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Effects of dopamine agonists on calling behavior in the green tree frog, *Hyla cinerea*

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HIGHLIGHTS

- We examine the role of dopamine in calling behavior of green tree frogs.
- Calling male frogs were treated with dopamine receptor-specific agonists or a control.
- Significant effects on calling behavior were detected and differed with treatment.
- D2-like receptor agonist quinpirole inhibits calling behavior in green tree frogs.

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ABSTRACT

Dopamine (DA) is an important neurotransmitter involved in social behaviors, such as courtship and pair-bonding. In the green tree frog (*Hyla cinerea*), calling behavior is the primary social behavior used for mate attraction, and is critical for the reproductive success of the species. Our study examined how DA influences advertisement calling behavior of the green tree frog. In a field environment, calling males were treated with either a DA receptor-specific agonist (SKF-38393 or quinpirole), a non-specific DA agonist (apomorphine), or a control Ringer's solution, and vocalizations were recorded after a 20 min post-injection period. Behavioral analyses focused on if and when the frogs called (call latency), and the number of calls produced during post-injection recordings (call rate). There were significant differences in all measurements that varied with treatment and/or dose. The results demonstrate that activation of D2-like receptors has an inhibitory effect on vocalization in the green tree frog, while the D1-like and non-specific DA agonists do not affect calling behavior. These findings coincide with behavioral data from other taxa, and support the function of D2-like receptors in the inhibition of certain social behaviors. Overall, the results suggest conservation for DA in social behaviors across vertebrates.

1. Introduction

Central dopamine (DA) systems mediate many forms of behaviors such as feeding [1], reward-seeking [2], locomotion [3], and energy expenditure [4]. DA influence on behavior has been examined across all major vertebrate taxa, including reptiles [5,6], birds [7–9], fish [10,11] and amphibians [12–14]. Moreover, central DA systems have been implicated in regulating different sociosexual behaviors across many vertebrate taxa, such as sexual behavior in rats [15,16] and birds [9,17], pair bonding in voles [18–20], and singing in birds [21–27]. In this study, we examined how DA functions in the social behavior of a more primitive vertebrate model by measuring the effects of DA receptor-specific agonists on advertisement calling behaviors of the green tree frog, *Hyla cinerea*, in its natural wetland environment.

Although research is limited on DA in the central nervous system and behavior outside of mammalian and avian studies, there are some general similarities across phyla in the neural areas for DA synthesis (midbrain) and axonal projections (striatal forebrain regions) [28]. Evidence has also shown conservation for the structure and function of DA receptors [29]. There are at least five different subtypes of metabotropic DA receptors (D_1–D_5) found across vertebrate species that are divided into two categories, D1-like and D2-like, based on the location in neuronal membrane and the induction of an excitatory or inhibitory response upon activation. D1-like receptors (D_1 and D_5) are located on dendrites, and mainly promote cell signaling by operating post-synaptically to initiate protein kinase A (PKA) synthesis [30], while D2-like receptors (D_2, D_3 and D_4) are found throughout the cell

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membrane and can function post-synaptically or as autoreceptors, depending on membrane location [29]; in either location, D2-like receptors decrease cell signaling by inhibiting PKA synthesis (post-synaptic) and neurotransmitter release (autoreceptor) [30]. The general localization for DA synthesis and receptor processes across vertebrates raise the question of whether the behavioral effects of the DA system are conserved as well.

One behavior regulated in part by DA in both mammals and birds is vocalization, particularly for social communication and reward. Rats express 50 kHz ultrasonic vocalizations associated with DA stimulation and drug use [31], as well as during social interactions [32,33], and in anticipation of play in young rats [34]. Avian singing and anuran calling behaviors are two examples of high energy vocal behaviors that are expressed under particular seasonal and hormonal states. Male birds exhibit vocal behaviors in the presence or absence of a mate (directed singing or undirected singing, respectively), and research supports DA is involved in these vocalizations. For example, levels of tyrosine hydroxylase-immunoreactive (TH-ir) cells are correlated with song production in a breeding context, i.e. in the presence of high testosterone levels and a female, in song- and motivation-specific/dopaminergic brain areas (Area X, medial preoptic area, and ventral tegmental area) in male European starlings [25]. Also Goodson et al. (2009) showed the number of TH-ir cells in the caudal VTA was significantly correlated to breeding context song production [22], and in vivo microdialysis measures show increased DA in Area X during directed song in the zebra finch [27]. Finally inhibiting DA reuptake increases singing, while blocking the D1 receptor decreases singing in the European starling [35].

