Serotonin Effects on Frequency Tuning of Inferior Colliculus Neurons

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Hurley, Laura M. and George D. Pollak. Serotonin effects on frequency tuning of inferior colliculus neurons. *J Neurophysiol* 85: 828–842, 2001. We investigated the modulatory effects of serotonin on the tuning of 114 neurons in the central nucleus of the inferior colliculus (ICc) of Mexican free-tailed bats and how serotonin-induced changes in tuning influenced responses to complex signals. We obtained a "response area" for each neuron, defined as the frequency range that evoked discharges and the spike counts evoked by those frequencies at a constant intensity. We then iontophoretically applied serotonin and compared response areas obtained before and during the application of serotonin. In 58 cells, we also assessed how serotonin-induced changes in response areas correlated with changes in the responses to brief frequency-modulated (FM) sweeps whose structure simulated natural echolocation calls. Serotonin profoundly changed tone-evoked spike counts in 60% of the neurons (68/114). In most neurons, serotonin exerted a gain control, facilitating or depressing the responses to all frequencies in their response areas. In many cells, serotonergic effects on tones were reflected in the responses to FM signals. The most interesting effects were in those cells in which serotonin selectively changed the responsiveness to only some frequencies in the neuron's response area and had little or no effect on other frequencies. This caused predictable changes in responses to the more complex FM sweeps whose spectral components passed through the neurons' response areas. Our results suggest that serotonin, whose release varies with behavioral state, functionally reconfigures the circuitry of the IC and may modulate the perception of acoustic signals under different behavioral states.

INTRODUCTION

The central nucleus of the inferior colliculus (ICc) is a nexus of the auditory system. It is the common target of projections from the majority of lower auditory nuclei (Oliver and Huerta 1992; Pollak and Casseday 1989; Ross and Pollak 1989; Roth et al. 1978), and it is strongly innervated by descending projections from the auditory cortex (Druga et al. 1997; Luethke at al. 1989; Saldaña et al. 1996; Winer et al. 1998). Consistent with its widespread afferent innervation, its efferent projections are also pervasive. It provides the principal source of innervation to the medial geniculate body (Winer 1992), and thus indirectly to the auditory cortex, and also provides prominent descending projections that feed back to many of the lower nuclei that provide it with its afferent innervation (Caicedo and Herbert 1993; Malmierca et al. 1996).

In addition to ascending and descending pathways of the auditory system, the ICc is also innervated by cholinergic, noradrenergic, and serotonergic fibers that originate from cell groups outside of the classical auditory system (Henderson and Sherriff 1991; Kaiser and Covey 1997; Klepper and Herbert 1991; Thompson et al. 1995; Thompson et al. 1994). Unlike the auditory system with its highly specified and restricted targets, these neuromodulatory systems derive from cell groups that project broadly throughout the auditory system and the rest of the brain (Campbell et al. 1987; Code and Carr 1994; DeFelipe et al. 1991; Klepper and Herbert 1991; Moore and Bloom 1979; Schafer et al. 1998; Steinbusch 1981; Vu and Tork 1992). The serotonergic innervation of the ICc, for example, originates largely from cells in the dorsal raphe nucleus (Klepper and Herbert 1991), which also projects to other auditory nuclei as well as to other regions of the nervous system. Rather than evoke discharges in auditory neurons, these transmitters usually modulate activity, either pre- or postsynaptically, and thus modify response features evoked by acoustic stimulation (Ebert and Ostwald 1992; Faingold et al. 1991; Farley et al. 1983; Fitzgerald and Sanes 1999; Habbicht and Vater 1996; Wang and Robertson 1997).

Here we report on the effects of serotonin on ICc neurons. There are two reasons why the influences of serotonin on the ICc are of particular interest. The first reason is that the activity of some serotonergic neurons in the dorsal raphe, the raphe nucleus that projects most strongly to the IC, varies markedly with an animal's arousal state and with the direction of attention to a stimulus (Jacobs and Fornal 1999; Trulson and Jacobs 1979). The second reason is that studies on other neural systems have shown that serotonin exerts profound neuromodulatory influences on circuits in both vertebrates and invertebrates (e.g., Harris-Warrick et al. 1992; Sillar et al. 1998). These features suggest that serotonin can not only modulate responses of auditory neurons but also can do so in accordance with the animal's behavioral state. Given the strategic position of the ICc in the auditory system, modulation of its response properties must significantly affect information processing throughout the auditory system.

The subjects for our studies are Mexican free-tailed bats (*Tadarida brasiliensis*). Previous studies have shown that while their brain stem auditory nuclei are greatly hypertrophied, the nuclei, circuitry and response properties of neurons in those nuclei are fundamentally similar to those reported for other, less specialized mammals (Bodenhamer et al. 1979; Grothe et al. 1994, 1997; Park et al. 1996, 1998; Pollak et al. 1977). The relative enlargement of their auditory system is related to the high premium bats place on hearing for their daily activities. These animals use acoustic signals not only for

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echolocation but also for social communication (Balcombe and McCracken 1992; Gelfand and McCracken 1986; Simmons et al. 1978, 1979). Because the features of those signals are known, we can evaluate the effects of serotonin on responses evoked by signals that simulate those normally used by these bats.

In a previous report, we showed that serotonin has substantial effects on a large proportion of the ICc neuronal population (Hurley and Pollak 1999). In that study, we evaluated the modulatory effects of serotonin on responses evoked by best-frequency tone bursts and brief frequency-modulated (FM) sweeps, which simulate bat echolocation calls. The surprising finding was that serotonin often modulates responses evoked by tone bursts differently than it modulates responses evoked by FM sweeps. The explanation we proposed was that serotonin may have complex modulatory effects, preferentially changing responses evoked by some frequencies in the neuron's tuning curve. Here we extend our initial observations by evaluating the influence of serotonin on the excitatory tuning of ICc neurons and by monitoring how those changes in tuning influence responses to brief FM signals.

METHODS

Electrodes

Recordings were made with "piggy back" multibarrel 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-barrel micropipette, pulled previously, that was glued to the multibarrel array so that the tip of the recording electrode was 10–20 μm from the blunted end of the multibarrel electrode. The recording electrodes had resistances of 8–15 MΩ. The recording electrode and the central barrel of the multibarrel electrode were filled with buffered 1 M NaCl and 2% Fast Green (pH 7.4). The Fast Green allowed the electrode to be easily visualized during placement over the inferior colliculus. The remaining barrels were each filled with serotonin creatinine sulfate (20 mM in 200 mM NaCl, pH 4) or with carrier vehicle (200 mM NaCl, pH 4). The barrels of the multibarrel electrode 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 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). Briefly, animals were anesthetized with methoxyflurane inhalation (Metofane, Mallinckrodt Veterinary, Mundelein, IL) and 0.02 mg/g body wt neuroleptic, Innovar-Vet (fentanyl and droperidol, Pitman-Moore), 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 inferior colliculus. The bat was then 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 multibarrel electrode was positioned over the IC under a dissecting microscope. The electrode was subsequently advanced through the brain from outside of the recording chamber using a piezoelectric microdrive (Burleigh 6000, Fishers, NY). Recordings were begun after the bats recovered from the anesthetic. The bats typically lie quietly in the restraining cushion and show no signs of pain or discomfort. Surgical recording sessions typically lasted 5–8 h, and water was provided with a dropper every 1–2 h. Supplementary doses of the neuroleptic 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. All experimental procedures were in accordance with a protocol approved by the University of Texas Institutional Animal Care and Use Committee.

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 ¼-in Brüel and 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. The custom-made loudspeaker has a wider dynamic range than the B&K loudspeaker. Its frequency response was flat ± 6 dB from 10 to 120 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 pinnae 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.

Tone bursts and downward sweeping FM signals having any desired duration as well as starting and terminal frequency were digitally generated by a Power Macintosh 7100/66 computer. Tone bursts were 5–20 ms in duration and had 0.2-ms rise-fall times. The FM signals had durations of 5–10 ms with 0.2-ms rise-fall times. All signals were presented at a rate of four per second. We also obtained 16 social communication calls emitted by Mexican free-tailed bats that were digitally stored in the computer. The call durations varied from about 16–35 ms.

Spikes were fed to a window discriminator and then to a Macintosh 7100 computer controlled by a real time clock. Peristimulus time (PST) histograms and rate-level functions were generated and graphically displayed. Unless otherwise noted, each PST histogram was generated from the discharges evoked by 20 presentations of a signal at a fixed intensity.

Once a unit was isolated, its best frequency (BF, the frequency to which the neuron was most sensitive) and the threshold at BF were determined audiovisually. Intensity was then set at 10 or 20 dB above threshold, and tone bursts having frequencies both above and below the neuron's BF were presented in steps varying in size from 200 Hz to 1 kHz pseudorandomly. The neuron's response area, defined as the range of frequencies that evoked discharges at a constant intensity, was obtained from the PST histograms generated by 20 presentations of each tone burst frequency (e.g., Fig. 1). A quantitative value for each response area was obtained by integrating the area under the graph of frequency versus spike count using Microcal Origin (Microcal, Northampton, MA). In each neuron, a response area was generated two to three times to ensure response stability. Only neurons whose response areas were stable over time were used to evaluate the effects of serotonin.

