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Effects of isotocin on social responses in a cooperatively breeding fish

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Oxytocin and its nonmammalian homologues play an important role in modulating a diverse array of social behaviours. Recently, it has been suggested that one of the key functions of oxytocin is to direct attention towards socially relevant stimuli, increase social motivation and guide social decision making. Here, we test whether an exogenous increase in isotocin (the teleost homologue of oxytocin) increases the response to social information in a cooperative breeder, the highly social cichlid fish, *Neolamprologus pulcher*. In our first experiment (a simulated territorial contest), we found that *N. pulcher* injected with isotocin were more sensitive to the size of their opponent regardless of whether their opponent was a live rival or a mirror image. Isotocin-treated fish fought in accordance with the size of their opponent whereas control fish fought according to their intrinsic aggressive propensity. In our second experiment (a social group context), we found that isotocin-treated *N. pulcher* were more responsive to aggressive feedback and produced more submissive displays (an important social signal in this species). These experiments provide evidence that isotocin increases responsiveness to social information and further support the function of the oxytocin family of nonapeptides as a highly conserved regulator of social behaviour across vertebrates.

The evolution of sociality represents one of the most enduring and important questions in behavioural biology (Székely et al. 2010). Why do some species show complex social behaviour, while other closely related species living in similar ecologies spend the majority of their lives in solitude? To answer this question, it is crucial that we develop an integrative perspective on social behaviour that includes a thorough understanding of the proximate mechanisms that generate social behaviour (Insel & Fernald 2004; Young 2009; Soares et al. 2010). The nonapeptide oxytocin (and its nonmammalian homologues; e.g. isotocin in teleost fish, mesotocin in birds and reptiles) represents a promising candidate system for the modulation of social behaviour (for recent reviews see: Donaldson & Young 2008; Goodson 2008; Lee et al. 2009; Ross & Young 2009; Goodson & Thompson 2010; Insel 2010).

Oxytocin acts both as a central neuromodulator and a peripheral hormone (Lee et al. 2009). In the periphery, oxytocin is involved in parturition and milk letdown (Lee et al. 2009). Centrally, oxytocin is essential for the regulation of behaviours related to reproduction, including pair bonding and parental care (Insel & Young 2001). A growing body of research has linked variation in oxytocin and its receptor to social behaviours outside of the realm of reproduction, including affiliation, attachment, trust, generosity, the formation of social memories and the suppression of social anxiety (MacDonald & MacDonald 2010). Taken together, this research suggests that the oxytocin system may be a very general mechanism involved in the regulation of social behaviour (Ross & Young 2009; Goodson & Thompson 2010).

The oxytocin system is highly pleiotropic, affecting an impressive diversity of behaviours across functional contexts (e.g. parental care, cooperation, aggregation, anxiety and aggression). One possible explanation for this functional diversity is that oxytocin may be centrally involved in a higher-order regulatory system with downstream effects on a wide variety of social behaviours transcending functional context (Ross & Young 2009; O'Connell & Hofmann 2011). Recently, a unifying principle has been proposed for the function of oxytocin as a central modulator of attention to social stimuli (Ross & Young 2009). Individuals or species with greater expression of oxytocin (higher circulating levels and/or greater receptor density) may be more attentive to socially relevant stimuli and as a result may be more socially motivated. In support of this idea, it seems that the effects of oxytocin manipulations are specific to explicitly social contexts, while other functionally similar but nonsocial behaviours remain unaffected (Nelson & Panksepp 1996; Ferguson et al. 2000; MacDonald & MacDonald 2010). For example, Kosfeld et al. (2005) found that humans treated with exogenous oxytocin were more accepting of risk in a socially framed economic game (which the authors interpreted as

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increased trust) but not so in a mathematically identical, but nonsocial, version of the game. In animal models, oxytocin suppresses fear associated with social interactions and activates reward centres in the brain (Insel & Shapiro 1992; Insel & Young 2001). Oxytocin appears to be important in the evaluation of the salience and valence of social stimuli, and thus is emerging as a key element of the neural machinery for social decision making (O'Connell & Hofmann 2011). Previous work in nonmammalian vertebrates suggests that the social functions of oxytocin may be evolutionarily ancient (Goodson et al. 2009). Thompson & Walton (2004) found that treatment with exogenous isotocin increased sociability in goldfish, consistent with a role for isotocin in increasing social motivation and interest in social stimuli. Similarly, Braida et al. (2012) found that zebrafish showed greater social motivation after treatment with isotocin. The characterization of the central function of oxytocin as a highly conserved and general regulator of attention to social stimuli and hence social motivation is intuitively satisfying in that it unifies many diverse findings on the social functions of oxytocin into a single conceptual framework.

