Seasonal variations in auditory processing in the inferior colliculus of
Eptesicus fuscus.

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Abstract:

Eptesicus fuscus is typical of temperate zone bats in that both sexes undergo marked seasonal changes in behavior, endocrine status, and reproductive status. Acoustic communication plays a key role in many seasonal behaviors. For example, males emit specialized vocalizations during mating in the fall, and females use different specialized vocalizations to communicate with infants in late spring. Bats of both sexes use echolocation for foraging during times of activity, but engage in little sound-directed behavior during torpor and hibernation in winter. Auditory processing might be expected to reflect these marked seasonal changes.

To explore the possibility that seasonal changes in hormonal status could drive functional plasticity in the central auditory system, we examined responses of single neurons in the inferior colliculus throughout the year.

The average first-spike latency in females varied seasonally, almost doubling in spring compared to other times of year. First-spike latencies in males remained relatively stable throughout the year. Latency jitter for both sexes was higher in winter and spring than in summer or fall.

Females had more burst responders than other discharge patterns throughout the year whereas males had more transient responders at all times of year except fall, when burst responses were the predominant type. The percentage of simple discharge patterns (sustained and transient) was higher in males than females in the spring and higher in females than males in the fall.

In females, the percentage of shortpass duration-tuned neurons doubled in summer and remained elevated through fall and early winter. In males, the percentage of shortpass duration-tuned cells increased in spring and the percentage of bandpass duration-tuned cells doubled in the fall.

These findings suggest that there are clear seasonal changes in basic response characteristics of midbrain auditory neurons in *Eptesicus*, especially in temporal response properties and duration sensitivity. Moreover, the pattern of changes is different in males and females, suggesting that hormone-driven plasticity adjusts central auditory processing to fit the characteristics of vocalizations specific to seasonal behavioral patterns.

Keywords: inferior colliculus, functional plasticity, seasonal variation, bat

Abbreviations:

Amplitude modulated tones (AM)

Best frequency (BF)

Estradiol (E2)

Auditory brainstem response (ABR)

Inferior colliculus (IC)
Sinusoidal frequency modulated (SFM)
Frequency response area (FRA)

Introduction:

Plasticity of sensory systems, including the auditory system, is important for adaptation and survival in fluctuating environments. Many temperate species adapt to seasonal environmental changes by altering their physiology and behavioral responses. Photoperiodicity drives changes in endocrine status; these hormonal changes in turn trigger behavioral modifications such as breeding, nesting, or food storage. It is reasonable to assume that sensory processing might be optimized to deal with the types of information that are of greatest behavioral relevance at each time of year. For example, during breeding season, sensory processing might be optimized to enhance signals that are important for seeking and recruiting mates.

Seasonal auditory plasticity has been shown in a number of vertebrate taxa including amphibians (Arch & Narins, 2009; Goense & Feng, 2005; Hillery, 1984; Walkowiak, 1980), fish (Sisneros, 2009) and birds (Caras et al. 2010, 2015), but few mammals (Harrison, 1965; Katbamna et al., 1993, Miranda et al., 2014). The studies in mammals did not deal with seasonal plasticity per se, but rather the effects of lowered body temperature or engaging in maternal care of pups.

In tree frogs, auditory thresholds drop and spontaneous firing rates increase during breeding season (Hillery, 1984). It is thought that stronger phaselocking to amplitude modulated tones (AM) and shifts in best frequency (BF) distributions during mating season help northern leopard frogs discriminate individual calls from intense background conspecific chorusing (Goense & Feng, 2005). Forlano, Sisneros, Rohmann, & Bass (2014) showed that peripheral auditory sensory neurons (sacculus hair cells) in midshipman fish were highly seasonally regulated, with gravid females exhibiting robust phaselocking to mating call frequencies compared to their nongravid counterparts. Recordings of auditory evoked potentials in female house sparrows during breeding season show enhanced frequency selectivity along with reduced temporal resolution (Gall et al., 2012). Female white-throated sparrows given estradiol (E2) and exposed to conspecific male song, show increased density of serotonin receptors on fibers that innervate the auditory system, suggesting that E2 affects the auditory system via serotonin. (Matragrano et al., 2012). Auditory brainstem response (ABR) studies in white-crowned sparrows have paradoxically shown increased thresholds and response latencies in both sexes when in breeding condition (Caras et al., 2010) but increased precision of spike timing in female sparrows given estrogen (Caras et al., 2015).

