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# Isotocin increases female avoidance of males in a coercive mating system: Assessing the social salience hypothesis of oxytocin in a fish species

Mary E. Ramsey[a,*], Dustin Fry[b], Molly E. Cummings[a]

[a] Section of Integrative Biology, University of Texas, Austin, TX 78712, USA
[b] Drexel University Dornsife School of Public Health, Department of Epidemiology and Biostatistics, Philadelphia, PA 19104, USA

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ABSTRACT

The nonapeptide oxytocin (and its fish homolog isotocin (IT)) is an evolutionarily-conserved hormone associated with social behaviors across most vertebrate taxa. Oxytocin has traditionally been regarded as a prosocial hormone, but studies on social cognition in mammalian models suggest it may play a more nuanced role in modulating social discrimination based on social salience and stimulus valence. Here we test IT and its role in regulating female social decision-making and anxiety behaviors in a live-bearing fish with a male coercive mating system. *Gambusia affinis* males engage in a forced mating strategy, with frequent harassment and attempted copulatory thrusts directed towards unwilling females. Exogenous IT produced anxiolytic responses in female *G. affinis* that altered exploration (time in center of tank) but not time in dark vs. light regions of the tank. Exogenous IT also produced context-specific changes in social tendency: IT-treated *G. affinis* females spent *less* time associating with males while association time with conspecific females was not altered. Further, while overall activity levels were not changed by IT treatment, the amount of social behaviors IT-treated females directed towards males, but not females, was reduced. Our results support the social salience hypothesis of oxytocin action in a teleost and suggest that oxytocin's critical input into social cognitive processing is conserved across vertebrate taxa.

## 1. Introduction

Oxytocin (OT) is a highly conserved nonapeptide known to regulate many aspects of social behavior. It is most commonly considered in prosocial arenas such as social approach and aggregation, reproduction, pair bonding and parental care (Donaldson and Young, 2008; Insel and Young, 2001; Lee et al., 2009; Ross and Young, 2009), including prosocial responses arising from anxiolytic and fear-reducing properties (Churchland and Winkielman, 2012; Neumann and Slattery, 2016). However, other experiments have found anti-social effects of OT such as increased aggression (Ferris et al., 1992; Winslow and Insel, 1991), context-dependent resource sharing towards strangers versus pairmates (Mustoe et al., 2015), reduced cooperation when prior social information is lacking (Declerck et al., 2010), altered social vigilance (Ebitz and Platt, 2014) and even social avoidance (Reddon et al., 2014) following treatment with exogenous OT.

In recent years, it has become increasingly apparent that OT is much more than a prosocial hormone, with accumulating evidence of its importance in various aspects of social cognition (Dore et al., 2013; Goodson and Thompson, 2010; Lee et al., 2009). Social cognition describes how an individual learns to make context-dependent adaptive choices through the process of acquiring, storing, accessing and acting on information from social encounters (Oliveira, 2013; Shettleworth, 2010; Taborsky and Oliviera, 2012). Social cognition processing influences a myriad of social decisions such as whether to join a group (Cheney et al., 1986; Goodson et al., 2009), strategies to resolve social conflict (Cheney et al., 1986; Cools et al., 2008; Emery et al., 2007; Fraser and Bugnyar, 2011), and mate choices (Cummings and Ramsey, 2015; Kavaliers and Choleris, 2017). OT modulates key aspects of social cognition such as social recognition, social memory, social vigilance and social discrimination (Bartz et al., 2011; Choleris et al., 2009; Dore et al., 2013; Ebitz and Platt, 2014; Goodson and Thompson, 2010; Lee et al., 2009), and it is these actions that may explain some of the anti-social effects of OT.

A particularly powerful framework to understand the pro- and anti-social actions of OT in social cognition is the social salience hypothesis of oxytocin (Bartz et al., 2011; Shamay-Tsoory and Abu-Akel, 2016). Originally conceived to explain the complex action of OT in humans (Shamay-Tsoory et al., 2009; Declerck et al., 2010), the social salience hypothesis of oxytocin synthesizes OT's multiple roles in prosocial/

* Corresponding author at: University of Texas, Section of Integrative Biology, 1 University Station C0990, Austin, TX 78712, USA.
*E-mail address:* mramsey@austin.utexas.edu (M.E. Ramsey).

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affiliative behaviors, anxiolytic effects, and group-identification into a framework emphasizing context-dependent behavioral responses to social cues. This view of OT action proposes that OT mediates social decision-making though modulating the salience, or attentional importance, of contextual (positively- or negatively-valenced) social cues (Shamay-Tsoory and Abu-Akel, 2016). In this way, OT may therefore sharpen the perceptional valence of other individuals within the social environment.

Social cognition and the need for complex social processing are not confined to mammalian systems and can be found in varying degrees across vertebrate taxa including birds (Emery et al., 2007), reptiles (Kis et al., 2015; Wilkinson et al., 2010) and fish (Bshary et al., 2014; Oliveira, 2013). OT and its non-mammalian homologs, including isotocin (IT) in teleost fishes, regulate social behaviors across vertebrates (Godwin and Thompson, 2012; Goodson and Thompson, 2010; Goodson et al., 2009), yet also exhibit some intriguing variation based on social system (Goodson and Thompson, 2010; Goodson et al., 2009; Insel and Shapiro, 1992). In fishes, IT has been implicated in sexually dimorphic reproductive behaviors in plainfin midshipman (Goodson and Bass, 2000), mutualism in cleaner wrasses (Cardoso et al., 2015), grouping/shoaling behavior in cichlids (O'Connor et al., 2016; Reddon et al., 2014), social approach decisions in goldfish and zebrafish (Braida et al., 2012; Thompson and Walton, 2004), and pair-bonding (O'Connor et al., 2016) and parenting (O'Connell et al., 2012) behaviors in cichlids. Most studies on teleosts outside of reproduction have focused on the prosocial and anxiolytic actions of IT (Braida et al., 2012; Thompson and Walton, 2004); however, evidence with group-living cichlids indicates exogenous IT can produce context-specific anti-social responses in aggression (Reddon et al., 2012), social discrimination and approach (Reddon et al., 2014), and group conflict and response to social perturbation (Hellmann et al., 2015), which suggests the social salience hypothesis of oxytocin may apply to teleosts as well.