In the current paper we set out to explore the role of the teleost oxytocin homologue, isotocin (IT), in the social behaviour of the cooperatively breeding cichlid fish, *Neolamprologus pulcher* (also known as *Neolamprologus brichardi*; Duftner et al. 2007). *Neolamprologus pulcher* is a small freshwater fish endemic to Lake Tanganyika, Africa, that forms permanent social groups containing a single dominant breeding pair and several (1–20) adult subordinate helpers (Taborsky & Limberger 1981; Balshine-Earn et al. 1998; Balshine et al. 2001; Heg et al. 2005; Wong & Balshine 2011a). Individual *Neolamprologus pulcher* engage in a rich variety of social behaviours and frequently interact with other members of their own group and with individuals in nearby groups (Taborsky 1984, 1985; Wong & Balshine 2011a). In an experimental context, *N. pulcher* are highly motivated to interact with conspecifics (Jordan et al. 2010; Reddon et al. 2011a). The social complexity of group life observed in *N. pulcher* is highly unusual amongst the fishes and presents an excellent opportunity to test the hypothesis that IT plays a general role in modulating responses to social information.

Here, we report the results of two experiments designed to investigate the role of IT as a regulator of social information use in *N. pulcher*. First, we explored the effects of an experimental increase in IT on behaviour in staged aggressive contests against both mirror images and live rivals. We investigated the general effect of IT on aggression and the effect of IT on opponent assessment (especially, how perceived opponent body size regulates aggression under IT administration compared to control). Opponent body size is an important determinant of contest dynamics in animals (Arnott & Elwood 2009), including *N. pulcher* (Mitchell et al. 2009; Reddon et al. 2011b), and is a vital component of mutual assessment models of contest behaviour, where the relative asymmetry of the contestants is the strongest predictor of fight dynamics and outcome (Parker 1974; Enquist & Leimar 1983; Arnott & Elwood 2009). We predicted that increasing IT would increase sensitivity to social information, and as a result, increase the importance of opponent assessment, thereby strengthening the correlation between opponent fighting ability and focal behaviour. Specifically, we expected that IT-treated fish would be less aggressive against more formidable opponents. In our second experiment, we explored the effects of an experimental increase in IT on social behaviour within a fish's normal social group. In particular, we were interested in the effects of IT on the regulation of aggressive, affiliative and submissive behaviours in permanent social groups where responses to social feedback from other group members are an important part of an individual's daily life. We predicted that experimentally increasing IT would increase responsiveness to social feedback from other group members, resulting in more dramatic responses to pro- and antisocial acts received from group members. Together, these two experiments increase our understanding of the role of IT as a regulator of social information use in a highly social, nonmammalian vertebrate.

GENERAL METHODS

Subjects and Housing

We used 55 adult *N. pulcher* (27 males and 28 females) in these experiments. Experimental fish were all laboratory-reared descendants of wild-caught fish. Each fish was used only once. The fish used in experiment 1 were housed in one of two 527-litre, mixed-sex communal aquaria ($183 \times 48 \times 60$ cm) prior to the experiment. These fish had been randomly assigned to these communal tanks as juveniles after being hatched within the social groups maintained in the laboratory. The fish used in experiment 2 were adult subordinate helpers from long-term social groups maintained in our laboratory. Each social group is housed in a 189-litre ($92 \times 41 \times 50$ cm) aquarium and consists of a single dominant breeding pair and several (mean: 10; range 7–15) subordinate adult helpers. Water temperature was maintained at 26 ± 2 °C. All fish were fed six times per week on commercially prepared cichlid flakes.

Dosage and Injections

Fish received intraperitoneal injections of isotocin (IT, 1 μg/g of body mass) dissolved in 0.9% saline and/or a 0.9% saline control. Injection volume was tailored to the mass of the fish (25 μl/g). The IT dose was based on previous nonapeptide research in other species (Propper & Dixon 1997; Semsar et al. 2001; Lema & Nevitt 2004; Santangelo & Bass 2006; Mennigen et al. 2008; Filby et al. 2010) and pilot testing in *N. pulcher* in our laboratory. Experiment 1 was a between-subjects design and each fish received only one of the two treatments (IT or saline control). Experiment 2 was a within-subjects design and each fish received both treatments separated by 7 days.

Ethical Note

The fish showed no adverse effects from the injections and resumed normal behaviour within a minute or two. No fish suffered any detectable injury or mortality as a result of the injections or behavioural testing. Focal fish were marked in experiment 2 with a dorsal fin clip to allow for visual identification. Fish recovered immediately from this procedure and showed no adverse effects from the marking. The methods for animal housing, handling and experimental protocols were assessed and approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol number 10-11-71) and adhere to the guidelines of the Canadian Council for Animal Care and ASAB/ABS Guidelines.