To our knowledge, seasonal plasticity independent of temperature has not been demonstrated in the auditory system of any mammal, although there are known to be seasonal changes in the size and function of other mammalian brain systems, especially the hippocampus and hypothalamus. Seasonal changes in cell proliferation have been described in the hypothalamus and thalamus of sheep (Migaud et al., 2011), the olfactory bulb of mice (Walton et al., 2012), and the hippocampus of mice (Burger et al., 2013). Keeley et al (2015) have shown that in the ground squirrel there are seasonal changes in the size of regions that are not

directly involved in seasonal breeding state, including certain cortical areas and the superior colliculus. Moreover, these changes are more pronounced in females than males (Keeley et al. 2015). The hippocampus of the ground squirrel also undergoes seasonal changes in size, and these changes are different in males and females (Burger et al., 2013).

There is some evidence for functional plasticity in these brain regions as well. The hypothalamus and cortex of the ground squirrel have been shown to undergo seasonal variations in gene expression (Schwartz et al., 2013). Walton et al. (2011) have shown sex differences and effects of photoperiod on LTP in the hippocampus of white-footed mice.

Temperate zone bats such as *Eptesicus fuscus*, show marked seasonal plasticity in behavior and are an ideal mammalian species in which to look for evidence of seasonal plasticity in auditory processing. *Eptesicus* is a highly social species that relies heavily on auditory cues for communication, navigation, and hunting prey. These bats undergo clear adaptation to seasonal changes in photoperiod and temperature. In the late summer and fall, they consume large numbers of insects, gaining brown fat stores, and mating before entering a state of torpor for the winter. During the winter, they slowly use their fat stores for energy, but remain quiet in their hibernacula. As the weather warms in springtime, the bats emerge from torpor to begin foraging, and gestating females form maternity roosts. At the height of spring, pups are born, and food consumption is maximal. Pups are weaned in summer between 4 – 8 weeks of age and colonies reorganize for the next fall mating season.

Sex steroid levels in Vespertilionid bats, including *Eptesicus*, peak for males in late summer just after gonadal development (Myotis, see Gustafson and Shemesh, 1976). In females, this peak is in the spring (Mendonca et al., 1996). *Eptesicus* actively mates throughout the fall and periodically in the winter when they awake from torpor.

Auditory processing in *Eptesicus fuscus* has been extensively studied (e.g., Covey and Casseday, 1999; Casseday et al., 2002; Covey, 2004; Covey and Carr, 2004), but to date it is not known whether there is any sort of seasonal auditory plasticity in these animals. This study examined a set of basic response properties in auditory neurons of the inferior colliculus (IC) of male and female bats at different times of year.

Methods:

All data used for analysis were obtained using procedures that have previously been described (e.g., Faure et al., 2003; Miller & Covey, 2011) but are summarized briefly below. All procedures were approved by the University of Washington Office of Animal Welfare. Bats in the University of Washington colony were initially collected from attic roosts of homes in North Carolina but subsequently bred in captivity at the University of Washington.

Seasonal Exposure

All bats were housed socially in colonies that were exposed to the local photoperiod and seasonal temperature variations similar to those they would experience in their natural environment. Heaters were used to recreate the consistent hot temperatures observed in maternity roosts, and were turned down in the autumn to trigger breeding before the bats transitioned into torpor. Colony temperatures were averaged over a 5-year period for winter, spring, summer, and fall quarters. Winter quarter included December, January, and February, when colony temperatures were at a minimum (high $14.1^{\circ}\text{C} \pm 2.3^{\circ}\text{C}$, low $9.4^{\circ}\text{C} \pm 1.9^{\circ}\text{C}$) and animals were observed to be in a state of torpor. Spring quarter included March, April, and May, when colony temperatures were gradually increased (high $24.4^{\circ}\text{C} \pm 5.7^{\circ}\text{C}$, low $15.9^{\circ}\text{C} \pm 4.5^{\circ}\text{C}$) and bats became more active, with individuals reorganizing into maternity groups and isolated groups of males. Approximately 75% of females typically gave birth in late April or early May. Summer quarter included June, July, and August, when colony temperatures were kept at a standard high (average high $32.7^{\circ}\text{C} \pm 3.5^{\circ}\text{C}$, low $22.0^{\circ}\text{C} \pm 2.9^{\circ}\text{C}$). Once pups were weaned (typically in June) colonies then reorganized into mixed sex groups of juveniles or adults. Autumn quarter included September, October, and November, when the temperature was gradually reduced, mating began, and the colony slowly went into torpor (average high $23.4^{\circ}\text{C} \pm 6.9^{\circ}\text{C}$, low $17.0^{\circ}\text{C} \pm 5.6^{\circ}\text{C}$). Bats intended for experiments were brought into an indoor climate-controlled environment (temperatures averaged 21°C), which was observed to arouse them from their torpor during winter. During the time when recordings were obtained, bats were kept at room temperature for up to two weeks, but were exposed to the seasonally appropriate light cycle.

