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α_1-Noradrenegic receptor antagonism disrupts female songbird responses to male song

Benjamin A. Pawlisch, Sharon A. Stevenson, Lauren V. Riters*

Department of Zoology, University of Wisconsin, Madison, WI, United States

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ABSTRACT

Female songbirds respond behaviorally to differences in male song structure. Past data suggest a complex role for norepinephrine in female responses to song. Here, we examined the effects of central infusions of the α_1-noradrenergic receptor antagonist prazosin on female European starling (*Sturnus vulgaris*) responses to nest boxes broadcasting male song. Prazosin disrupted female preferential responses to male starling song over the less biologically relevant purple martin (*Progne subis*) song. Prazosin decreased female responses to male starling song in a linear dose–response fashion; whereas, it affected responses to purple martin song in a U-shaped dose–response fashion. Results suggest that the role of norepinephrine in female responses to male song differs depending upon drug dose and the biological relevance of the song stimulus.

Vocal communication is a critical component of social behavior for many animal species. In seasonally breeding songbirds, males sing to attract mates and defend territories during the breeding season, and females respond selectively to particular songs by approaching or displaying copulation solicitation [15,21]. The function of vocal communication and the neural regulation of vocal production are well-studied in male songbirds [24]. The auditory processing and perception of song are also increasingly well-studied [24], but little is known about neural circuits underlying selective behavioral responses of females to male song.

Noradrenergic projections are implicated in arousal, attention, decision-making, and sexual behavior in mammals [2,5,9]. During the breeding season, female songbirds attend to sensory information from, become aroused by, and then selectively approach certain males. Therefore, norepinephrine (NE) is likely a key neurotransmitter in the regulation of female responses to male song.

Past studies implicate NE in female responses to male song, but have produced conflicting results. In female starlings, the NE-specific neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) *increased* female approach responses to nest boxes broadcasting male song [20]. However, in female canaries, DSP-4 treatment *decreased* copulation solicitation displays in response to sexually-stimulating male song [1]. In zebra finches, DSP-4 *decreased* female behavioral responses to complex song and, although not the focus of this study, it appeared to *increase* responses to less-preferred male songs [22].

The conflicting results of past studies make it difficult to interpret the role of NE in selective behavioral responses to male song. The differences in DSP-4 effects on female behavior in prior studies may be explained by differences in the extent to which DSP-4 depleted NE activity during testing. When DSP-4 is peripherally administered, it has wide-ranging, yet non-uniform effects on populations of NE neurons, and subjects may have compensatory responses over time [3,4,23]. Furthermore, studies using DSP-4 do not provide insight into specific NE receptor subtypes involved in female responses to male song. Although studies using DSP-4 have been instrumental in establishing a role for NE in female responses to male song, more specific NE manipulations are needed to advance this area of research.

Past research in rats implicates α_1-NE receptors in female sexual responses to male stimuli [12–14,16], suggesting these receptors may also regulate female starling responses to male courtship song. Here, we examined effects of central administration of three doses of the α_1-NE antagonist prazosin on female starling behavioral responses to nest boxes broadcasting purple martin (*Progne subis*) song (a stimulus of low biological relevance) and male starling courtship song (a stimulus of high biological relevance).

Six female European starlings (*Sturnus vulgaris*) were trapped on a farm outside of Madison, Wisconsin during the winter of 2008. Females were housed five birds to a cage (47 cm h × 47 cm w × 91 cm l) in single-sex groups on an 11 h light

* Corresponding author at: Department of Zoology, 428 Birge Hall, 430 Lincoln Drive, University of Wisconsin, Madison, WI 53706, United States. Tel.: +1 608 262 6506; fax: +1 608 265 6320.
E-mail address: LVRiters@wisc.edu (L.V. Riters).
URL: http://www.wisc.edu/zoology/faculty/fac/Rit/Rit.html (L.V. Riters).

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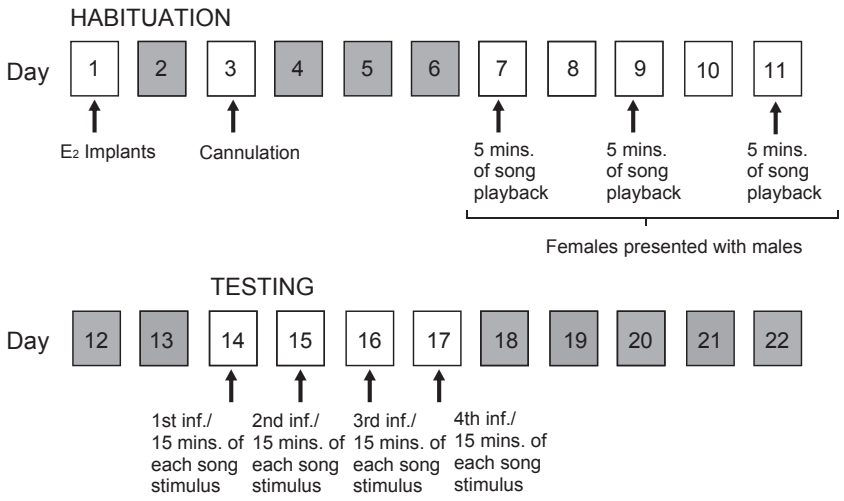

Fig. 1. Diagram illustrating the order and timing of experimental procedures. Each box represents one day. Females were habituated on days 1–13 and tested on days 14–17. Arrows indicate days on which manipulations took place. During repeated testing, females received four infusions of vehicle and doses of prazosin in a counterbalanced order and were sequentially played long starling song and purple martin dawn song. See text for additional experimental details.

