mM NaCl, pH 4). The barrels of the multibarreled pipette were connected via silver–silver chloride wires to a six-channel microiontophoresis constant current generator (Medical Systems Neurophore, BH-2: Greenvale, NY). The central barrel was connected to the sum channel in order to balance current in the drug barrels and reduce current effects on the recorded neuron. The recording electrode was connected via a silver–silver chloride wire to a Dagan AC amplifier (model 2400: Minneapolis, MN). When a drug was not being applied, a retention current of -15 nA was applied to each drug barrel to prevent leakage.

Surgical and recording procedures

Surgical and electrophysiological procedures were conducted as described previously (Hurley and Pollak 1999, 2001). Briefly, animals were anesthetized by methoxyflurane or isoflurane inhalation (Metofane, Mallinckrodt Veterinary: Mundelein, IL; IsoFlo, Abbott Laboratories: Chicago, IL) or 0.02 mg/gm body weight neuroleptic, Innovar-Vet (fentanyl and droperidol, Pitman-Moore, Inc.), injected intraperitoneally. The skin and muscle overlying the skull was reflected after the topical application of 2% Lidocaine (Elkins-Sinn: Cherry Hill, NJ) and a small hole was then drilled over the IC. The bat was transferred to a heated, sound attenuated recording chamber, where it was placed in a

restraining cushion constructed of foam molded to the animal's body. The restraining cushion was attached to a platform mounted on a custom-made stereotaxic instrument (Schuller et al. 1986). A small metal rod was cemented to the skull and then attached to a bar mounted on the stereotaxic instrument to ensure a uniform positioning of the head. A ground electrode was placed between the reflected muscle and the skin. A multibarreled electrode was positioned over the IC under a dissecting microscope. The electrode was subsequently advanced through the brain from outside the recording chamber using a piezoelectric microdrive (Burleigh 6000: Fishers, NY).

Recordings were begun after the bats recovered from the anesthetic. Recovery was assessed by response to gentle touch on the ears and the ability to drink water offered from a dropper. The bats typically lie quietly in the restraining cushion and show no signs of pain or discomfort. Surgical recording sessions lasted 5–8 h, and water was provided with a dropper every 1–2 h. Supplementary sub-anesthetic doses of the neuroleptic or more commonly, a mixture of ketamine (120 mg/kg) and xylazine (5 mg/kg) were given if the bat struggled or otherwise appeared in discomfort. If the bat continued to show signs of discomfort, recordings were terminated and the bat was returned to its cage. Bats were usually used for two recording sessions. All experimental procedures were in accordance with a protocol approved by the University of Texas Institutional Animal Care and Use Committee, and follow the guidelines within the Guide for the Care and Use of Laboratory Animals (NIH publication 85–23, revised 1996).

To assess whether the surgical or supplementary anesthesia altered the observed effects of serotonin, two analyses were performed using the responses to tones at best frequency or to linear FM sweeps. First, the average effect of serotonin on spike count for the first three neurons recorded after surgery were compared with the average effect of serotonin on spike count for later neurons. There was no significant difference in the average effect of serotonin on the spike counts of neurons in these two groups ($P = .91$, 2-tailed unpaired test). Second, we compared the effects of serotonin on spike count for neurons recorded soon after the administration of supplementary anesthesia ($n = 12$) versus the effect of serotonin on spike count for neurons recorded more than 3 h after supplementary anesthesia, or soon after direct observations in which bats were judged to be awake because of responsiveness to disturbance, or rapid breathing ($n = 54$). There was no significant difference in the average effect of serotonin on spike count between these groups ($P = .79$, 2-tailed unpaired test).

A total of 90 neurons were recorded from 28 bats; 54 of these neurons were recorded in 12 bats in which the main experimental focus was examining responses to bat calls, and 36 neurons were recorded in 16 bats that were also used in separate experiments. To determine whether there was substantial variability in the effects of

serotonin among bats, the average effects of serotonin on responses to tone bursts or FM sweeps were compared in six animals in which at least five neurons were used for this study. Among these six animals, there were no significant differences in the effects of serotonin on spike count (ANOVA, $F(18,4) = 1.68$, $P = .2$).

Acoustic stimulation, processing of spike trains and iontophoresis

At the start of each experiment, a loudspeaker was placed in the ear contralateral to the side from which recordings were made. The loudspeaker was either a 1/4 inch Brüel & Kjaer (B&K) microphone biased with 200 V DC and driven as a loudspeaker, or a custom made loudspeaker (Schuller 1997). The B&K loudspeaker was flat within ± 5 dB from 18 kHz to at least 60 kHz and the custom made loudspeaker was flat ± 6 dB from 10 to about 90 kHz, where harmonic distortions were at least 34 dB below the fundamental frequency. The B&K loudspeaker with the windscreen attached or the custom loudspeaker was inserted into the funnel formed by the bat's pinna, and positioned adjacent to the external auditory meatus. The pinna was folded over the housing of the loudspeaker and wrapped with Scotch tape. The binaural cross-talk with this arrangement was attenuated by 35–40 dB.

Spikes were fed to a window discriminator and then to a Macintosh 7100 computer controlled by a real time clock. Peristimulus time histograms (PSTHs) and rate-level functions were generated and graphically displayed. Each PSTH was generated from the discharges evoked by 20 presentations of a signal at a fixed intensity. A neuron was considered to respond to a sound if it fired at least five action potentials to these 20 presentations; this was the response criterion.

Two types of acoustic signals were presented to each neuron: tone bursts and a suite of 16–17 species-specific calls. All signals were presented at a rate of 4 per second and were presented in pseudo-random order. Tone bursts were digitally generated by a Power Macintosh 7100/66 computer, were 5–20 ms in duration and had 0.2 ms rise-fall times. Tones were used to determine the best frequency (BF), threshold at BF, and tuning curve for each neuron. The species-specific calls were 16 social communication calls (C1–C16) and one pair of echolocation calls (E1) emitted by Mexican free-tailed bats and digitally sampled at over twice the maximum frequency contained in the calls. Each call had a broad bandwidth and a different spectro-temporal structure. Most calls had either upward or downward frequency modulations, or both upward and downward modulations, and all the calls had two or more harmonics. Although calls were sampled from a database collected during mother–pup interactions, the behavioral contexts of the calls, and whether they are representative exclusively of mother–pup interactions or more generally of intraspecific communication, is unknown. The 17 calls allowed us to

present to each neuron a varied class of complex, species-specific sounds of types likely to be heard by Mexican free-tailed bats.

Most IC cells were selective for calls in that they responded to only some calls and not to others, even though the calls to which they failed to respond had suprathreshold energy in their tuning curves, as has been previously demonstrated (Klug et al. 2002; Pollak et al. 2003). To quantify selectivity, we calculated a selectivity value for each neuron, defined as the number of calls to which a neuron responded out of all of those presented. Each selectivity value was calculated from the number of presented calls that a given neuron did not respond to, divided by one less than the total number of calls that were presented, multiplied by 100. With this definition, a neuron responding to just one call out of 16 or 17 would have 100% selectivity, and a neuron responding to 16 out of 16, or to 17 out of 17 calls would have a 0% selectivity.

