Rapid steroid influences on visually guided sexual behavior in male goldfish

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The ability of steroid hormones to rapidly influence cell physiology through nongenomic mechanisms raises the possibility that these molecules may play a role in the dynamic regulation of social behavior, particularly in species in which social stimuli can rapidly influence circulating steroid levels. We therefore tested if testosterone (T), which increases in male goldfish in response to sexual stimuli, can rapidly influence approach responses towards females. Injections of T stimulated approach responses towards the visual cues of females 30–45 min after the injection but did not stimulate approach responses towards stimulus males or affect general activity, indicating that the effect is stimulus-specific and not a secondary consequence of increased arousal. Estradiol produced the same effect 30–45 min and even 10–25 min after administration, and treatment with the aromatase inhibitor fadrozole blocked exogenous T's behavioral effect, indicating that T's rapid stimulation of visual approach responses depends on aromatization. We suggest that T surges induced by sexual stimuli, including preovulatory pheromones, rapidly prime males to mate by increasing sensitivity within visual pathways that guide approach responses towards females and/or by increasing the motivation to approach potential mates through actions within traditional limbic circuits.

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Introduction

Social stimuli, particularly aggressive and sexual stimuli, often stimulate rapid increases in sex hormones in vertebrate animals. Such acute changes in sex steroid levels can influence subsequent social encounters, likely through genomic mechanisms that involve changes in gene transcription associated with intracellular steroid receptors (Trainor et al., 2004). Additionally, such steroid elevations may modulate the immediate expression of ongoing behavior through more rapid mechanisms mediated by membrane receptors (reviewed in Balthazart and Ball, 2006; Bass and Remage-Healey, 2008). Thus, in addition to slowly sculpting neural pathways associated with behavioral control, sex steroids are also capable of acting as dynamic regulators of those pathways through rapid, nongenomic mechanisms.

However, with the exception of work in toadfish and plain midshipmen showing that sex steroids can rapidly influence hindbrain pattern generators involved in the production of motor output related to social communication (Remage-Healey and Bass, 2006; Remage-Healey and Bass, 2007), very little is known about where and how within the brain sex steroids act to rapidly influence social behavior. One interesting possibility is that sex steroids may rapidly modulate sensory mechanisms that facilitate the processing of social stimuli. It has been demonstrated that chronic steroid treatments can influence how animals perceive sensory information related to social communication by acting on early stages of sensory detection and processing. For example, chronic androgen treatment alters the tuning of primary electroreceptive sensory afferents in weakly electric fish (Keller et al., 1986) and stingrays (Sisneros and Tricas, 2000) and selectively increases the magnitude and sensitivity of the electroolfactogram response to a putative sex pheromone in a Southeast Asian cyprinid, the tinfoil barb (Cardwell et al., 1995). However, it is not known whether sex steroids can rapidly modulate sensory processes and thus influence social perception in ways that have immediate behavioral consequences.

The importance of olfactory signals for social communication has been well described in many vertebrate species, including goldfish. However, visual cues are also important for social communication in this species, particularly in sexual contexts. Male goldfish follow ovulating females more than nonovulating females, even after ablation of the olfactory tract (Partridge et al., 1976), and males preferentially approach female over male visual stimuli in choice tests during the breeding season (Thompson et al., 2004). That visual processes related to reproduction may be influenced by sex steroids is suggested by the presence of high levels of aromatase, as well as androgen and estrogen receptors, in regions of the brain involved in the detection of (retina) and orientation towards (optic tectum) visual stimuli (Gelinas and Callard, 1993; Gelinas and Callard, 1997). In fact, androgen treatments that masculinize reproductive behavior in female goldfish also induce selective approach responses towards female visual stimuli (Thompson et al., 2004). Interestingly, exposure to preovulatory females causes a T surge in males (Kobayashi et al., 1986) that could rapidly influence those visual responses. We therefore tested if T can rapidly influence male approach responses towards females in an experimental paradigm in which only visual...
cues were present. To determine the biochemical pathway associated with any such influences, we also tested whether T produces rapid behavioral effects through its conversion by aromatase to estradiol (E2).

Methods

Subjects

Adult comet goldfish (Carassius auratus) 12–16 cm and 25–50 g were kept in same-sex tanks of circulating dechlorinated tap water in controlled environmental conditions. Fish tested in nonbreeding conditions were kept on a 12:12-hr light/dark cycle at 15 °C, and fish tested in reproductive condition were kept on a 16:8-hr light/dark cycle at 20 °C. The animals were fed commercial goldfish pellets once daily. All procedures were in accordance with federal regulations established for the use of vertebrate animals in research and were approved by Bowdoin IRB.