Poeciliid fishes are an ideal model system to test the social salience hypothesis of oxytocin/isotocin in a non-mammalian model. Poeciliids are small, live-bearing freshwater fishes with internal fertilization and sperm storage, both of which contribute to intense levels of sexual selection and conflict (Bisazza, 1993; Cummings, 2018; Farr, 1989). Indeed, poeciliids have long been models for sexual selection studies due to the variety and character of their mating systems (Bisazza, 1993; Farr, 1989). In some species, males are elaborately ornamented and court females for mating opportunities (Bisazza, 1993; Farr, 1989; Ryan and Rosenthal, 2001). However, in more than half of poecilid species, males are highly coercive and engage in extremely high levels of sexual harassment and forced copulatory attempts (Bisazza, 1993; Farr, 1989). Male coercion involves chasing after females from behind and gonopodial thrusting (swinging their intromittent organ (gonopodium)) towards the female gonopore and attempting to gain purchase for possible insemination using gonopodial hooks and barbs. This level of sexual harassment is detrimental to females, reducing foraging opportunities and increasing risk of injury and disease (Magurran and Garcia, 2000; Magurran, 2011). In these species with high levels of sexual conflict, males represent a negative social valence and females avoid male attention, often by aggregating with other females (Dadda et al., 2005; Dadda, 2015) or engaging in other avoidance behaviors such as fleeing or orienting themselves around physical barriers that prevent male copulatory thrusts (Magurran and Garcia, 2000). Following the social salience of oxytocin/isotocin model within the context of a coercive mating system, IT may facilitate adaptive social decisions by increasing female social vigilance, thereby reducing unwanted interactions with males.

Here we test the role of exogenous IT on female social decision-making in *Gambusia affinis*, a poecilid species with a male-coercive mating system. We injected female fish with IT or saline control and measured behavioral responses in a nonsocial anxiety assay as well as two non-contact social decision-making contexts. We tested the anxiolytic role of IT in these fish with a scototaxis (light/dark preference) trial. In accordance with its known action in reducing anxiety, we predicted exogenous IT would reduce anxiety-related behaviors in *G. affinis* females such that IT-injected females would spend increased time in the white zone and increase exploration into the center of the tank compared to saline-injected controls. We then tested the social salience vs. prosociality hypotheses of isotocin in two social decision-making contexts including (i) females exposed to conspecific coercive males, and (ii) females exposed to conspecific females. If IT increases the social salience of behavioral cues and enhances perception of social valence, we would predict opposing responses in females exposed to males vs. females. Specifically, we predicted decreased social preference for coercive males and increased social preference for conspecific females in IT-injected females and no change in saline-injected controls. Alternatively, if IT acts solely as a prosocial activator in this fish system, we instead would predict increased sociality (association time) across both social treatments (conspecific females and conspecific coercive males). Finally, if IT is acting solely as an anxiolytic agent, we would expect reduced sociality towards both the aversive (male) and non-aversive (female) social exposure groups because decreased social distance (aggregation) can be an anxiety response in teleosts (Stewart et al., 2012).

## 2. Methods

*Gambusia affinis* females (n = 80) were wild-caught from a pond at Brackenridge Field Laboratories (University of Texas). Female sizes ranged from 22 mm to 39.7 mm. All females were sexually mature (average size at sexual maturity in *G. affinis* females is 17–20 mm; Pyke, 2005) and possessed an anal spot, which is a marker of ovarian cycling (Peden, 1973). Fish were housed in mixed sex community tanks in the laboratory until females were isolated for each experiment (n = 40 for scototaxis trials; n = 40 for social context trials). For both experiments, females were isolated for 7 days prior to baseline behavior pre-testing. This isolation ensured that all individuals experienced the same housing conditions and recent social experiences prior to entering into an experiment.

After pre-test, females were returned to home isolation tanks for 7 days prior to pharmacological manipulation and post-treatment behavior testing. All animal experimental procedures were approved by IACUC (#AUP-2013-00156) at the University of Texas at Austin.

### 2.1. Anxiety behavior testing

Female *G. affinis* (n = 40) were tested in a scototaxis (light/dark preference) assay to assess pre-treatment baseline vs. post-treatment anxiety and exploration responses. Scototaxis is a standard anxiety vs. exploration test for many vertebrate species including teleosts (Maximino et al., 2010a) and has been optimized in our lab for poeciliids (*G. affinis*: Etheredge et al., 2018; *Xiphophorus nigrensis*: Ramsey et al., 2014). Pharmacological validation of scototaxis has been conducted in zebrafish (Maximino et al., 2011) and pharmacological manipulations with known anxiolytic properties (MK-801) altered scototaxis response in another poecilid species (Ramsey et al., 2014). Briefly, fish were placed into a felt-lined 50 cm × 25 cm × 31 cm scototaxis testing aquarium bisected into white vs. black compartments (Fig. 1A). We used white and black felt to create a matte, non-reflective surface for each compartment. Before each trial, we added fresh reservoir water (dechlorinated tap water, the source of home tank water for all fish) to a depth of 10 cm. The focal fish was placed into a central compartment (10 cm wide with 5 cm of white backing, 5 cm black) bounded by removable white and black felt barriers that matched each respective end color. The testing tank was on the floor and illuminated by standard overhead fluorescent fixtures 2.4 m above the tank, with lux ranging from 320 on the white side to 286 on the black side of the testing tank. Each fish was allowed to habituate to the central compartment for 5 min. As the trial began, the interior barriers were removed and focal fish behavior was measured for 20 min. To