Surgery

Prior to electrophysiological recording, bats underwent surgery to attach a small titanium post to the skull. Bats were initially given a dose of Buprenorphine (0.025mg/kg , SQ), then anesthetized with isoflurane (2-3% $\sim 1\text{L/min}$ O_2) and placed into an adjustable stereotaxic apparatus to stabilize the head. Bupivacaine (0.25mg/kg , SQ) was injected locally prior to making an incision in the skin, reflecting the temporal muscles, and cleaning the skull overlying the cortex and IC. The post was placed anterior to the IC and attached using cyanoacrylate. The bat was allowed a minimum of 24 hours to recover before beginning physiology.

Electrophysiological recording

The temperature of the recording booth was maintained at a constant $27.7\text{--}28.8^{\circ}\text{C}$.

Each bat was used in 2 to 8 recording sessions lasting $\sim 6\text{ hr/day}$. Experiments were terminated if the bat showed signs of restlessness or discomfort. Between sessions, the opening in the skull was covered with Gelfoam and coated with sterile petroleum jelly. Bats were housed in individual cages in a temperature- and humidity-controlled environment and were given *ad libitum* access to food and water.

Electrophysiology recordings were conducted inside a double-walled, sound-attenuating booth (Industrial Acoustics Co., Inc, New York, NY), with ambient temperature maintained at 27.7-28.8 C. Prior to recording, the bat was given a subcutaneous injection of a neuroleptic (19.1 mg/kg of 1:1 Fentanyl/Droperidol mixture) and placed in a foam-lined holder that was suspended within a stereotaxic frame (ASI Instruments, Warren, MI) mounted on a floating vibration isolation table (TMC, Inc., Peabody, MA). The bat's head was immobilized by attaching the metal post to a micromanipulator (David Kopf Instruments, Tujunga, CA). A chlorided silver wire was placed under the temporal muscle to serve as a ground. On the first recording session, a small hole was made in the bone over the IC, and the dura was opened. On subsequent sessions, no additional opening was made on that side and bats were only lightly tranquilized to calm them during placement in the body and head holder. Bats were awake for the duration of the recording session. Once the bat was awake, a single glass microelectrode (10-40 M Ω , filled with 0.15M (0.9%) sodium chloride, 5% biodextran amine, 10000 MW, Invitrogen, Carlsbad, CA, or 2.5% Chicago Sky Blue dye) was lowered into the central nucleus of the IC using a hydraulic micropositioner (David Kopf Instruments model 650). Electrodes were advanced while presenting a search sound to elicit neuronal responses. Action potentials were resolved at 10x via a Neuroprobe Amplifier before being bandpass filtered and further amplified by a spike pre-conditioner (TDT PC1, 300 Hz – 7 kHz filtering). The output of the PC1 was passed to a spike discriminator (TDT SD1) and event timer (TDT ET1) synchronized to a timing generator (TDT TG6) and logged on a computer. Stimulus generation and on-line data visualization were controlled by custom software. Spike times were displayed as dot rasters ordered by the acoustic parameter that was randomized during testing.

