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by

Sarah E. Earp

Donna L. Maney
Adviser

Neuroscience and Behavioral Biology

Donna L. Maney

Adviser

Darryl Neill

Committee Member

Kristin F. Wendland

Committee Member

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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Is music uniquely human? This question has been debated among musicians and scientists alike. One example of a possible non-human form of music is birdsong, which is believed by some to be music, and perceived by others as simple animal vocalization. Attempts to compare song to music have usually focused on the qualities of the sound itself, such as melody and rhythm. It may be more informative, however, to compare the experiences of the intended listeners. Previous studies show that areas in the neural reward circuit, including the nucleus accumbens (nAc) and the ventral tegmental area (VTA) respond in humans listening to pleasurable music. I investigated whether a bird's experience of birdsong is similar to the rewarding experience of music listening in humans by exploring whether the nAc and VTA respond in white-throated sparrows listening to song. I studied neural responses of female and male white-throated sparrows (*Zonotrichia albicollis*) exposed to either courtship song or a control sound. Half of the birds were treated with reproductive hormones to simulate the breeding condition, and the others were given a placebo and acted as controls. I looked at neural responses by labeling the immediate early gene (IEG) product Egr-1, which indicates new protein synthesis in response to a stimulus. Egr-1 expression was quantified in the nAc and the VTA. I found that females in breeding condition had a higher IEG response to song than to the control sound, whereas the females that were not in breeding condition and all males had similar Egr-1 responses to song and control sounds. Overall, my results suggest that birdsong is like human music in that reward circuits respond in the listener. In this seasonally breeding songbird, that parallel can be drawn only for females during the breeding season, when song is expected to have positive valence.

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Introduction

The debate over whether the song of birds is music has been explored by many scientists and musicians. There are many perspectives from which this question can be approached, especially in light of the fact that music has no singular, accepted definition. In past studies, researchers have raised questions ranging from whether song is an evolutionary precursor to music to whether melodies in song and music share similar tonal relationships. Because researchers have approached this question in different ways, many different conclusions about the relationship between birdsong and music have been reached.

Comparing the Structures of Song and Music

Is song an evolutionary precursor to music? This question is a major topic of debate in the field of evolutionary musicology—or the study of the origins of music. Wallin et al. (2001) argued that song is unlikely to be evolutionarily homologous to music, as birds are very distant evolutionary ancestors to humans. According to Wallin, if song were a precursor to human music, the genes that encode musical vocalization would have had to be present in the genome while remaining unexpressed for thousands of years of evolution, which seems unlikely. Rather, Wallin proposes that, just as several independent forms of animal song have arisen throughout the animal kingdom, human music may have spontaneously developed rather than evolving from the song of animals such as birds.

Although it is unlikely that music gave rise directly to song, it is possible that, from an evolutionary perspective, music may serve the same ultimate function in humans that song serves in birds. For example, just as song plays a role in sexual selection (Catchpole & Slater, 1995; Darwin 1871), it is possible that music serves a similar role in humans (Miller, 2001). Slater

(2001) suggests that sexual selection is one explanation for the rich variety in birdsong, supporting the idea that—although song may not be an evolutionary precursor to music—the acoustically pleasing tonal and rhythmic qualities of song that make it sound musical to the human ear could be a result of parallel evolutionary paths and may serve the same ultimate purpose.

The beautiful embellishments of birdsong that make it so appealing to the human ear may result from a common ultimate function of song and music, but just how similar are the tonal, melodic, rhythmic, and organizational qualities of song to those of music? Hall-Craggs (1969) suggested that humans perceive birdsong as music because song and music share several melodic and rhythmic qualities. Dobson and Lemon (1977) characterized tonal relationships found in white-throated sparrow (*Zonotrichia albicollis*) song and found them to be unlike those of Western music. In order to perform this analysis, the researchers recorded the songs of 58 white-throated sparrows and measured the tonal relationships between the temporally adjacent notes and found that they did not align with those seen in Western scales. Other species, however, may produce timbres, rhythmic variations, and modes similar to those found in music (Baptista & Keister, 2005). An example of a species with a distinctly musical song is the musician wren (*Cyphorhinus arada*), whose elaborate song nearly fits into a Western musical scale (Slater, 2001). In general, since the tonal features of song vary by species, the song of only some songbirds shares tonal qualities with human music.

For the species that do sing a music-like song, researchers have asked whether these songs have a syntactical organization similar to that found in music. In music, a recognizable musical idea made up of a short sequence of notes is known as a motif, and a musically directional string of many motifs, like words in a sentence, is called a theme (Clendinning &

Marvin, 2011). According to Marler (2001), the songs of some songbird species appear to be organized syntactically. The swamp sparrow (*Melospiza Georgiana*), for example, has a repertoire of several acoustic units which may be combined in many ways to form song, like musical notes combining to form a motif. Although the swamp sparrow's song shares the motivic organization of music, and some songbirds seem to recognize syntactical organization in song (Gentner et al., 2006), songbirds do not organize many strings of acoustic units into more complex musical themes (Marler, 2001). From this perspective, although song is structurally similar to the short, simple motifs found in music, it is not as complex as human music because the motifs are not organized into tonally directional musical themes.

Human music is creative, but is song creative as well? Marler (2001) argued that song is like music in that it allows for creative recombination of sounds. Each male winter wren (*Troglodytes hiemalis*), for example, learns a set of up to ten songs, each of which consists of a unique sequence of acoustic phonemes. Although each male will learn phonemes from other males, he will combine these phonemes in a novel way, creating his own individual song. Because the components of song are combined in unique ways by each male, rather than mimicked from the tutor males, the song of the winter wren is arguably a form of creative combination of musical ideas. Hartshorne (2008) argues, however, that the songs of many other bird species, such as the monotonous song of the European yellowhammer (*Emberiza citrinella*) and the two note melody of the black-capped chickadee (*Parus atricapillus*), are short and stereotyped. Although there are individual variations in song, each individual repeats his own song with little variability, contrasting heavily with the rich variation in human music.

Comparing Responses in the Listener

Song, like music, involves the transmission of a signal from a sender to a receiver. In nature, the function of a signal, such as song, depends on the response of the receiver. The above analyses have focused on the structure of the signal rather than the response that the signal elicits in the listener. Rather than analyzing the structure of the signal itself—i.e., song or music—it may be helpful to consider the responses in the recipient of the signal, or the listener. In humans, hearing music elicits feelings of pleasure or reward. Here, I sought to better understand whether hearing song is rewarding in birds. If it is rewarding, then, in this way, song would be similar to music.

Behavioral Responses to Song

To examine whether hearing song is rewarding to songbirds, researchers have looked for a phonotaxic or a reinforcing effect of song. Eriksson and Wallin (1986) demonstrated a phonotaxic effect of song on female pied flycatchers (*Ficedula hypoleuca*) and collard flycatchers (*Ficedula albicollis*) in the field. Hypothesizing that male song is directly involved in female mate attraction, these investigators fashioned nest-box traps that contained male dummies and speakers playing recorded male song. Out of ten females caught in the nest-box traps, nine were caught during male song playback, while only one was caught in a nest box without song playback. These results suggest that song has a phonotaxic effect on females in the field, suggesting that it is rewarding to approach. In a study of European starlings (*Sturnus vulgaris*), Gentner and Hulse (2000) constructed nest boxes that would play either male song or another stimulus, such as song played in reverse order, when a female perched on the box. The females preferred to perch on the nest-boxes that played song, indicating that song acts as a reinforcing stimulus, supporting the idea that song is rewarding to these females.

Not only will songbirds approach song, but they will also work to hear it. Operant conditioning paradigms have been used to test whether a stimulus is rewarding; for example, it is well-established that pigeons (*Columba livia*) will peck a key to receive a rewarding food stimulus (Dews 1955, Brown & Jenkins, 1968, Staddon & Frank, 1975). Riebel (2000) demonstrated that female zebra finches (*Taeniopygia guttata*) will peck a key to trigger a playback of conspecific song, indicating that hearing song is rewarding to these birds. Females in this study demonstrated a preference for male songs to which they were exposed during rearing, but even females with little song exposure during rearing would peck to hear male song. Overall, overwhelming evidence suggests that female songbirds have a preference for the song of conspecific males over other sounds (reviewed by Byers & Kroodsma, 2009).