EXPERIMENT 1: TERRITORIAL AGGRESSION

Methods

Thirty-six *N. pulcher* (18 females, 18 males; mean standard length = 51.1 mm, range 39.4–62.8 mm) were used in this experiment. Fish were tested for aggressive tendencies in two contexts, first against their mirror image and then against a live same-sex rival across a transparent barrier. Fish were tested in a 38-litre aquarium divided into two compartments by a pair of barriers, one transparent and one opaque, running down the centre (Fig. 1). The far ends of the aquarium were covered with a mirror hidden from

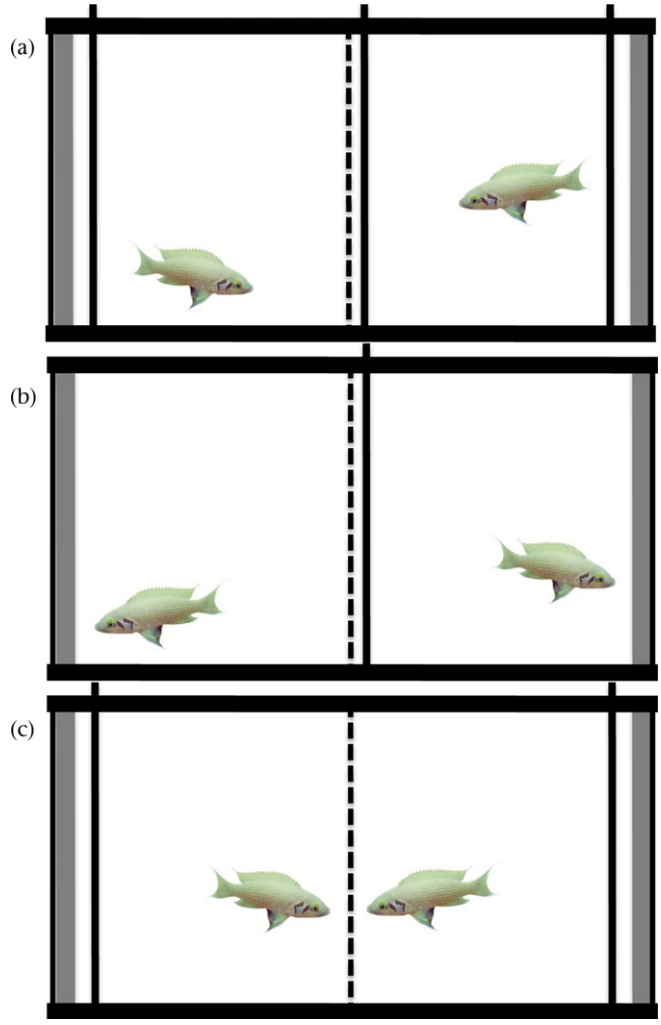

Figure 1. Schematic representation of the territorial aggression testing apparatus used in experiment 1 during the (a) acclimation period, (b) mirror trial and (c) live rival trial. Black line: opaque partitions; broken line: transparent partition; grey line: mirror.

each focal fish by an opaque barrier. We placed a pair of sex-matched fish in each aquarium, one fish in each aquarium half. Individuals were separated from their opponent by the opaque and transparent barriers (Fig. 1a). We allowed each pair of fish a 3 h acclimation period. Aside from sex matching, experimental pairs were chosen randomly, one fish from each of two holding tanks so that all pairs were unfamiliar with one another. The pairs were not size-matched because we wanted to be able to separate statistically the effects of focal and opponent body size on aggression (see Arnott & Elwood 2009). However, the fish were all within the range of asymmetries that would naturally fight (1–12% difference in standard length). There was no significant difference between the average size of IT and saline-treated fish (Welch's test: $t_{33.64} = 0.06$, $P = 0.95$).

We administered an injection of IT to one of the two fish in each experimental pair while the other was given a control injection of saline. Which of the two fish received IT was determined randomly by coin-flip. Fish were given 5 min to recover from the injection and then the barriers covering the mirror were lifted and the fish were allowed to interact with their mirror image for 10 min (Fig. 1b). The barriers were then reinserted, covering up the mirrors, and 1 min later the rival trial began. To do so, we raised the opaque barrier between the two fish, allowing the fish to see and interact with the other pair member (a same-sex fish that had received the opposite treatment) across the transparent barrier for 10 min (Fig. 1c). Both the mirror and the rival trials were videorecorded for subsequent analysis.

The mirror and rival trials were scored from the videorecordings by a trained observer who was blind to the sex and treatment condition of the fish. We assessed the total number of aggressive acts delivered to the mirror and to the rival. We scored the following aggressive behaviours: puffed throats, where the focal fish approaches it opponent with its opercula flared outwards; aggressive head down posture, where the focal fish approaches its opponent with its head angled downward; lateral displays, where the focal fish presents its lateral aspect to its opponent with its head angled downward and/or its unpaired fins held erect; rams, where the focal fish swims quickly towards its opponent and hits its head against the barrier or the mirror but no obvious bite is taken; and bites, where the focal fish makes a biting motion against the barrier or mirror directed towards its opponent (for further descriptions of the behavioural repertoire of this species, see: Taborsky 1984; Buchner et al. 2004; Sopinka et al. 2009). We did not observe any clear escalation of aggressive behaviours (sensu Enquist et al. 1990) in these staged aggressive encounters that lacked physical contact, so we chose to combine all aggressive acts into a single aggression score.