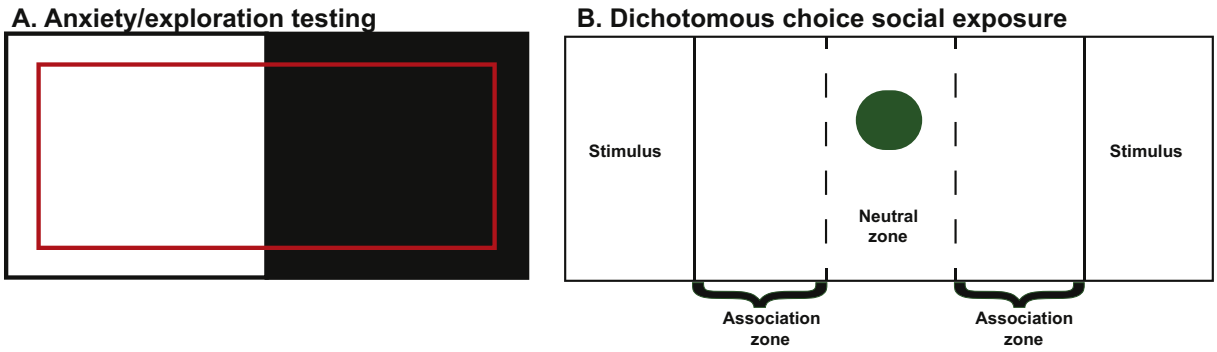


**Fig. 1.** Testing apparatus for A. anxiety/exploration (scototaxis) tank, and B. dichotomous choice testing tank. Red box in A. indicates 2 cm thigmotaxis (edges) versus center zones in the scototaxis tank. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

avoid cross-contamination, the testing tank was rinsed and water was replaced for each trial. At the end of each testing day the tank was thoroughly rinsed and allowed to dry overnight before being used again. After the pretest scototaxis trial, females were returned to their home tanks for 7 days until pharmacological treatment and posttesting. Females were randomly assigned to either saline control or one of three IT dose treatment groups (n = 10 per group). Each trial was recorded by an overhead camera and scored by an individual blind to treatment. All scototaxis videos were processed through Pixel Conduit software (https://pixelconduit.com/) to embed a scoring grid with explicit thigmotaxis/exploration zones (Fig. 1A).

Scototaxis, like many experimental paradigms designed to measure anxiety, often measure some combination of anxiety, boldness, exploratory tendencies, neophobia and stress (Maximino et al., 2010b; Greggor et al., 2015). Because the relationship between all of these responses is often quite complicated (e.g. Maaswinkel et al., 2013; Etheredge et al., 2018), it is recommended to measure multiple behavioral responses during the experimental procedure (Perals et al., 2017). To evaluate anxiety, we measured the latency to enter the white zone along with the proportion of time spent in black (more anxious) and white (less anxious) sides of the tank. We measured exploration and boldness as time in the center regions of the tank (> 2 cm distance from the tank edge; see also Fig. 1A). We report total exploration tendency (time spent in center regions regardless of tank background color) because in our experiment proportion of time in center was consistent in both black and white regions of the tank (Supplemental Fig. 1; correlation proportion in the center of the tank on the black vs. white side of the tank (S (sum of all squared rank differences) = 13,081, $p < 2.2e-16$, rho = 0.84). Overall, pretest proportion in white and center were not significantly correlated (p = 0.07). All trials took place between Feb-May 2015. All scototaxis trials were conducted between 10:45 am and 6:10 pm with fish held on a 12:12 light cycle 7 am–7 pm.

### 2.2. Dichotomous choice behavior testing

*Gambusia affinis* females (n = 40) were isolated for a 7-day period prior to initial behavior pre-testing. Female behavior was measured in non-contact dichotomous choice trials conducted as in Cummings et al. (2008), Lynch et al. (2012), Ramsey et al. (2012), Ramsey et al. (2014), Wang et al. (2014), and Wong et al. (2012). Testing occurred in a 120 cm × 30 cm × 48 cm tank consisting of 24 cm stimulus compartments at either end (separated by transparent plexiglass barriers) and a central focal area consisting of 24 cm association zones adjacent to each stimulus area and a 24 cm central neutral zone containing a plastic plant (Fig. 1B). Trials were video recorded from a side-angle view for later scoring. For male-exposed trials (N = 20), stimuli pairs consisted of a large and small male *G. affinis* male (LM-SM pairing). Male sizes ranged from 27.7 to 23.8 mm SL for larger males and 21.6 to 20.7 mm SL for smaller males with an average size difference between the males of 4.6 mm (range 3.1–6.1 mm size difference). For female-exposed trials (N = 20), stimuli pairs consisted of a large and small female (LF-SF pairing). Female sizes ranged from 34.2 to 32.4 mm SL for larger females and 28.7 to 27.3 mm SL for smaller females (average size difference in female stimuli pair 5.3 mm). Trials lasted 30 min, and midway through the trial the stimuli were switched to control for side bias. Behaviors were scored for the entire 30-minute trial. After the pretest dichotomous choice trial, females were returned to their home tanks for 7 days until pharmacological treatment and posttesting. In the posttest trial, females were tested with the same stimulus pairing as they were during the pre-test trial. Females were randomly assigned to either saline control or isotocin (IT) treatment groups (n = 10 control, n = 10 treat for both male-exposed (LM-SM) and female-exposed (LF-SF) trial types). All trials took place between June and October 2015. All fish were held on 12:12 7 am–7 pm light cycle. All female exposure trials were conducted between 1:15 pm and 6:50 pm. 35 of 40 male exposure trials were conducted between 11:30 am and 6:30 pm, however due to technical error 5 male exposure trials (4 pretests (2 pre-saline and 2-pre-IT), 1 IT posttest) were collected outside of the daylight cycle period. We tested our male exposure pretest behavioral measures for differences in trial time within and outside of the daylight cycle and found no effect (total association: $t = -1.56$, p = 0.15; total activity: W = 30, p = 0.55; total social behaviors: W = 34.5, p = 0.83; the ratio of social behaviors by total activity W = 40, p = 0.87). Removing these individuals did not change the direction of our results, and modeling pretest vs. posttest behavioral changes for all females tested outside the daytime cycle indicated no effect of within vs. outside the daylight cycle (total association Chi-Sq 2.15, p = 0.14; total activity Chi-Sq 0.0007, p = 0.98; total social behaviors Chi-Sq 0.52, p = 0.47; ratio social behaviors to total activity Chi-Sq 0.21, p = 0.65). Therefore we include all individuals in our behavior measures.