Drugs

For all of our experiments, the control, vehicle injections consisted of 100 μL of a teleost Ringer + 0.1% methanol solution. T was prepared by first making a 30 mg/mL T stock solution by dissolving T powder (Sigma Aldrich) in pure methanol. To prepare the injection mixture, we dissolved the stock in teleost Ringer solution (1:1000) to obtain a final concentration of 0.03 μg/μL T in saline + 0.1% methanol. Fish were intraperitoneally injected with 100 μL of the T injection solution, which resulted in a final T dosage of 3 μg/fish. Previous studies have shown that this dose leads to elevated T concentrations within the physiological range 30–45 min after injection (Huggard et al., 1996; Schreck and Hopwood, 1974). Similarly, a 30 mg/mL solution of estradiol (E2; Sigma) was dissolved in methanol. This stock was also dissolved in teleost Ringer (1:1000) to a final concentration of 0.03 μg/μL E2 in saline + 0.1% methanol in Exp 2a; in Exp 2b a water soluble form of E2 was dissolved directly into saline, and saline was used for control injections. Fish were injected IP with 100 μL of the E2 injection solution, again resulting in a dose of 3 μg/fish. We used the same dose of E2 as T to ensure elevations of local E2 within the brain that would approach the maximal levels that could have been produced by the local aromatization of our T injections, although we recognized that this would result in supraphysiological levels of peripheral E2. Fadrozole (FAD; 3.5 mg/mL; supplied by Novartis) was dissolved in the same vehicle (saline + 0.1% methanol). Fish were injected IP with 100 μL of the FAD solution, resulting in a dosage of 350 μg/fish. This dose, which is approximately 12 mg/kg of fish, was shown in a previous study to rapidly block T actions in teleost fish (Remage-Healey and Bass, 2007).

Testing apparatus

Experimental test tanks had three separate sections, each divided by acrylic Plexiglas (Polymershapes, Chicago, IL). The middle experimental section was 70 L, and the two side compartments used to hold stimulus fish were 18 L each. Animals were able to see between sections, but the Plexiglas partitions were sealed to prevent water and thus chemical exchange. Dual, full-spectrum light bulbs (Reptisun 5.0, Zoomed, CA) were hung above each tank. Behavioral measurements were recorded with black and white video cameras. No observers were in the experimental room during behavioral testing, except for the motor activity recordings in Experiment 2 (see below). All behavioral testing sessions were carried out in the afternoon.

Experiment 1: Effects of acute T injections on male visual approach responses

Approach responses to female visual stimuli were measured at two times of year. The first test took place in June, when fish were in reproductive condition, as evidenced by the presence of secondary sexual characteristics (tubercles, expressible milt). Because of a high variability in levels of social approach across fish, we used a design in which all fish were tested twice, once after vehicle injections and once after steroid injections, which enabled us to control for that baseline variability (see statistics). However, because steroids may have long-lasting effects, we could not employ a counterbalanced design. Therefore, on the first day of testing, all fish received control IP injections of the vehicle, and on the second test day, 48 hr later, control fish again received vehicle injections, but experimental fish received steroid injections. After all injections fish were placed in the center of the 70 L rectangular test tank described above. After a 15 min habituation, the time the fish spent in proximity (nose within 1 cm) to each Plexiglas barrier was recorded for a 15 min baseline by the software program Limelighet®. A stimulus fish was then put in the stimulus tank on the side where the subject had spent the least amount of time during the baseline recording period, which forced the fish to move away from its preferred side to approach the stimulus fish. The time spent in proximity to the Plexiglas partition separating the subject from the stimulus fish was then measured for 15 min. Thus, social approach behavior was recorded 30–45 min after the injections on both test days. Proximity scores were calculated by subtracting the baseline score from the time spent in proximity to the same side when the stimulus fish was present. We then repeated the same test, but used male fish as stimuli instead of females. We also repeated the test later in the summer (August), when fish were no longer in reproductive condition, again using females as stimuli.

We also tested the effects of T injections on motor activity in fish that likewise did not display secondary sexual characteristics indicative of fish in reproductive condition. For that experiment, all fish were injected IP with vehicle on the first test day, as before, and again placed in the center compartment of the test tank. Baseline side preferences were measured 15 min later, and 30 min later, activity was measured for 15 min by counting the number of times the fish’s nose crossed each of 2 lines marked on the side of the tank that separated it into thirds. Immediately after the activity test, and thus 45 min after the injections, a stimulus female was added to the least preferred side compartment, and proximity to that side was measured, as described previously, for 15 min. On the second test day, all fish were injected with 3 μg T, the same dose that effectively stimulated approach responses towards females in the previous experiment. Motor activity tests were again conducted 30–45 min after the injections and thus during the same time interval when we measured T effects on social approach in the previous experiments, and social proximity was again measured 45–60 min after the injections.

Experiment 2: Rapid effects of E2 on male approach towards females

Two experiments were done to see if E2, like T, can stimulate social approach responses towards female visual stimuli. Both experiments were done in the same testing apparatus using the same general procedures. The first of these experiments was performed in November when fish were not in reproductive condition. All fish were injected with vehicle on the first test day, and the control group was again injected with vehicle on the second test day, 48 hr later, whereas the experimental group was injected with 3 μg E2. We then repeated the experiment in April in fish that were in reproductive condition (expressing milt), but measured behavioral responses 10–25 min after injections to see how quickly E2 could stimulate social approach responses.

Experiment 3: Effects of an aromatase inhibitor on T’s behavioral effects

The goal of this experiment was to test if treatment with the aromatase inhibitor fadrozole (FAD) before a T injection would keep the androgen from stimulating social approach responses towards a
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