This experiment was analysed using SPSS 20.0 (SPSS Inc., Chicago, IL, U.S.A.) for Macintosh. We computed aggressive acts per minute for each individual's mirror and rival trial and investigated differences between the treatment and control fish. We also examined the correlations between aggressive rates and the standard length (SL) of each fish and the SL of its opponent to determine whether IT affects the use of own and/or opponent size information in *N. pulcher* (Taylor & Elwood 2003). In the case of the mirror trials, the fish's own SL was equal to it perceived opponent's SL (as a fish fighting against a mirror is its own perceived opponent; Rowland 1999; Desjardins & Fernald 2010). Submissive and affiliative acts were rarely observed in this experimental contest context, which is devoid of physical contact between contestants and so these behaviours were not analysed in this first experiment.

We used a linear mixed model (LMM), which treated the contesting pair as the experimental unit (following the recommendation of Briffa & Elwood 2010), with sex as a between-experimental units factor and treatment as a within-experimental units factor to compare aggression rate produced by the IT-treated and the control fish in the live rival trials. Although the two fish could not see one another during the mirror trials, we elected to use a more conservative LMM approach, treating pairs of fish in the same aquarium as a single experimental unit to account for the fact that we could not conclusively exclude the possibility of auditory or olfactory cues being passed across the barriers between the pairs of fish during the time that fish interacted with their mirror images. We included treatment as a within-experimental units factor and sex as a between-experimental units factor in the mirror trials.

We used GLM models, treating body size of the focal fish and its opponent as continuous predictors, to examine the relationship between self and opponent body size and aggression rate for each of the two treatments in the live rival trials (following Taylor & Elwood 2003). We chose not to use a composite measure of the asymmetry between the two contestants because asymmetry measures can lead to erroneous conclusions when analysing contest data (for thorough discussions of this issue, see: Taylor & Elwood 2003; Arnott & Elwood 2009; Briffa & Elwood 2009; Reddon et al. 2011b). Reddon et al. (2011b) found that opponent size was the strongest predictor of contest duration and intensity in unrestrained *N. pulcher* contests. Therefore, size asymmetry may not be appropriate for analysing *N. pulcher* contests. If the

asymmetry in size between the two competitors was an important factor determining the contestants' behaviour, then we would expect to observe a positive effect of own body size on aggression and a negative relationship between opponent body size and aggression (Taylor & Elwood 2003). We used GLM models to explore the relationship between aggression and own body size for each of the two treatment groups during the mirror aggression trials. We also used GLM models to assess whether aggression rate in the mirror trial was a good predictor of aggression in the subsequent live rival trial for each of the two treatments.

One pair did not engage in aggressive behaviour during the live rival trial. Because we could not assign this lack of aggression to one fish or the other, we excluded the data from this trial. The residuals from all of our models did not depart significantly from a normal distribution (all Shapiro–Wilk test: $W < 0.97$, all $P > 0.20$) justifying our use of parametric analyses.

RESULTS

Isotocin-treated and control fish had similar rates of aggression against their mirror images (LMM effect of treatment: $F_{1,31.78} = 0.13$, $P = 0.72$), males and females did not differ in aggression against their mirror image (LMM effect of sex: $F_{1,31.78} = 2.43$, $P = 0.13$) and there was no interaction between sex and treatment on aggression against a mirror (LMM sex*treatment effect: $F_{1,31.78} = 0.29$, $P = 0.60$). Isotocin- and control-treated fish were equally aggressive against a live rival (LMM effect of treatment: $F_{1,26.12} = 1.86$, $P = 0.19$), although females were more aggressive, on average, than males (LMM effect of sex: $F_{1,26.12} = 4.50$, $P = 0.04$). There was no significant interaction between treatment and sex on aggression against a live rival (LMM sex*treatment effect: $F_{1,26.12} = 0.009$, $P = 0.93$).

Contrary to our prediction, IT-treated fish showed a positive correlation between their rates of aggression and their own body size (the size of their perceived opponent) in the mirror trials (GLM effect of SL: $F_{1,16} = 8.30$, $P = 0.01$; Fig. 2a). Fish that received a control injection of saline in the mirror trials showed no correlation between rates of aggression and perceived opponent size (GLM effect of SL: $F_{1,16} = 1.35$, $P = 0.26$; Fig. 2b).

Contrary to our original prediction, isotocin-injected fish also showed more aggression against larger live opponents (GLM effect of opponent SL: $F_{1,16} = 6.14$, $P = 0.03$; Fig. 3a), while control fish showed no such response (GLM effect of opponent SL: $F_{1,16} = 0.14$, $P = 0.72$; Fig. 3b). Aggression rates against a live rival were unrelated to a fish's own body size in the IT-treated fish (GLM effect of SL: $F_{1,16} = 1.16$, $P = 0.62$) or in the saline-injected control fish ($F_{1,16} = 0.14$, $P = 0.72$). Hence, own body size did not correlate with aggressiveness in *N. pulcher*, regardless of isotocin treatment, indicating that competitor size asymmetry was not driving the relationships observed with opponent body size.