The studies mentioned above support the idea that song is an attractive stimulus to female songbirds. Other studies reveal that this finding may not pertain as strongly to males. For example, Dobson and Petrinovich (1973) found that whereas almost all of the female white-crowned sparrows (*Zonotrichia leucophrys*) in their study demonstrated a strong preference for male song over silence, only some males did. The finding of high individual variation in males was also reported by Stevenson-Hinde and Roper (1975), who found that some male chaffinches (*Fringilla coelebs*) showed increased rates of perching when perching was reinforced by playback of conspecific male song, whereas many others did not. The results of another study by Stevenson-Hinde (1972) suggested that a reinforcing effect of song was exaggerated in first-year male chaffinches treated with testosterone (T), indicating a possible link between hormone levels and reinforcing effect of song.

Neural Responses to Music

Overall, behavioral evidence has suggested strongly that songbirds find song rewarding, as they will approach song and will perform tasks reinforced by song. Humans will perform similar behavioral tasks—such as listening to a radio or attending a concert in order to hear music— that indicate that they find music rewarding. Researchers using brain imaging techniques have reported that the pleasurable feelings associated with music listening are associated with neural responses in brain regions that make up a reward circuit (Blood & Zatorre, 2001; Brown et al., 2004). For example, functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) in humans listening to music have shown blood-oxygen-level-dependent (BOLD) or PET responses in the nucleus accumbens (nAc) and ventral tegmental area (VTA; Blood & Zatorre, 2001; Brown et al., 2004; Mitterschiffthaler et al., 2007). In humans, these areas show an elevated BOLD or PET response to music listening especially when the music elicits "strongly pleasant feelings" (Brown et al., 2004) or immensely pleasurable "chills" in the listener (Blood & Zatorre, 2001). Because elevated responses in the nAc and VTA are consistently observed concurrent with what participants describe as a rewarding experience, these responses are likely to be causally linked to the rewarding experience of music listening in humans.

The Neural Reward Circuit

The nAc and VTA are connected as components of a reward circuit in humans and in other vertebrates. A major component of this circuit was first discovered when Olds and Milner (1954) found that hooded rats (*Rattus norvegicus*) would press a lever in order to receive electrical stimulation within the septal region of the brain. They would self-stimulate this region rather than eat or sleep, indicating that electrical stimulation in this area is immensely satisfying, even more so than rewarding behaviors that are naturally-occurring.

It was later discovered that electrical stimulation of the septal area causes dopamine release from the VTA into the nAc (Fiorino, 1993), suggesting that this release is involved in behavioral reward. The involvement of dopamine in behavioral reward was further supported by studies showing that rats will press a lever to self-administer dopamine receptor agonists to the nAc (Ikemoto et al., 1997). Dopaminergic neurons project from the VTA to the nAc in vertebrates, including rats, humans, and birds such as the pigeon, chicken (*Gallus gallus domesticus*), and budgerigar (*Melopsittacus undulates*; reviewed by Oades & Halliday, 1987; Spanagel &Weiss, 1999; Fig. 1). It is these dopaminergic neurons that are thought to create a functionally connected reward circuit. Overall, natural reward— as well as reward associated with drugs of abuse (reviewed by Wise & Rompre 1989)—is believed to be associated with the release of dopamine in the nAc from these neurons.

Mapping Neural Responses

In order to compare the responses in songbirds to those seen during music listening in humans, it is important first to understand the techniques used to measure those responses in both humans and non-human models. In humans, non-invasive brain imaging techniques such as fMRI and PET scanning have been used to assess neural responses to music. Functional MRI scanning uses magnetic resonance technology to produce a static image of the brain in addition to a map of the real-time location of oxygen-rich blood. The blood-oxygen-level-dependent (BOLD) signal produced by fMRI imaging allows changes in the flow of oxygen-rich blood to be mapped over the static image of the brain. Blood flow within the brain is thought to be associated with neural activity (reviewed by Huettel et al., 2004). The arrival of oxygen-rich blood to a region in the brain is most closely associated with summation of synaptic inputs by neurons in the region, as evidenced by correlation between BOLD signals and local field potentials (reviewed by Arthurs & Boniface, 2002). A high-amplitude BOLD signal in a brain region is therefore associated with firing of many neurons projecting to that area.

PET scanning functions similarly to fMRI in that it traces blood flow through the brain. PET scans trace the movement of metabolically-active radioactive molecules, such as fluorodeoxyglucose, injected into the participant, enabling identification of the areas in which these molecules are metabolized. Because BOLD and PET signals depict similar events—movement of blood within the brain—PET signals, like BOLD signals, are thought to reflect the integration of synaptic activity in a brain region (reviewed by Tagamets & Horowitz, 2001). The magnitude of PET signals has been correlated with dopaminergic activity in several brain regions, including the striatum (reviewed by Fisher et al., 1995), making PET especially relevant as a functional imaging tool for the reward circuit.

In non-human animals, it is possible to look at responses in the reward circuit at the cellular level. One way to do so is by labeling immediate early gene (IEG) protein products, the levels of which indicate that a neuron is responding to a stimulus with new protein synthesis, in brain tissue. Like BOLD and PET signals, the presence of IEGs in a brain region occurs soon after the onset of a stimulus and disappears afterward. Although the BOLD and PET signals last for mere seconds, IEG expression can endure for minutes or even hours. When brain tissue is collected within this time period after the stimulus presentation, antibodies against IEG products can be used to map stimulus-induced responses (reviewed by Sheng & Greenberg, 1990). IEG labeling has been used in the past to visualize responses in the avian reward circuit, as investigators have shown IEG expression in the VTA associated with song production in male European starlings (Heimovics & Riters, 2007) and white-throated sparrows (Maney et al., 2003). IEG induction in the reward circuit appears to occur simultaneously with behavioral reward. For example, levels of IEG mRNA were elevated in the nAc of rats during self-administration or seeking of drugs of abuse associated with reward (Burton et al., 2009; Schmidt et al., 2005; Valjent et al., 2001) or during the presentation of rewarding food cues (Flagel et al., 2011). Rewarding electrical self-stimulation of the brain was accompanied by expression of IEG mRNA in the VTA (Marcangione & Rompre, 2008). Further, expression of the IEG product Egr-1 in the reward circuit seems to be necessary for behavioral reward, as rats did not respond to conditioned cues associated with rewarding drugs of abuse when Egr-1 was disabled (Valjent et al., 2006). In young male zebra finches, Egr-1 mRNA is present in the VTA during exposure to a singing male (Nordeen at al., 2009). This finding indicates that Egr-1 expression is induced by stimuli that also elicit behavioral reward in these animals.

The Natural Function of Song: Study Predictions

Involvement of the reward pathway during human music listening may provide some insight into subjective experience and allow us to explore the same phenomena in other species. Because humans and songbirds show similar behavioral responses to music and song, respectively, I predicted that the neural response to song in the songbird reward pathway would resemble that in humans listening to music. The current study is divided into three parts in order to compare sound-induced responses in the reward circuit under varying conditions. In the first study (Study 1) I aimed to demonstrate higher Egr-1 induction—and thus greater neural response—in the nAc and VTA in female birds exposed to song than in those exposed to silence. Because females usually demonstrate a behavioral preference for song (reviewed by Byers &

Kroodsma, 2009), I predicted that female white-throated sparrows exposed to song would have higher levels of Egr-1 expression in the reward pathway than those exposed to silence.

For the female listener, song functions as a courtship signal in the breeding season and an aggressive signal outside the breeding season (Eriksson & Wallin, 1986; Baptista et al., 1993; Maney & Goodson, 2011). Therefore, in Study 2, I predicted that hearing song would induce higher levels of Egr-1 expression in the reward pathway than hearing a control sound, but only in animals with breeding-typical levels of ovarian hormones. Outside of the breeding season, I predicted that hearing song would not induce as much Egr-1 because song in this context does not function as a courtship signal that would be adaptive to approach.