To assess the tuning of response regions, we measured the low- and high-frequency borders of each response region before and during application of serotonin. Since ICc neurons had little or no spontaneous activity, we considered the low- and high-frequency borders as lowest
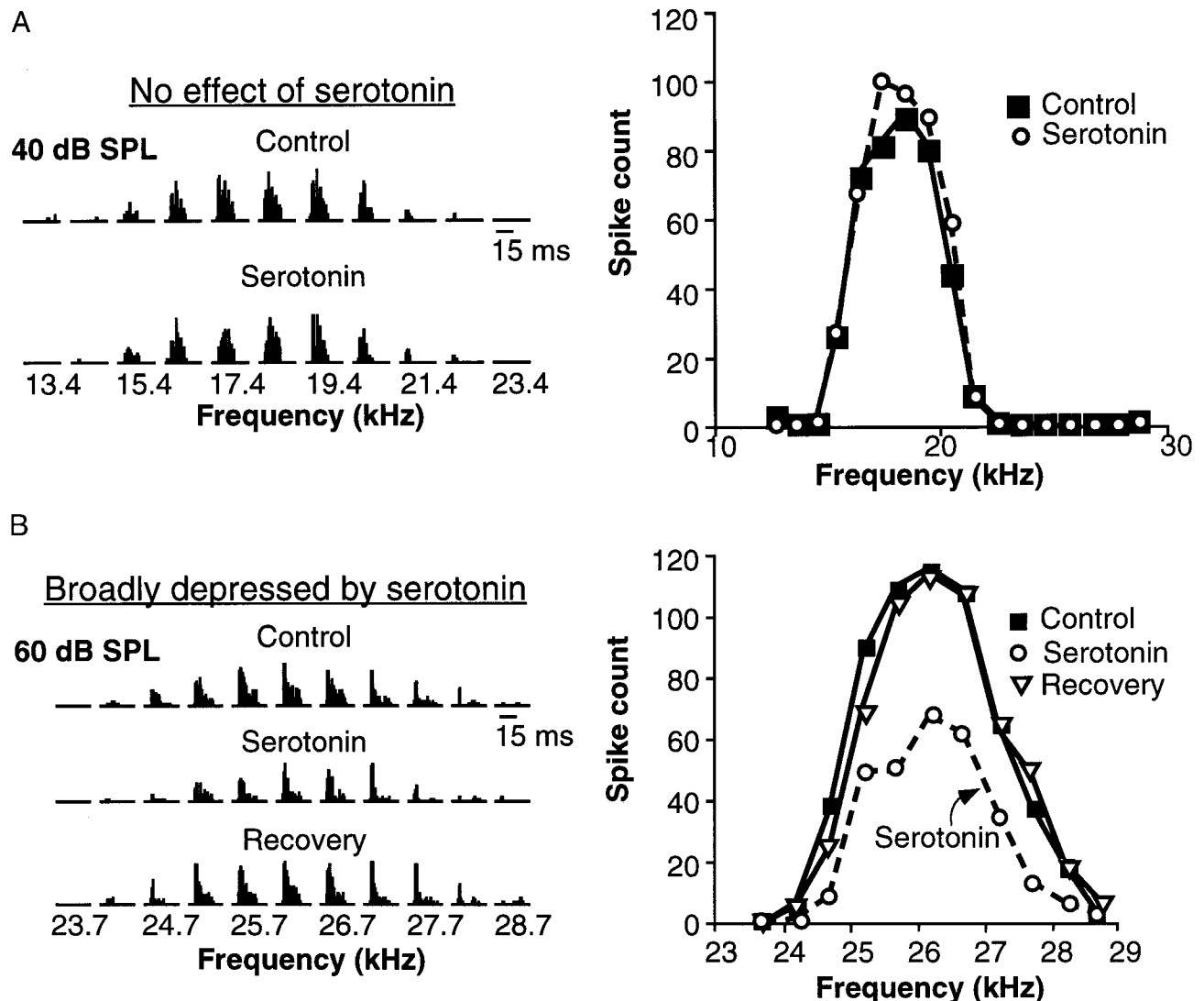
FIG. 1. Comparison of a neuron in which serotonin had no effect (A) and another neuron (B) in which serotonin broadly depressed spike counts. Note that serotonin depressed the spike counts at all frequencies to which the neuron responded and that the depressed spike counts recovered about 10 min after the application of serotonin was stopped. Shown on the *left* in both A and B are peri-stimulus (PST) histograms of responses to tone bursts having a range of frequencies but constant sound intensity (40 dB SPL in A and 60 dB SPL in B). *Right*: the response areas in both A and B. The response areas are the same data as in the PST histograms plotted as spike counts vs. frequency. The ejection current was 50 nA in A and 75 nA in B.

and highest frequencies that evoked five stimulus-locked spikes/20 stimuli. The difference in frequency between the low- and high-frequency borders is the width of the response region in kilohertz.

Following the acquisition of these data, serotonin was iontophoretically applied. During serotonin application, rate-level functions were monitored until spike counts stabilized. Once spike counts were stable, the same stimuli were presented again, and responses were obtained for comparison with those obtained before the application of serotonin. In 39 neurons, vehicle alone was applied but it did not affect responsiveness as did serotonin (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.

We assigned a serotonin-induced change in response area to those neurons in which the integrated values of the response areas obtained before and during serotonin differed by 30% or more. Our criterion for a change in the tuning of the response region was that the width of the serotonin response region had to be at least 20% broader or 20% narrower than the width of the control response region. We also assessed whether serotonin-induced changes in tuning occurred at both the low- and high-frequency borders or whether it occurred disproportionately at one end of the frequency range. This was accomplished by subtracting the frequencies of the high-frequency borders in the serotonin and control response regions and then subtracting the frequencies of the low-frequency borders in the serotonin and control response regions. This provides a value, in kilohertz, of the serotonin-induced change in the high-frequency border and a value, in kilohertz, of the change at the low-frequency border. The difference between these two values provide an index indicative of any asymmetry associated with changes in tuning. The asymmetry index was then normalized by dividing it by the width (in kHz) of the control response region. An asymmetry index of 0.0 indicates an equal change at both the high and low borders of the response region, while values greater than 0.0 indicate a larger change at one border than at the other.

Statistical comparisons of mean values were Student's t-tests performed with Statview 4.0 (Abacus Concepts, Berkeley, CA).

RESULTS

Serotonin was iontophoretically applied to 114 neurons in the ICc. The BFs ranged among our sample from 12 to 55 kHz, although the majority of cells had BFs between 18 and 30 kHz. Almost all IC cells had little or no spontaneous activity, thereby facilitating the identification of sound-evoked activity. With few exceptions, serotonin had no obvious effect on spontaneous activity in most cells, and we did not examine this feature in greater detail. A response area, a plot of the spike counts evoked by excitatory frequencies at a constant intensity, was generated for each cell (e.g., Fig. 1A, right). The effects of serotonin were determined by comparing the response area of each neuron before serotonin was applied to the response area obtained during the application of serotonin (Fig. 1).

Serotonin had no discernible effect on 40% (46/114) of the neurons in our sample. In these neurons, the response areas obtained during the application of serotonin were within 30% of the control response areas. An example is shown in Fig. 1A. In 60% of the neurons (68/114), serotonin changed the response area by at least 30% (e.g., Fig. 1B). The changes were due either to a serotonin-induced depression of tone-evoked discharges, which was the most common effect, or to a facilitation of spike counts. These changes in response area were always due to depression or facilitation of tone-evoked responses and were sometimes, but not always, accompanied by expansions or contractions in the borders of the response area. However, in none of the cells was a change in response area due only to an expansion or contraction of the frequency borders.

Whether or not a neuron was influenced by serotonin was not correlated with BF or temporal discharge pattern. The BFs of neurons whose response areas were unchanged by serotonin ranged from 12 to 50.2 kHz, where most had BFs from 18 to 30 kHz. This was not significantly different from the BFs of cells whose response areas were changed by serotonin ($P = 0.709$, 2-tailed unpaired t-test). Similarly there was also no difference in the effects of serotonin on neurons that responded to BF tones with a phasic compared with a sustained discharge pattern. Thus of the neurons affected by serotonin, 72% (49/68) were phasic while 28% (19/68) were sustained. These percentages of phasic and sustained neurons affected by serotonin did not appear different from the percentage of phasic (61%; 28/46) and sustained (39%; 18/46) neurons that were not affected by serotonin. This lack of correlation among response properties and serotonergic effects is consistent with our previous findings (Hurley and Pollak 1999).

Serotonin had two main types of effects

All neurons whose response areas were changed by serotonin were placed into one of two classes. We refer to one class of neurons (51/68, 75%) as "broadly affected," because serotonin changed the spike counts evoked by all frequencies in their response areas (Figs. 1B and 2, A and B). The responses of most (38/51, 75%) broadly affected neurons were depressed by serotonin. In a smaller number of neurons (13/51, 25%), serotonin facilitated responses (e.g., Fig. 2B). None of the 51 broadly affected neurons exhibited a serotonin-induced depression at some frequencies and facilitation at others. On average, the decrease in the response area for the 38 broadly depressed neurons was $60 \pm 3.1\%$, while the average increase in response

area was $60 \pm 17\%$ for the 13 broadly facilitated neurons (Fig. 3A).