In IT-treated fish, the rate of aggression against a mirror and against a live rival was not significantly correlated (GLM effect of mirror aggression: $F_{1,15} = 1.02$, $P = 0.33$; Fig. 4a). However, in control fish, rates of aggression against a mirror and against a live rival were strongly positively correlated (GLM effect of mirror aggression: $F_{1,15} = 10.82$, $P = 0.005$; Fig. 4b), indicating that *N. pulcher* were consistently aggressive across stimuli in the control condition.

EXPERIMENT 2: BEHAVIOUR IN SOCIAL GROUPS

Methods

Each of the 19 fish used in this experiment (10 females, 9 males; mean standard length = 47.5 mm, range 40.1–56.8 mm) were given an IT and a control saline treatment, 1 week apart. The order of treatment was counterbalanced across subjects. Only one fish per social group was used in this experiment, and all observations took place within the focal fishes established social groups in their home tank. Focal animals were all mid-ranked helpers (mean rank = 6.5, range 5–8) with an average group size of 10 adults (range 7–15). We chose to concentrate on mid-ranked helpers because these fish produce aggression against other group members ranked below them and they receive aggression from those ranked above. All fish were weighed, measured, individually marked by fin clips and sexed by examination of their external genitalia 1 week prior to the onset of experimentation.

Each fish was observed for 10 min prior to each injection. We counted all aggressive behaviour (see Experiment 1), submissive behaviour (submissive postures, where one fish raises its head upwards towards another, presenting its ventral aspect to the other fish; submissive displays, during which the focal fish assumes a submissive posture and performs a quivering motion with its tail or whole body) and affiliative behaviour (soft touches, where one fish touches the other gently with its head; parallel swims, where

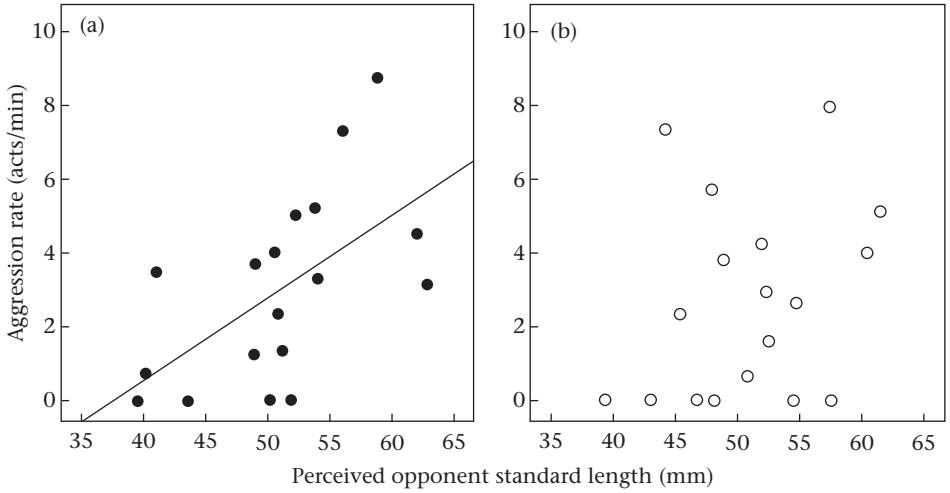

Figure 2. Relation between aggression rate and body size during mirror image aggression trials for (a) isotocin (IT)-treated *N. pulcher* ($P = 0.01$) and (b) saline-treated control fish ($P = 0.26$).

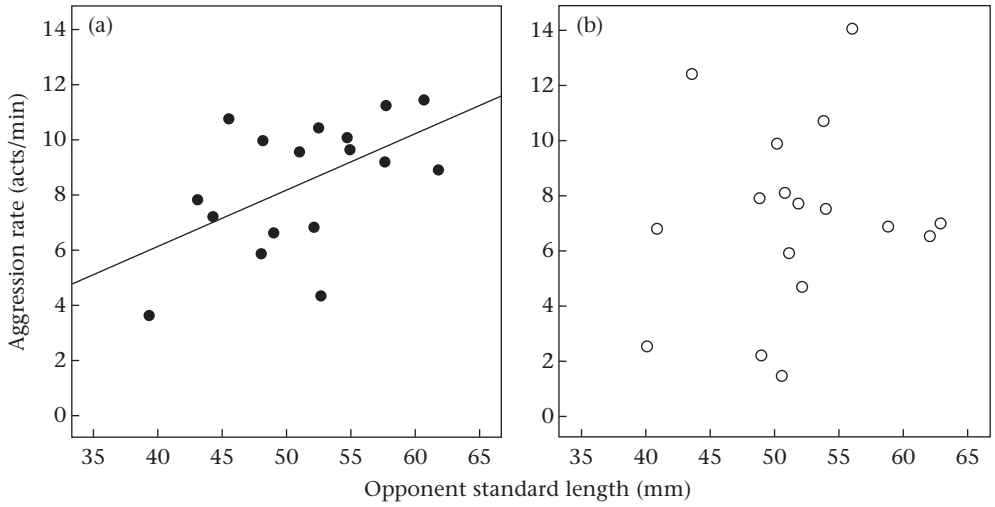

Figure 3. Relation between aggression rate and opponent body size for (a) isotocin (IT)-treated *N. pulcher* ($P = 0.03$) and (b) saline-treated control fish ($P = 0.72$).