We categorized female sociality based on proximity to a stimulus fish (time in association zone) versus neutral (time spent in center zone). As in Ramsey et al. (2014), fish activity measures were considered social if oriented towards the stimulus barrier (up/downs, laterals, glide swims) or neutral if not oriented towards a stimulus barrier (transits across the tank, vertical or lateral swims towards the front or back of the tank). Total association is the summed time a focal fish spent in either association zone. We tested for size preferences by measuring time with the larger stimulus vs. time with the smaller stimulus. Total social behaviors are the sum of all socially-directed behaviors measured during a trial. Total activity is the sum of all social and neutral behaviors measured during the trial. The ratio of social behaviors to total activity is the total number of social behaviors divided by total activity. All trials were scored live and videotaped. Final scoring for each trial was conducted by individuals blind to treatment group on videotaped trials recorded via Microsoft LiveCam (https://www.microsoft.com/accessories/en-us/products/webcams/lifecam-studio/q2f-00013) cameras with no post-processing.

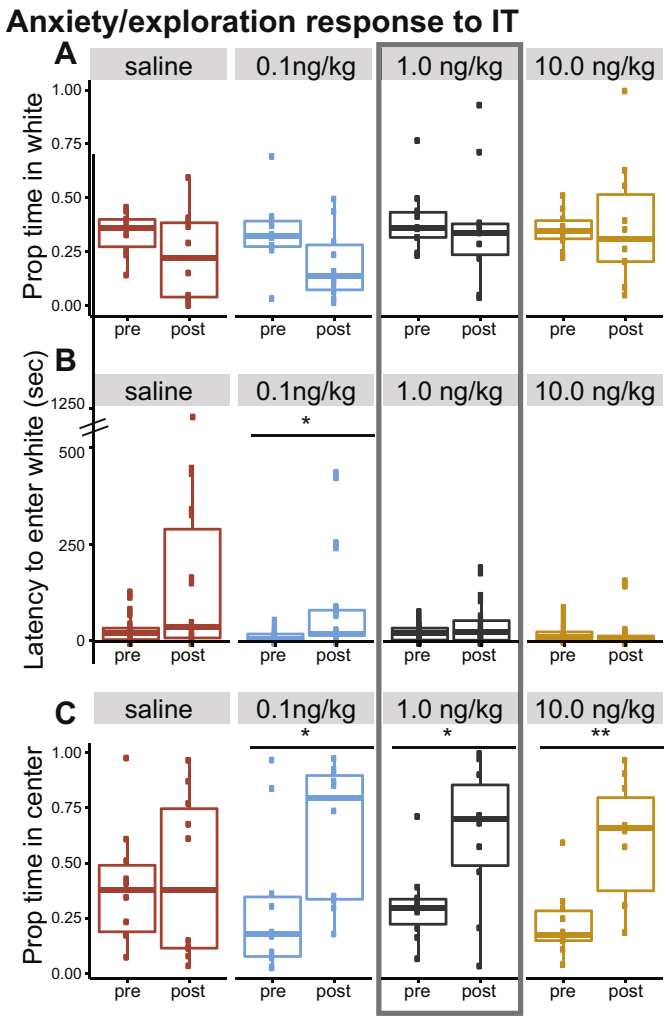


**Fig. 2.** Anxiety-exploration following exogenous IT dose-response experiment. A. IT did not significantly alter anxiety (proportion time in white) by pre vs. posttest (GLMER ChiSq 2.77, p = 0.10) or treatment dose (GLMER ChiSq 4.96 p = 0.17). Looking within each dose, low IT reduced proportion time in white during post- vs. pre-treatment assays although it did not survive FDR correction (saline p = 0.38 FDR p = 0.50; low IT p = 0.04 FDR p = 0.15; med IT p = 0.11 FDR p = 0.21, high IT p = 0.85 FDR p = 0.85). B. IT significantly altered latency to enter the white region of the testing tank pre vs. posttest (GLMER Chi-Sq 8.83, p = 0.003) but treatment was not significant (Chi-Sq 6.23 p = 0.10). Wilcoxon tests on pre vs. post response within each treatment indicate an increased latency to enter white at the low IT dose (saline p = 0.09 FDR p = 0.18, low IT p = 0.009 FDR p = 0.04, med IT p = 0.28 FDR p = 0.37, high IT p = 0.84 FDR p = 0.84. C. IT altered exploration tendency. There was a significant main effect of pre vs. posttest (Chi-Sq 11.77, p = 0.0006) but not treatment type (Chi-Sq 4.35, p = 0.23) with a significant interaction between pre vs. posttest and treatment type (Chi-Sq 8.20, p = 0.04). Wilcoxon signed rank tests indicate no significant change pre vs. posttest within the saline group (p = 0.77 FDR p = 0.77), while posttest time in center was higher than pretest for all three doses of IT (low IT p = 0.01 FDR p = 0.01; med IT p = 0.01 FDR p = 0.01; high IT p = 0.002 FDR p = 0.008). Box plots indicate 1st and 3rd quartiles, the line indicates the median, and whiskers indicate high and low values. Circles indicate data points. For all panels, saline (0 ng/kg; red), low dose IT (0.1 ng/kg; blue), med dose IT (1 ng/kg; black), high dose IT (10 ng/kg; yellow). Box indicates the IT dosage chosen for subsequent social discrimination tests (med IT). For each comparison, we conducted glmer with main effects of dose or pre-vs. post treatment and fishID as random effect. Post-hoc within group comparisons are paired Wilcoxon signed rank tests. FDR = p-value for Wilcoxon tests following Benjamini-Hochberg correction for multiple comparisons. *p < 0.05, **p < 0.01. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

### 2.3. Pharmacology

Isotocin (H-2520, Bachem) was resuspended in 0.9% saline and stored in 10 μg/μl stock solution aliquots at −20 °C until use. On the day of treatment, IT stock solution was thawed on ice and serially diluted using sterile 0.9% saline solution to working concentration (see below). Excess reagent was discarded and fresh aliquots were used each treatment day. IT dose range and recovery period were modified from other exogenous IT experiments using zebrafish (Braida et al., 2011; 0.1 ng/kg–10 ng/kg IT in 2 μl/g body weight volume) and cichlids (Reddon et al., 2012; 1 μg/g IT in 25 μl/g body weight volume).