The other class of neurons (17/68, 25%), which we refer to as focally affected neurons, exhibited serotonin effects that were focused only on portions of their response areas (e.g., Fig. 2, C and D). These neurons also exhibited at least a 30% change in their response areas, but serotonin had little or no effect on the spike counts evoked by some frequencies, whereas the spike counts evoked by other frequencies were profoundly changed. Focally affected neurons, like broadly affected neurons, could be either depressed or facilitated by serotonin, but most were depressed (14 neurons were depressed but only 3 were facilitated). On average, the decrease in the response area for the 14 focally depressed neurons was $51.2 \pm 4\%$, while the average increase in response area was $70 \pm 24\%$ for the 3 focally facilitated neurons (Fig. 3A). In the following text we discuss additional features affected by serotonin, first for broadly affected and then for focally affected neurons.

Broadly affected neurons

The changes in the spike counts induced by serotonin varied markedly among the population of broadly affected neurons. The depression in some neurons, such as the neuron in Fig. 1B, was moderate, and all frequencies in the response area were depressed by about the same degree. In other broadly affected neurons, the serotonin-induced changes in spike counts were more profound. The depression of the neuron in Fig. 2A, for example, was far greater than the neuron in Fig. 1B. In the neuron in Fig. 2A, each frequency in the response area was either completely or almost completely depressed by serotonin. In yet other neurons, serotonin changed the spike counts evoked by all frequencies in the response area, but the degree to which each frequency was affected was not uniform. The neuron in Fig. 2B is an example of a serotonin-induced facilitation where the degree of facilitation was nonuniform in that the responses to some frequencies were facilitated more than the responses evoked by other frequencies. The nonuniformity is illustrated by comparing the facilitation at 24.6 kHz to the facilitation at 25.6 kHz. Both frequencies evoked only two spikes in the control condition (*), but serotonin facilitated responses to 25.6 kHz by 450% (from 2 to 9 spikes) while responses to 24.6 kHz were facilitated by 1,350% (from 2 to 27 spikes). Although responses to some frequencies were facilitated to a much greater degree than were responses to other frequencies, the neuron was classified as broadly affected because the responses to every frequency in the response area were facilitated.

Serotonin also changed the width of the response areas of many broadly depressed neurons, although changes in width were usually smaller than were the changes in spike counts. We were unable to measure response area widths in 11 neurons due to extreme serotonin-evoked depression of spike counts. However, 59% (16/27) of the cells that we measured had serotonin-induced reductions of 20% or more in the widths of their response areas. These tuning changes were typically due to contractions on both the high- and low-frequency borders of the response areas. This can be seen in Fig. 3B, which shows that the asymmetry index for almost all broadly depressed neurons was below 0.2 and for most it was between 0.0 and

Broadly Affected Neurons
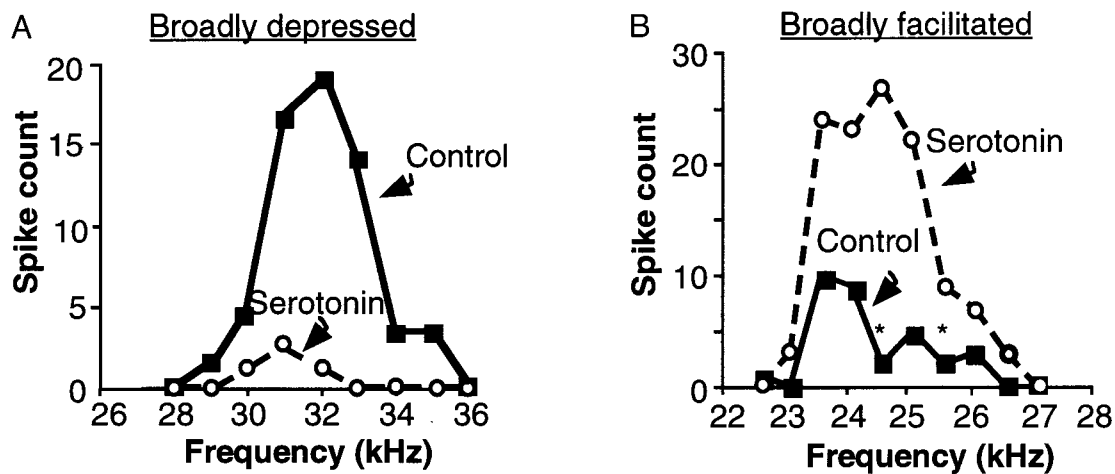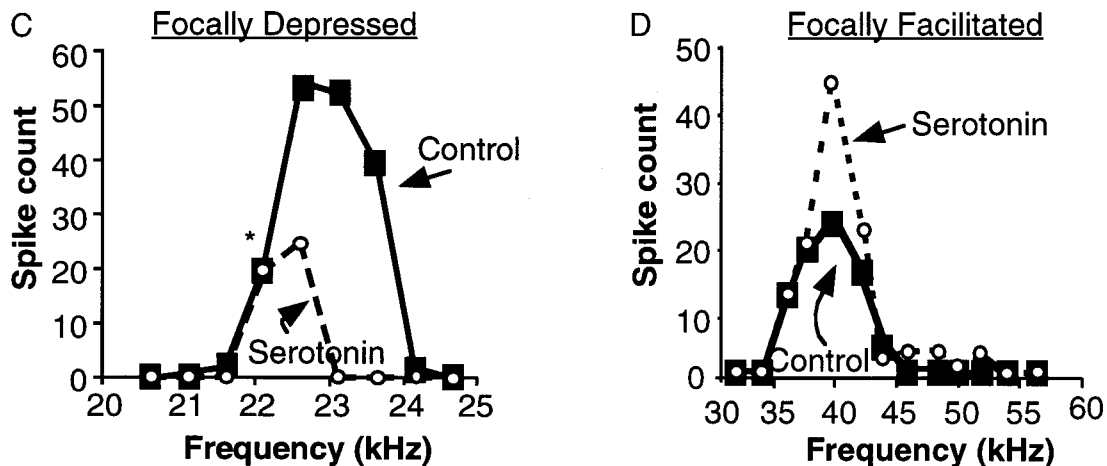

Focally Affected Neurons

FIG. 2. Examples of broad and focal effects of serotonin on response areas of 4 neurons. *A* and *B*: examples of a broadly depressed neuron (*A*) and a broadly facilitated neuron (*B*). Both neurons were classified as broadly affected because serotonin changed the spike counts evoked by all frequencies in their response areas. For the broadly facilitated neuron, the facilitation was substantially greater at 23.6–25.0 kHz than at 25.6–26.6 kHz. *, spike counts evoked by 24.6 and 25.6 kHz in the control condition. Note the difference in the serotonin-induced facilitation at these 2 frequencies. All sound intensities were 30 dB SPL in *A* and 50 dB SPL in *B*. Ejection current was 60 nA in *A* and 80 nA in *B*. *C* and *D*: examples of a focally depressed neuron (*C*) and a focally facilitated neuron (*D*). For the focally depressed neuron (*C*), the distinguishing feature was that serotonin had no effect on responses to 22 kHz (*), but responses to higher frequencies were strongly or completely depressed. Signals were 40 dB SPL. Ejection current was 80 nA. In the focally facilitated neuron in *D,* facilitation was evoked only at 40–42 kHz while other frequencies in the response area were unaffected by serotonin. Signal intensities were 30 dB SPL. Ejection current was 80 nA.

0.1. We also observed changes in the frequency borders in 23% (3 of 13) of the broadly facilitated neurons. In these neurons, serotonin caused the response areas to widen by 20% or more.

Focally affected neurons

The feature that distinguished focal serotonin effects from broad effects was that for focal neurons, responses evoked by some frequencies in the response area were only slightly changed, or not changed at all by serotonin, whereas the responses to other frequencies were markedly changed. As was the case for broadly affected neurons, the degree to which spike counts were changed by serotonin varied among the population. In some neurons, the frequencies affected by serotonin were depressed by about half at most, whereas in other neurons the responses of frequencies affected were completely or almost completely depressed. An example of a focal neuron that was strongly depressed is shown in Fig. 2*C*. The neuron's control response area encompassed frequencies from about 21.6 to 24.1 kHz, but the most effective frequency, the frequency that evoked the highest spike count, was 22.6 kHz. The significant feature is that serotonin did not change the spike count evoked by 22.1 kHz (*), although the spike count evoked by 22.6 kHz, the most

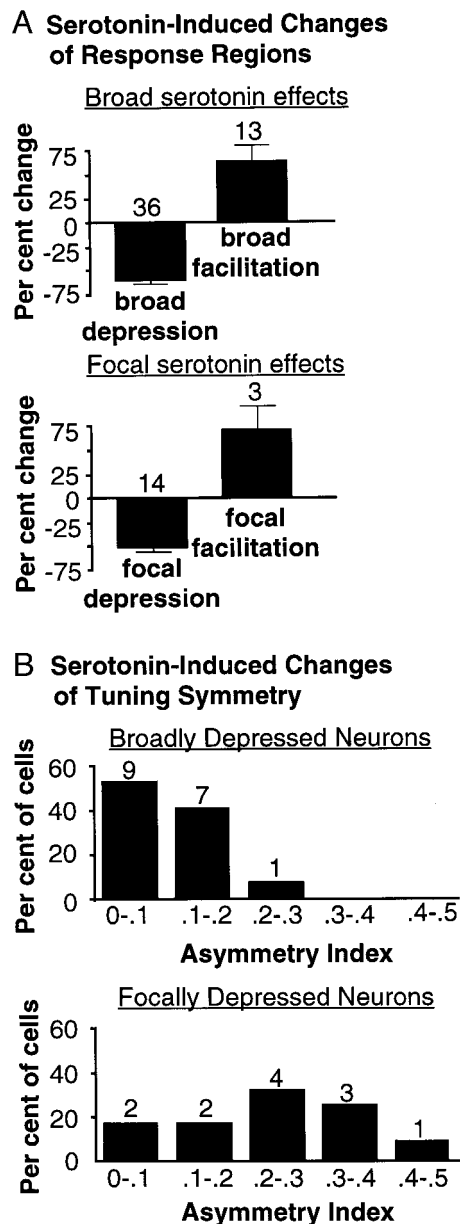## A Serotonin-Induced Changes of Response Regions