two fish swim closely together side by side; follows, where the signalling fish swims closely behind another group member), both given by and received by the focal fish (for a detailed ethogram for this species, see Sopinka et al. 2009). We counted aggressive and affiliative behaviours as the number of these acts produced by the focal fish in 10 min. We calculated the number of submissive behaviours produced per aggressive acts received because submission is most often produced in response to an aggressive act received directly from another conspecific. Following the 10 min pre-injection observation period, we quickly captured the focal fish and injected it with either IT or a saline control. The experimenter delivering the injection and observing the fish was blind to the substance being injected. After the injection, the focal fish was allowed 5 min to recover from handling and injection within its social group. We then observed the focal fish for a 10 min post-injection period during which we recorded the same behavioural measures as in the pre-injection observation. One week later, each focal fish was given the other injection using the same procedure (10 min pre- and post-injection observations). For each of the two injections we calculated the change in the behaviour (aggressive, submissive or affiliative) of the focal fish as follows: (behaviour after injection) − (behaviour before injection), and then compared these values between the IT and control treatments. We also compared the pre- versus post-injection change in total number of behaviours produced across classes of behaviour between the IT and control treatments as a measure of change in total activity level.

We used generalized linear mixed models (GLMM) with an identity link function to analyse this experiment. We included treatment as a within-subjects fixed effect, sex as a between-subjects fixed effect and subject identity as a random effect. This experiment was analysed using SPSS 20.0 for Macintosh.

RESULTS

Fish showed greater submission when they were treated with IT compared to saline (GLMM effect of treatment: $F_{1,35} = 7.05$, $P = 0.01$; Fig. 5a). The fish showed no significant change in their level of aggressive or affiliative behaviour when treated with IT compared to control (aggression: $F_{1,35} = 2.15$, $P = 0.15$; Fig. 5b; affiliation: $F_{1,35} = 0.34$, $P = 0.53$; Fig. 5c). There was no change in overall activity following IT compared to the control treatments ($F_{1,35} = 0.03$, $P = 0.87$; Fig. 5d) and no effect of sex (all $P > 0.05$) in this experiment.

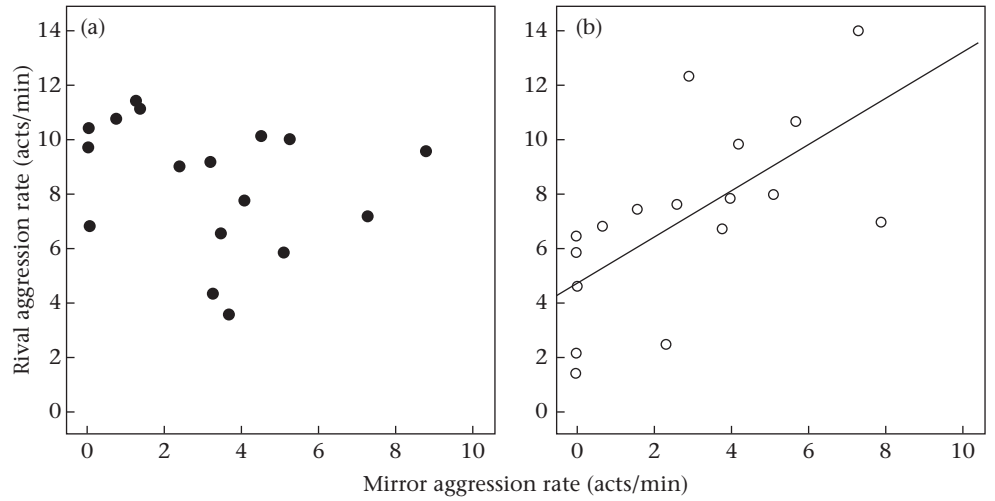

Figure 4. Relation between aggression rate in live rival territorial contest trials and the mirror image aggression trials for (a) isotocin (IT)-treated *N. pulcher* ($P = 0.33$) and (b) control fish ($P = 0.005$).