Seven days after pretesting, females were i.m. injected with either saline control or IT solution. Fish were weighed immediately prior to injection to calculate injection volume (20 μl/g body weight volume). Fish were i.m. injected with the syringe (Becton Dickinson, 3/10 $cm^3$ insulin syringe) into the largest part of the caudal muscle on the left side of each fish. Following injection fish were immediately placed into a recovery tank for a 10-minute recovery period. Next fish were placed into the posttest treatment arena for a 5-minute habituation followed by behavior testing. For dichotomous choice behavior trials, fish were injected with either 0.9% saline solution or 1 ng/kg IT in 0.9% saline. For the scototaxis behavior trials, fish were injected with either 0.9% saline or one of three IT doses: 0.1 ng/kg, 1 ng/kg, or 10 ng/kg.

### 2.4. Statistical analysis

In our scototaxis/anxiety assays, we used GLMER models with trial type (pre vs. posttest) and treatment type (saline, low IT, med IT, high IT) as fixed effects and fish ID as random effect. For each measure we used AIC values to assess the best fit distribution family for testing GLMER model parameters, and we report effect size for our GLMER models with marginal (fixed effects only) and conditional (fixed plus random effects) $R^2$ calculations. For proportion time in white, we modeled with a Gaussian distribution. For latency to enter white side, we used negative binomial distribution. For both proportion in white and latency to enter the white side, a comparison of AIC values indicated a model with no interaction term was the best fit. For proportion in center of tank (exploration measure), we model with a beta distribution. A comparison of AIC values indicated a model including an interaction between pre vs. posttest and treatment was the best fit, allowing a test for differential exploration tendency following IT treatment vs. saline controls. To determine overall activity levels we also measured number of crosses into the white (Supplemental Fig. 2, GLMER model with Gaussian distribution). We report Wald Chi-square test on GLMER model fit for all GLMER model estimations (Type II for models with no interaction and Type III for models that include an interaction term). To examine within-group response patterns we performed paired Wilcoxon signed rank tests of pre vs. posttest performance for the four treatments (saline, low IT, med IT, high IT) with Benjamini-Hochberg FDR correction for each behavioral measure. All statistical analysis was conducted using R version 3.4.3 and R studio version 1.1.383 with lmer, car, MASS, ggplot2, glmTMB, and fitdistrplus packages. GLMER was conducted using the 'MCMCglmm' v. 2.26 package. GLMER effect size estimates were conducted using sjstats and r2gmm packages.

For pairwise comparisons of behaviors in our dichotomous social context choice tests, we used paired *t*-tests (total association with males, time with large stimulus, time with small stimulus) or paired Wilcoxon signed rank test (all other behavioral measures) depending on whether our measures violated the assumption of normal distribution as determined with a Shapiro-Wilks Test. We report effect size using Cohen's d for pairwise comparisons using an online effect size calculator (https://www.memory.psych.mun.ca/models/stats/effect_size.shtml).

## 3. Results

### 3.1. IT dose response: anxiety/exploration

We measured anxiety responses following treatment with either saline control or injection with low (0.1 ng/kg), medium (1 ng/kg), or high (10 ng/kg) doses of exogenous IT. We assessed three anxiety/exploration measures: proportion of time spent in the white region of a black and white tank (scototaxis response), latency to enter white region, and proportion of time spent in the center vs. the edges of the tank (exploration response). Type II Wald Chi-square tests on model fit for proportion time in the white side of the tank indicates no significant effect of IT injection on pre vs. posttest or treatment type on scototaxis response (Fig. 2A; GLMER modeling (gamma distribution) main effect pre vs. post Chi-Sq 2.77, p = 0.10; main effect treatment Chi-Sq 4.96, p = 0.17; marginal $R^2$ (main effects) = 0.09, conditional $R^2$ (includes random effects) = 0.22). Although there was also no indication of an interaction between pre vs. posttest and treatment type on proportion time on the white side of the tank, a Wilcoxon signed rank test within each treatment indicated a decrease in proportion time on the white side at the low IT dose although it did not remain significant following FDR correction (pre vs. posttest low IT V = 48, p = 0.037; FDR p = 0.15). There were no differences in proportion time in white at the other doses (saline V = 37, p = 0.38 FDR p = 0.50; med IT V = 44, p = 0.11 FDR p = 0.21, high IT V = 30, p = 0.85 FDR p = 0.85).

Latency to enter the white side of the tank was significantly altered pre vs. posttest (Fig. 2B; Type II Wald GLMER (nbinom) Chi-Sq pre vs. posttest 8.83, p = 0.003) but not by treatment type (Chi-Sq treatment 6.23 p = 0.10), with overall model effect size $R^2$ = 0.135. Due to low variance in our random term we do not report a conditional $R^2$. Looking within each treatment, Wilcoxon signed rank test identified a significant increase in latency for the low IT dose (V = 0, p = 0.009 FDR p = 0.04). There was no difference in latency to cross white in pre vs. posttest for saline treated females or the other IT doses (saline V = 10.5, p = 0.09 FDR p = 0.18, med IT V = 16, p = 0.28 FDR p = 0.37, high IT V = 30, p = 0.84 FDR p = 0.84). As a measure of overall activity changes, we also looked at total crosses into the white side of the tank. Crosses into white were significantly reduced following injection, including saline control and all three IT doses (Supp. Fig. 2; Type III Wald Chi-square test on GLMER model fit (Gaussian distribution with pre vs. posttreat by treatment with an interaction term) indicated a significant main effect pre vs. posttest (Chi-Sq 11.8, p = 0.0006), but no main effect of treatment (Chi-Sq 0.40, p = 0.94). While the model increased fit with an interaction term, the interaction between pre vs. posttest and treatment was not significant (Chi-Sq 1.46, p = 0.69). The effect size for the crosses to white model was $R^2$ = 0.28 (marginal) and $R^2$ = 0.56 (conditional).

Isotocin injections induced a significant change in exploration response (proportion time in center of tank) in *G. affinis* females (Fig. 2C). GLMER (beta distribution) modeling indicated a significant main effect of pre vs. posttest (Chi-Sq 11.77, p = 0.0006) but not treatment (Chi-Sq 4.35, p = 0.23). There was a significant interaction between pre vs. posttest response and treatment type (Chi-Sq 8.20, p = 0.04). Looking within each treatment, Wilcoxon signed rank tests indicate no significant change pre vs. posttest within the saline group (V = 24, p = 0.77 FDR p = 0.77), while posttest time in center was higher than pretest for all three doses of IT (low IT V = 3, p = 0.01 FDR p = 0.01; med IT V = 3, p = 0.01 FDR p = 0.01; high IT V = 0, p = 0.002 FDR p = 0.008).