Following the acquisition of these data, serotonin was iontophoretically applied. To ensure that the responses of the neurons to sound remained stable over time, repeated measurements were taken of the neurons' responses to recorded calls, or of responses to sets of tones encompassing the neurons' excitatory tuning ranges. Neurons with unstable responses over time were rejected from the data set. Serotonin was iontophoresed using currents that did not exceed 90 nA. Because monoamines can have qualitatively different effects at different dosages (Devilbiss and Waterhouse 2000), we tested 11 neurons with levels of iontophoretic current as low as 25 nA, in addition to the higher levels of 75–90 nA that were more typically used. Of these 11 neurons, 3 showed no effect of any dose of serotonin, 4 showed incremental increases at each dose of serotonin, and 4 showed plateaus at the highest doses of serotonin, even when serotonin had selective effects on particular calls. In no case did we observe a change in the nature of the effects of serotonin with increasing iontophoretic current. This is similar to our previous finding that the level of iontophoretic current does not qualitatively alter the effects of serotonin on frequency tuning (Hurley and Pollak 2001), given a starting concentration of 20 mM in the iontophoresis pipette.

Neurons were also usually similarly responsive to multiple applications of serotonin. In eight neurons, serotonin was applied twice with a recovery or vehicle application intervening. Of these seven neurons, serotonin had the same effect in six, whether this was a decrease in the response to calls ($n=4$), or the lack of an effect ($n=3$). In only one neuron did serotonin decrease the responses to calls during its first application but not during its second.

During serotonin application, responses to stimuli were monitored until spike counts stabilized, and an experimental dataset was collected, a process that usually took from 3 min to 15 min, depending on the size of the dataset. Iontophoresis of the carrier solution, 200 mM NaCl, does not by itself affect neuronal re-

sponses as serotonin does (Hurley and Pollak 1999). The ejection current was then switched off. If contact with the neuron was maintained, recovery data were obtained by presenting the same suite of stimuli until the responses were similar to those obtained before serotonin was applied. Neurons typically recovered within 5–15 min.

Results

Serotonin changes the responses to recorded vocalizations

The effects of iontophoresing serotonin on the responses to a suite of 16–17 species-specific calls was evaluated in 90 IC neurons. Sixteen of the calls were communication calls, and one was a pair of echolocation calls (Fig. 1). For each neuron, the criterion for a serotonin-induced change in a call response was a 50% change in the spike counts evoked by the same call in the control condition. Serotonin changed responses to one or more calls in 68% of the neurons (61/90). The most common effect of serotonin was to depress the spike counts evoked by one or more calls compared to the spike counts evoked by the same calls before serotonin was applied. Of the 61 neurons whose responses were changed by serotonin, there was a reduction in the spike counts to one or more calls in 72% (44/61) of those neurons. In 38% of neurons (23/61), serotonin caused an increase in the spike counts evoked by one or more calls, and thus facilitated the responses to those calls. The reason that the total percentage is greater than 100% is that in certain

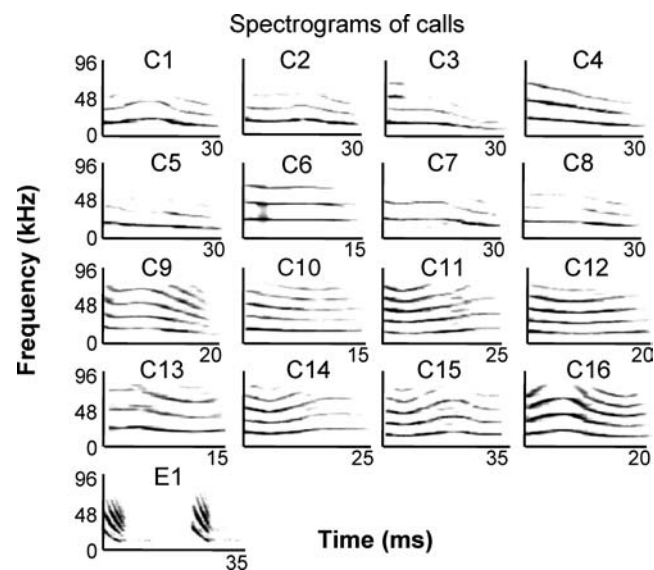
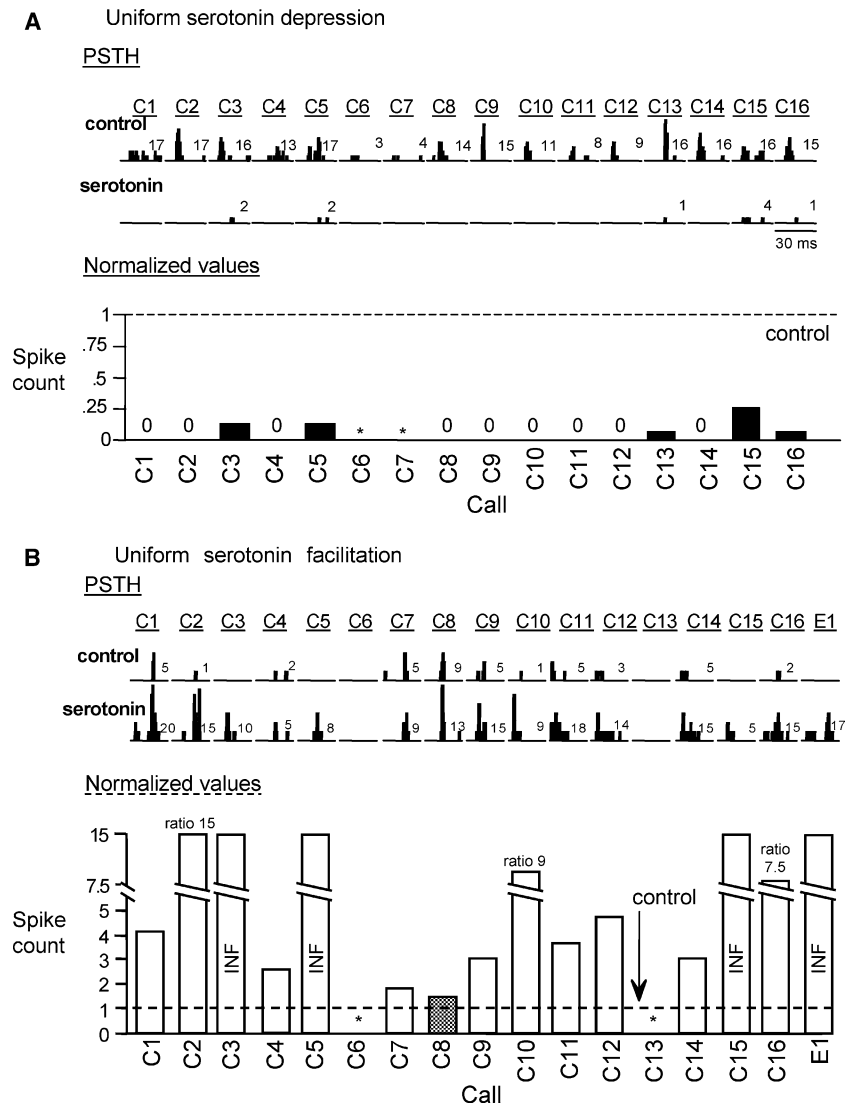