FIG. 3. Population measures of serotonin-induced changes in response area and tuning. *A*: changes in response areas primarily reflect serotonin-induced changes in spike counts and are shown as the mean percent changes in the response areas for broadly and focally affected neurons. Numbers *above* each column are the sample size for each category, and error bars represent SE. *B*: a comparison of serotonin-induced changes in the frequency borders of the response areas (i.e., tuning) of broadly depressed and focally depressed neurons. Index of tuning asymmetry indicates whether serotonin-induced changes in tuning occurred at both the low- and high-frequency borders of the response areas or whether it occurred disproportionately at one frequency border. An asymmetry index of 0.0 indicates an equal change at both the high and low borders of the response area, while values greater than 0.0 indicate a larger change in one border than on the other. The population of focally depressed neurons had higher asymmetry indices than broadly depressed neurons. This shows that focally depressed neurons had a higher incidence of tuning changes at either the high or low borders of their response areas than did the broadly depressed neurons. Numbers *above* each column represent the sample size. For reference, the focally depressed neuron in Fig. 2C had an index of asymmetry of 0.32.

effective frequency, was strongly depressed and higher frequencies were completely depressed. Thus serotonin only depressed discharges evoked by some frequencies in the

response area and had no effect on the responses evoked by other frequencies.

Serotonin also changed width of response areas by at least 20% in 64% (9/14) of the focally depressed neurons. The tuning changes in the focally depressed neurons differed in one important respect from the changes in tuning for broadly depressed neurons: for focally depressed neurons, the contractions of frequency borders were usually confined to one end of the frequency range, whereas in most broadly depressed neurons there were contractions in both ends of the frequency range. This asymmetry in tuning is shown in Fig. 3B. A large number of focally depressed neurons had an asymmetry index greater than 0.2, which contrasts markedly with the low indices of asymmetry calculated for broadly depressed neurons.

Serotonin-induced effects were similar at different intensities

The serotonin-induced effects described above were obtained at one sound intensity, usually at 10 or 20 dB above the threshold at the neuron's BF. In 42 neurons, we also documented serotonin-induced effects on response areas at two to three intensities. In 30 of 42 neurons (71%), the effects of serotonin observed at one intensity were similar at the other intensities we presented. In the other 12 cells, serotonin only changed discharge rates at one intensity but at other intensities serotonin had no effect in that the discharge rates were similar to those obtained at the same intensity before serotonin was applied.

Two examples of neurons in which the effects of serotonin were similar as intensity was increased are shown in Fig. 4. The broadly affected neuron in Fig. 4A, for example, was strongly depressed when the sound intensity was 20 dB sound pressure level (SPL). When the intensity was increased by 20 dB, to 40 dB SPL, the borders of the response area expanded, but serotonin still strongly depressed responses to all frequencies in the response area. A similar pattern can be seen in the focally facilitated neuron in Fig. 4B. At 30 dB SPL, serotonin only facilitated responses to 40–42 kHz and had little or no effect on the spike counts evoked by other frequencies. When intensity was increased by 20 dB, to 50 dB SPL, facilitation was still confined to 40–42 kHz, and this occurred although the response area expanded at the higher intensities, especially on the high-frequency side. Thus most neurons that displayed focal serotonin-induced effects at one intensity were focally affected at other intensities, and most neurons that were broadly changed at 10–20 dB above threshold also displayed broad changes at higher intensities.

Dose dependence

The data we presented in the preceding text were obtained when serotonin was applied with one ejection current, although the ejection current was different for each neuron. To evaluate the dose dependence of serotonin effects, we applied different dosages of serotonin in 12 neurons. In most neurons, the effects of serotonin increased with dosage. We illustrate these results with three representative neurons in Fig. 5. For the neuron in Fig. 5A, a control response area was first obtained, followed by two additional response areas, each generated with a different dosage of serotonin. The lower dosage caused a small response depression that was limited to only a portion of the response
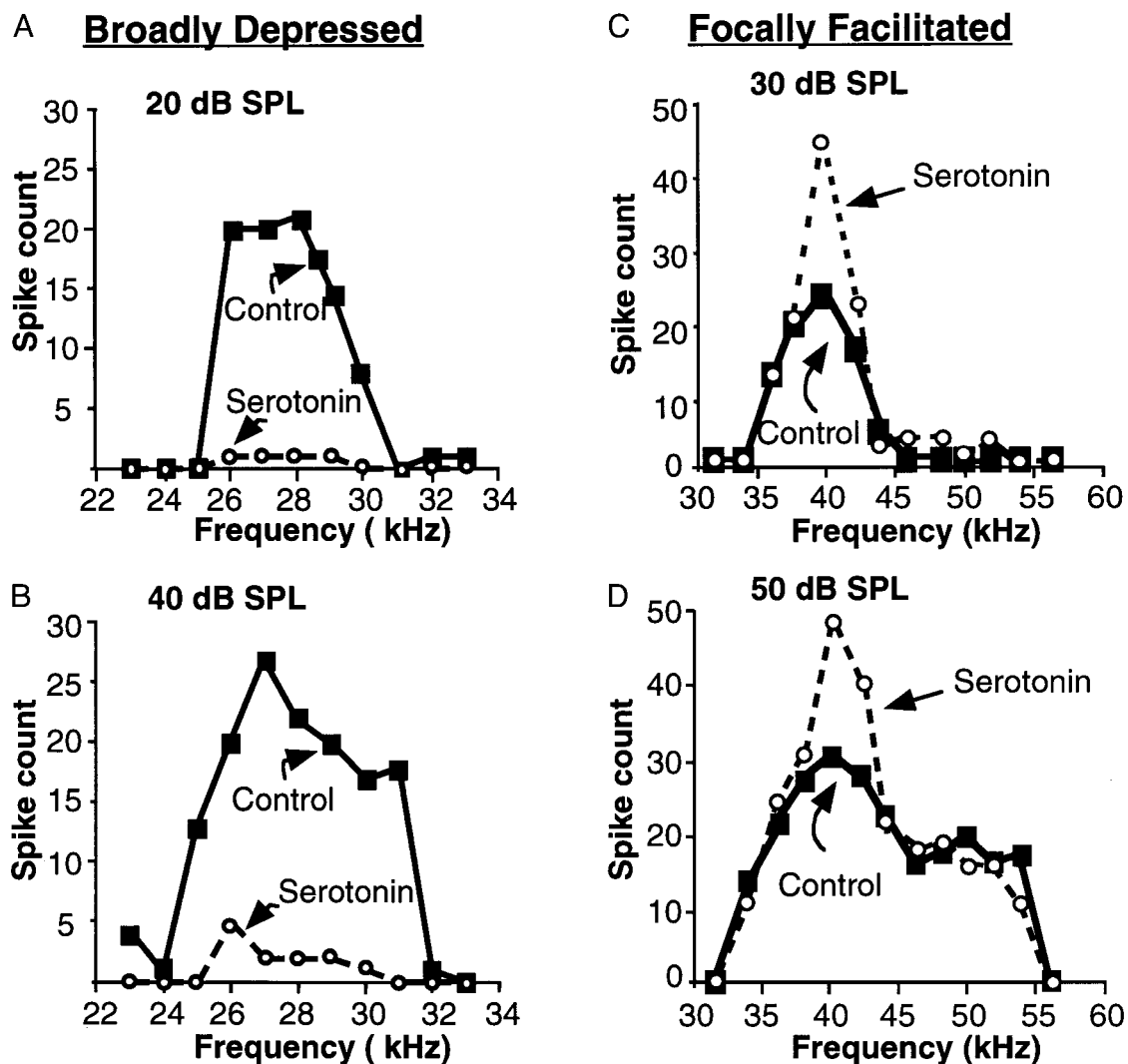
FIG. 4. Serotonin-induced changes in response areas were similar at different sound intensities. *A* and *B*: 2 response areas from a neuron that was broadly depressed by serotonin. Each response area was generated with a different sound intensity, but both were similar in that the responses were strongly depressed and the strong depression encompassed all frequencies in the response areas. Ejection current was 50 nA. *C* and *D*: response areas from a focally facilitated neuron. This is the same neuron as shown in Fig. 2*D*. When sound intensity was 30 dB SPL (*top right*), facilitative effects of serotonin were focused on the responses to 40–42 kHz and had little or no effect on responses evoked by other frequencies. When intensity was increased by 20 dB, to 50 dB SPL, facilitation was still confined to 40–42 kHz, and this occurred although the response area expanded at the higher intensities especially on the high-frequency side. Ejection current was 80 nA.