### 3.2. IT injection reduces social behaviors towards males but not females

*G. affinis* females reduced total time spent with males following treatment with exogenous IT whereas saline-injected females showed no such reduction (total association time pre vs. posttest saline t = 1.19 p = 0.26, Cohen's d 0.38; IT t = 3.48 p = 0.007, Cohen's d 1.10; Fig. 3A). Exogenous IT did not alter overall activity levels (pre vs. posttest saline V = 29 p = 0.92, Cohen's d 0.12; IT V = 37 p = 0.38, Cohen's d 0.41; Fig. 3B); however, it did significantly reduce the number of social behaviors directed towards males (pre vs. posttest saline V = 28 p = 1, Cohen's d 0.25; IT V = 47 p = 0.05, Cohen's d 0.61; Fig. 3C) as well as the ratio of social behaviors/total activity (saline V = 31 p = 0.77, Cohen's d 0.03; IT V = 49, p = 0.03, Cohen's d 0.81; Fig. 3D), whereas social behaviors were not reduced in the posttest for saline controls. We tested for size-based association biases for one male stimulus over another and found no evidence for bias towards the large male in saline control or IT-treated females (time with LM vs. SM saline pretest t = −0.24, p = 0.82; saline post treatment t = −1.74, p = 0.12; IT pretest t = 1.46, p = 0.18; IT post treatment t = 0.63, p = 0.55).

In contrast, saline but not IT reduced total association time for *G. affinis* females exposed to other females (total association time pre vs. posttest saline V = 48 p = 0.04, Cohen's d 0.71; IT V = 30 p = 0.20, Cohen's d 0.40; Fig. 4A). Total activity (pre vs. posttest saline V = 30, p = 0.85, Cohen's d 0.04; IT V = 28, p = 1, Cohen's d 0.18; Fig. 4B) was not altered in females exposed to saline or exogenous IT, nor were total social behaviors (pre vs. post saline V = 26, p = 0.92, Cohen's d 0.10; IT V = 28, p = 1, Cohen's d 0.30; Fig. 4C) or the ratio of social behaviors to total activity (pre vs. post saline V = 33, p = 0.625, Cohen's d 0.26; IT V = 36, p = 0.4, Cohen's d 0.253; Fig. 4D). Female *G. affinis* fish did not exhibit size bias in association time with female conspecifics (time with LF vs. SF saline pretest t = 0.47, p = 0.65; saline post treatment t = 0.20, p = 0.84; IT pretest t = 0.29, p = 0.78, IT post treatment t = 1.15, p = 0.28).

## 4. Discussion

Our results support the role of IT in producing anxiolytic effects (increasing exploration and boldness) as well as mediating social salience and valence assessment of social stimuli in female fish from a coercive mating system. Female *G. affinis* fish treated with exogenous IT were more likely to explore the center of tank regions in a threatening nonsocial environment (scototaxis tank). Meanwhile females were less likely to spend time with or direct social behaviors towards coercive males in social environments. However, this effect was context-specific, and contrary to the prosocial or anxiolytic hypotheses predictions, there was no change in association time or social behaviors towards female conspecifics.

If IT was modulating social approach decisions solely through anxiolytic effects in our experiment, we would have expected to see altered shoaling response across all social treatments (female and male conspecifics). Instead, time with females was not altered following IT treatment. Across mammals there is substantial evidence revealing the anxiolytic effects of exogenous OT (Lee et al., 2009; Neumann and Slattery, 2016; Windle et al., 1997), and research in humans has suggested that the increase in prosociality following exogenous OT is a result of the anxiolytic effects of OT on the dopaminergic system (Kirsch et al., 2005; Domes et al., 2007; Shamay-Tsoory and Abu-Akel, 2016). However, in our experiment the lack of association between IT-induced anxiolytic effects and increased sociality (no increased approach towards conspecifics) may be due to species-specific differences in the function of prosociality. Previous research with zebrafish has shown a positive association between anxiolytic and prosocial behavioral responses to exogenous IT (Braida et al., 2011). Yet, poeciliids, like many small fish, are known to aggregate under stressful conditions (Dadda et al., 2005; Dadda, 2015; Heinen-Kay et al., 2016). Hence, if IT reduces stress reactivity, then it is plausible that exogenous IT might actually result in reduced shoaling response. In cichlid fish, for example, lowered anxiety following exogenous IT treatment may have contributed to reduced approach towards a larger shoal (Reddon et al., 2014). However we did not find reduced sociality across both social contexts, suggesting that the anxiolytic effects of IT are not the dominant effect modulating social responses in this species.

