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SHORT COMMUNICATION

Effect of isotocin on shoaling behaviour of the Guppy (*Poecilia reticulata*)

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Abstract

Pro-social effects of oxytocin and its homologues are well-documented in birds and mammals. However, in fishes, the effect of isotocin, the homologue of oxytocin, on social behaviour is less clear. Studies in fishes have generally shown no effect of isotocin on social behaviours or even an anti-social effect. In our study, we measured association preference for conspecifics in 92 adult guppies (46 females and 46 males), half of which were injected with isotocin and the other half with an isotocin antagonist. We found that individuals injected with isotocin spent 29% more time associating with conspecifics than individuals injected with an isotocin antagonist. The effect of isotocin on association time did not differ between males and females. Our study provides some of the first evidence of a pro-social effects of isotocin in a fish and suggests that in fishes, isotocin may have a homologous role to oxytocin, at least in promoting shoaling behaviour.

Keywords Isotocin · Oxytocin · Nonapeptide · Social behaviour · Shoaling · Guppy

Introduction

Research into neuro-behavioural mechanisms underlying social behaviours has suggested an important role for oxytocin and its homologues. Many studies have shown that oxytocin has pro-social effects (for review, see Goodson 2013). For example, in mammals, oxytocin administration increases maternal behaviour (Pedersen et al. 1982; McCarthy 1990), cooperative behaviour (Harmon et al. 2002; Madden and Clutton-Brock 2011) and the amount of time spent in close proximity with a conspecific (Smith et al. 2010). In birds, administration of mesotocin, the avian homologue of oxytocin, increases time spent with a group of conspecifics (Goodson et al. 2009) and altruistic behaviour (Duque et al. 2018).

The effect of oxytocin may differ between the sexes at least for some social behaviours (for review, see Dumais and Veenema 2016). For example, in the prairie vole (*Microtus ochrogaster*) oxytocin administration increases the amount of time that females spend in proximity to an adult of the opposite sex, but has no effect on the amount of time that males spend in proximity to an adult of the opposite sex (Cushing and Carter 2000). In humans, oxytocin administration improves kinship recognition in females but not in males, and improves the recognition of which of two interacting characters won a competition in males but not females (Fischer-Shofty et al. 2013). These sex-specific effects may occur because females are more sensitive to oxytocin, as the sex-steroid estrogen has been linked to the up-regulation of the expression of oxytocin receptors (Larcher et al. 1995; Carter 2007).

Although the pro-social effects of oxytocin and mesotocin on behaviour are well studied in mammals and birds, studies in fishes have provided only weak evidence for a pro-social effect of isotocin. In goldfish (*Carassius auratus*), isotocin administration increases the time spent associating with another conspecific, but only when the authors examined the subset of the subjects ($n = 6$ of 13) that had the lowest baseline association values (Thompson and Walton 2004). Some other studies in fishes have shown no effect of isotocin on social behaviour or even an anti-social effect. In daffodil cichlid (*Neolamprologus pulcher*), for example, the level of isotocin in the brain is negatively correlated with affiliative behaviour (Reddon et al. 2015). Furthermore, in this cichlid, isotocin administration reduces association with conspecifics, whereas administration of an isotocin antagonist increases association with conspecifics (Reddon et al. 2014).

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In zebrafish (*Danio rerio*), Lindeyer et al. (2015) found no effect of isotocin or its antagonist on shoaling behaviour, and Braida et al. (2012) found that, whereas isotocin administration at intermediate doses increases social preference for phenotypically similar conspecifics, isotocin administration at low or high doses has the opposite effect.

It is also unclear if the effect of isotocin on social behaviour is sex-biased in fishes. In mosquitofish (*Gambusia affinis*), isotocin administration decreases association time with conspecifics in males but not females (Ramsey et al. 2019). In plainfin midshipman (*Porichthys notatus*), Goodson and Bass (2000) showed that isotocin administration decreases vocalization in females but did not affect vocalization in males that acoustically court females. These initial studies suggest that there could be differences in the effect of isotocin between the sexes. Certainly, more studies are needed in fishes to more fully understand the effect of isotocin on social behaviour.