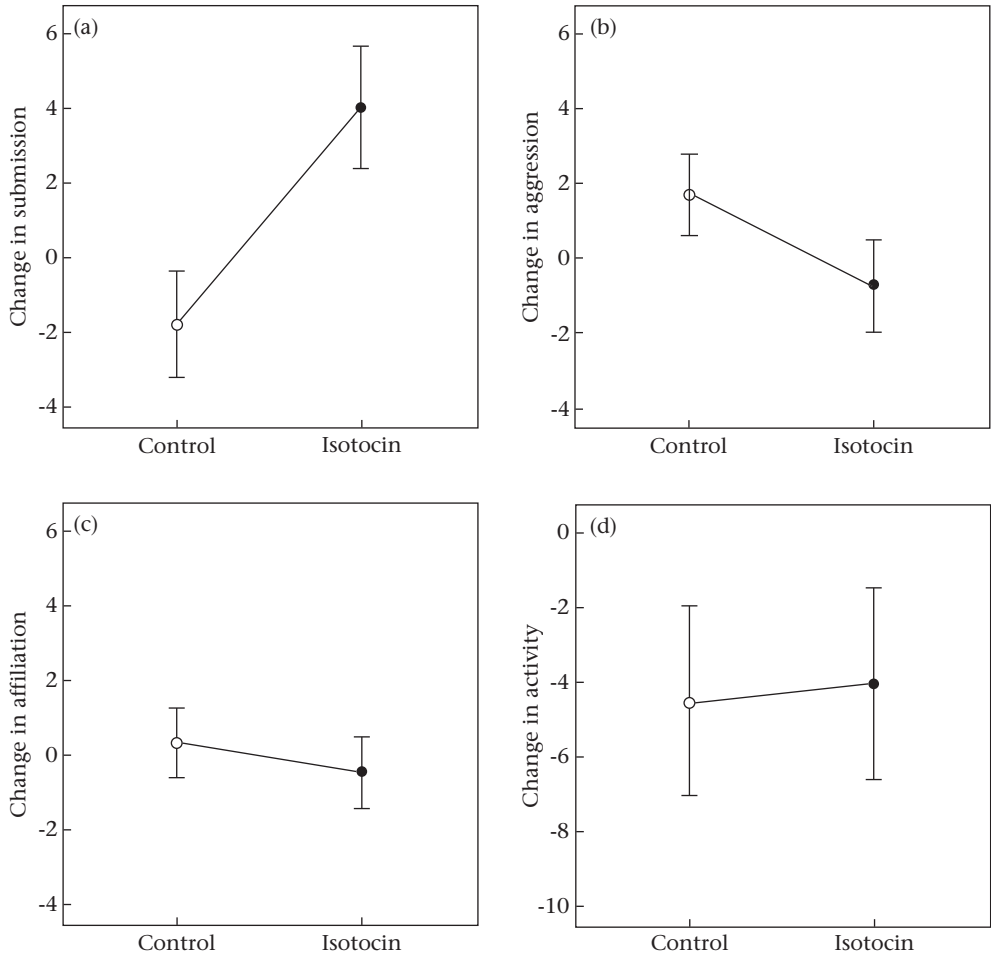

Figure 5. Change in (a) submission ($P = 0.01$), (b) aggression ($P = 0.15$), (c) affiliation ($P = 0.53$) and (d) activity ($P = 0.87$) following injections of isotocin (IT) and saline (control) in *N. pulcher* within their social group.

DISCUSSION

Here we present results of two controlled experiments representing two social contexts, which collectively suggest that experimental increases in IT level enhances sensitivity to social stimuli. In our first experiment we found that *N. pulcher* injected with IT were more sensitive to the size of their perceived opponent and were more aggressive when facing a large opponent. IT-treated fish appeared to make fighting decisions in accordance with their opponents' perceived competitive ability (which is well indicated by body size in this species; Reddon et al. 2011b). In contrast, control fish behaved in accordance with their own intrinsic aggressivity. Aggressive rates for control fish were correlated across the mirror and the live rival trials. Consistent with these results, previous research has also indicated cross-contextual consistency (Riebli et al. 2011) and temporal stability (Chervet et al. 2011) in aggressiveness in unmanipulated *N. pulcher*.

There are at least two reasons why aggression in IT-treated fish is best explained by increased assessment of their perceived opponent (their mirror image) rather than by knowledge of their own fighting abilities. First, there was no correlation between aggression and a given fish's own body size during the rival trials, only a correlation between aggression and opponent body size. It seems logical that the same assessment mechanisms would be in play in a fight against a mirror image and a live rival. Second, previously we showed that assessment of opponent strength is an important determinant of *N. pulcher* contest dynamics, whereas assessment of own strength is relatively unimportant (Reddon et al. 2011b). Although we cannot definitively rule out the possibility that the behavioural effects we observed were driven by the behaviour of the saline-injected opponent and not by the treatment itself, the convergent evidence from the mirror assay (showing the same pattern) suggests this interpretation is most parsimonious.

It is possible that the mirror trial affected the behaviour observed in the rival trial, perhaps by priming the fish to be more aggressive against the rival. However, given that all fish in the aggression experiment received the two assays in the same order, the sequence effects could not have driven the differences we found between the treatments.