Sound presentation

Acoustical stimuli were synthesized in the same manner as previously described (Faure et al. 2003). Stimuli were presented to the contralateral ear via a Brüel&Kjær (B&K) type 4135 1/4-inch condenser microphone modified for use as a loudspeaker with a circuit to correct for nonlinearities in the transfer function (Frederiksen, 1977). The transducer was positioned so that its diaphragm was ~1 mm in front of the external auditory meatus. The output of the loudspeaker, measured with a B&K type 4138 1/8-inch condenser microphone calibrated with a B&K Type 4220 sound level calibrator, is expressed in decibels of sound pressure level (SPL root mean square with regard to 20 μ Pa) equivalent to the peak amplitude of continuous tones of the same frequency (Stappells et al, 1982). The transfer function of the transducer was flat ± 5 dB from 26 to 118 kHz (measured 1 to 134 kHz). All signals had rise-fall times of 0.4 ms and were presented at a repetition rate of 2 or 3 pulses per second. Stimuli included pure tones, broadband noise, frequency sweeps and sinusoidally frequency-modulated tones (SFM). Cross talk between the two ears was tested as previously described by (Ehrlich et al. 1997).

Response characteristics

Single units were identified visually on a Tektronix oscilloscope and had a consistent spike waveform and minimum signal to noise ratio of 3:1. Once isolated,

we conducted routine tests to characterize its response properties. Initial tests were run to determine whether it favored pure tones, directional frequency sweeps, or sinusoidal frequency modulations (SFM). Search stimuli consisted of a pure tone (PT) followed by an SFM stimulus. If neurons responded to the PT, or had the strongest response to PT, they were classified as such. If neurons did not respond to a PT but did respond to the FM sweeps, they were tested to determine the best depth, center frequency, and direction of the FM sweep. If they did not respond, or responded poorly, to single FM sweeps, they were tested with SFM to determine the best modulation depth, center frequency, and modulation rate. A few neurons only responded to broadband noise or showed stimulus-specific adaptation, requiring ongoing change in stimulus parameters in order to respond. These classes of neurons were excluded from the present analysis.

Neurons were characterized by sound-evoked firing patterns, selectivity for stimulus type, duration sensitivity, and other response properties. If the unit responded to pure tones, the best frequency, threshold, and frequency response area (FRA) were determined. If the strongest response was to sweeps (any neuron that did NOT respond to 0 kHz sweep depth), we determined the best sweep depth, center frequency, and threshold. For neurons that were SFM selective (Yue et al. 2007), we varied modulation rate and depth. All neurons that responded to pure tones were tested for duration sensitivity at BF, 10 dB above minimum threshold. Shortpass neurons fired to short stimulus durations up to a certain threshold and then did not fire to stimuli of longer durations. Bandpass neurons fired to an intermediate range of stimulus durations but did not fire to stimuli above and below this range. Longpass neurons responded only when the stimulus duration reached a certain length and fired to all durations longer than this minimum. Allpass neurons responded to stimuli of any duration. (Aubie et al. 2014) Responses were categorized by discharge pattern (burst: short interspike interval with a short period of repeated firing unrelated to the duration of the stimulus; sustained: firing for the duration of the stimulus; transient: 1-2 spikes fired in response to a stimulus; onset-offset: transient response to the onset of the stimulus followed by a multi-spike response to the offset of the stimulus; prolonged: a response that continues beyond the duration of the stimulus by an amount longer than would be predicted by response latency; and irregular: weak firing that did not fit into any of the previous five categories.) We measured first spike latency and latency jitter to BF at 10 dB above threshold. Rate-level functions were constructed to look for intensity sensitivity; monotonic neurons were classified as responding at increasing or stable rates with increasing sound level, whereas nonmonotonic neurons were defined as those for which responses decreased by 10% or more at sound levels higher than the peak response (e.g., Covey, 1993) sometimes not responding at all to the loudest intensities presented. Neurons were also characterized for their response to the onset or offset of a stimulus as determined in duration recordings.

Statistical analysis

For quantitative data, two-way ANOVAs were run in SPSS (v19.0, IBM) to determine if there were interactions between sex and season, followed by t tests and one-way

ANOVAs needed. For qualitative data, chi-squared tests were run in Prism (v6.0, Graphpad).

Results:

A total of 673 neurons were analyzed from 95 females and 78 males, spanning all four seasons. No data were obtained from females during the month of May, when they typically gave birth and nursed their young.

Thresholds and frequency tuning properties

For 363 neurons that responded to pure tones, we determined best frequency, threshold, and constructed the FRA. Figure 1A and B shows the distribution of thresholds as a function of frequency in males and females. Although the general distribution was similar for both sexes, females had more neurons with thresholds below 20 dB (dotted line). Differences in average minimum threshold between sex and season were not significantly different.