Fig. 1 Spectrograms (frequency vs. time plots) of the Mexican free-tailed bat calls that were used as auditory stimuli. The calls all contain multiple harmonics and vary nonlinearly in frequency and intensity over time. *C* designates communication calls and *E* designates echolocation calls in all figures

neurons the responses to some calls were depressed by serotonin while the responses evoked by other calls were facilitated, as described below.

Fig. 2 Serotonergic effects on call responses. **a** Serotonin uniformly decreases responses to calls in one neuron. **PSTH**: At the top of the figure, summed PSTHs of a neuron's response to 20 presentations of 16 different communication calls are plotted in the control and in serotonin. Calls were presented at 30 dB above tone threshold. Serotonin reduced the responses to all 14 calls that evoked responses above the criterion of five spikes. **Normalized values**: Spike count values from A are plotted as ratios of spike count in serotonin/control. A value of 1, indicated by the dashed line, indicates no change in the presence of serotonin. *Asterisks* mark calls that did not evoke responses above the criterion value in the control or in serotonin. *Filled bars* represent depression by 50% or more. *Zeros* indicate total suppression of call responses by serotonin. **b** Serotonin uniformly increases responses to calls in a different neuron. Note that for four calls, calls C3, C5, C15 and E1, neurons did not respond in the control but did in serotonin. This is denoted by the text 'INF' in the normalized value plots for these calls to indicate infinite ratios. *Open bars* represent facilitation by 50% or more, and *stippled bars* represent a change of less than 50%. Otherwise, conventions are the same as in **a**. Calls were presented at 30 dB above tone threshold for this neuron

In the 61 neurons whose responses were altered by serotonin, the effects of serotonin on the responses evoked by the calls were relatively uniform in some neurons, while in other neurons they were more complex. The uniform effects of serotonin were seen in 26% of the neurons (16/61). In each of these neurons, serotonin had similar effects on the responses to all calls that initially elicited spikes, either depressing or facilitating the response to each call. Two examples are shown in Fig. 2. The neuron in Fig. 2a responded to 14 of 16 calls above the criterion level (5 discharges to 20 presentations of a call) in the control condition. During the application of serotonin, the responses to each of the 14 calls that originally elicited a response were substantially or completely depressed, such that the responses to each of the 14 calls were below our threshold criterion as well as below the control spike count (Fig. 2a). The neuron in Fig. 2b responded to 6 of 17 calls in the control condition. When serotonin was applied, the responses to each of the 6 calls were greater than in the control, and the responses to all but one of these calls (call C8) was at least 50% greater during serotonin iontophoresis. In



addition, several calls that either failed to evoke any response (calls C3, C5, and E1) or evoked only responses below the criterion value (calls C2, C4, C10, C12, and C16) in the control, evoked responses above the criterion value when serotonin was applied. For some calls then, serotonin caused the facilitation of a response that was not apparent in the control condition.

Serotonin had more variable effects in 74% of the neurons (45/61). In these neurons, the effects of serotonin varied from call to call, and serotonin depressed the responses to some calls, had no effect on responses to other calls, and even facilitated the responses to yet other calls. These variable effects of serotonin are illustrated in Fig. 3. The neuron in this figure responded to 8 of 17 calls before serotonin was applied. Serotonin either completely or strongly suppressed the responses to four calls (C2, C5, C7 and C8), had less than a 50% effect on responses to four calls (C1, C4, C10, and C14), and facilitated the responses to four calls (C11, C12, C15, and C16). However, we also point out that the effects of serotonin varied more continuously than these categories would suggest. For example, the responses to call C4 increased by about 44% with serotonin, from 9 spikes to 13 spikes, while facilitation of other calls raised what was a response below the criterion level in the control (1 or 2 spikes) to a vigorous response with serotonin, e.g., calls C11 and C12. Together, the neurons in Figs. 2 and 3 illustrate the two main effects, uniform and variable, that serotonin had on the responses to the variety of calls that we presented.

The effects of serotonin on selectivity to calls

As is apparent in Figs. 2 and 3, serotonin changed the set of calls to which individual neurons responded, or their selectivity. To quantify this change, a value of

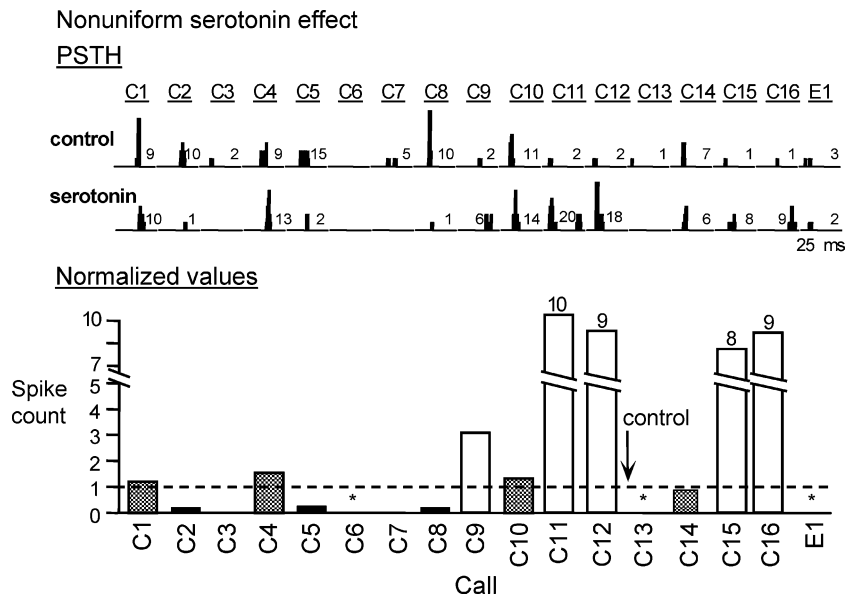
selectivity was calculated for each neuron in response to calls that were 30–40 dB above threshold for the response to a tone at best frequency (see Methods). Two neurons having different selectivities are illustrated in Fig. 4a. Neuron 1 responded to 12 out of 16 calls and therefore had a relatively low selectivity of 27%. Neuron 2 responded to only 1 of 16 calls and therefore had the highest possible selectivity of 100%.