area, to frequencies ranging from 23.5 to 24.5 kHz. This had the form of a focal depression, although we would not classify this as a serotonin-induced effect because the depression did not cause a 30% reduction in response area. When the dosage was doubled, not only did the depression increase, but the range of affected frequencies expanded to encompass all frequencies in the response area (i.e., the neuron was broadly depressed at the higher dosage). A similar change in depression with dosage was seen in the neuron in Fig. 5*B*. In this neuron, an ejection current of 50 nA depressed responses to higher frequencies (25–27.5 kHz) but had little or no effect on responses to lower frequencies (22.5–24.5 kHz). Thus the lower dosage produced a focal depression. Increasing the ejection current to 80 nA increased the depression. The higher frequencies were depressed even more than they were with the lower ejection current, but now the lower frequencies were also

depressed, although not as strongly as the higher frequencies. Because serotonin depressed all frequencies in the response area, we would classify this as a broad depression. Thus this neuron was focally depressed by lower dosages and broadly depressed by a higher dosage. The neuron in Fig. 5*C* illustrates focal effects that were unchanged with increasing dosage. In this neuron, the lower dose of serotonin (60 nA) only depressed responses on the high-frequency side of the response area, at 27–27.5 kHz, and had little or no effect on lower frequencies (25–26.5 kHz). Increasing the dose to 90 nA produced no change over that produced by the lower dose. Thus the focus of the depression on responses evoked by 27–27.5 kHz and the amount of depression were virtually unchanged as dosage increased. The absence of any further effect as dosage increased suggests that the serotonergic receptors were saturated when the ejection current was 60 nA.
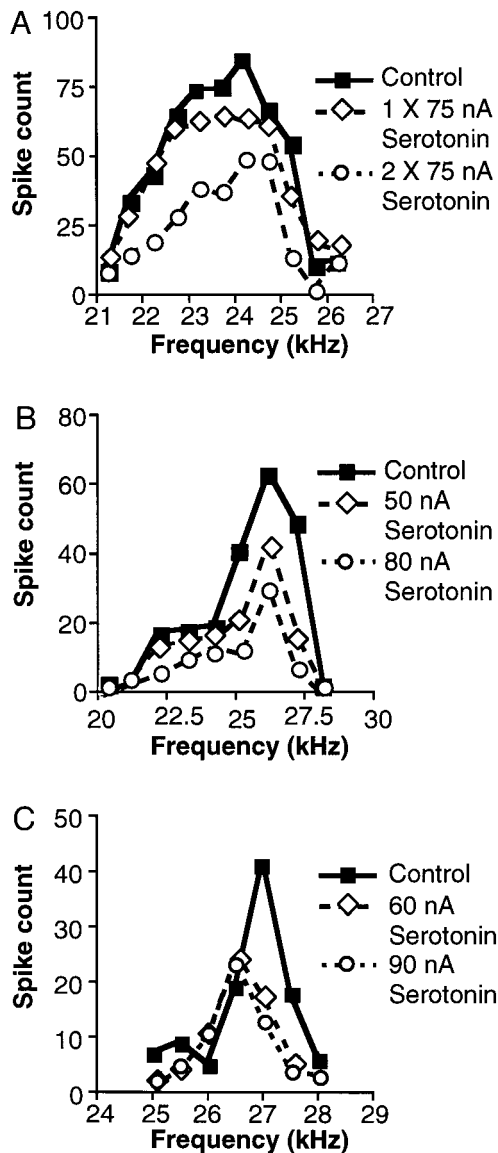
FIG. 5. Dose-dependent effects of serotonin on the response areas of 3 neurons. *A*: slight depression of responses limited to frequencies ranging from 23 to 24.5 kHz when serotonin was ejected from 1 barrel of the multibarrel pipette with an ejection current of 75 nA. A larger depression that encompassed all frequencies in the response area occurred when serotonin was ejected from 2 barrels of the multibarrel pipette, each with an ejection current of 75 nA. Signal intensity was 50 dB SPL. *B*: another neuron showing an enhanced depression by serotonin when ejection current was increased from 50 to 80 nA. With the lower ejection current (50 nA), the high frequencies of the response area (25–27.5 kHz) were depressed while the lower frequencies (below 25 kHz) were hardly affected. The higher ejection current (80 nA) caused a further depression of the high frequencies and a depression of the lower frequencies as well. Signal intensity was 50 dB SPL. *C*: focally depressed neuron in which the depression was unchanged with an increasing ejection current of 60–90 nA, suggesting that receptors were saturated at lower dosage. Signal intensity was 0 dB SPL.

Responsiveness to spectrally complex signals

In the preceding sections, we showed that serotonin changed the responses to tonal frequencies that comprised the response areas of many ICc neurons. Here we show that serotonin also changed responses to complex signals that had frequency components that stimulated all or portions of the neuron's response area. Additionally, the serotonin-induced changes in the re-

sponses to the complex signals were, in most but not all neurons, consistent with the changes in the response areas generated with tone bursts.

Complex signals of particular importance to bats are brief, downward-sweeping FM chirps because these are the signals many bats, including Mexican free-tailed bats, use for echolocation. Consequently, we presented a series of brief FM sweeps, each encompassing different and sometimes overlapping frequency ranges within the neuron's response area. We then evaluated the degree to which the serotonin-induced changes in the neuron's response area correlated with serotonin-induced changes in their responsiveness to the FM signals.

The serotonin-induced changes in responsiveness evoked by FM sweeps were consistent with the changes in the response areas in 45 of 58 neurons (77%). In focally affected neurons, serotonin-induced changes in responses to FM sweeps were often selective in that responses to some FM sweeps were changed differently than were responses to other FM sweeps. We illustrate these changes in response selectivity with the neuron in Fig. 6. The control response area was generated with 30 dB SPL tone bursts and had two portions: an effective high-frequency portion, from about 24 to 21 kHz, and a less effective portion at lower frequencies, from about 17 to 19 kHz. The two portions were separated at 20 kHz, where there was a slight decline in spike count. Three FM sweeps, each at 30 dB SPL, were presented. FM 1, which swept through the entire response area, evoked two discharge peaks in the PST histogram. The larger first peak was most likely evoked as the signal swept through the most effective, high-frequency portion of the response area. The second, smaller peak (*) presumably was evoked as the signal passed through the less-effective low-frequency portion. The few discharges in the gap between the peaks may have occurred as the signal swept through 20 kHz, where there was a dip in the response area. FM 2 only swept through the high-frequency portion of the response area and evoked a single peak in the PST histogram with a spike count smaller than that evoked by FM 1. FM 3 only swept through the less-effective low-frequency portion of the response area and evoked a single discharge peak at a lower spike count than FM 2.

Serotonin strongly depressed only the lower frequencies of the response area and had little effect on the high frequencies. The depression of the low frequencies corresponds closely with the changes in responses to the FM signals. When serotonin was applied, FM 1 evoked only one discharge peak, and thus a lower spike count than the control spike count (31 compared with 23 spikes). Presumably as the signal swept through the low frequencies, responses were no longer evoked because the excitation provided by the low frequencies was depressed by serotonin. In contrast, neither the discharge pattern nor the spike count evoked by FM 2 was depressed by serotonin. This absence of any change in responses to FM 2, whose spectrum was confined to the higher frequencies of the response area, corresponds closely with the absence of any substantial serotonin-induced change in responses to these frequencies. Finally, with serotonin, there was almost no response to FM 3; this corresponds closely with the strong serotonin-induced depression of the frequencies in the FM 3 sweep. In short, the changes in the response area induced by serotonin are in concordance with the serotonin-induced changes in the responses evoked by the three FM signals that we presented.
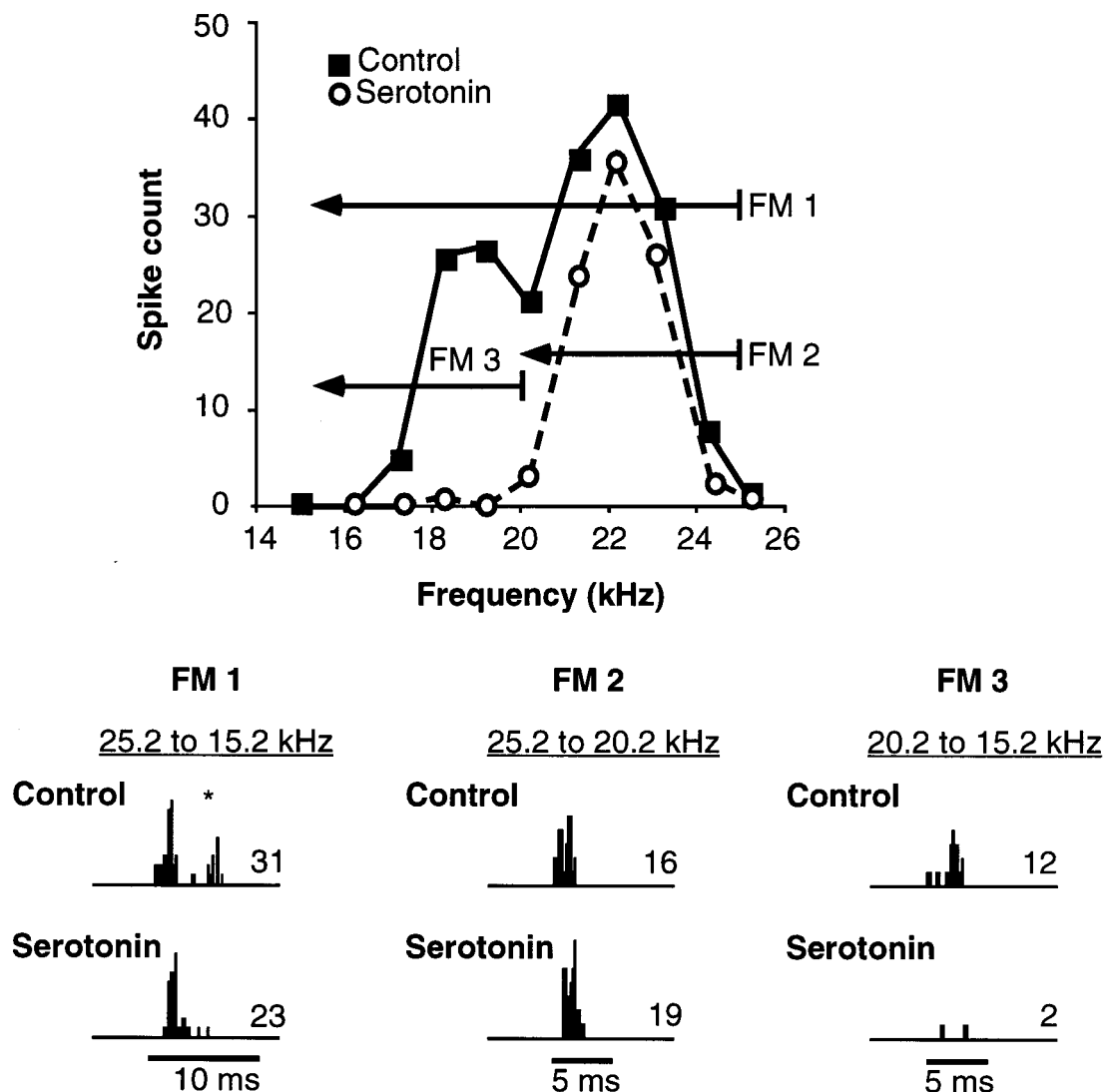
FIG. 6.   Focally depressed neuron in which serotonin-induced changes in the response area correlated closely with changes in response selectivity for 3 FM sweeps. FM 1 swept downward from 25.2 to 15.2 kHz, FM 2 from 25.5 to 20.2 kHz, and FM 3 from 20.2 to 15.2 kHz. *Top*: the response areas before serotonin was applied (control) and while serotonin was applied (serotonin). Serotonin strongly depressed the lower frequencies of the response area and had little effect on the higher frequencies. The placement of each ← on the ordinate indicates the spike count evoked by that sweep (e.g., in control condition, FM1 evoked 31 spikes). *Bottom*: PST histograms evoked by FM 1, FM 2, and FM 3 under control (*top histograms*) and serotonin (*bottom histograms*) conditions. FM 1 was 10 ms in duration while FM 2 and FM 3 were 5 ms in duration. Sound intensities of tone bursts and FM sweeps were 30 dB SPL. Ejection current was 70 nA.