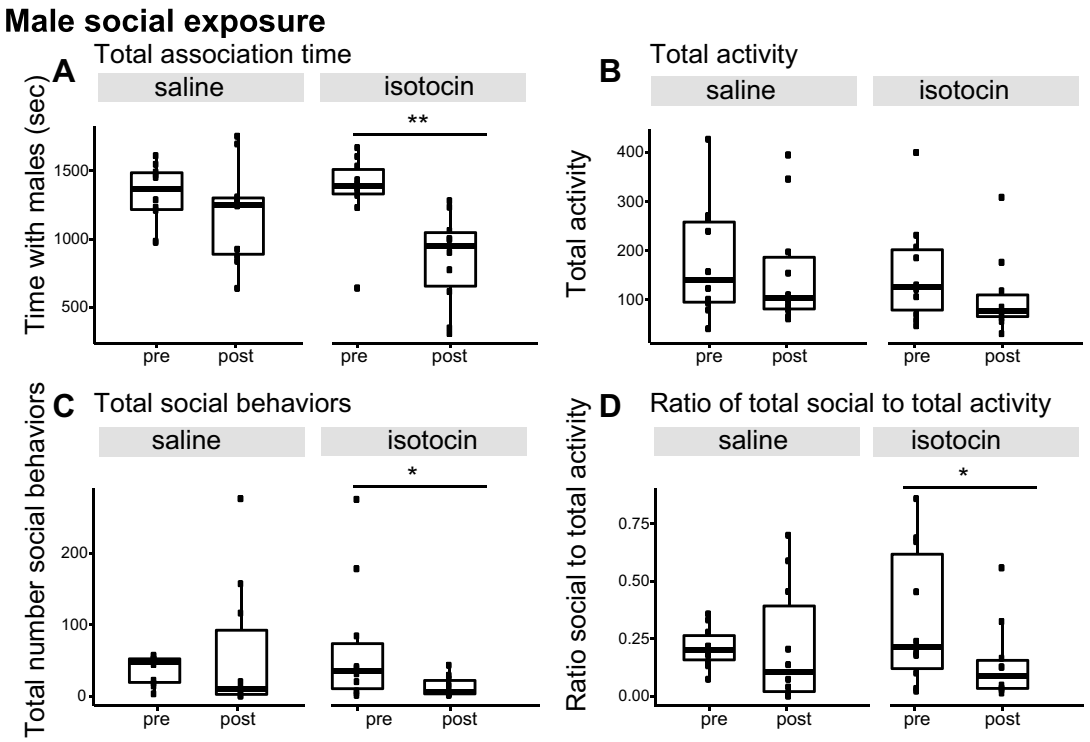


**Fig. 3.** Male social exposure: Social decisions towards males were altered by exogenous IT. A. Association time with coercive males was reduced in *G. affinis* females treated with IT but not saline vehicle control (saline p = 0.27; IT p = 0.007). B. Total activity levels were not altered by IT (saline p = 0.85; IT p = 1). C. Total number of social behaviors were reduced following IT treatment (saline p = 1; IT p = 0.05). D. The ratio of the number of social behaviors to overall total activity was reduced following IT treatment (saline p = 0.77; IT p = 0.03). Box plots indicate 1st and 3rd quartiles, the line indicates the median, and whiskers indicate high and low values. Circles indicate data points. For all panels, saline (0 ng/kg) and IT (med dose 1 ng/kg). *p < 0.05, **p < 0.01).

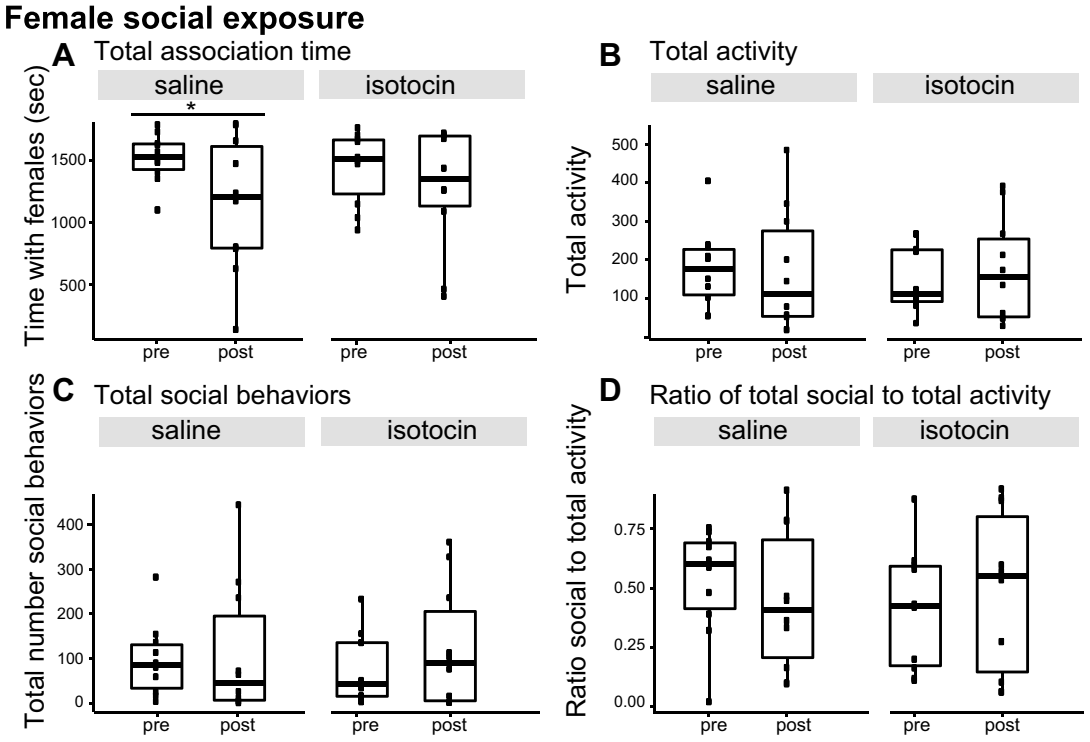


**Fig. 4.** Female social exposure: Social decisions towards females were not altered by exogenous IT. A. Association time with conspecific females was not altered in *G. affinis* females treated with IT (saline p = 0.04; IT p = 0.19). B. Total activity levels were not altered by IT (saline p = 0.85; IT p = 1). C. Total number of social behaviors towards conspecific females were not altered by IT (saline p = 0.92; IT p = 1). D. The ratio of the number of social behaviors to overall total activity towards conspecific females was not altered following IT (saline p = 0.63; IT p = 0.43). Box plots indicate 1st and 3rd quartiles, the line indicates the median, and whiskers indicate high and low values. Circles indicate data points. For all panels, saline (0 ng/kg) and IT (med dose 1 ng/kg). *p < 0.05, **p < 0.01.

The context-dependent social approach results we observed in *G. affinis* fish are more consistent with the social salience hypothesis of oxytocin (Shamay-Tsoory and Abu-Akel, 2016). If IT is modulating the attentional orienting responses based on the salience of the social stimuli, then we would expect a stronger behavioral response by females in the male-exposed group than the female-exposed group following exogenous IT because of the high levels of sexual conflict within the male coercive *G. affinis* mating system (Cummings, 2018; Magurran, 2011; Wang et al., 2015). In response to high rates of male harassment (Bisazza, 1993; Farr, 1989; Magurran, 2011), females have evolved behavioral avoidance responses to resist unwanted matings that necessitate high levels of vigilance towards approaching males (Dadda et al., 2005; Dadda, 2015; Darden and Croft, 2008). In our experiment, wild-caught females were held in mixed sex communal tanks until isolation 2 weeks prior to final treatment and testing. *G. affinis* females store sperm, and like many other poeciliids are only briefly receptive to male attention as virgins and immediately after giving birth to a brood. As with most poecilid species, multiple insemination is extremely common in *G. affinis* (Chesser et al., 1984), and even in the absence of males oocytes can begin developing and undergo fertilization with stored sperm within days of parturition (Koya et al., 2000). This means that females in our experiment were likely pregnant and resistant to male attention. Our results suggest that exogenous IT may have facilitated this resistance by enhancing the salience of unwanted male attention and increasing avoidance of males under our experimental conditions.