The selectivity values of most IC neurons were high and averaged $74 \pm 3.0\%$. The selectivity values of the 90 neurons in our sample are plotted in Fig. 4b. Of the 90 neurons tested with multiple calls, 20% (18/90) had selectivities of 100%; these neurons responded to just one call, though not all neurons responded to the same call. Fully a third of neurons (33%, or 30) had selectivities in the expanded range of 90–100%. A smaller percentage, 19% (17/90), had selectivities of 80–90%. The least selective neurons comprised 48% (43/90) of our sample. These neurons had selectivities of less than 80% and responded to four or more calls.

Although serotonin changed the responses to one or more calls in 61 neurons, it changed the selectivity values in only 47 of those 61 neurons. In the other 14 neurons, serotonin changed the responses to calls, either depressing or facilitating the responses to some calls, but the effects were such that they did not change the number of calls to which the neurons responded above or below the criterion level, and therefore did not change the selectivities of those neurons. Thus serotonin changed the selectivity values in 77% of the neurons affected by serotonin, and in about 52% of our entire sample of 90 IC neurons.

Because the dominant effect of serotonin was to depress responsiveness, the majority of 38 of the 47 neurons whose selectivities were influenced by serotonin responded to fewer calls than in the control conditions, and thus they became more selective (Fig. 5a). These

Fig. 3 A neuron showing mixed effects of serotonin on different calls. Conventions are the same as in Fig. 2. Serotonin suppresses the responses to three calls, calls C2, C5 and C8, facilitates the responses to five calls, calls C9, C11, C12, C15 and C16, and has little effect on the responses to four calls, calls C1, C4, C10 and C14. Numbers above the bars represent the ratio of spike counts in serotonin versus the control. Calls were presented at 40 dB above tone threshold



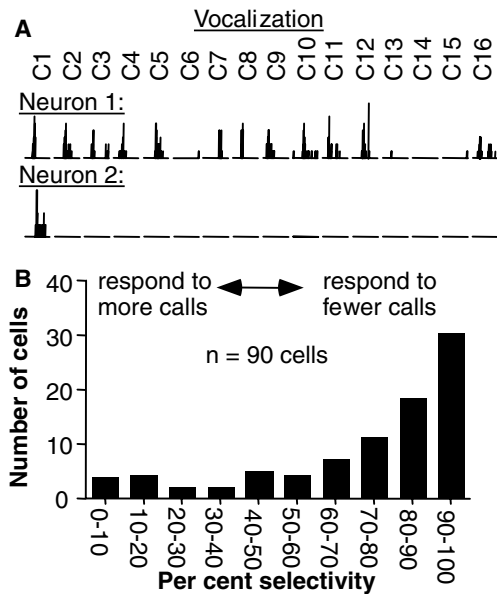


Fig. 4 Selectivity for calls. **a** IC neurons show a range of selectivities for calls. Two individual IC neurons had very different selectivities in the control: neuron 1 responded to 12 of 16 calls, while neuron 2 responded to only one. The calls were presented at 50 dB above threshold for neuron 1 and 40 dB above threshold for neuron 2. **b** Selectivities of the IC neuron population. Selectivity index is plotted against number of neurons, where selectivity = number of calls eliciting no response / (total number of presented calls - 1). Most IC neurons were 80% selective or more

neurons responded on average to 2.7 fewer calls during iontophoresis of serotonin than they did in the control condition. This translated into an average selectivity increase of 21% (Fig. 5b). However, in 9 of the 47 neurons, serotonin actually increased the number of calls to which the neurons responded, a result of serotonin's facilitatory effects on those IC cells (Fig. 5a). These cells responded on average to 3.2 additional calls, which in turn reduced the average selectivity of the 9 cells by 16% (Fig. 5b). As mentioned, 14 neurons of the 61 showing serotonin-evoked changes in spike count did not show parallel changes in selectivity (Fig. 5a).

Serotonin changes the population responses to calls

We next explored how the observed serotonin-evoked changes in the selectivities of single neurons could be expected to change the responses of the population of neurons across the IC to individual calls. To provide a flavor for the complex and variable changes in population responses that can be induced by serotonin, Fig. 6 shows the control and serotonin-induced responses that were evoked by two calls, call C1 and call C13, in a sample of 15 IC neurons. The normalized spike counts evoked by each call are shown as bar graphs in Fig. 6a, with the absolute spike counts shown above each bar. For convenience, the neurons are arranged, from top to bottom, according to the effect of serotonin on call C1: the responses of the top five neurons were completely

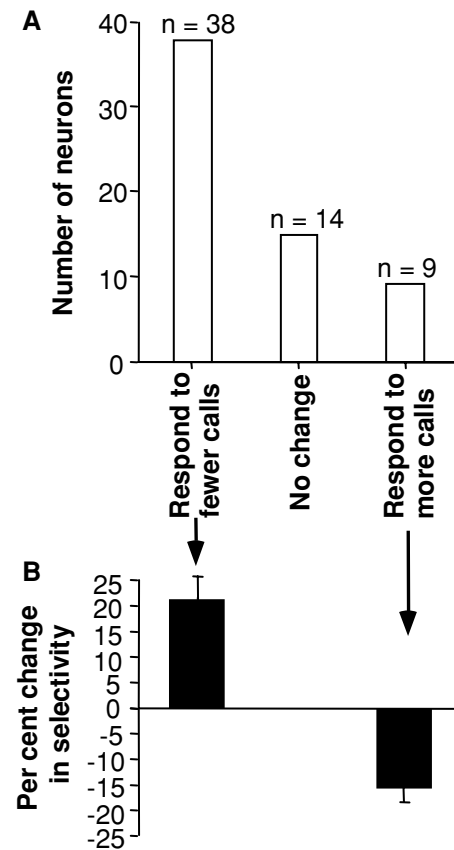


Fig. 5 Serotonin changes selectivity for calls. **a** Of the 61 neurons whose responses were affected by serotonin, 38 neurons responded to fewer calls, 14 neurons responded to the same number of calls, and 9 neurons responded to more calls in serotonin. Neurons in the 'no change' category showed serotonin-evoked spike changes that did not cross the criterion value for responses. **b** Per cent changes in selectivity for neurons that responded to fewer calls ($n=38$) and to more calls ($n=9$) in serotonin. Error bars represent standard errors of the mean

suppressed by serotonin, neuron 6 was weakly suppressed, the next two neurons (7–8) were facilitated by serotonin, and the responses of the next four neurons (9–13) were changed by less than 50%. These neurons are representative in that the percentage of the 15 neurons whose spike counts were depressed (46.1%), facilitated (15.4%) and unchanged (38.5%) by serotonin are similar to the percentages showing these effects in our sample of 90 IC neurons. Thus, the activity profiles of the 15 neurons evoked by call C1, viewed down the control column and down the serotonin column, are different, as they must be because in many of the neurons serotonin changed the response to call C1. The same is true for the activity profile evoked in the 15 neurons by call C13, so that the profile in the presence of serotonin is different from the control profile.