In most broadly depressed neurons, responsiveness to FM signals was depressed by serotonin. Unlike focal neurons, however, the responses to all the FM signals that we presented were depressed in most, but not all, of these neurons (see following text). An example is shown in Fig. 7. Three FM sweeps were presented to this neuron, each with a duration of 10 ms and each at 60 dB SPL, the same intensity as the tones used to generate the response area. Each FM signal swept through different portions of the response area, and each evoked more or less vigorous discharges. Serotonin strongly depressed responses evoked by all of the FM sweeps. As with the focally affected neuron described in the preceding text, the effects of serotonin on FM sweeps in this broadly affected neuron were consistent with its effects on tonal frequencies.

While there was a close correspondence between the effects of serotonin on response areas and FM sweeps in the vast majority of neurons, in a few neurons serotonergic effects on FM sweeps were not in agreement with its effects on tonal frequencies. One example is the broadly depressed neuron in Fig. 8. Before serotonin was applied, the frequencies evoking the highest spike counts ranged from 50 to 55 kHz, and the responses to these frequencies were most strongly depressed by serotonin (Fig. 8, *top*). Consistent with this depression, the responses to FM 1, a 10-ms FM that swept from 53.6 to 48.6 kHz, were also depressed by serotonin. The control spike count was 35 spikes and was reduced by serotonin to 21 spikes. The surprising finding was that the responses to FM 2, a 10-ms FM signal that swept from 58.6 to 48.6 kHz, were hardly affected at all by serotonin. The control spike count was 27 spikes and was 23 spikes with serotonin. Based on the serotonin-induced changes in the response area, the prediction would be that
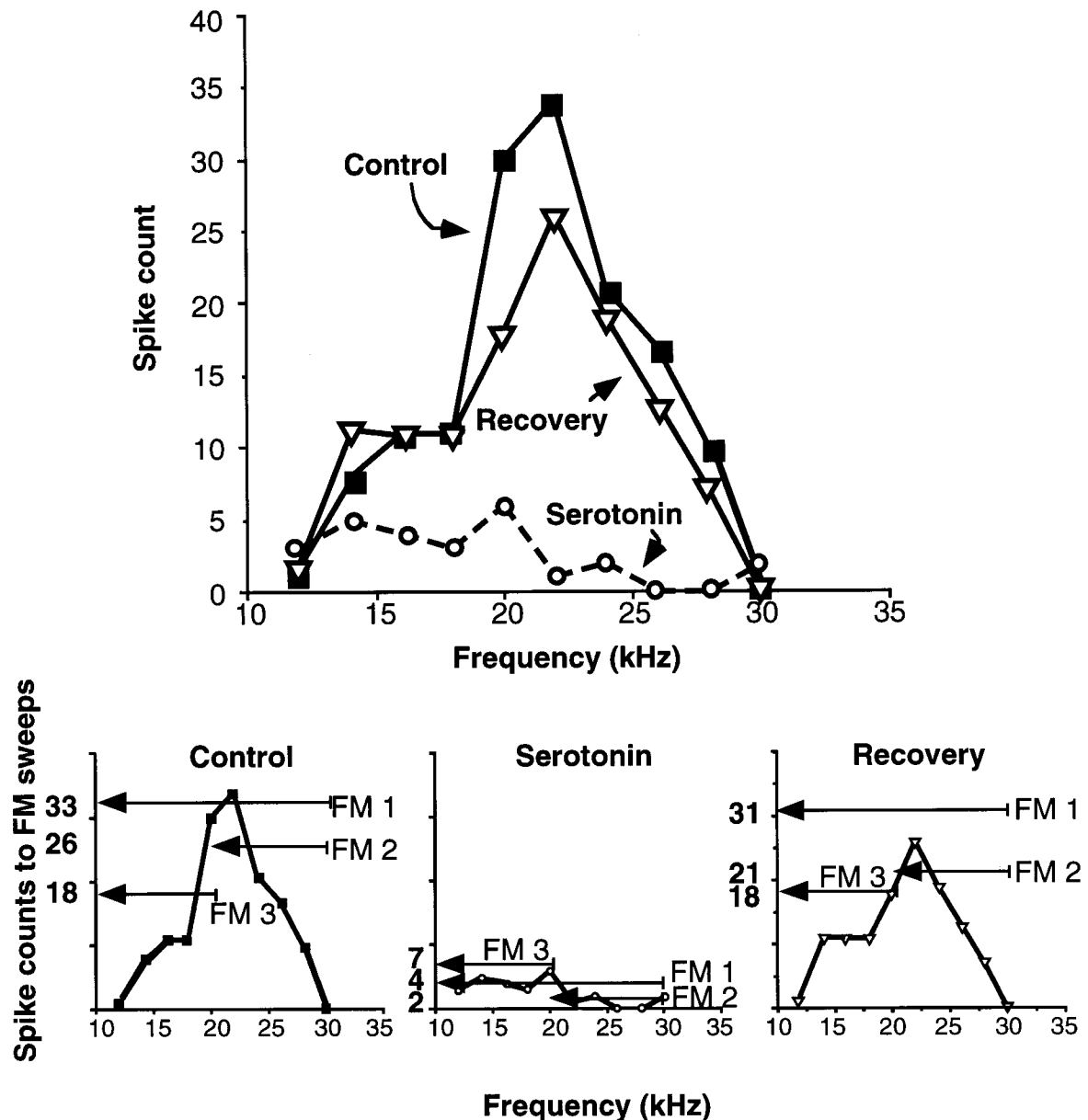
FIG. 7. Broadly depressed neuron in which serotonin-induced changes in the response area correlated closely with changes in responses to FM sweeps. *Top*: the response areas before serotonin was applied (control), while serotonin was applied (serotonin), and 10 min after termination of serotonin (recovery). *Bottom*: the control, serotonin, and recovery response areas separately, with arrows representing 3 downward FM sweeps. The length of each arrow indicates the extent of each sweep: FM1 swept downward from 30 to 10 kHz, FM 2 from 30 to 20 kHz, and FM 3 from 20 to 10 kHz. The placement of each arrow on the ordinate indicates the spike count evoked by that sweep (e.g., in control condition, FM 1 evoked 33 spikes). Serotonin caused a broad depression throughout the response region; frequencies at and above the most effective frequency (22.5 kHz) were completely depressed, while frequencies below the most effective frequency were strongly but not completely depressed. The spike counts evoked by the 3 FM sweeps in the control and recovery conditions were similar, but the responses to each FM sweep were strongly depressed by serotonin. Thus responses evoked by FM 1 and FM 2 were almost completely depressed in serotonin, while FM 3 evoked a weak response. The greater depression of responses to FM 1 and FM 2 was most likely due to the stronger depression of higher frequencies in the response region, thereby all but eliminating any excitation when spectral components of FM 1 and FM 2 swept through those frequencies. Ejection current was 70 nA. All signal intensities were 60 dB SPL.

spike count evoked by FM 2 should have been substantially reduced by serotonin, and yet the spike count was hardly changed.