To the adult male listener, song is usually perceived as an aggressive signal and may not be adaptive to approach (reviewed by Catchpole & Slater, 1995). Therefore, in Study 3, I predicted that males—regardless of whether they are in the breeding condition— would be unlikely to show higher Egr-1 induction to song than to a control sound. Overall, by comparing the neural responses of songbirds listening to song to the response in the same pathway in humans listening to music, I hoped to show similarities between songbirds and humans at the level of the neural reward circuit and therefore similarities between birdsong and human music.

Methods

Experimental Design

I presented recordings of auditory stimuli, consisting of conspecific male song or frequency-matched tones, to white-throated sparrows. I first conducted a preliminary study, here referred to as Study 1, to test whether a song chorus known to strongly elicit copulation solicitation display (CSD) behavior (Maney, unpublished manuscript) would induce Egr-1 in the reward circuit at higher levels than silence. Because I hypothesized that females in the breeding condition would be more likely than other females to have elevated Egr-1 induction in the reward circuit, I treated all of the females in Study 1 with estradiol (E2). In Study 2, I examined the selectivity of the Egr-1 response in females with differing levels of E2, and in Study 3 I examined the selectivity of the Egr-1 response in males with differing levels of T.

Animals

Forty-eight female and 23 male white-throated sparrows were captured during fall or spring migration. Sex of the animals was confirmed via laparotomy or PCR, and at the start of the study, ovaries and testes were in a regressed state. Before the start of the study, birds were held on short day length for approximately 4 months or longer in order to bring reproductive hormones to the low levels found outside of the breeding condition, as done in previous studies in the lab (Maney et al. 2008).

Study 1

Hormone Treatment

At the onset of the study, birds were photostimulated by exposing them to a long day length (18L:6D). Several days after the onset of photostimulation, birds were moved to individual cages inside large walk-in sound-attenuated booths accommodating two birds housed in individual adjacent cages. During this time, the birds continued to be exposed to a long day length. In captivity, despite the stimulatory effect of long days on ovarian development, plasma levels of E2 do not increase to normal breeding levels in this species (Moore, 1983; Maney et al., 2007; Lake et al., 2008). In order to reproduce normal breeding levels of E2, at least one week after photostimulation, each bird received one subcutaneous 12 mm silastic implant (ID 1.47

mm, OD 1.96 mm, Dow Corning, Midland, MI, USA) sealed at both ends with A-100-S Type A medical adhesive (Factor 2, Lakeside, AZ, USA) containing 17β-estradiol (Steraloids, Newport, RI, USA). This dose has been shown to elevate plasma E2 to physiological breeding levels in females of this species (Maney et al., 2006; 2008; 2009). These implants maintain constant, breeding levels of E2 from day 2 for at least 80 days (Moore, 1983).

Sound Stimulus

Recordings of white-throated sparrow songs were obtained from the Cornell Lab of Ornithology Library of Natural Sounds. The songs were combined to produce a chorus of male song such that there were very few breaks between singing, and the songs of different males often overlapped. This type of auditory stimulus was used because it strongly elicits CSD (Maney, unpublished observations).

Stimulus Presentation and Tissue Collection

On the afternoon prior to stimulus presentation, two singly-housed birds were placed within a sound-attenuation booth equipped with microphone, speaker and video camera. All booths were identical. All stimuli were delivered at a peak level of 70 dB measured at the bird's cage. Stimulus presentation took place at least 2 days after treatment, at which point plasma E2 levels should be stable (Moore, 1983). On the day of stimulus presentation, at one hour after lights on, birds heard either nothing (n=8), a 4 min recording of male conspecific song (n = 8), or the 4 min recording repeated seven times with three min of silence between the recordings (n=5). All stimuli were delivered via speakers located inside the booths. Video recordings of all females made during the first 4 minutes of stimulus presentation were later scored for copulation solicitation events, defined as tail lifts, wing quivers or vocalizations characteristic of CSD (see

Maney et al., 2003). Sixty minutes following the onset of the stimulus presentation, birds were deeply anesthetized with isoflurane (Abbott Laboratories, North Chicago, IL, USA) and decapitated. Their brains were harvested, fixed, and sectioned at 50μm as previously described (Maney et al., 2003; 2005).

Histology

Brain tissue was processed for immunohistochemistry as previously described (Maney et al., 2003). Briefly, every third 50-μm section was incubated with an antibody against Egr-1 (anti-egr-1; Santa Cruz Biotechnology, Santa Cruz, CA, USA), which was subsequently labeled using a biotinylated secondary antibody and a preformed avidin: biotinylated enzyme complex (Vector, Burlingame, CA, USA). Labeling was visualized using diaminobenzidine enhanced with nickel (see Shu et al., 1988; Maney et al., 2003). Sections were mounted onto gelatin-coated slides, dehydrated, and coverslipped in DPX.

Quantification of Egr-1 immunoreactivity

The VTA and nAc were identified in coronal sections with reference to Stokes et al. (1974), Maney et al. (2008), and Husband and Shimizu (2011). Egr-1 immunoreactivity was quantified in three consecutive sections, 100μm apart, in the nAc and six consecutive sections in the VTA. Egr-1 was quantified in these regions on one side of the brain, chosen at random except when one side was damaged; in these cases the intact side was chosen. Images of the nAc and VTA were acquired with a 4× objective using a Leica DFC480 camera attached to a Zeiss Axioskop microscope. All images were approximately 4.4 MB. The light level on the microscope was set exactly the same for each picture. Egr-1 immunoreactive (ir) cell nuclei in the nAc were quantified in each image by a blind observer using the thresholding feature in

Image J (National Institutes of Health) as previously described (Maney et al., 2003). Briefly, the particles with an optical density higher than a threshold value were counted within each region of interest. Because of variability in background staining among brains, this threshold was set manually for each image such that clusters of pixels highlighted by the computer program agreed with what the observer considered to be labeled nuclei. For the nAc, these clusters were counted inside a 0.16-mm^2 circle placed at the rostroventral edge of the lateral ventricle within the nAc (Husband & Shimizu, 2011; Balint & Csillag, 2007; Fig. 2A). For the VTA, Egr-1-ir cells were counted within the entire region of interest (Fig. 2B) at 10× magnification. The area of the VTA traced in each bird was calculated by using the free trace feature of ImageJ. The areas of the four tracings were summed to find the total area sampled within the VTA in each bird. For both the nAc and the VTA, the number of labeled nuclei was divided by the total area sampled to calculate the number of labeled cells per unit area.

Statistical Analysis

The values for cells per unit area in the nAc and VTA were square root transformed to normalize their distribution, then entered into a MANOVA (SPSS). One missing data point for the VTA was generated in SPSS using the series mean. When significant main effects or interactions were found, I then performed univariate F-tests and, when appropriate, Scheffe tests to make pairwise comparisons within treatments between stimuli and within stimulus between treatments. To test whether the animals' own behavior could explain Egr-1 expression, Spearman correlation tests were used to assess relationships between CSD behavior in females or song in males and the number of Egr-1-positive cells per unit area in both regions for each group. I also tested whether Egr-1 expression in the nAc was correlated with Egr-1 expression in the VTA by using Pearson's correlation tests for each group.

Study 2

Hormone Manipulation

Throughout this study, birds were housed on a short photoperiod (10L:14D) to prevent gonadal recrudescence and the elevation of endogenous E2. All birds received subcutaneous silastic capsules, 12 mm in length, as in Study 1. In this study, 11 birds received empty capsules, and 12 birds received capsules containing E2. After hormone treatment began, birds were housed in individual cages, four per sound-attenuation booth, for a period averaging 9 days before stimulus presentation (see "Sound Stimuli", below). Hormone treatment was heterogeneous within these housing groups.

Sound stimuli

New song presentations were created for Study 2. In these presentations, songs were played one at a time every 15 seconds in order to simulate a more natural rate of repetition. Songs were presented from 14 different males in order to minimize habituation to the song stimuli (Stripling et al., 1997). Recordings of white-throated sparrow songs were downloaded in Real Player format (RealNetworks, Seattle, WA, USA) from the Borror Laboratory of Bioacoustics birdsong database and converted to AIFF format using AudioHijack (Rogue Amoeba Software, Cranbury, NJ, USA). All remaining audio processing was done with Audacity (Carnegie Mellon Computer Music Group, Pittsburgh, PA, USA). Background noise was removed. The recordings were edited such that there was 15 s of silence between each song and each recording lasted for exactly 3 min. These 3-min segments, each containing the songs of a unique male, were spliced together to form stimulus presentations such that the identity of the singer changed to a novel male every 3 min. Each presentation contained the songs of 14 males and totaled 42 min in duration. The 3-min segments from each male were presented in an order determined by a balanced Latin Square, such that each bird presented with song stimuli heard a unique order of singing males with no single male's songs preceding or following another's more than once.