(L): 13 h dark (D) cycle. Starlings captured in winter months respond to long day lengths with increases in steroid hormones and sexual responsivity, a physiological state known as photosensitivity. Photosensitive starlings maintained on 11L:13D treated with estradiol become reproductively active [8,20]. All procedures were in accordance with the University of Wisconsin Institutional Animal Care and Use Committee and the National Institutes of Health Guidelines.

Females received subcutaneous implants of estradiol to facilitate reproductive behavior and then were returned to cages in single-sex groups. Each bird was anesthetized with isofluorane gas and implanted subcutaneously posterior to the last rib. Females received two 18 mm silastic implants (1.47 mm i.d., 1.96 mm o.d.; Dow Corning, Midland, MI) filled for approximately 14 mm with 17-beta-estradiol (Sigma, St. Louis, MO). All implants were present at sacrifice and had hormone remaining.

Each female received a cannula targeting the subarachnoid space just dorsal to the habenula and ventrolateral to the rostral most portion of the cerebellum. This site was selected because cerebrospinal fluid (CSF) from this area diffuses throughout the brain [18], and we could reliably implant the cannula tip in this area. The cannula surgeries were performed two days after estradiol implantation (Fig. 1). Each female was anesthetized with isofluorane gas and stereotaxically implanted with a unilateral, 26 gauge guide cannula (Plastics One, Akron, OH) aimed at the subarachnoid space (−4.8 mm A/P, ±1.0 mm M/L, −7.1 mm D/V, angled at 4°). The anterior/posterior coordinates were relative to the ear bar, and the dorsal/ventral and medial/lateral coordinates were based on the midvein. Three stainless steel screws and dental acrylic were used to secure the guide cannula. The stylet, a stainless steel wire, was inserted in each cannula directly after surgery to prevent occlusion.

Directly after cannula surgery, females were moved into testing cages (51 cm h × 51 cm w × 97 cm l). Testing cages consisted of two perches, a nest box, food, and water (additional details in [17]). Females were allowed a period of ten to eleven days to recover from surgery and adjust to their cages prior to testing. During the final week of habituation, females were played starling song from a nest box in their cage for 5 min on three alternating days, and, thereafter, male starlings were presented to females on a total of five days prior to testing (Fig. 1). Males were released inside the room for 20 min and then were released into the cages of females for 20 min. The playback of starling song and introduction of males into the testing areas were included to increase the female responsivity to song stimuli. The day before testing, females were anesthetized and infused with saline to familiarize them to the injection procedure.

On each test day, females were anesthetized with isofluorane gas, and vehicle, a low, medium, or high dose of the α_1-NE receptor antagonist prazosin (Sigma P7791) was centrally infused. Prazosin was dissolved in 0.9% sterile saline at 0.2 nmol (low dose), 2.0 nmol (medium dose), and 20 nmol (high dose). The treatment order was counterbalanced in a repeated measures design. Every female received each dose, with a fourth of the females receiving vehicle, a fourth receiving the low dose, a fourth receiving the medium dose, and the final fourth receiving the high dose on a test day. The drugs were infused using a 33 gauge cannula, which was attached to a Hamilton syringe with PE50 tubing (Plastics One). The volume and rate of the infusion were controlled by using an injection pump (Harvard Apparatus, Holliston, MA). The pump was set to infuse at 0.100 μl/min until 0.30 μl of drug was infused, which was double-checked by reading the syringe volume before and after injection. The cannula was left within the guide for 5 min to allow the drug to diffuse out from around the cannula tip.

After recovering from anesthesia for 15 min, 30 min of long starling and purple martin song stimuli were sequentially broadcast from a speaker within the nest box. The long starling songs were recorded from five testosterone-treated male starlings, singing courtship song in response to a female. Recordings were made using Avisoft Recorder software (Berlin, Germany). The distribution of all of the full songs sung by the males was plotted, and those songs longer than the interquartile range were used to compose long song stimuli (46–64 s; mean 51.76 ± sem 0.99 s). Male purple martin (*P. subis*) dawn song was used to compose song stimuli that were natural (unlike tones), yet less biologically relevant than male starling song. Dawn songs from a Purple Martin Dawnsong CD (Purple Martin Conservation Association, Erie, PA) were edited to match the mean length of all of the starling songs. The CD consisted of a single, 74 min track of dawn song, which was broken into 24 purple martin songs (mean 39.5 ± sem 0.01 s). Long starling or purple martin songs were put together into 15 min song stimuli, which were made with the same duration of song and silence. Four songs were randomly chosen from the compilation of purple martin or long starling songs. These four songs were repeated four times for long starling song stimuli and five times for purple martin song stimuli. For starling song stimuli, individuals contributed more than one song within a song stimulus, but no single song was included in more than one stimulus. For purple martin song