Both concordance and nonconcordance with serotonin-induced changes in response areas were also seen with responses to communication calls. In three broadly depressed neurons, we did not present FM sweeps but rather presented 16 different communication calls used by Mexican free-tailed bats after obtaining a response area for each neuron. The communication calls we presented had durations ranging from 16 to 35 ms, and each had several harmonics as well as frequency and intensity modulations that occurred throughout the calls. We point out that the very small sample cannot represent the effects of serotonin on responses to communication calls among the

population. Rather the results are presented to further underscore the findings of broadly depressed neurons obtained with FM sweeps and to show that these phenomena occur with the actual signals these animals use. In two of the neurons, serotonin depressed the responses to the communication calls to which it previously responded as would be predicted by the broad serotonin-induced depression of their response areas (not shown). In the third neuron, however, serotonin had effects that could not have been predicted from its broad depressive effect on the neuron's response area (Fig. 9). In the control condition, the neuron was highly selective in that it responded to only one of the 16 communication calls that we presented, *call A1,* and was unresponsive to the other communication calls, including *call D2.* Although the neuron responded to *call A1* and not to *call D2,* portions of each of the two calls, shown in Fig. 9, encroached on the neuron's response area. During the application of serotonin, the neuron no longer responded to *call A1,* a result consistent with the depressive effect of serotonin on its

FIG. 9. A broadly depressed neuron in which the effects of serotonin on responses to social communication calls were evaluated. *Top*: the response areas before (control) and during application of serotonin. *Middle*: responses to *calls A1* and *D2* before serotonin was applied (control), while it was applied (serotonin), and after recovery from serotonin. *Bottom*: sonograms of *call A1* and *D2*. - - -, bracket for the frequencies of the control response area. The duration of *call A1* was 31 ms and duration of *call D2* was 16 ms. Before serotonin was applied, the neuron responded to *call A1*. It did not respond to *call D2* or to any of the other 14 calls that we presented. Portions of both *calls A1* and *D2* encroached on the neuron's response area (*bottom*). Application of serotonin completely depressed responses to *call A1,* which was consistent with the broad depressive effect of serotonin on the response area. However, during application of serotonin the neuron responded to *call D2,* although it did not respond to this call before serotonin was applied. Ten minutes after termination of serotonin (recovery), the neuron again responded to *call A1* and was again unresponsive to *call D2*. Signal intensity was 40 dB SPL for *call A1* and 20 dB SPL for *call D2*. Ejection current was 50 nA.
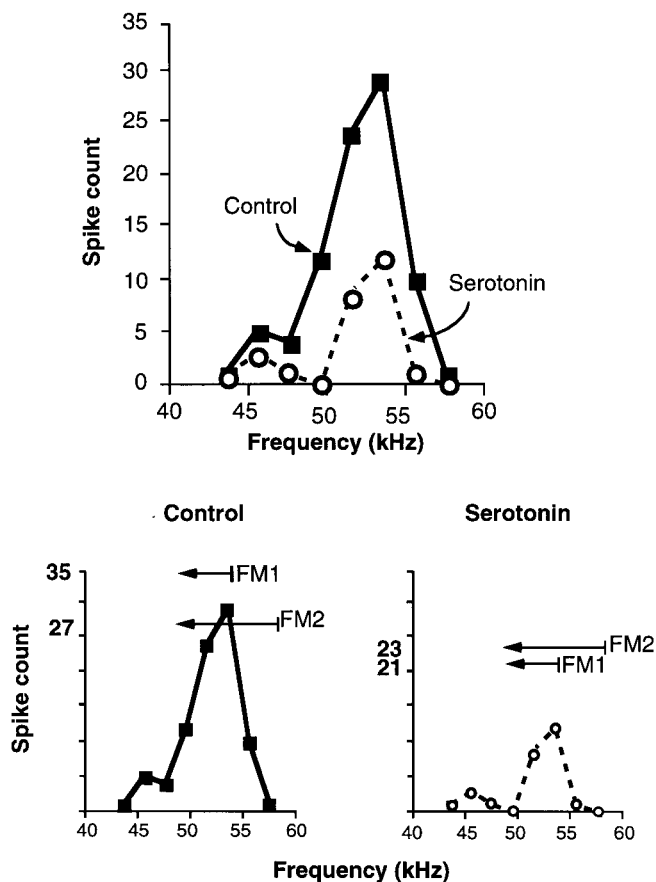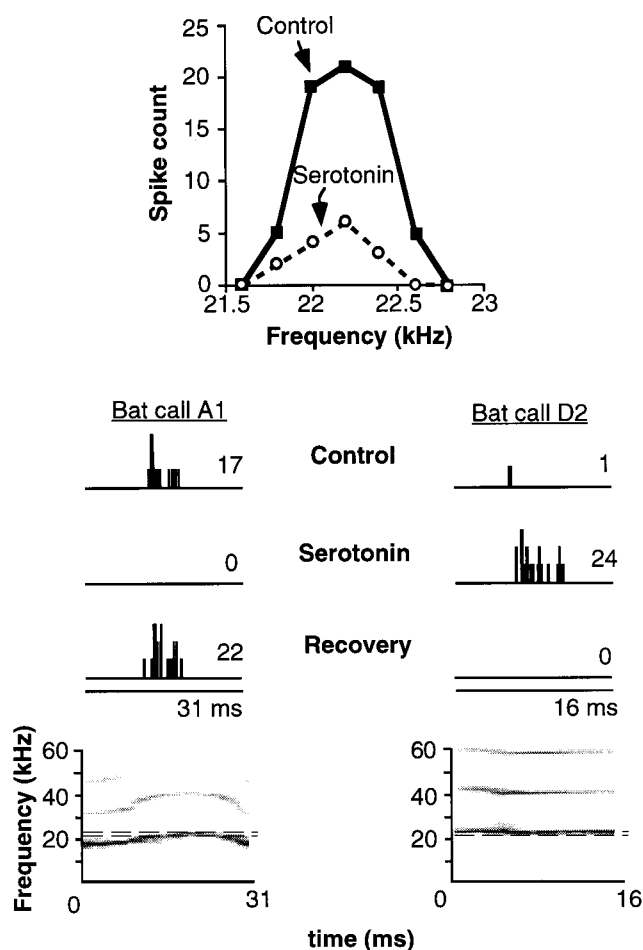

FIG. 8. A broadly depressed neuron in which the serotonin-induced changes in response area were consistent with the changes in responses to 1 FM sweep but not with the responses to another FM sweep. *Top*: the response areas before (control) and during application of serotonin. *Bottom*: the control and serotonin response regions separately with arrows representing 2 downward FM sweeps. The length of each arrow indicates the extent of each sweep. FM 1 swept downward from 53.8 to 48.8 kHz and FM 2 swept from 58.8 to 48.8 kHz. The placement of each arrow on the ordinate indicates the spike count evoked by that sweep (e.g., in the control condition, FM 1 evoked 35 spike). Both FM sweeps were 10 ms in duration and both swept through the most effective frequencies in the response area. Note that spike counts evoked by FM 1 were depressed considerably by serotonin while the spike counts evoked by FM 2 were hardly affected. Sound intensities were 60 dB SPL. Ejection current was 50 nA.

response area. However, the neuron now responded briskly to *call D2.* Within 5 min of terminating serotonin, the neuron recovered and again responded briskly to *call A1* but not to *call D2.* Thus although serotonin produced a strong response depression to all tones in the response area, it allowed the neuron to respond to a signal to which it was previously unresponsive.

DISCUSSION

Here we showed that serotonin has a significant influence on acoustically evoked responses of many ICc neurons. In the majority of neurons, serotonin exerted a gain control, depressing or facilitating the overall level of responsiveness. We refer

to these as broadly affected neurons. In a smaller number of neurons, that we refer to as focally affected, serotonin changed responses evoked by only some frequencies but not other frequencies. For most neurons of both types, there was a close correlation between the effects of serotonin on responses to tones and on responses to brief FM sweeps that encroached on the neuron's frequency response area. Of particular interest are focal neurons in which serotonin-induced changes in tuning corresponded closely to the serotonin-induced changes in response selectivity for different FM signals.

These results are consistent with other studies of serotonergic influences on auditory neurons as well as with our previous study. In two auditory nuclei below the level of the inferior colliculus, the cochlear nucleus and ventral nucleus of the trapezoid body, serotonin facilitates and depresses responses evoked by tone bursts (Ebert and Ostwald 1992) or injected current (Wang and Robertson 1997). In these studies, serotonergic effects on tuning or on more complex stimuli were not reported. A review by Faingold et al. (1991) briefly mentioned that serotonin had only depressive effects on a small number (6) of ICc neurons. The majority of neurons that we studied were also depressed by serotonin, and in this regard our results are in agreement.