In this study, I controlled for the presentation of an audio stimulus by exposing half of the birds to synthetic tones, a non-song sound that does not typically induce a CSD response (Maney et al, 2006; Sanford et al., 2010). Each bird hearing tones heard a unique sequence that was matched to one of the song sequences described above with regard to several parameters of sound. For each of the 14 recordings of males, the frequency of each whistle (note) in one song was measured using AudioXplorer (Arizona Software, San Francisco, CA, USA). Songs usually contained five distinct frequencies. For each song, eight sinusoidal tones were generated at these frequencies and arranged in a random order 200 ms apart. This resulted in tone sequences that matched individual songs in the average number of onsets and offsets as well as total sound energy at each frequency. Tone sequences were spliced together as for the song stimuli, with 15 s of silence between each eight-tone sequence, in an order determined by a balanced Latin Square.

Data Collection and Analysis

Playbacks were conducted as described for Study 1. The type of stimulus (song or tones) was balanced across treatment group so that six E2-treated and six placebo-treated birds heard song, and six E2-treated and five placebo-treated birds heard tones. Video and audio recordings were made during stimulus presentation and later scored for CSD events as described for Study 1. Tissue collection, immunohistochemistry, and data collection were as described for Study 1.

Cell counts per unit area were square root transformed and entered into a two-way MANOVA to determine the effects of hormone treatment and stimulus type, and the interaction between the two. Spearman correlation tests were used to look for relationships between CSD behavior and the level of Egr-1 induction in each region. To determine whether Egr-1 induction was related in the two areas, Pearson's correlation tests were performed.

Study 3

The design of Study 3 was identical to that of Study 2, except that I used males instead of females, and I manipulated T instead of E2. Eleven birds received empty capsules, and 12 birds received capsules containing T (Steraloids). The length of the capsules was 15 mm. This dose of T brings plasma levels within a breeding-typical range within 7 days (Maney et al., 2009). After hormone treatment began, birds were housed four per sound-attenuation booth for 7 days. As in Study 2, hormone treatment was heterogeneous within these housing groups. Animals in this study were exposed to the same sound stimuli as those in Study 2. The type of sound stimulus heard was balanced between the treatment groups, so that six T-treated birds were exposed to song, six placebo-treated birds were exposed to song, six T-treated birds were exposed to tones, and five placebo-treated birds were exposed to tones.

The remaining portions of Study 3, including stimulus presentation, tissue collection, immunohistochemistry, quantification of Egr-1-ir cells, and statistical analysis were identical to those of Study 2. Spearman's correlations were used to test for relationships between the number of songs given by the males in the study and Egr-1 induction in each area. I determined whether Egr-1 induction was correlated in the two regions of interest by using Pearson correlation tests.

Results

The results of each study are summarized below. I found that in females, hearing song induced a Egr-1 response in both areas of the reward circuit (Study 1). This response was selective for song over tones, however, only in females with breeding-typical levels of E2 (Study 2). In males, responses to song and tones were similar regardless of whether the males were treated with T (Study 3).

Study 1

The results of Study 1 are depicted in Fig. 3. A MANOVA performed on cell counts in the nAc and VTA revealed a significant effect of song exposure (Wilks' lambda $F_{4\ 36} = 4.42$, $p = 0.007$). Post-hoc univariate F-tests showed a significant effect both in the nAc ($F_{2,\ 19} = 5.698$, $p = 0.012$) and in the VTA ($F_{2,\ 19} = 7.787$, $p = 0.003$). Post-hoc Scheffe tests revealed that the Egr-1 response in both brain areas was higher after seven 4-min exposures to song than after a single 4-min exposure (nAc $p = 0.022$; VTA $p = 0.023$) or silence (nAc $p = 0.024$; VTA $p = 0.004$). There was no correlation between the number of CSD events and the number of Egr-1-ir cells in the nAc, indicating that the effect of the stimulus cannot be explained by the birds' own behavioral responses. There was a significant correlation between Egr-1 expression in the nAc and in the VTA ($R^2 = 0.343$; $p = 0.004$), but it was clearly due to the effect of stimulus on each region separately. When the analyses were limited to within-stimulus groups, the correlation disappeared (all p values > 0.148).

Study 2

The results of Study 2 are depicted in Fig. 4. A MANOVA performed on the data obtained from the nAc and VTA revealed a significant effect of stimulus (Wilks' lambda $F_{2,}$

$_{18}$=6.379, p = 0.008) and an interaction between stimulus and treatment (Wilks' lambda $F_{2,18}$ =4.647, p = 0.024). Post-hoc univariate F-tests showed a significant effect of stimulus in the nAc ($F_{1, 19}$= 4.855, p = 0.040) and an effect of treatment ($F_{1, 19}$=6.099, p = 0.023) and stimulus ($F_{1, 19}$=12.313, p = 0.002) in the VTA. Univariate F-tests also revealed a significant interaction between stimulus and treatment in the nAc ($F_{1, 19}$ = 9.652, p = 0.006), but not in the VTA ($F_{1, 19}$ = 2.043, p = 0.169). Post-hoc pairwise comparisons revealed a significantly higher Egr-1 response to song than to tones in E2-treated birds for both brain regions (nAc p = 0.037; VTA $p < 0.001$) and a higher response to song in E2 treated birds than in untreated birds (nAc p = 0.038, VTA p = 0.027). In the birds that performed CSD (E2-treated birds hearing song), there were no significant correlations between CSD behavior and Egr-1 expression in either brain region (both p values > 0.266). A Pearson's correlation test revealed a significant correlation between Egr-1 expression in the nAc and in the VTA (R^2 = 0.681464, p = 0.015), but this correlation was clearly driven by the effect of hearing song on both regions independently. When the stimulus groups were assessed individually, no significant correlations between Egr-1 expression and behavior were found (all p values > 0.178).

Study 3

The results of Study 3 are depicted in Fig. 5. A MANOVA performed on the data obtained from the nAc and VTA revealed a significant effect of treatment (Wilks' lambda $F_{2, 18}$ = 5.205, p = 0.016), but no significant effect of stimulus ($F_{2, 18}$ = 0.078, p = 0.925) and no interaction between stimulus and treatment ($F_{2, 18}$ = 0.125, p = 0.883). Post-hoc univariate F-tests revealed a significant effect of treatment in the VTA ($F_{1, 19}$ = 8.528, p = 0.009) and a trend in the nAc ($F_{1, 19}$ = 3.445, p = 0.079). Post-hoc pairwise comparisons revealed that this effect was significant only in the VTA in birds exposed to tones (p = 0.031). The number of songs given by the males during the stimulus presentation was correlated with Egr-1 expression in the VTA ($R^2 = 0.396$, $p = 0.001$) but not the nAc ($R^2 = -0.12$, $p = 0.621$). When the groups were assessed individually, correlations between Egr-1 expression in the VTA and song production remained significant only in birds treated with T (song: $R^2 = 0.861$; $p = 0.008$; tones: $R^2 = 0.972$, $p < 0.001$). Egr-1 expression in the nAc was not correlated with that in the VTA either when considering all birds together or each group individually (all $p > 0.136$).

Discussion

Summary of Results

In Study 1, I showed that hearing song induced Egr-1 in the nAc and VTA. The Egr-1 response in these regions was consistent with what is seen in other animals experiencing reward (reviewed by Wise & Rompre 1989). Study 1 served as a preliminary study, as I looked for a song-induced response in the reward circuit before manipulating hormonal state and sound stimuli. Because I did see a higher Egr-1 response to song than to silence, I proceeded to Studies 2 and 3.

In Study 2, I demonstrated that the Egr-1 response in both the nAc and the VTA was selective for song over a behaviorally irrelevant control sound, but only in females treated with E2. This finding suggests that breeding-typical levels of E2 are required for the reward pathway to respond selectively to song, which is a courtship signal. Further, E2 appears to enhance responses to song, as song-induced Egr-1 expression in both regions was higher in treated than untreated females. Overall, from Study 2, I concluded that the Egr-1 response to song is modulated by reproductive state.