Although associating with males can be costly, female *G. affinis* fish did spend time associating with coercive males, particularly in the pretest trials, and the variance in some behavioral measures is greater in the pre- than posttest assays. This was presumably due to individual variation in response to exposure to a novel testing tank coupled with exposure to potentially threatening coercive males as well as a possible indirect effect of trial time on some pre-test activity measures. Decisions to associate with males presumably represent a tradeoff between the potential for sexual harassment versus an aggregation response to a potentially threatening environment (here a novel testing tank). A promising future direction would be to test the importance of experience in mediating male avoidance by testing behavioral responses to coercive males in virgin versus pregnant females.

Exogenous IT did not increase female association with or social behaviors towards other females (a presumably rewarding social stimuli). This may be due to a ceiling effect of this behavior (female pretest association time was quite high), or that conspecific females represent relatively less salient social stimuli to females than conspecific males. Interestingly, association time with other females was reduced following saline treatment, so it is possible IT manifested a mild prosocial effect by rescuing association time back to pre-test levels in IT-injected females. Female poecilids benefit from shoaling behavior (e.g. social learning (Laland and Reader, 1997); protection from predators (Magurran and Garcia, 2000) and harassing males (Dadda et al., 2005; Dadda, 2015)). However, there are also drawbacks to shoaling including foraging competition (Ward et al., 2006) and increased exposure to disease (Magurran and Garcia, 2000). It is possible that our experimental design did not induce motivation to approach other females, and future experiments testing other measures of social approach such as shoal discrimination, or designs that include an explicit pro-social motivator to approach other females (e.g. threat of harassing male) might show a prosocial effect with exogenous IT.

As part of the social salience hypothesis of oxytocin, Shamay-Tsoory and Abu-Akel (2016) proposed that OT alters the attentional mechanisms in the dopaminergic system that are involved in assigning salience and directing attention to relevant social information. As the dopaminergic system is centrally involved in processing both aversive and rewarding events (Bromberg-Martin et al., 2010), OT may enhance the attentional orientation and relevant behavioral responses to diverse social stimuli. In mammals, the interaction between dopaminergic and neuropeptide circuitry has long been established for mating decisions (Young and Wang, 2004), and increasing evidence supports their interplay in mediating social recognition and appropriate behavioral responses to potentially threatening social interactions (Skuse and Gallagher, 2009; Shamay-Tsoory and Abu-Akel, 2016). Much of the dopaminergic-neuropeptide circuitry implicated in social decision-making are conserved across vertebrates including teleosts (O'Connell and Hofmann, 2011; O'Connell and Hofmann, 2012; Oliveira, 2013), and key nodes implicated in social salience and reward processing (e.g. Dm, basolateral amygdala homolog, Dl, hippocampus homolog, and POA) have been identified in mate preference decisions in another poecilid species (Wong et al., 2012; Wong and Cummings, 2014). While our behavioral results provide initial support for the social salience hypothesis of oxytocin in teleosts, future studies will allow us to test whether the explicit mechanisms of this hypothesis are conserved between mammals and teleosts. In particular, it is critical to test how nonapeptide–dopaminergic systems may enhance this attention to diverse social stimuli and help coordinate the relevant behavioral response towards both aversive and rewarding stimuli. Specifically, we can test whether exogenous IT in fish leads to increases in dopamine release, or whether it leads to enhanced activation of IT receptors in the amygdala (which plays a central role in salience detection in mammals (Adolphs and Spezio, 2006; Phelps and LeDoux, 2005)) as fish experience diverse social interactions.

In addition to exploring the specific neural mechanisms underlying IT/OT's role in regulating social salience, an examination of the diurnal patterns associated with this nonapeptide-mediated response will be important. Diurnal rhythms are known to affect multiple behavioral measures (reviewed in Nelson, 1990), including female receptivity (e.g. Delville et al., 1986) and multiple behaviors including spawning in fish (Reebs, 2002). Female poeciliids, however, exhibit a facultatively dissociated reproductive cycle (Ramsey et al., 2011), and studies with guppies have shown that poeciliids mating interactions and other activities (e.g. feeding) may occur outside predicted diurnal patterns (Archard et al., 2009; Fraser et al., 2004). Although we found no effect of time of day in our pharmacological manipulations, our measurement window was quite broad and it is known that IT varies on a diurnal cycle (Gozdowska et al., 2006). Hence, it is possible that our measurement series missed an underlying diurnal pattern to these processes. Future studies explicitly testing behavioral and physiological cycling (IT, steroid hormones) would be a welcome addition to the field.

In summary, we found that exogenous IT produced context-specific divergent responses in female fish from a coercive mating system that support the conservation of the social salience hypothesis of oxytocin in teleosts. We found that exogenous IT exerted anxiolytic effects in nonsocial conditions as measured by an increase in exploration into the center region of an anxiety/exploration assay. We found that exogenous IT enhanced social decision-making in contexts with divergent social valence and salience as IT-treated females decreased time spent with coercive males but did not alter time with conspecific females. We propose that poeciliids are a system where we can explore the role of IT in influencing social salience assignment and development of social cognition using a highly important interaction (mating decisions) with direct consequences to individual fitness. We can take advantage of the diversity of poecilid mating systems that range from taxa with 100% coercive to those featuring different courting male phenotypes (Cummings and Ramsey, 2015; Cummings, 2018) to understand why exogenous IT led to anxiolytic effects in threatening nonsocial conditions (our scototaxis trials) yet enhanced social vigilance (avoidance behavior) in threatening social conditions (coercive males).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2019.03.001.

## Data accessibility

The dataset supporting this article will be available at Mendeley Data.

## Author contributions

MER and DF conceived the study, designed the experiment, collected and analyzed the data. MER, DF and MEC interpreted the results. MEC advised regarding the experimental design and interpretation of the results. MER wrote the first draft of the manuscript, which was then revised by all authors. All authors approved the final version of the manuscript.

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