We measured Q values for 334 neurons that responded to pure tones or sweeps and had complete FRAs. The Q value is calculated as BF/breadth of tuning at a given level above minimum threshold, and provides a measure of frequency specificity. Figure 1C shows the average of Q10 and Q20 values for males and females. Q values differed significantly between quarters ($F_{3, 326} = 2.916$, $p = 0.034$), but we did not detect an effect of sex or an interaction (all $F_{3, 326} = 1.859$, $p > 0.136$). Post hoc analysis revealed that the Q values in summer and autumn quarter were significantly different from each other, but did not differ with other quarters. Although not significant, the Q values are lowest for both sexes in fall and winter, suggesting that neurons are, on the whole, more broadly tuned to frequency during this time.

Discharge patterns and temporal properties.

First spike latencies were examined for 378 neurons that responded to the onset of a pure tone stimulus. Significant differences were noted for both sex and season (two way ANOVA, $F_{3, 370} = 4.983$, $p = 0.002$). Figure 2a shows that average latency values for females peaked in spring (mean 19.3 ms) while those for males remained constant (mean 11.1 ms). The average latency increase for females in spring was just over 8 ms. This increase was statistically significant with respect to other times of year (one way ANOVA, $F_{3, 208} = 7.781$, $p < 0.001$) and with respect to males in the spring (Ttest, $t = 2.321$, $p = 0.020$). First spike latency jitter (Figure 2b) was largest in spring for both sexes, but was only statistically significant across seasons in females (oneway ANOVA, season $F_{3, 370} = 5.789$, $p = 0.001$). Post hoc analysis showed that spring jitter was significantly different than that in summer or autumn.

Figure 3 shows the distribution of the three most common classes of discharge patterns found for both females (N=310) and males (N=285), along with examples of each class. In addition to burst, sustained, and transient responders, we observed neurons that responded to the stimulus in an onset/offset, prolonged, and irregular

pattern, as well as other patterns such as pauser, buildup, and others. However these categories were encountered far less often so that the sample sizes would have been too small to perform any reliable statistical analyses. Overall, females had more burst-type responses than males at all times of year except autumn (chi square, $p=0.0154$). Sustained responses in females were most common in spring (chi square, $p<0.001$) and were uncommon at other times of year. Transient responses in females decreased significantly in spring (chi square for females/all discharge patterns; $p<0.0001$). Burst responses were not significantly different between the sexes; but there was a difference between males and females for sustained ($p=0.001$) and transient ($p=0.0196$) responses.

Males had more transient responses than females except in fall (chi square $p=0.0196$). Burst responses peaked in summer for females and in fall for males, but these seasonal differences were not statistically significant (chi square, $p = 0.2496$).

Throughout the year, females ($N=210$) had a higher proportion of offset responses than males ($N=180$, figure 4, see figure 3A dot raster for an example of an offset response). There was no significant difference for onset responses (examples in figure 3B and 3C dot rasters), but offset responses were significantly different between males and females (chi square, $p < 0.001$).

Duration sensitivity

Duration sensitivity (as measured 10 dB above minimum threshold) was examined in 223 neurons in females, and 194 neurons of males. Figure 5 shows the distribution of different forms of duration sensitivity along with example plots from neurons classified into each group. Females had more shortpass neurons than males at all times of year. In females, bandpass neurons were least common in spring and summer. In males, bandpass neurons were uncommon in every season except fall when the proportion increased considerably. Longpass neurons were most common in females during winter and spring, but in summer and fall for males. Both males and females had significantly different duration tuning throughout the year, (chi square; female $p=0.0007$, male $p=0.024$) and bandpass and longpass neurons differed significantly between the sexes (chi square, $p=0.0121$ and $p=0.0007$ respectively).

Other response properties

Rate-level functions were examined in 273 neurons in females and 235 neurons in males. Figure 6 shows the distribution of monotonic and non-monotonic rate-level functions along with example plots illustrating each type. There was no significant difference throughout the year for females or between males and females. Males differed significantly throughout the year (chi square, $p=0.0014$) with non-monotonic rate-level functions peaking in the summer and monotonic rate-level functions peaking in the fall.