Although serotonin would alter the population response to each of the calls we used, this finding does not address the question of whether serotonin would change the relative population activity for different calls. To compare the response disparities of the control and the

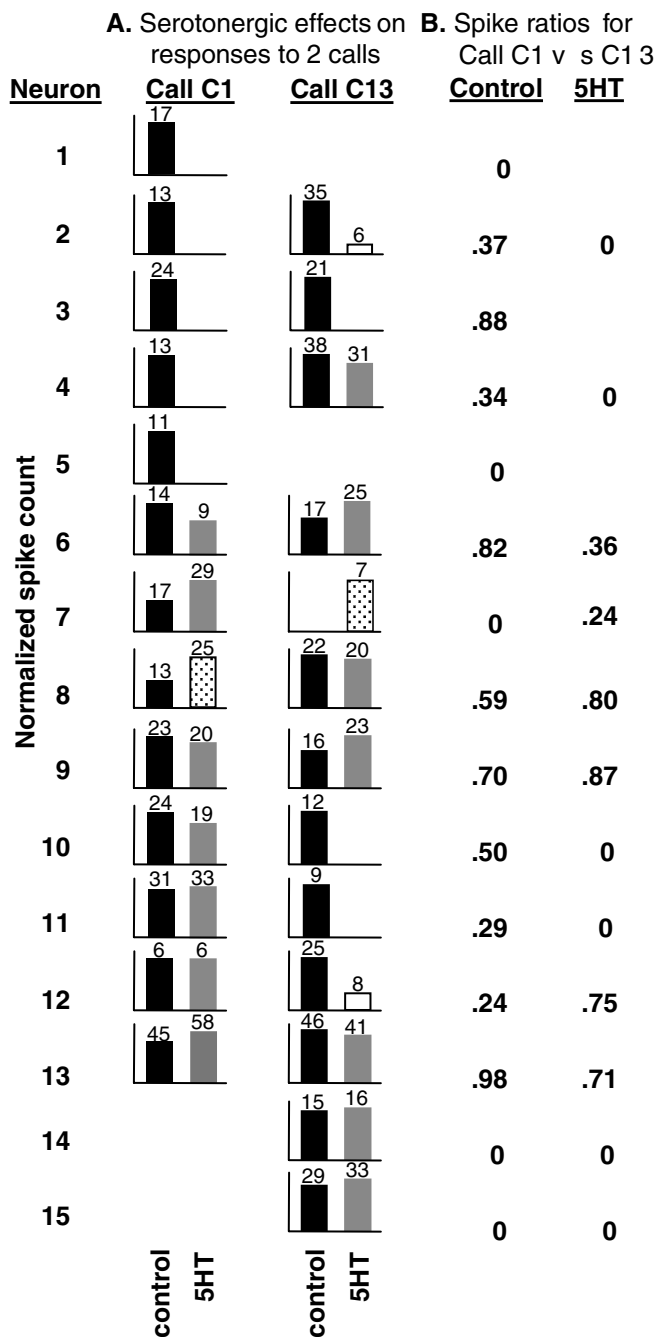


Fig. 6 Comparison of serotonergic effects on two calls. **a** Comparison of spike counts in control versus serotonin for call C1 and call C13 in a sample of 15 neurons. Spike counts are normalized to the highest value, either in the control or in serotonin. All control spike counts are represented by *filled black columns*. *Unfilled columns* represent spike counts that were decreased by at least 50% in serotonin relative to the control, *stippled columns* represent spike counts that increased by at least 50% in serotonin relative to the control, and *gray columns* represent spike counts that changed by less than 50% relative to the control. Serotonin had different effects on responses to different calls, even for individual neurons such as neuron 8. **b** Spike ratios are the ratio of spike counts in response to calls C1 and C13 in the control and in the presence of serotonin

serotonin-induced population responses for different calls, we quantified the overall similarity of the population response to two different calls with a similarity index (SI). The SI incorporates the difference in spike-counts evoked by two calls, with higher values indicating greater response similarity. The first component of this index was to compute for each neuron the ratio of the spike counts evoked by two calls, such as calls C1 and C13. The larger the ratio, the more similar were the spike-counts evoked by the two calls. Neurons that responded to both calls with the same spike count had a ratio of 1.0 whereas neurons that responded to only one call and failed to respond to the other had a ratio of 0. Neurons that failed to respond to either of the calls were not assigned a ratio. The spike ratios for each neuron in the control and in the presence of serotonin are presented in Fig. 6b. The SI is simply the mean spike ratio for all neurons. Because the SI incorporates the ratio rather than the absolute values of spike counts, serotonin could decrease the spike count but still increase the similarity index between two different calls.

For the sample set of 15 neurons in Fig. 6a, the SI for the control condition was 0.38, but in the presence of serotonin, the SI was 0.31, representing an 18% decrease in the similarities between calls C1 and C13 in the presence of serotonin relative to the control. The main point here is that the control SI was larger than the SI in the presence of serotonin, suggesting that the population responses evoked by the two calls were more similar in the control than when serotonin was applied. Stated differently, the population responses for the two calls in the presence of serotonin were more disparate than they were in the control condition.

We calculated the SI for all possible pairs of calls across all 90 neurons we recorded, resulting in an SI value for 136 pairwise comparisons in the control, and an equal number in the presence of serotonin. We measured the percent change in the SI between the control and in the presence of serotonin for each of these pairwise comparisons. Among the 136 percentage values that resulted, the majority were negative ($n=83$), representing a decrease in the similarity between the pairs of calls in the presence of serotonin. A minority of the values were positive ($n=44$), indicating an increase in the similarity between the pairs of calls in the presence of serotonin, and a few values were null ($n=9$). We condensed these values into an average for each call versus the 16 other calls in our stimulus repertoire. These average values are shown in Table 1. For example, across our entire neuronal population, the spike ratio of call C1 relative to the other 16 calls was 28.8% lower in the presence of serotonin than in the control. Like call C1, many other calls had a reduced average SI in the presence of serotonin, most notably calls C2, C6, C8, C13, and E1, which had average decreases in SI ranging from 22.5% (call C6) to 42.9% (call E1). For some calls,

Table 1 Effects of serotonin on the average similarity index

Call	% Change in SI (mean \pm S.E.M.)
C1	-28.8 \pm 4.7
C2	-40.2 \pm 3.0
C3	-3.7 \pm 9.1
C4	7.4 \pm 6.4
C5	1.0 \pm 6.2
C6	-22.5 \pm 7.0
C7	-3.2 \pm 7.3
C8	-23.0 \pm 7.0
C9	-2.4 \pm 7.9
C10	12.4 \pm 10.3
C11	16.6 \pm 10.6
C12	-6.0 \pm 9.2
C13	-40.4 \pm 6.0
C14	0 \pm 8.2
C15	-8.3 \pm 5.1
C16	0.1 \pm 6.3
E1	-42.9 \pm 7.6

the average SI did increase in the presence of serotonin. The two largest increases in average SI, for calls C10 and C11, were only 12.4% and 16.6%, respectively, and had relatively large SE. Thus, there was variation in the effects of serotonin on the average SI between calls, and the echolocation call along with several communication calls showed the largest decrease relative to the other calls. However, whether the variation in the effects of serotonin on the average SI correlates with differences in the behavioral functions of most of these calls is unknown. In general, within our sample of 90 IC neurons, the activity profiles evoked by different recorded calls were usually made more distinct by the iontophoretic application of serotonin. The implications of this finding are discussed below.