The guppy (*Poecilia reticulata*) is a small live-bearing fish with internal fertilization that is abundant in Trinidad (Houde 1997). Guppies often form social groups referred to as shoals, but the propensity to shoal differs among populations and between the sexes. The variation in shoaling behaviour among populations has been linked to predation regime, with guppies from populations with high levels of predation showing a higher propensity to shoal with conspecifics than guppies from populations with low levels of predation (Magurran and Seghers 1994a). At sexual maturity, female guppies are about three times larger than males, and females also show a greater preference for shoaling than males (Magurran and Seghers 1994b). Guppies offer an excellent system to examine the effect of isotocin on shoaling behaviour, with the opportunity to test whether or not isotocin administration leads to differences in shoaling behaviour, and whether or not these effects differ between the sexes.

Methods

Experimental design

The experimental animals were drawn from a laboratory-reared population of guppies that were originally collected from the low-predation Paria River in Trinidad (for additional collection details, see Hain et al. 2016). This population has been maintained in 200 L aquariums that contain approximately 100 adult guppies and similar numbers of males and females. Fish were kept at 25 ± 1 °C and on a 12:12 h light:dark cycle. Fish were fed ad libitum daily with live brine shrimp nauplii (Canadian Aqua Farm, Canada) and TetraMin tropical fish flake food (TETRA Werke Melle, Germany).

To obtain baseline shoaling behaviour measurements prior to the experimental manipulation of isotocin levels, a standard dichotomous choice behavioural trial protocol was used. Following Hain and Neff (2007), a test tank (34 cm length × 19 cm width × 15 cm water depth) was divided into three chambers by adding two plastic barriers 8 cm from each end of the tank. The barriers were clear and permeable to odours. On the center chamber (18 cm in length), vertical lines were drawn 5 cm from each plastic barrier to indicate association preference zones for each end of the tank. For each behavioural trial, a group of six stimulus fish were placed in one end-chamber whereas no fish were placed in the other end-chamber. The stimulus fish were all adult fish of the same sex as the test fish, with a total of 30 males and 30 females used as stimulus fish. Males were identified by their colourful body and rod-shaped anal fin, whereas females were identified by their large body and the absence of male colouration (Houde 1997). Females may have mated prior to the start of trials, and their pregnancy status was not assessed. The stimulus fish were placed in the stimulus chamber of the test tank 15 min before the test fish to let them acclimate to the test tank and to allow any chemical cues to accumulate in the water (following Hain et al. 2016). Shoal location (left or right side) was randomized across trials. For each trial, a single test fish was released in the center chamber and its behaviour was recorded for 20 min using a digital camcorder placed in front of the tank. A total of 92 adult individuals (46 females and 46 males) were used as test fish. Test fish were never previously used as stimulus fish. Stimulus fish were reused across multiple trials.

Isotocin (Bachem, Torrance, USA) and atosiban (an oxytocin/isotocin receptor antagonist; Cardoso et al. 2015) (Sigma-Aldrich, Oakville, Canada) were dissolved in sterile 0.9% saline at a concentration of 1 mg/ml and stored at − 20 °C until the day of injection. Immediately following the baseline shoaling behaviour assessment, half of the test fish were injected with isotocin and the other half with isotocin antagonist. For injection, a test fish was removed from the tank and weighed by placing them in a pre-weighed container of water. Fish were then immobilized using a pair of cloth-covered forceps and given an intraperitoneal injection using a 5 µl Hamilton Neuros-Syringe with a 33G needle (Hamilton Company, Canada) at a dose of 10 µg/g fish body mass. The dosage follows a similar study on zebrafish (Lindeyer et al. 2015). Following injection, the fish was placed in a post-injection recovery tank for 5 min to allow the injected substance to reach the brain (Lindeyer et al. 2015; Ramsey et al. 2019). The fish was then returned to the center chamber of the test tank and the post-injection shoaling behaviour was recorded for 20 min. The water of the test tank was replaced with fresh water after each trial.