Best stimulus type was examined in 310 neurons in females and 285 neurons in males. Figure 7 shows that the majority of neurons responded to pure tones

throughout the year, but the percentage was highest for males in summer and for females in spring ($P < 0.0001$, chi-square). The percentage of sweep-selective cells was highest in autumn and winter for both males and females (chi square, $P = 0.0092$). Males overall had more SFM-selective neurons than females, although they made up less than 10% of all neurons in both sexes (chi square, $P = 0.0127$). The absence of SFM-selective neurons in spring may be due to the fact that recordings were generally not performed in females in the late stages of pregnancy, so the sample size was relatively small.

Discussion

The data presented here show that a number of features of IC neurons' sound-evoked responses vary according to season, in both male and female bats. However, the patterns of variation are not parallel for the two sexes. These findings provide some of the first evidence for sex-related and seasonal differences in central auditory function in any mammal.

It is known that temperature can affect auditory processing (Harrison 1965, Howell et al., 1975, Katbamna et al., 1993) so all recording sessions were conducted with animals that had been maintained at a standard ambient temperature of approximately $[20.0-22.2^{\circ}\text{C}]$ for at least 48 hours, and recordings were performed at a constant ambient temperature of $27.7-28.8^{\circ}\text{C}$. It is possible that some reversal of seasonal changes occurred during the time that bats were held in the lab, but this would mean that any observations of seasonal differences were on the conservative side. We do not know whether females recorded from in late winter or early spring were pregnant, but typically at least 75% of females in the colony gave birth each year. Bats chosen for electrophysiology were over 1 year old, so all females were in breeding condition, and it is likely that most were pregnant. Although bats were awake throughout the recording session it is possible that some residual effects of the analgesics could have interacted with hormonal state. However, if there were any residual effects, they would have been consistent across seasons, and would probably not have affected the results.

Gonadal steroid hormones are known to modulate the activity of multiple brain systems. GnRH neurons in the mammalian hypothalamus are regulated by sex hormones (e.g., Radovick et al., 2012). In humans, elevated estrogen and androgen levels are known to be correlated with increased latencies in peaks of the ABR corresponding to responses at the midbrain and higher levels. (Elkind-Hirsch et al., 1992; 1994), suggesting that responses of neurons in the central auditory system may be modulated by hormones, including those that vary seasonally as well as cyclically.

Some response properties were similar for males and females, and these did not change seasonally. Most notably, there was little difference in breadth of frequency tuning across the year, although there was a slight trend for Q values to be higher in spring and summer when bats were most active. This might appear to be

inconsistent with the increased frequency selectivity (and decreased temporal resolution) that has been seen in female house sparrows during breeding season (Gall et al., 2012), but because bats mate in fall with gestation delayed until spring, it is difficult to compare them with a species that mates and raises offspring during a single season. Minimum thresholds were, on average, significantly lower in females than males in every season except summer, when males' thresholds were as low as those of females. Summer is the season when bats of both sexes spend the most time echolocating during feeding, so it may be advantageous to males to have maximal auditory sensitivity during this time.

The most pronounced differences between males and females and across seasons were in temporal response patterns. In females, first-spike latency was longest and latency jitter was greatest during spring when most female bats were pregnant, suggesting that latency and firing precision might be influenced by hormonal state. It is possible that changes in latency and increased jitter could affect the responses of delay-tuned neurons in the IC and higher levels, and that these changes could be correlated with a slowing of hunting behavior, particularly during the later stages of pregnancy when the mothers' weight has increased to the point that they are reluctant to fly. By summer, latency and latency jitter have decreased, consistent with the lactating mothers' need to forage efficiently at a time when energy requirements have approximately doubled (Neuweiler, 2000).

The finding of increased latency and jitter is consistent with the finding in sparrows (Caras et al., 2012; 2015) that ABR latency was longest during breeding season. It is also consistent with the finding that estradiol increases the density of serotonin receptors in the central auditory system of birds (Matragrano, 2012) and the finding that serotonin increases response latency in some IC neurons in the bat (Hurley and Pollak, 2005). It appears that a decrease in speed and temporal precision of responses to sound during pregnancy may be a characteristic of female warm-blooded vertebrates. In fish or amphibians, phaselocking to sound becomes more precise during breeding season, or at least during the mating phase when identification of species-specific mating calls is important (Grilliot et al., 2014). *Eptesicus* mates in the fall, and during this time the temporal precision of responses is greater than in the spring. This is consistent with the increased temporal precision seen in auditory responses of fish and amphibians during mating season, suggesting that high temporal precision is important for processing of mating signals in at least some mammals.