Discussion

Two main findings emerge from these experiments. First, when considering the effects of serotonin on the responses of individual neurons to single calls, the most common effect (in 44 of 90 neurons) was a decrease in the spike count evoked by the call. As a result of this, for most neurons serotonin increased the selectivity for calls, in that most neurons affected by serotonin responded to fewer calls in the presence of serotonin (38 neurons). Increases in responses to calls, although less common, were not rare, with 23 neurons showing increases in spike count in response to at least one call. This resulted in a decrease in selectivity for a few of the neurons that responded to serotonin (9 neurons). Many responses to calls were also unaffected by serotonin, resulting in selectivities for calls that were unchanged in the presence of serotonin for 43 neurons.

Our second major finding was that individual neurons did not necessarily show a single type of response to serotonin. In fact, 50% of all neurons recorded showed mixed effects, in that serotonin could decrease the response of a neuron to some calls, but have no effect or

even opposite effects on responses to different calls (Fig. 3). Below we consider the range of serotonergic effects in light of their potential mechanisms, functional consequences, and relation to previous hypotheses on the role of serotonin within sensory systems.

Comparison of serotonergic effects on tone and call evoked responses

In previous studies we showed that serotonin often has a powerful effect on responses to tone bursts that comprise the neuron's excitatory tuning curve (Hurley and Pollak 1999, 2001). The serotonergic influences on the more complex species-specific calls we observed here were in some cases consistent with the serotonin-induced changes of tone burst responses we observed in our previous studies but in other cases were not, as discussed below. In our previous studies we found that the most common effect of serotonin on responses to tone bursts was a broad depression or facilitation of spike counts evoked by all frequencies that previously drove that neuron. These broad depressive or facilitative effects are consistent with the uniform depression or facilitation of responses to communication calls that we observed in this study.

In our earlier studies we also found a smaller number of neurons in which serotonergic effects on tone-evoked responses were more complex than the common, broad effects of serotonin (Hurley and Pollak 2001). We called this pattern of changes 'focal' because in these neurons serotonin strongly depressed or facilitated responses to only some frequencies. Such focal serotonergic effects on tone responses are consistent with neurons presented here in which serotonin differentially suppressed or facilitated responses to some calls while having no effect on the responses to other calls. The putative linkage between the focal effect on tones and the differential effects on calls is that serotonin should only suppress or facilitate responses to calls that have energy concentrated in the frequencies suppressed or facilitated by serotonin, respectively. Conversely, other calls should be unaffected because those calls have energy concentrated in frequencies that are unaffected by serotonin.

However, in our previous studies we did not observe both a serotonin-induced facilitation of responses to some tonal frequencies and depression of other tone-evoked responses in the same neuron. Thus, the mixed serotonergic effects on call-evoked responses in the same neuron, where some calls were depressed, some were facilitated and some were not affected, were more complex than any of the effects we had observed with tones. This could be due to the fact that the responses of IC neurons to recorded vocalizations cannot always be predicted based on excitatory responses to tones. Inhibitory inputs are also likely to shape responses to complex sounds like calls, because when inhibitory inputs are blocked, responses to vocalizations become more predictable (Klug et al. 2002). Thus, the effect of serotonin on inhibitory inputs, among other factors,

may need to be measured to fully account for the complex effects of serotonin on responses to vocalizations.

Serotonin as a suppressor of activity

We have shown that serotonin increases the selectivity of neurons for calls. However, one could argue that any agent that decreased spike counts would increase the selectivity of individual neurons and also of the population activity for calls. In considering this possibility, it is useful to compare the effects of serotonin with those of an inhibitory agent such as GABA acting on GABA_A receptors. A previous study examined the effects of GABA on the selectivity of IC neurons in the free-tailed bat to vocalizations by blocking GABA_A receptors with iontophoretically applied bicuculline (Klug et al. 2002). Although blocking GABA_A receptors did decrease selectivity for vocalizations, two main differences emerge in the effects of GABA and serotonin in a comparison of these two studies. The first of these differences is that the effect of blocking GABA receptors is more widespread than the effect of applying serotonin. A second difference between the effects of serotonin and GABA is that serotonin causes a larger range of effects. Thus, serotonin and GABA show qualitative as well as quantitative differences, and are released under different circumstances. These differences apply even though serotonin may act in part through targeting GABAergic neurons within the IC (Peruzzi and Dut 2004). From these considerations we conclude that, even if serotonin did have purely suppressive effects, it would cause a different pattern of suppression than GABA. Moreover, across neurons and across different calls within single neurons, serotonin appears to be a more selective agent than GABA. This type of selectivity is typical of neuromodulators within sensory systems in general, because the effects of a given neuromodulator depend on both the type of receptor and the role of the targeted neuron within the neural circuitry (Hurley et al. 2004).

Serotonin enhances the signal to noise ratio evoked by calls

Based on our results we propose that serotonin, by changing the responsiveness of individual neurons to a call, also changes the profile of the population response evoked by that call. These changes would generally serve to enhance or amplify differences in the population responses evoked by different calls.

Even in the absence of serotonin, IC neurons display a wide diversity of selectivities for calls, with some neurons responding to many calls and others responding to only a few. When a different call is presented, a pattern of activity that shares some of the same neurons and also incorporates additional neurons, will result. Thus, due to the diverse selectivities among the IC population each call should generate a unique spatio-temporal pattern of activity in the IC (Klug et al. 2002; Suta et al.

2003). When, in addition to selectivity, response magnitude is factored in, the population response evoked by a given call becomes more distinctive than the population response evoked by any other call, as shown in Fig. 6.

In the presence of serotonin, the population response to a call would be represented by a group of neurons with unchanged responses, a large number of neurons with diminished or totally suppressed responses, and a small number of neurons with increased responses relative to the population response before serotonin. Thus, serotonin would sculpt the pattern of population activity evoked by a particular call (Fig. 6a). In fact, our data suggest that, when the similarity in spike counts as well as the identities of responding neurons are considered, the patterns of activity evoked by different calls would become more distinct in the presence of serotonin, because serotonin alters the responses to different calls from neuron to neuron. That is, for each call, a serotonin-evoked sculpting of population activity would result in a more divergent and less ambiguous response to each call (Fig 6b, Table 1). This effect would be greatest when serotonin levels are expected to be highest, during high levels of behavioral arousal. It is not unreasonable to speculate that in free-tailed bats, high levels of alertness would occur in hunting and social contexts, but whether serotonin release within the auditory system varies in these situations is unknown.