Statistical analysis

Body mass was compared between sexes using a *t* test. As in Hain and Neff (2007), a test fish was considered to be shoaling when its head was in the association zone associated with the group of six stimulus fish. For each trial, the time spent in the association zone with the group of six stimulus fish was calculated as a proportion of the total duration of the trial (i.e. time spent in association zone/20 min). The proportion of time spent in the association zone was analyzed separately for the pre- and post-injection behavioural observations using linear models that included sex, treatment, and sex × treatment interaction as factors. The location of the group of stimulus fish (left vs right) was included as a random effect but was not significant (pre-injection $p = 1.00$; post-injection $p = 0.22$) and was removed from the final models. Quantile plots indicated that the time spent in the association zone was well represented by a normal distribution. Statistical analysis was conducted using JMP statistical software version 4.0.2.

Ethical note

Experimental methods used in this study were approved by the Western University Animal Care Committee (Protocol 2018-084).

Results

As expected, body mass was significantly higher for females (mean ± SD; 0.41 ± 0.11 g) than for males (0.13 ± 0.04 g; $t_{90} = 15.4$, $p < 0.001$).

Before the isotocin and isotocin antagonist injections, females spent significantly more time in the association zone adjacent to the six stimulus fish (hereafter referred to as the "association zone") than males ($F_{1,88} = 5.86$, $p = 0.02$; Fig. 1a). Before the injections, there was no significant difference in the time spent in the association zone for fish assigned to the different treatments ($F_{1,88} = 0.03$, $p = 0.87$; Fig. 1a), and no significant interaction between treatment and sex ($F_{1,88} = 0.33$, $p = 0.57$; Fig. 1a).

After the isotocin and isotocin antagonist injections, females again spent more time in the association zone than males ($F_{1,88} = 11.37$, $p < 0.001$; Fig. 1b). Individuals injected with isotocin spent significantly more time in the association zone than individuals injected with isotocin antagonist ($F_{1,88} = 6.93$, $p = 0.01$; Fig. 1b). The amount of time in the association zone differed about twice as much between the isotocin and isotocin antagonist groups in males compared to females, albeit both sexes spent more time in the association zone after injection with isotocin than the isotocin antagonist, and the interaction between treatment and sex was not significant ($F_{1,88} = 1.15$, $p = 0.29$; Fig. 1b).

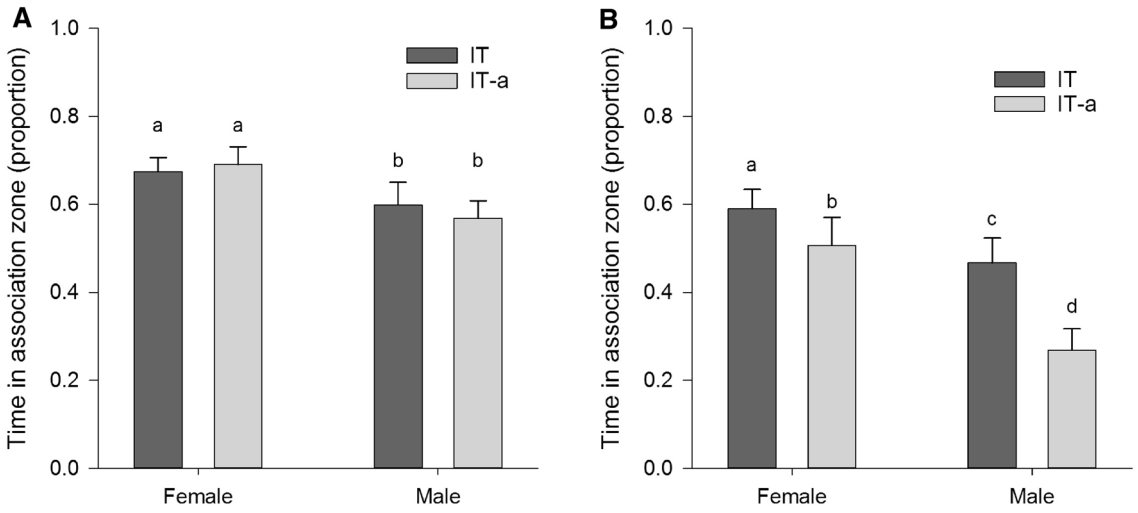

Fig. 1 The time test fish spent in the association zone with a group of six stimulus fish during a dichotomous trial in guppies (*Poecilia reticulata*). Plots show mean ± SE for females and males. Behaviour prior to isotocin (IT) and isotocin antagonist (IT-a) injection are presented in (**a**) and behaviour after IT and IT-a injection are presented in (**b**). Different letters above the bars indicate significant differences between groups