In Study 3, I predicted that Egr-1 responses in both regions of interest would be similar in all four groups of males because song delivers an aggressive message to all male listeners. I found that the response to song and tones did not differ and that T treatment had no effect on this result. These findings support my hypothesis that the reward circuit in males does not respond selectively to song either inside or outside the breeding season. Although the Egr-1 response was not itself affected by sound stimulus, it did depend on T-treatment; in the VTA, Egr-1 expression was higher in the males receiving T than in those with blank implants. This finding is consistent with others suggesting that gonadal steroids alone, independent of social stimuli, can induce IEG activity in the VTA in Japanese quail (*Coturnix japonica*; Charlier et al., 2005) and Syrian hamsters (*Mesocricetus auratus*; DiMeo & Wood, 2006).

Comparing Neural Responses in the Reward Circuit

In the present study, I aimed to compare my findings in songbirds with those in humans listening to pleasurable music. Many imaging studies have been conducted in humans listening to music (Table 1), and although each revealed a unique pattern of neural responses to pleasurable music, most showed clear responses in either the nAc or VTA or both. Responses in these regions—components of the reward circuit—are concurrent with reported feelings of enjoyment or pleasurable chills during music listening (Blood and Zatorre, 2001; Brown et al., 2004; Koelsch et al., 2006; Menon and Levitin, 2005; Mitterschiffthaler et al., 2007; Montag et al. 2011). In other vertebrates, IEG responses in the nAc and VTA are concurrent with rewarding stimuli, such as administration of rewarding drugs (Burton et al., 2009; Schmidt et al., 2005; Valjent et al., 2001). Because of the evidence that female songbirds in breeding condition find song rewarding (reviewed by Byers & Kroodsma, 2009; Gentner & Hulse, 2000; Reiner, 2000; Eriksson & Wallin, 1986) I predicted that song-induced Egr-1 expression in sexually receptive females would be high in the nAc and VTA. In my study, I found that song does induce Egr-1 in these regions and that when E2 is at breeding levels, this response is selective for song over a behaviorally irrelevant sound. These findings indicate that, just as hearing music elevates BOLD responses and PET signals in the reward circuit in humans, song elevate Egr-1 responses in the same circuit in birds. Similar neural responses in the two species may indicate that the experience of listening to song in birds may be similar to the rewarding experience of listening to music in humans.

Comparing Responses in the nAc: Anticipation of Reward?

Neural responses in the nAc, especially those involving dopamine, are associated with behavioral reward in both mammals and birds (reviewed by Oades & Halliday, 1987; Wise & Rompre 1989). For example, rats will repeatedly press a lever to self-administer DA agonists into the nAc (Hoebel et al., 1983; Philips et al., 1994). Thus, the response in the nAc in songbirds may indicate that song is pleasurable to them. Some research, however, suggests that responses in the nAc may indicate anticipation of a rewarding experience rather than actually experiencing it as a consummatory reward (reviewed by Ikemoto & Panksepp, 1999). For example, Ikemoto and Panksepp (1996) reported that intra-nAc injections of dopamine antagonists did not affect rats' consumption of sugar water, a stimulus known to induce operant responses (Gibson et al.,1965), placed in front of them. When the rats were required to work for access to the reward—a task that they would consistently complete before injections of antagonists—the injections interfered with their motivation to do so. Responses in the nAc associated with anticipation of reward are found also in primates. Schultz et al. (1992) showed that electrical activity in dopaminergic neurons projecting to the nAc increased in macaques (*Macaca fascicularis*) presented with stimuli that cue reward, and Knuston et al (2001) found that BOLD responses in the human nAc occur during anticipation of monetary rewards. These results suggest that the nAc may be involved in reward-seeking behavior rather than consummatory reward.

Like in other vertebrates, the nAc may be involved in appetitive reward in birds. To the female white-throated sparrow in breeding condition, male conspecific song indicates the presence of a mating opportunity. Female pied and collared flycatchers in breeding condition exposed to song approach the source of song, indicating that the stimulus is attractive, and thus possibly rewarding, to them (Eriksson & Wallin, 1986). This approach behavior is similar to responses in other organisms while they seek out a reward, and is disabled when an antagonist is injected into the nAc (Ikemoto & Panksepp, 1996). Therefore, the nAc plays a fundamental role in regulating this approach behavior. Thus, the nAc response in the female songbird listener may be due to her anticipation of and approach of the rewarding experience of mating.

The nAc may respond during the anticipation of reward in humans. For example, Knutson et al. (2001) found that human participants showed increased BOLD responses in the nAc while they were anticipating monetary reward. In the context of anticipation of reward, the reasons underlying the nAc response when listening to music are less clear. The structure of music may offer one explanation. Music is built on the interplay of consonance and dissonance. In Western music, phrases may contain dissonance, but at the end of a musical phrase, the melodic and harmonic lines usually lead to a pleasing, consonant cadence (Clendinning & Marvin, 2011). Thus, the listener may experience anticipation while the musical line leads to the cadence and reward upon the arrival of the cadence. Salimpoor et al. (2011) examined neural responses to music during times of “peak emotional arousal,” defined by the onset of pleasurable chills, and during the “anticipation” phase of music, or the time period before experiencing chills. Although previous studies suggested that the nAc is involved in appetitive components of reward (reviewed by Ikemoto & Panksepp, 1999), “anticipation” was associated with BOLD responses in the caudate nucleus, not the nAc. In contrast, responses in the nAc were associated with “peak emotional arousal,” or consummatory reward. Overall, this study suggests that, in humans listening to music, the nAc responds during the experience of reward rather than anticipation.

Comparing Responses in the VTA

Whether it is induced by anticipation or by reward itself, activity in the nAc is controlled largely by inputs from the VTA (Oades & Halliday, 1987; Spanagel &Weiss, 1999). Elevated BOLD responses in the VTA are concurrent with administration of cocaine in human addicts (Breiter et al., 1997). VTA responses have been associated with administration of rewarding drugs of abuse in other animals as well (reviewed by Bardo, 1998), suggesting that, like the nAc, the VTA responses to rewarding stimuli are conserved across species. Further, the VTA appears to be necessary for experiencing reward, as rats will no longer self-administer cocaine following lesions of the VTA (Roberts & Koob, 1982). Blood and Zatorre (2001) and Menon and Levitin (2005) reported BOLD responses in the VTA in humans listening to pleasurable music. I showed that the VTA responds selectively to song (see also Maney et al., 2008). My findings thus indicate that, like music, song may be pleasurable to the listener.

Comparing Neural Responses Outside of the Reward Circuit

The nAc and VTA are not the only regions of the human brain that respond during music listening. Other regions have been shown to respond, and those responses and can be compared to those in the homologous regions in songbirds listening to song. Many of the regions that respond in humans (Table 1) are located in the cortex, an area in which the homologies with the avian brain are not as well established as in subcortical areas. Because avian-human cortical homologies are somewhat controversial, I have focused here on subcortical structures such as the amygdala, hippocampus, regions of the hypothalamus, and the caudate nucleus (for avian homologies see Berk and Butler,1981; Cheng et al.,1999; Colombo and Broadbent, 2000; Huber and Crosby,1929; Reiner et al., 1994; Zeier and Karten,1971).