Similar to avian song behavior, male anurans express “advertisement” and “aggressive” calls during the mating season under certain environmental and physiological conditions to attract mates and to maintain a calling territory [36–38]. While it has been shown that 6-OHDA lesions to the DA system impair the phonotaxis response to advertisement calls in female anurans [39], the role of DA in male anuran vocal communication is unknown. Based on what is known about DA in bird song behaviors and sociosexual studies in mammals, we hypothesized that DA is involved in the social behavior of calling in anurans. Using the green tree frog (*H. cinerea*) as our model, we tested how D1- and D2-like receptor agonists affect advertisement calling during the mating season in a field environment. Pharmacological testing shows anurans possess two different types of DA receptors with affinities similar to those seen in mammals for DA receptor-specific drugs [40]. Therefore based on the different transduction pathways of DA receptors, we predicted a D1-like agonist would increase calling, and a D2-like agonist would decrease calling if DA is involved in this anuran social behavior. We used the D1-like agonist SKF-38393 (SKF), the D2-like agonist quinpirole (QUIN), the non-specific DA agonist, apomorphine (APO), and amphibian Ringer's solution (control). Our results show a receptor-specific effect of DA on calling behaviors, such that D2 receptor activation inhibits calling. Considering amphibians diverged from a common aquatic vertebrate ancestor over 300 million years ago [38,41,42], these results contribute to the evolutionary understanding of how DA is involved in the social behaviors of tetrapods.

2. Materials and methods

2.1. Subjects

Eighty-six breeding-condition male green tree frogs, *H. cinerea*, were used in field experiments conducted in June 2008 and June-August 2009 (Atlanta, GA). Only stationary and vocalizing males producing strong, steady calls were selected. Mean snout-vent length (SVL) was 41.7 ± 1.7 mm across all subjects. In 2009, mean body weight was 4.4 ± 0.12 g. Body weights were not measured in 2008.

2.2. General procedures and sampling

Experiments began each night once the breeding chorus was established (~2100 h), and continued until the chorus became weak (~2400 h). To facilitate a continual chorus, a recording of a green tree frog chorus (recorded from previous years) was broadcast using a portable CD player and field speaker (Sony CD Walkman Model D-NS505, Saul Mineroff SME SC-A9). Water and air temperatures were recorded at the start and end of each night.

Each subject was tested using the following procedure: The experimenter used a flashlight and headlamp to visually locate a prominently calling individual male green tree frog. Calls of each male were recorded for five minutes (Baseline recording) using a microphone held 20–70 cm from the male (Marantz PMD420 field recorder, Sennheiser ME80 directional microphone with K3-U power module and MZW 415 wind screen). After the baseline recording, the subject was hand-caught and given an intraperitoneal injection of one of the following treatments dissolved in 100 μl of amphibian Ringer's solution: 1 μg or 10 μg APO, 5 μg or 50 μg QUIN, 5 μg or 50 μg SKF, or amphibian Ringer's solution alone (control). All drugs were purchased from Sigma Chemical Co. (St. Louis, MO). Injections were coded to be blind to the experimenter. The subject was replaced to its original location and observed for 20 min. Call latency (time at which the subject resumed calling following treatment), as well as behavioral changes and movements were noted. No abnormal motor behaviors were observed during the post-injection period following any of the treatments, and this factor was dropped from further analyses.

Following the 20-min post-injection period, actively calling subjects were recorded for an additional 5 min (post-injection recording) and silent subjects were observed for 5 additional minutes. If a silent subject resumed calling at any point within the 5-min observation period, a 5-min post-injection recording was made. At the end of the observation period, subjects were re-caught, measured for body length and weight, and released at their original locations.

2.3. Analysis

Call recordings were analyzed by two independent scorers who counted the number of calls per each minute of the 5-min recordings. Approximately 50% of all recordings were counted by both scorers (inter-rater reliability, Pearson's $r = 0.921$). Baseline and post-injection call rates (mean number of calls per minute) were determined by averaging the calls quantified by each scorer for each minute. Call rates were then adjusted for temperature to a standard of 25.5 °C based on a regression equation for air temperature and call rate across all testing nights in 2008 and 2009. Call rate adjustments were necessary because the call rates of frogs have been shown to correlate with air temperature [43], and the average air temperature differed significantly between 2008 and 2009 field seasons (27.6 ± 0.4 and 23.3 ± 0.2 °C, respectively). Twenty-three males received APO ($n_1 = 11$, $n_{10} = 12$), 22 males received SKF-38393 ($n_5 = 10$, $n_{50} = 12$), 19 males received QUIN ($n_5 = 10$, $n_{50} = 9$), and 22 males received control injections. Six subjects in 2009 were eliminated from the final analysis due to insufficient baseline call rates (<15 calls/min), unclear recordings due to technical problems, or post-injection mating.

To examine general changes in calling behavior, baseline and post-injection call rates were first compared within subjects across groups using a paired *t*-test. Because of extremely skewed distributions and violations of parametric requirements, normalcy could not be met with the post-injection recording data, and baseline and post-injection call rates were analyzed separately for treatment differences with adjusted alpha levels. In order to determine if there were any pre-treatment differences, baseline call rates between treatment groups were compared using a parametric one-way independent ANOVA. Post-injection call rates were analyzed for treatment