Discussion

Oxytocin and its homologues have been reported to regulate social behaviour, acting as a pro-social modulator in mammals and birds (for review see Goodson 2013). Studies in fishes, on the other hand, have provided little support for pro-social effects of isotocin (e.g. Braida et al. 2012; Reddon et al. 2014; Lindeyer et al. 2015; Ramsey et al. 2019). Here, we provide some of the first evidence of pro-social effects of isotocin in a fish, showing that the time spent in proximity to conspecifics was 29% higher in guppies that received isotocin than in guppies that received an isotocin antagonist. Several factors might explain why our results contrast with earlier studies in fish. First, the pro-social effects of isotocin might depend on the dosage of isotocin administered. Indeed, there is some evidence in zebrafish that the effects of isotocin on social behaviour have a non-linear relationship with dosage (Braida et al. 2012). However, all the dosages used by Braida et al. (2012) were much lower than our study, and another study also in zebrafish that matched our dosage found no effect of isotocin on association behaviour (Lindeyer et al. 2015). Nevertheless, it is possible that only a specific dosage range will elicit social behaviour in a particular species of fish. Second, the differences among studies might reflect the social behaviour being measured and its context. Several of the studies that found no pro-social effect of isotocin compared association with groups of different sizes (Thompson and Walton 2004; Reddon et al. 2014) or groups that differed in body colouration (Braida et al. 2012), such that the studies were not clearly comparing a pro-social to non-social behaviour. In contrast, our study compared association with a group of six versus no conspecifics, providing a clear contrast between a pro-social and non-social choice. Regardless, we present some of the first evidence that isotocin has a homologous role to oxytocin in promoting social behaviour in a fish. More research is needed to better understand dosage and context-dependent effects.

Social behaviours may be expressed similarly in both sexes or may be sex biased. In our study, before and after isotocin and isotocin antagonist administration, females spent more time with a group of same-sex conspecifics than males. Importantly, the effects of isotocin and its antagonist did not differ between sexes, with both male and female guppies showing greater shoaling after administration of isotocin than administration of the isotocin antagonist, albeit there was a non-significant trend towards a greater magnitude of effect in males than in females. This result suggests that both sexes in guppies share a common relationship between isotocin and shoaling behaviour and it is unlikely that isotocin regulates social behaviour in only one sex. In contrast, in mosquitofish, the effects of isotocin administration differed between sexes, with only males having reduced association with conspecifics following isotocin administration (Ramsey et al. 2019). Sex-specific responses to isotocin were also described in plainfin midshipman in which isotocin suppresses vocalizations in females but not males that acoustically court females (Goodson and Bass 2000). It remains unknown what leads to the differences among species and behaviours in how the response to isotocin differs between sexes.

One potential limitation in the interpretation of our data is the absence of a control group that received an injection without an active compound (i.e. saline alone). Instead we used the pre-injection behaviour for a baseline measurement. However, we saw an overall decrease in the time spent in the association zone from the pre-injection to the post-injection observations across all groups. This decline might reflect a depressive effect of the handling and injection on social behaviour, or a decline in social interest as the test fish became acclimated to the test conditions. Regardless of its cause, the decline in association following injection means that it is not possible to confidently distinguish between three explanations that are consistent with our observations: 1) isotocin injections increased association and the isotocin antagonist had no effect; 2) isotocin injections increased association and the isotocin antagonist decreased association; or 3) isotocin injections had no effect and the isotocin antagonist decreased association. Although distinguishing between these explanations is an interesting question that is worthy of further investigation, this limitation does not alter our conclusion that isotocin is positively associated with social behaviour in guppies.

In summary, although it has been shown that oxytocin and its homologues act as a pro-social modulator in mammals and birds, studies in fishes have provided mixed results. Our study provides some of the first evidence of a pro-social effect of isotocin, and also suggests that isotocin and its antagonist affect the behaviour of both females and males similarly. Our findings about shoaling in guppies contrast with some earlier studies on the effect of isotocin in fishes. Several factors might explain this contrast, including dosage and behaviour or context-specific effects of isotocin.

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