Hypothalamus

In humans listening to music, BOLD responses in the nAc and the VTA were strongly correlated with responses in the hypothalamus (Menon and Levitin, 2005). These authors proposed that the correlation occurs because the hypothalamus is responsible for executing the physiological responses, such as chills and changes in breathing and heart rate, associated with the rewarding experience of music listening. The nAc, VTA, and hypothalamus are extensively connected (reviewed by Zahm, 2000), adding further support to the idea that the hypothalamus may help modulate the reward response to music in humans *via* its connectivity to the nAc and VTA. Although each individual nucleus of the hypothalamus regulates distinct physiological responses (reviewed by Lammers & Lohman, 1974; Nolte, 1988), these investigators quantified responses in the hypothalamus as a whole. By looking at responses of the entire hypothalamus only, the investigators left the responses in the individual nuclei unclear. In white-throated sparrows, Maney et al. (2007; 2008) found selective Egr-1 responses in the medial preoptic area, the mediobasal hypothalamus, and the ventromedial hypothalamus. These regions of the hypothalamus may regulate physiological responses associated with song. For example, in ferrets (*Mustela putorius furo*), the medial preoptic area responds to sexual stimuli such pheromones with increased expression of the IEG FOS (Kelliher et al.,1998) and in European starlings may be involved in the motivation to sing (Schroeder & Riters, 2006). The mediobasal hypothalamus is involved in the release of gonadotropin-releasing hormone in response to song (Maney et al., 2007). In males the lateral portion of the ventromedial hypothalamus responds to territorial intrusions only during the breeding season, which suggests that it plays a role in aggressive responses to song when the context is appropriate (Goodson & Evans, 2004; Goodson et al., 2005). In females, however, this region responds selectively to song both inside and outside of the breeding season, indicating a possible sex differences in its function (Maney et al., 2008). Overall, evidence suggests that in humans and birds, responses in the hypothalamus may be linked to physiological responses to music and song, respectively.

Caudate Nucleus and Striatum

The caudate nucleus, which like the nAc is part of the basal ganglia, may also be involved in experiencing reward. For example, Knutson et al. (2005) found BOLD responses in the caudate during anticipation of monetary reward in humans. The caudate also responds while learning difficult activities that may lead to a monetary reward (Haruno et al., 2004). Overall, associations with monetary reward—which is a secondary, or learned reward—indicate that the caudate may be associated with responses to rewards other than the primary rewards such as food, water, and copulation. The caudate responds in humans listening to pleasurable music (Berns et al., 2010; Blood & Zatorre, 2001; Koelsch et al., 2006; Montag et al. 2011). Berns et al. (2010) found that the intensities of BOLD responses in the caudate were strongly correlated with participants' ratings of the likeability of a song. If songbirds show homologous responses to song, we would expect to see song-induced Egr-1 expression in the avian homolog of the caudate (Reiner et al., 2004). Using methods similar to those in Study 1, Maney et al. (2008; unpublished manuscript) found that the IEG responses in both the lateral and medial striatum are not greater to song than to silence. Therefore, to the extent that we can compare these structures in humans and songbirds, they do not seem to respond similarly to music and song, respectively.

Amygdala

Whereas the BOLD signal increases during music listening in some brain regions, it decreases in others. Blood and Zatorre (2001) found for example that pleasurable music causes a decrease in the PET signal in the human amygdala. This effect is not unique to pleasurable music, as decreased BOLD responses in the amygdala have been associated with other types of reward or extremely pleasant emotions (Breiter et al., 1997; Ketter et al., 1996). The association between decreased responses in the amygdala and pleasure may be explained by inhibitory input from neurons originating in the nAc (Bardo, 1998). Increased responses of one region may lead to decreased responses in the other. Blood and Zatorre (2001) found further that the PET signal in the amygdala was inversely related to those in the ventral striatum, likely including the nAc, and midbrain structures such as the VTA, supporting the idea that activity in reward centers may affect the amygdala. Like the hypothalamus, the amygdala is a heterogeneous collection of distinct cell types with unique functions (reviewed by Swanson & Petrovich, 1998). For example, the basolateral amygdala has been shown to respond to stimuli with negative or fearful valence (reviewed by Fanselow & LeDoux), whereas the medial amygdala responds to sexual stimuli (Baum & Everitt, 1992). Although Blood and Zatorre found local decreases in PET signals in the amygdala in general, they did not differentiate among the nuclei it contains. Thus, the PET response they observed in the amygdala may not be indicative of the responses of its individual components. Using methods similar to those in the present study, Maney et al. (2008) found that the Egr-1 response is higher to song than to tones in the nucleus taeniae (TnA), the homologue of the mammalian medial amygdala (Cheng et al.,1999; Zeier and Karten,1971). In rats, this region is known to respond to sexual stimuli such as sexually receptive members of the opposite sex (Baum & Everitt, 1992). In birds, song-induced IEG expression in the TnA may thus be associated with the sexual message of song to the listener. Because the individual components of the human amygdala have not been imaged during music listening, it is difficult to compare its response to music to the response to song in birds.

Hippocampus

Hearing pleasurable music decreases the PET signal not only in amygdala, but also in the hippocampus (Blood and Zatorre, 2001). Blood and Zatorre (2001) found a decrease in regional cerebral blood flow (rCBF) in the hippocampus that, like the decrease in amygdala, was proportional to an increase in rCBF in structures such as the nAc and VTA. Inhibitory connections between the nAc and the hippocampus may allow the nAc to dampen hippocampal responses during music listening in humans (Bardo, 1998). In contrast, hearing song elevates IEG expression in the avian hippocampus (Bailey et al., 2002; Maney et al., 2008). In zebra finches, IEG responses in the hippocampus are induced by sexual stimuli such as a first courtship (Sadananda & Bischof, 2002) and hearing a mate's song (Vignal et al., 2008). This evidence suggests that responses in the hippocampus may stem from its involvement in processing sexual stimuli—a function that may not be served in humans listening to music. Overall, whereas decreases in rCBF are found in the hippocampus in humans listening to music, IEG induction is increased in this region in birds listening to song.

Overview: Comparing Neural Responses

My analysis of neural responses to music and song has revealed that some brain regions respond similarly humans and songbirds, whereas other regions do not. The hypothalamus and the caudate nucleus respond in both humans and songbirds (Bailey et al., 2002; Maney et al., 2008; Menon and Levitin, 2005). Activity in other regions, such as the amygdala and the hippocampus, appears to be inhibited by pleasant music in humans (Berns et al., 2010; Blood & Zatorre, 2001; Brown et al., 2004; Koelsch et al., 2006) but stimulated by song in songbirds. Further, song-induced IEG responses in other regions that respond to music, such as the cerebellum (Brown et al., 2004), remain relatively unexplored in songbirds. Investigating whether there is a selective IEG response to song in this region and others would allow for a more informative comparison of subjective experiences in humans and birds.

A factor to consider when comparing the studies done in humans with my own study is the use of different tools—PET or BOLD signals in humans and Egr-1 induction in birds—to measure neural responses. BOLD and PET signals indicate integration of graded potentials at a region of interest, which may not always correspond with subsequent neuron firing (reviewed by Tagamets & Horowitz, 2001). Egr-1 induction indicates cellular responses that lead to new protein synthesis, which, like graded potentials, is not always linked to firing. Because the tools used measure different components of the neural response to stimuli, it is difficult to determine whether the observed similarities and differences in neural responses between humans and birds accurately reflect similarities and differences in underlying neural firing at the region of interest.

Sex, Hormonal State, and Social Context

A notable feature of the Egr-1 response in the reward circuit in my study was a dependence on sex and hormonal state. Sex and hormonal state may contribute to a social context upon which the interpretation of the signal by the listener is based. For example, females in breeding condition respond to song with CSD (Searcy & Marler, 1981) and/or phonotaxis

(Eriksson & Wallin, 1986), which is consistent with the idea that females interpret song as a mating signal. In contrast, males respond to song with countersong and aggressive approach, indicating that song functions as an aggressive signal to a male listener (reviewed by Catchpole & Slater, 1995). In humans, however, the function of music is not as clear. Behavioral preferences for music do not appear to vary according to reproductive state. Both men and women seek out music, and changes in endocrine state associated with the menstrual cycle do not appear to change music-seeking behavior. Although we may not think that women with high levels of E2 are more likely to attend concerts, a number of studies suggest that hormones may actually affect music-associated reward. Panksepp (1995) found that women are more likely than men to report feelings of pleasurable "chills" when listening to music, indicating that, like female songbirds who show behavioral preferences for song (reviewed by Byers & Kroodsma, 2009; Gentner & Hulse, 2000; Reiner, 2000; Eriksson & Wallin, 1986), women may perceive music as more pleasurable than their male counterparts do. Music perception may also be affected by endocrine state. Sanders and Wenmoth (1998) found, for example, that perception of music chords by women differs according to the phase of the menstrual cycle. Specifically, these investigators found that women in the menses phase, during which E2 levels are low, scored higher on a dichotic listening task than those in the midluteal phase, during which E2 levels are high. Ovarian hormones may therefore affect auditory processing and cognition. Future research on music preferences will be necessary to better understand the extent to which they are altered by endocrine state.