The interpretation that serotonin alters the population response to calls is consistent with findings in other sensory systems. For example, cholinergic inputs to the olfactory bulb increase the discriminability of odorants in a model of neural activity, reflecting the behavior of cholinergic-lesioned animals (Linster and Cleland 2002). In barrel cortex, multielectrode arrays demonstrate that an alpha 2 adrenergic agonist can also emphasize the representation of directional deflection of a whisker (Devilbiss and Waterhouse 2002). Likewise, behavioral arousal suppresses the representation of whisker deflection in the barrel cortex, leading to the ‘focusing’ of somatosensory inputs (Castro-Alamancos 2002). Thus, in accordance with our results, the central representation of a stimulus in multiple sensory modalities is a dynamic entity subject to alteration by various neuromodulators and by behavioral states.

In addition to changing the selectivity of neurons for species-specific vocalizations, however, serotonin is likely to alter other aspects of stimulus coding within the IC (Hurley et al. 2002). This is because of the widespread distribution of serotonergic fibers within the IC (Hurley and Thompson 2001), as well as the wide range of neurons that are responsive to serotonin (Hurley and Pollak 1999, 2001). The neural responses evoked by many types of behaviorally relevant signals are potential targets for serotonergic modulation because they rely on either the magnitude or timing of neural responses, both of which are properties modulated by serotonin (Hurley and Pollak 1999, 2002). Serotonin within the IC has also been implicated in behaviors such as fear responses

(Melo and Brandao 1995). In light of these considerations, we feel that the involvement of serotonin in altering responses to vocalizations must represent only one facet of serotonergic function within the IC.

Acknowledgements The authors would like to thank Carl Resler for technical assistance. Work was supported in part by NIH grants DC-20068 and DC-00391. Experiments comply with the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, revised 1996), and were approved by the University of Texas Animal Care and Use Committee.

References

- Adams J (1979) Ascending projections to the inferior colliculus. *J Comp Neurol* 183:519–538
- Aitkin L (1986) The auditory midbrain: structure and function in the central auditory pathway. Humana Press, Clifton
- Andersen R, Snyder R, Merzenich M (1980) The topographic organization of corticocollicular projections from physiologically identified loci in the AI, AII, and anterior auditory cortical fields of the cat. *J Comp Neurol* 191:479–494
- Balcombe J, McCracken G (1992) Vocal recognition in Mexican free-tailed bats: do pups recognize mothers? *Anim Behav* 43:79–87
- Bauer E, Klug A, Pollak G (2002) Spectral determination of responses to species-specific calls in the dorsal nucleus of the lateral lemniscus. *J Neurophysiol* 88:1955–1967
- Beyerl B (1978) Afferent projections to the central nucleus of the inferior colliculus in the rat. *Brain Res* 145:209–223
- Bodenhamer R, Pollak G, Marsh D (1979) Coding of fine frequency information by echoranging neurons in the inferior colliculus of the Mexican free-tailed bat. *Brain Res* 171:530–535
- Brunso-Bechtold J, Thompson G, Masterton R (1981) HRP study of the organization of auditory afferents ascending to central nucleus of inferior colliculus in cat. *J Comp Neurol* 197:705–722
- Bunin M, Wightman R (1998) Quantitative evaluation of 5-hydroxytryptamine (serotonin) neuronal release and uptake: an investigation of extrasynaptic transmission. *J Neurosci* 18:4854–4860
- Castro-Alamancos M (2002) Role of thalamocortical sensory suppression during arousal: focusing sensory inputs in neocortex. *J Neurosci* 22:9651–9655
- Devilbiss D, Waterhouse B (2000) Norepinephrine exhibits two distinct profiles of action on sensory cortical neuron responses to excitatory synaptic stimuli. *Synapse* 37:273–282
- Devilbiss D, Waterhouse B (2002) Determination and quantification of pharmacological, physiological, or behavioral manipulations on ensembles of simultaneously recorded neurons in functionally related neural circuits. *J Neurosci Methods* 121:181–198
- Ebert U, Ostwald J (1992) Serotonin modulates auditory information processing in the cochlear nucleus of the rat. *Neurosci Lett* 145:51–54
- French B, Lollar A (1998) Observations on the reproductive behavior of captive *Tadarida brasiliensis mexicana* (Chiroptera: Molossididae). *Southw Natural* 43:484–490
- French B, Lollar A (2000) Communication among Mexican free-tailed bats. *Bats: Bat Conserv Internat* 18:1–4
- Gelfand D, McCracken G (1986) Individual variation in the isolation calls of Mexican free-tailed bat pups (*Tadarida brasiliensis mexicana*). *Anim Behav* 34:1078–1086
- Grothe B, Schweizer H, Pollak G, Schuller G, Rosemann C (1994) Anatomy and projection patterns of the superior olivary complex in the Mexican free-tailed bat, *Tadarida brasiliensis mexicana*. *J Comp Neurol* 343:630–646
- Grothe B, Park T, Schuller G (1997) Medial superior olive in the free-tailed bat: response to pure tones and amplitude-modulated tones. *J Neurophysiol* 77:1553–1565
- Havey D, Caspary D (1980) A simple technique for constructing 'piggy-back' multibarrel microelectrodes. *Electroencephalogr Clin Neurophysiol* 48:249–251
- Henderson Z, Sherriff F (1991) Distribution of choline acetyltransferase immunoreactive axons and terminals in the rat and ferret brainstem. *J Comp Neurol* 314:147–163
- Herbert H, Aschoff A, Ostwald J (1991) Topography of projections from the auditory cortex to the inferior colliculus in the rat. *J Comp Neurol* 304:103–122
- Hoyer D, Hannon J, Martin G (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* 71:533–554
- Hurley L, Pollak G (1999) Serotonin differentially modulates responses to tones and frequency-modulated sweeps in the inferior colliculus. *J Neurosci* 19:8071–8082
- Hurley L, Pollak G (2001) Serotonin effects on frequency tuning of inferior colliculus neurons. *J Neurophysiol* 85:828–842
- Hurley L, Pollak G (2002) Serotonin effects on latencies of inferior colliculus neurons. *SFN Abstr* 32:762.9
- Hurley L, Thompson A (2001) Serotonergic innervation of the auditory brainstem of the Mexican free-tailed bat, *Tadarida brasiliensis*. *J Comp Neurol* 435:78–88
- Hurley L, Thompson A, Pollak G (2002a) Serotonin in the inferior colliculus. *Hear Res* 168:1–11
- Hurley L, Devilbiss D, Waterhouse B (2004) A matter of focus: monoaminergic modulation of stimulus coding in mammalian sensory networks. *Curr Opin Neurobiol* 14:488–495
- Kaiser A, Covey E (1997) 5-HT innervation of the auditory pathway in birds and bats. In: Syka J (ed) *Acoustical signal processing in the central auditory system*. Plenum, New York, pp 71–78
- Klepper A, Herbert H (1991) Distribution and origin of noradrenergic and serotonergic fibers in the cochlear nucleus and inferior colliculus of the rat. *Brain Res* 557:190–201
- Klug A, Bauer E, Hanson J, Hurley L, Meitzen J, Pollak G (2002) Response selectivity for species-specific calls in the inferior colliculus of Mexican free-tailed bats is generated by inhibition. *J Neurophysiol* 88:1941–1954
- Kössl M, Vater M (1989) Noradrenaline enhances temporal auditory contrast and neuronal timing precision in the cochlear nucleus of the mustached bat. *J Neurosci* 9:4169–4178
- Linster C, Cleland T (2002) Cholinergic modulation of sensory representations in the olfactory bulb. *Neural Netw* 15:709–717
- Luethke L, Krubitzer L, Kaas J (1989) Connections of primary auditory cortex in the New World monkey, *Saguinus*. *J Comp Neurol* 285:487–513
- Manunta Y, Edeline J (1999) Effects of noradrenaline on frequency tuning of auditory cortex neurons during wakefulness and slow-wave sleep. *Eur J Neurosci* 11:2134–2150
- Melo L, Brandao M (1995) Role of 5-HT_{1A} and 5-HT₂ receptors in the aversion induced by electrical stimulation of inferior colliculus. *Pharmacol Biochem Behav* 51:317–321
- Olazábal U, Moore J (1989) Nigroreticular projection to the inferior colliculus: horseradish peroxidase transport and tyrosine hydroxylase immunohistochemical studies in rats, cats, and bats. *J Comp Neurol* 282:98–118
- Oliver D, Huerta M (1992) Inferior and superior colliculi. In: Webster D, Popper A (eds) *The Mammalian Auditory Pathway: Neuroanatomy*. Springer, Berlin Heidelberg New York, pp 168–222
- Park T, Grothe B, Pollak G, Schuller G, Koch U (1996) Neural delays shape selectivity to interaural intensity differences in the lateral superior olive. *J Neurosci* 16:6554–6566
- Park T, Klug A, Oswald J, Grothe B (1998) A novel circuit in the bat's midbrain recruits neurons into sound localization processing. *Naturwissenschaften* 85:176–179
- Peruzzi D, Dut A (2004) GABA, serotonin and serotonin receptors in the rat inferior colliculus. *Brain Res* 998:247–250