We found significant changes in the proportion of different discharge patterns and duration sensitivity types throughout the year, with sustained responses and longpass duration-sensitive neurons peaking in females during the spring, at around the time when they give birth. One possible explanation is that for the first week or two of an infant bat's life, it emits longduration isolation calls (Monroy et al., 2011), so neurons that are selective for long durations and respond throughout the call would be ideally suited to transmit information about infant bats' vocalizations. It is notable that during summer, the proportion of SFM-selective neurons in females

appears to increase. Infant bats often use SFM-components in their communication calls during this time, so it is likely that females raising young would benefit from enhanced sensitivity to the SFM pattern.

In summer and fall, the proportion of sustained responses in females decreases, with burst and transient responses becoming the predominant types. It is likely that these shorter responses are better suited to echolocation during feeding and/or processing of male mating calls, which are relatively short in duration. In males, the relative proportions of burst and transient neurons change throughout the year, with transient responses exceeding burst responses except in fall when the proportions reverse. Females do not appear to produce any sort of mating vocalizations. In fact, males tend to mate with torpid females (based on unpublished observations in our breeding colony), so it is not clear why this change in sensitivity would be advantageous during this time unless it were to enhance processing of males' antagonistic vocalizations during competition and females' antagonistic vocalizations during mating.

The finding that females have a higher proportion of offset responses than males probably reflects the fact that females have more shortpassduration-tuned neurons than males throughout the year, and more bandpass neurons in fall. (Figure 5) Both shortpass and bandpass neurons respond at the offset of a sound, so an increased proportion of these duration-tuning types would necessarily be reflected in a larger number of offset responses. It remains an open question whether the higher proportion of duration tuned neurons is due to differences in GABAergic or glycinergic inhibition, nor is it clear why females would have more duration-tuned neurons than males, but it may have to do with greater pressure to be successful in foraging, especially early in pregnancy or while lactating.

To our knowledge, this is the first demonstration of seasonal changes in auditory processing in the mammalian midbrain. It is likely that these changes are driven by the interactive influence of hormonal levels, seasonal light-dark cycles, temperature cycles, and behavioral requirements. As in birds, fish, and amphibians, the observed changes appear to optimize processing of those signals that are most relevant in each season.

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Figure Legends:

Figure 1. Scatterplots of BF versus threshold and averaged Q10 and Q20 values for individual neurons in males and females. A) BF vs threshold for females. B) BF vs threshold for males. C) averaged Q values for both sexes across the year. Asterisks indicate statistical significance between summer and autumn at $p < 0.05$ level.

Figure 2. A) First spike latencies with standard errors for males and females across the year. B) Latency jitter with standard errors for both sexes across the year. Double asterisk indicates statistical significance between spring and all other seasons for females at $p < 0.001$ level.

Figure 3. Distribution of common types of discharge patterns in response to best frequency at 10 dB above threshold in males and females seasonally. Dot rasters illustrating each type of discharge pattern are shown to the right of the distributions. Horizontal bars indicate stimulus duration. Note that the Y-axis is scaled differently in each bar graph for ease of viewing. A) Burst responses. B) Sustained responses. C) Transient responses.

Figure 4. Distribution of onset responses (A) and offset responses (B) in males and females. Note that the Y-axis is scaled differently in each panel for ease of viewing. During all seasons the difference between offset responses in males and females was statistically significant at a level of $p < 0.001$. C) Total population of neurons in each sex/season. For this and subsequent figures, population distributions are virtually identical.

Figure 5. Distribution of different forms of duration sensitivity in males and females across the year. Plots illustrate examples of each duration class. Note that the Y-axis is scaled differently in each panel for ease of viewing. A) Allpass neurons; B) Shortpass; C) Bandpass; D) Longpass.

Figure 6. Distribution of rate-level functions in males and females across the year, along with example plots. A) Monotonic; B non-monotonic.

Figure 7. Selectivity for stimulus type in males and females across the year. Note that the Y-axis is scaled differently in each panel for ease of viewing. A) Pure tones; B) Frequency sweeps; C) Sinusoidal frequency modulation.

figure 1

figure 2

figure 3

figure 4

figure 5

figure 5

figure 6