Sex and hormonal state are clearly important components of the social context in which white-throated sparrows interpret signals such as song (Maney & Pinaud 2011), but they may not be as important for humans interpreting music. Rather than examining the effects of hormonal state and sex on music enjoyment in humans, we might instead look at the role of other types of social context. In a study by Berns et al. (2010), adolescents rated clips of music, and after being told how their peers had rated the music, were told to rate it again. The peers' opinions of a music clip significantly affected the individuals' rating of the music. The participants found music that was "popular" with their peers to be more enjoyable. Overall, this study offers behavioral evidence that the pleasure associated with a piece of music depends on social context in humans. These investigators found that context-modulated changes in likeability of music were correlated with responses in cortical structures such as anterior cingulate and anterior insula, providing a possible neural substrate for this phenomenon. Because social context, as defined by endocrine state, alters song-induced responses of the nAc and VTA in songbirds, these areas could also be involved in context-dependent reward in humans. Responses in these regions were not reported by Berns et al. (2010). Future studies should test whether music-induced responses in the human reward pathway are modulated by social context.

Neural Responses in the Performer

To compare song to music, I examined the effect of these signals on the listeners. Song and music can also be compared with respect to their effects on the performer. Some of the same brain areas respond in humans performing music and male songbirds performing song. For example, IEGs are induced in the song control circuit in singing white-throated sparrows (Maney et al., 2003), and BOLD responses are induced in motor areas controlling performance-related muscles in performing musicians (Meister et al., 2004). Some regions of the brain, however, respond differently in the human and songbird performer. Several hypothalamic regions and the TnA respond in singing white-throated sparrows (Maney et al., 2007; 2008), but the PET responses in the same regions are decreased in performing jazz musicians (Limb & Braun, 2008).

Hara et al. (2007) and Maney et al. (2003) showed that IEG products are up-regulated in the VTA in singing male zebra finches but no corresponding increase in activity has been reported in that region in performing humans. The present study is consistent with an increase in IEG activity in the VTA, as the number of songs given by males correlates with Egr-1 expression in that area. Overall, with the exception of motor areas involved in performing, most of the brain regions examined in performers respond differently in songbird and humans.

Conclusion

In the present study, I showed that song is similar to music in that they both induce responses in the same components of the neural reward circuit—the nAc and the VTA—in the listener. These responses suggest that both songbird and human listeners may experience reward, or the anticipation of reward. Whereas these results suggest that the experience of song and music may be similar to the songbird and human listener, they do not necessarily answer the question of whether music in uniquely human. Other species show similar behavioral responses to sexually-motivated vocalizations that lack the aesthetic beauty of song to the human listener. For example, Goodson and Adkins-Regan (1997) found that female Japanese quail have a phonotaxic response to conspecific male calls, which are not songlike. In other animals, male vocalizations have a phonotaxic effect on females. For example, males of some species of frogs and toads use vocalizations to advertise themselves to potential mates (reviewed by Narins et al., 2007). In species that court using vocalizations, females will approach calling males or recordings of "advertisement calls" (Littlejohn & Michaud, 1959; Zeyl & Laberge, 2011). A brain region involved in reward, the dorsal striatum, was found to respond to male vocalizations with increased Egr-1 expression in male túngara frogs (*Engystomops pustulosus*; Hoke et al., 2007) suggesting that, although the responses in the nAc and VTA during these behaviors have not been described, the underlying neural effects in these species may be similar to those seen in songbirds exposed to song. Overall, my findings do show that music and birdsong may elicit similar experiences in the listeners; however, these experiences may be shared also by quail and frogs listening to sounds that bear much less resemblance to music. If my results support the hypothesis that song is music, therefore, they also suggest that many other sounds, some of which do not share the aesthetic beauty of birdsong, may also qualify.

Song has been compared to music in the past, but for the first time, I compared the responses in the recipient of the signal—the listener. I found that the neural reward circuit most often reported to respond to music in humans also responded to song in songbirds, indicating that, at least in this way, song and music are similar. It is possible that the responses in these regions may underlie behavioral preference for these sounds. I found that in white-throated sparrows, this response is highly dependent on sex and endocrine state, as only females in breeding condition showed elevated Egr-1 responses to song compared with a control sound. Future research on the effects of hormones, sex, or social context on music-associated reward in humans, as well as the neural substrates that may underlie this phenomenon, would provide a more thorough understanding of how these factors modulate music perception in humans.

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Table 1. Regions of the human brain that show changes in blood oxygen-level-dependent (BOLD) responses or positron-emission topography (PET) signals in response to pleasurable music.

Brain region in humans	**References**
Amygdala	Blood and Zatorre, 2001
Anterior cingulate	Brown et al., 2004 (prefrontal); Mitterschiffthaler et al., 2007; Pereira et al. 2011 (rostral)
Caudate nucleus	Berns et al., 2010; Blood & Zatorre, 2001; Koelsch et al., 2006; Montag et al., 2011; Salimpoor et al., 2011
Dorsal striatum	Mitterschiffthaler et al., 2007
Cerebellum	Brown et al., 2004
Heschl's gyrus	Koelsch et al., 2006
Hippocampus	Brown et al., 2004
Hypothalamus	Menon and Levitin, 2005
Inferior frontal gyrus	Koelsch et al., 2006; Pereira et al. 2011
Insula	Brattico et al. 2011; Brown et al., 2004 (anterior); Koelsch et al., 2006 (anterior superior); Menon and Levitin, 2005; Montag et al., 2011
Medial frontal gyrus	Pereira et al. 2011
Orbitofrontal cortex	Blood and Zatorre, 2001
Parahippocampal gyrus	Mitterschiffthaler et al., 2007
Retrosplenial cortex	Brown et al., 2004
Rolandic operculum	Koelsch et al., 2006
Subcallosal cingulate gyrus	Brown et al., 2004
Superior frontal gyrus	Pereira et al. 2011
Ventral medial prefrontal cortex	Blood and Zatorre, 2001
Ventral striatum (including nAc)	Blood and Zatorre, 2001; Brown et al., 2004; Koelsch et al., 2006; Menon and Levitin, 2005; Mitterschiffthaler et al., 2007; Montag et al., 2011; Salimpoor et al., 2011
Ventral tegmental area (midbrain)	Blood and Zatorre, 2001; Menon and Levitin, 2005

FIGURES

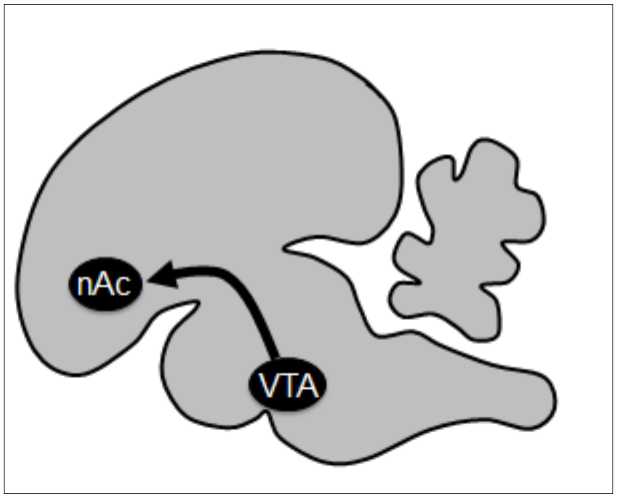

Fig. 1. The neural reward circuit. This image depicts the reward circuit in a sagittal section of the avian brain, with the beak to the left. The same pathway exists in the human brain. Dopaminergic neurons project from the VTA to the nAc, and, when releasing dopamine into the nAc, are associated with behavioral reward.