- Pollak G, Marsh D, Bodenhamer R, Souther A (1977) Characteristics of phasic on neurons in inferior colliculus of unanesthetized bats with observations relating to mechanisms for echo ranging. *J Neurophysiol* 40:926–942
- Pollak G, Klug A, Bauer E (2003) Processing and representation of species-specific communication calls in the auditory system of bats. *Int Rev Neurobiol* 56:83–121
- Rasmussen K, Strecker R, Jacobs B (1986) Single unit response of noradrenergic, serotonergic and dopaminergic neurons in freely moving cats to simple sensory stimuli. *Brain Res* 369:336–340
- Ross L, Pollak G, Zook J (1988) Origin of ascending projections to an isofrequency region of the mustache bat's inferior colliculus. *J Comp Neurol* 270:488–505
- Roth G, Aitkin L, Andersen R, Merzenich M (1978) Some features of the spatial organization of the central nucleus of the inferior colliculus of the cat. *J Comp Neurol* 182:661–680
- Schmidt S, Thaller J (1994) Temporal auditory summation in the echolocating bat, *Tadarida brasiliensis*. *Hear Res* 77:125–134
- Schuller G (1997) A cheap earphone for small animals with good frequency response in the ultrasonic frequency range. *J Neurosci Methods* 71:187–190
- Schuller G, Radtke-Schuller S, Betz M (1986) A stereotaxic method for small animals using experimentally determined reference profiles. *J Neurosci Methods* 18:339–350
- Simmons J, Lavender W, Lavender B, Childs J, Hulebak K, Rigden M, Sherman J, Woolman B, O'Farrell M (1978) Echolocation by free-tailed bats (*Tadarida*). *J Comp Physiol* 125:291–299
- Simmons J, Fenton M, O'Farrell M (1979) Echolocation and pursuit of prey by bats. *Science* 203:16–21
- Steinbusch H (1981) Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* 6:557–618
- Suta D, Kvasnak E, Popelar J, Syka J (2003) Representation of species-specific vocalizations in the inferior colliculus of the guinea pig. *J Neurophysiol* 90:3794–3808
- Thompson A, Hurley L (2004) Dense serotonergic innervation of principal nuclei of the superior olivary complex in mouse. *Neurosci Lett* 356:179–182
- Thompson G, Thompson A, Garrett K, Britton B (1994) Serotonin and serotonin receptors in the central auditory system. *Otolaryngol Head Neck Surg* 110:93–102
- Thompson A, Moore K, Thompson G (1995) Distribution and origin of serotonergic afferents to guinea pig cochlear nucleus. *J Comp Neurol* 351:104–116
- Trulson M (1985) Simultaneous recording of dorsal raphe unit activity and serotonin release in the striatum using voltammetry in awake, behaving cats. *Life Sci* 37:2199–2204
- Trulson M, Jacobs B (1979) Raphe unit activity in freely moving cats: correlation with level of behavioral arousal. *Brain Res* 163:135–150
- Trulson M, Trulson V (1982) Differential effects of phasic auditory and visual stimuli on serotonergic neurons in the nucleus raphe dorsalis and nucleus raphe pallidus in freely moving cats. *Neurosci Lett* 32:137–142
- Vater M, Kössl M, Horn A (1992) GAD- and GABA-immunoreactivity in the ascending auditory pathway of horseshoe and mustached bats. *J Comp Neurol* 325:183–206
- Wang X, Kadia S (2001) Differential representation of species-specific primate vocalizations in the auditory cortices of marmoset and cat. *J Neurophysiol* 86:2616–2620
- Wang X, Robertson D (1997) Effects of bioamines and peptides on neurones in the ventral nucleus of trapezoid body and rostral periolivary regions of the rat superior olivary complex: an in vitro investigation. *Hear Res* 106:20–28
- Winer J, Larue D, Diehl J, Hefti B (1998) Auditory cortical projections to the cat inferior colliculus. *J Comp Neurol* 400:147–174
- Zook J, Casseday J (1982) Origin of ascending projections to inferior colliculus in the mustache bat, *Pteronotus parnellii*. *J Comp Neurol* 207:14–28