The results reported here also offer partial explanations for some of the results we obtained in our previous study of serotonergic influences on ICc neurons (Hurley and Pollak 1999). As mentioned in the INTRODUCTION, we previously reported the effects of serotonin on responses to tone bursts, but those tones were only presented at the neuron's BF. Consistent with the present results, we found that serotonin depressed discharges to tone bursts in the majority of neurons and facilitated discharges in a smaller percentage of cells. In our previous study, we also presented both BF tone bursts and brief FM signals to many neurons. The surprising finding was that in some neurons, serotonin had a differential effect on the responses to BF tones compared with responses to FM sweeps. Additionally, serotonin affected the responses evoked by FM sweeps that had particular temporal or spectral structures differently from the responses evoked by FM sweeps having different structures. The present results suggest that at least some of those cells were focally affected by serotonin. The focally affected neuron in Fig. 6, for example, illustrates several of those differential effects. In this neuron, serotonin had virtually no effect on BF tones. However, the discharges evoked by two of the FM signals (FM 1 and 3) were depressed by serotonin, whereas serotonin had no effect on responses to FM 2. In this neuron, then, serotonin affected responses to BF tones differently than it did to some FM sweeps, and it also differentially influenced the responses evoked by FM sweeps that had different spectral structures. The explanation we offer for these results is straightforward: responses evoked by some FM signals were depressed because those FM signals swept through the portion of the response area that was depressed by serotonin, while the responses to other FM signals were not depressed because the frequencies in those FM sweeps were confined to the region of the response area that was not affected by serotonin.

Focal effects of serotonin, however, cannot explain all observations. In our previous study, we reported that in some neurons, the responses to BF tones were profoundly depressed by serotonin yet the responses evoked by FM signals that swept through the BF were unaffected by serotonin. These results are similar to those found for the neuron shown in Fig. 8. How serotonin can strongly suppress responses to BF tones, or to the frequencies in the entire response area as in Fig. 8, and not affect responses to signals that sweep through that frequency is difficult to explain. Similarly, shown in Fig. 9 is a neuron in which serotonin strongly depressed all frequencies in its response area. The remarkable feature of this neuron is that serotonin depressed responses to a communication call (*call A1*) that the neuron had previously responded to, yet serotonin allowed the neuron to respond to a communication call (*call D2*) to which it was previously unresponsive. How serotonin can, at once, produce a strong depression of all excitatory frequencies and at the same time unmask a responsiveness to a spectrally complex communication call is also difficult to explain. But while we cannot offer an explanation for these results, they illustrate that serotonin can have surprising and strikingly complex influences on at least some ICc cells.

Comparison of serotonergic and cholinergic effects on ICc neurons

The other neuromodulator in the IC that has been described in detail is acetylcholine (ACh). The IC contains high levels of both nicotinic and muscarinic ACh receptors (Morley et al. 1977; Rotter et al. 1979; Schwartz et al. 1982; Wamsley et al. 1981). The application of ACh, like serotonin, changes discharge rates evoked by tone bursts in IC neurons. Habbicht and Vater (1996) reported that ACh changed discharge rates in 53% of the ICc neurons tested, which is close to the proportion of ICc neurons (60%) affected by serotonin. In an earlier study, Farley et al. (1983) reported that 91% of IC neurons were affected by ACh and 81% were affected by the ACh agonist, carbachol. The reported effects of ACh on IC neurons are somewhat different from the effects of serotonin. Of the ICc neurons influenced by serotonin in this study, the majority (76%) were depressed and a smaller proportion (24%) were facilitated. The effects of ACh reported by both Habbicht and Vater and Farley et al. are exactly the opposite; most neurons were facilitated by ACh and a smaller number were depressed. Another difference is that tuning curves were not changed by ACh. The effects of ACh covered the whole response area without changing the borders of the tuning curves (Habbicht and Vater 1996). The finding that ACh affected all excitatory frequencies is similar to the effects of serotonin on broadly affected neurons. However, none of the neurons reported by Habbicht and Vater displayed a contraction or expansion of frequency borders with ACh, whereas changes in the borders of the response area were seen in some ICc neurons broadly affected by serotonin and in many ICc neurons that were focally affected by serotonin.

Endogenous sources of serotonin may have dynamic effects on ICc neurons

As mentioned previously, the activity levels of serotonergic neurons in the dorsal raphe, the main source of serotonergic input to the IC, vary markedly with behavioral states. Studies that monitored single-unit activity of dorsal raphe neurons revealed that discharge rates increase markedly as animals awaken from sleep (Trulson and Jacobs 1979), and response

rates in some neurons are then further modulated when animals are presented with sounds and direct their attention to a particular stimulus (Heym et al. 1982; Jacobs and Fornal 1999; Trulson and Trulson 1982).

Although we did not monitor or control the state of alertness of the animals in this study, whenever the animals were checked, they responded to light touch, and when offered water, they would either drink or actively reject the water. Since previous studies have shown that serotonergic neurons are active in awake animals (Trulson and Jacobs 1979), we assume that many ICc neurons received a basal level of endogenous serotonergic input. Thus the changes in responsiveness that we observed were presumably due to the exogenously applied serotonin acting in tandem with an endogenous input. This argument, together with the assumption that activity of serotonergic neurons varies with behavioral states, suggests alternate interpretations for the results we obtained. One result that can have alternative interpretations concerns cells in which we observed no effects of exogenously applied serotonin (e.g., Fig. 1A). While it is possible that the serotonin we applied did not reach receptors on these cells, or that the cells had no serotonin receptors, another possibility is that they did, in fact, receive the serotonin we applied but that their receptors were already saturated due to the endogenous serotonergic innervation. If this proves to be the case, then the population of ICc cells under serotonergic influences is larger than our data indicate. In future studies, we will test this possibility by applying blockers of serotonergic receptors to determine whether cells are subject to ongoing endogenous modulation by serotonin.

An alternative interpretation can also be given to our categorization of serotonergic effects as either broad or focal. We feel that broad and focal effects almost certainly represent two ends of a continuum rather than two absolute categories. To be sure, many cells had pronounced effects that clearly were either broad, such as the neuron in Fig. 2A, or focal, such as the neuron in Fig. 6. This is also supported by the neuron in Fig. 5C in which focal effects were unchanged with higher dosages of serotonin. However, in many neurons that were classified as broadly affected by serotonin, the serotonergic effect on response magnitude evoked by some frequencies was greater than it was on other frequencies (e.g., Fig. 2B). It seems possible, for instance, that had we applied lower dosages to these broadly affected cells, the frequencies that were lesser affected may not have been affected at all. Thus such a cell would be broadly affected with higher doses of serotonin and focally affected with lower doses. The dependency on dosage for generating broad or focal effects in at least some neurons is supported by the dose-dependent depression of responses observed in two neurons in Fig. 5. Indeed, with the lower dosage of serotonin, we would classify the neuron in Fig. 5B as focally affected, but with the higher dosage we would classify it as broadly affected. While not conclusive, these arguments suggest that under behavioral states that produce different levels of serotonergic activity, serotonin might affect the responses to one signal differently than responses to another signal having a different acoustic structure, in a manner analogous to the differential responses evoked by various FM signals of the neuron in Fig. 6.

Serotonergic activity functionally reconfigures the circuitry of auditory system

Modulatory effects of serotonin have been shown to functionally reconfigure a wide range of neural circuits, from the stomatogastric ganglion of decapod crustaceans (Harris-Warrick et al. 1992) to the swim motor circuit in *Xenopus* tadpoles (Sillar et al. 1998). The circuit reconfigurations are not due to changes in connectivity but rather are consequences of changes in the responses of the neurons in the circuit. Serotonergic receptors act either through G proteins, which then open or close ion channels, or act directly through serotonin-gated ion channels (Gyermek 1995; Jacobs and Azmitia 1992; Lucas and Hen 1995; Martin and Humphrey 1994; Peroutka 1994). The recruitment or closure of channels changes the response of neurons in the circuit, and thus the activity of the entire circuit is shifted from one functional mode to another (Harris-Warrick et al. 1992; Sillar et al. 1998). In a similar manner, populations of ICc neurons could respond differently to acoustic signals when serotonin is present compared with when it is absent, and thus serotonin would functionally reconfigure the circuitry of the ICc.

Thus it is possible that the ICc population response to a particular sound depends, in part, on whether the animal is alert, whether it is passively listening, or whether it is directing its attention toward a particular sound source. Bats employ a wide diversity of acoustic signals for both echolocation and for communication (Balcombe and McCracken 1992; Gelfand and McCracken 1986; Simmons et al. 1978, 1979). Assuming that serotonergic activity varies with behavioral state, then the way in which many ICc neurons respond to natural signals may also vary with behavioral state.

While this study focused on serotonergic influences on the ICc, we again point out that serotonin, together with other neuromodulators, acts not only in the ICc, but along the entire auditory system, from cochlea to cortex (Campbell et al. 1987; DeFelipe et al. 1991; Gil-Loyzaga et al. 1997; Klepper and Herbert 1991; Vu and Tork 1992). The actions of neuromodulators in lower centers presumably alters the information presented to the ICc (Ebert and Ostwald 1992; Fitzgerald and Sanes 1999; Wang and Robertson 1997). The additional modulation that then occurs in the ICc itself changes the information presented to its principal targets in the medial geniculate body, which then influences the information the medial geniculate conveys to the auditory cortex. This suggests that the impact of neuromodulation must be profound and that the response properties observed in neurophysiological studies of the auditory system may be substantially different from those that actually occur as animals interact with their environment.

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