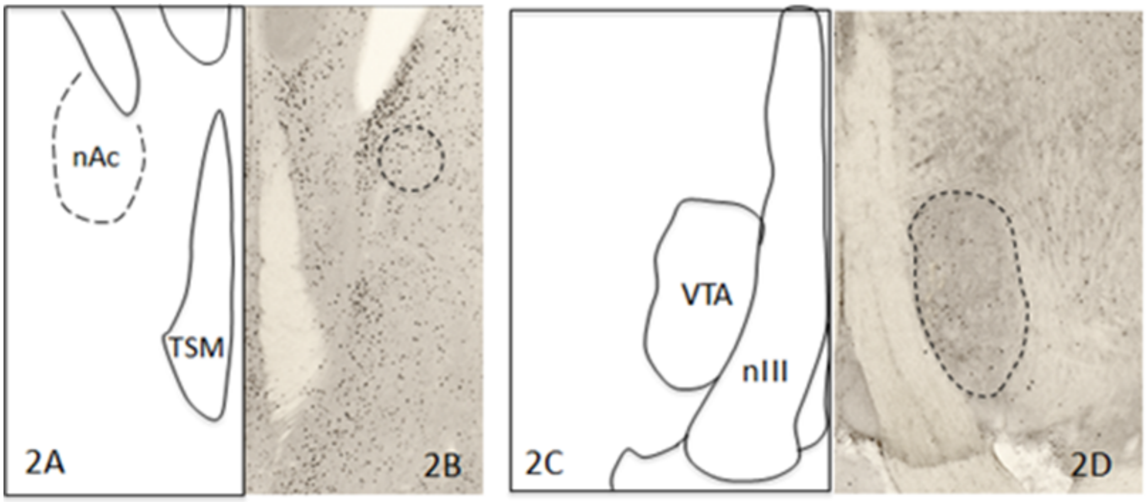

Figure 2. Egr-1 immunoreactivity was quantified in coronal sections. Figs. 2A and 2C show a schematic representation of major landmarks with the midline to the left. Figs. 2B and 2D show photomicrographs, acquired with a 4× objective, of Egr-1 immunoreactivity. Dotted lines in Figs. 2B and 2D encircle the areas sampled within the nucleus accumbens (nAc) and the ventral tegmental area (VTA), respectively. In both Fig. 2B and 2D, the midline is to the right. nIII, third cranial nerve; TSM, septopallio-mesencephalic tract.

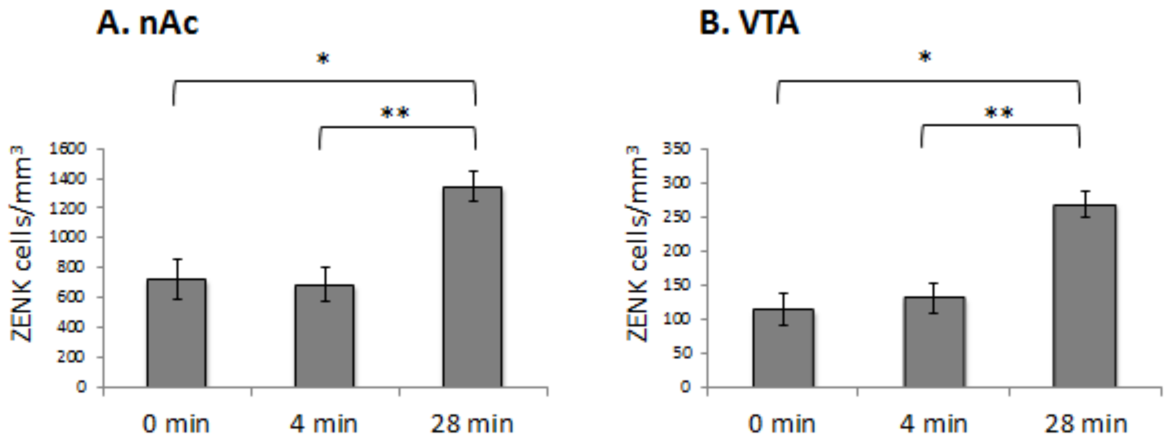

Figure 3. Song-induced Egr-1 responses in the nAc (A) and VTA (B) in E2-treated female white-throated sparrows. In both the nAc and VTA, the Egr-1 response to seven four-min exposures to song (total 28 min) was higher than to a single four-min exposure (**nAc p = 0.022; VTA p = 0.023) or silence (*nAc p = 0.024; VTA p = 0.004).

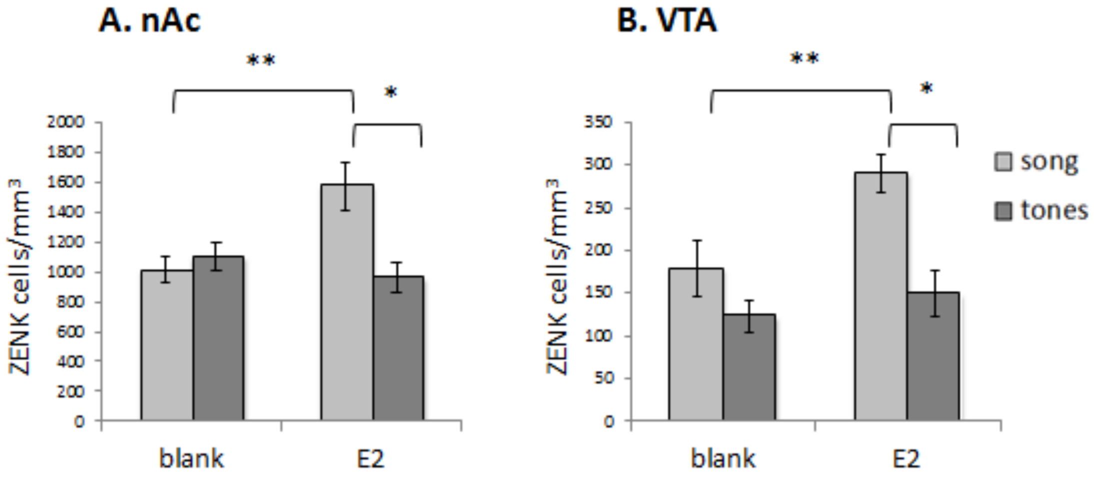

Figure 4. Egr-1 responses in the nAc (A) and VTA (B) in female white-throated sparrows. In both regions of interest, the Egr-1 response to song was significantly higher than to tones in E2-treated birds (*nAc $p = 0.037$; VTA $p < 0.001$) and higher to song in E2-treated birds than in untreated birds (**nAc $p = 0.038$, VTA $p = 0.027$).

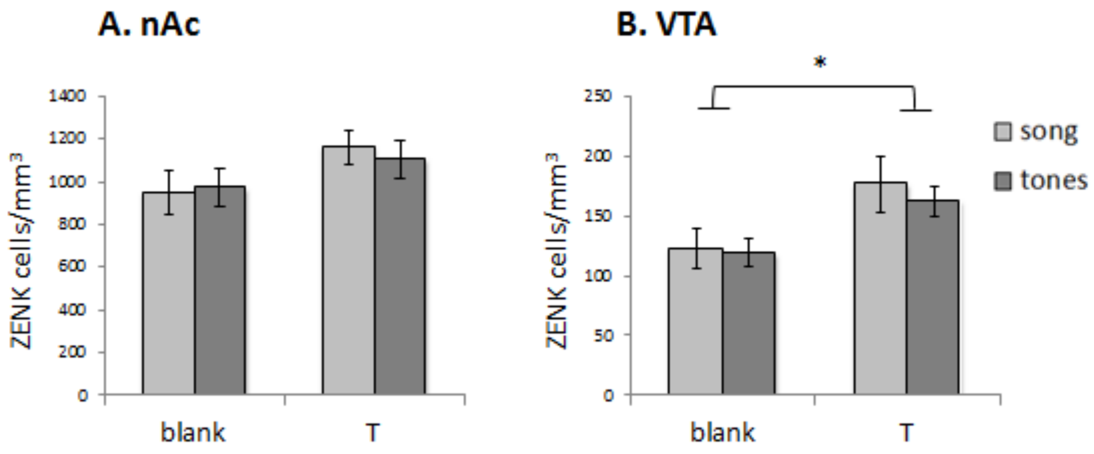

Figure 5. Egr-1 responses in the nAc (A) and VTA (B) in male white-throated sparrows. In the VTA, T-treatment increased Egr-1 expression regardless of stimulus group, and a similar trend was present in the nAc (nAc $p = 0.079$; *VTA $p = 0.009$). The response to song was no higher than the response to tones in either region for either treatment group.