Contrary to our prediction, increased opponent body size was correlated positively with increased aggressive behaviour in IT-treated fish during our territorial contest trials. While Reddon et al. (2011b) found that opponent size was the strongest factor influencing the decision to relent in *N. pulcher* contests, large opponent body size was associated with faster acquiescence times and reduced aggressive intensity. Importantly, in those experiments, fish had full physical access (Reddon et al. 2011b), while the fish in the current study were limited to visual displays and noncontact interactions only. It is possible that visual information acts as an 'approach' signal in these fish, while tactile feedback from their opponents provides a 'withdraw' signal (as has been shown in other species; e.g. Rillich et al. 2007). If this is the case, then we

would expect that in fights with physical contact, IT-treated fish would approach faster but also relent faster and show less aggression overall than control fish fighting against large opponents. Visual signals need not necessarily motivate approach and can result in withdrawal responses in many species (Hurd & Enquist 2001), so this prediction will need to be carefully tested in a future study. Nevertheless, our results do show that IT-treated *N. pulcher* are more sensitive to opponent body size than are saline-treated control fish, suggesting that this neuropeptide may be important in opponent assessment and contest decision making.

In our second experiment, fish treated with IT showed increased submission when challenged aggressively. This was a specific change in behaviour, as levels of aggression, affiliation and activity remained unchanged. Submissive displays are an important social signal in *N. pulcher* thought to appease dominant group members, stabilize the social hierarchy and reduce the probability of eviction (Bergmüller & Taborsky 2005; Wong & Balshine 2011a, b). Increased submission rates suggest that IT enhancement results in greater sensitivity to within-group conflict and to the social hierarchy in general. Early life social experiences could have an important organizational effect on the isotocin system and result in life-long behavioural variation in *N. pulcher* (Arnold & Taborsky 2010; Taborsky et al. 2012). Taken together, our results provide evidence of increased social sensitivity in fish that experience an experimental increase in an oxytocin homologue and, therefore, provide support for the hypothesis that oxytocin acts to increase the salience of social stimuli (Ross & Young 2009; Soares et al. 2010; O'Connell & Hofmann 2011).

Interestingly, our injections were peripheral and yet the behavioural changes observed were consistent with a central effect. Traditionally, the effects of oxytocin on behaviour have been revealed by using central administrations or peripherally administered blockers with high transmission into the brain (Thompson & Walton 2004; Goodson et al. 2009; Lukas et al. 2011; Oldfield & Hofmann 2011). However, our study joins a growing literature showing that peripheral nonapeptide administration can lead to behavioural changes (e.g. Propper & Dixon 1997; Semsar et al. 2001; Lema & Nevitt 2004; Ring et al. 2006; Santangelo & Bass 2006; Mennigen et al. 2008; Filby et al. 2010; Madden & Clutton-Brock 2011; Braida et al. 2012). There are at least two possible explanations for how our peripherally administered isotocin could have had centrally mediated effects. First, peripheral administrations of oxytocin might penetrate the blood–brain barrier and reach central receptors (Banks & Kastin 1985a, b; Ring et al. 2006). In male mice, peripheral oxytocin injections had behavioural effects and, surprisingly, these could be blocked by central infusions of an oxytocin antagonist, suggesting that the effects of peripheral oxytocin administrations on behaviour are mediated directly by their action on central receptors (Ring et al. 2006). Furthermore, the blood–brain barrier may be much more permeable to neuropeptides in fish than in mammals (Bernstein & Streicher 1965; Olson et al. 1978). Second, the effects we observed may in fact be mediated by action of peripheral receptors (Goodson & Thompson 2010), as the same populations of neurons in the brain may be part of both the central and the peripheral nonapeptide systems, suggesting a tight integration of the central and peripheral actions of nonapeptides (Ross & Young 2009; Ross et al. 2009; Goodson & Thompson 2010). It is therefore possible that exogenous IT binds to peripheral receptors that exert a secondary effect on behaviour through central IT production or some other mechanism. Although we did not measure isotocin levels in the blood or brain, the behavioural effects we observed coupled with prior results using similar doses of nonapeptides in other fish species (e.g. Semsar et al. 2001; Santangelo & Bass 2006; Mennigen et al. 2008; Filby et al. 2010) suggest that our treatment was appropriate.

Each of our two experiments has interesting implications for the role of oxytocin and its homologues in the architecture of social behaviour, and the results of each experiment suggest important follow-up studies. The results of our territorial aggression experiment indicate that IT may be important in opponent assessment. If true, then we would expect animals treated with oxytocin/isotocin to assess one another more effectively and as a result, have shorter, less costly contests. Similarly, an oxytocin antagonist should result in reduced sensitivity to social information, impaired assessment and longer, more costly contests. These predictions require testing, but should provide a valuable window into the effects of the oxytocin system in regulating contest behaviour and territorial aggression. Our results suggest that oxytocin may be a key neurobiological mechanism underlying decision making in resource contests, which would be an important contribution to understanding the evolution of fighting behaviour in animals (Arnott & Elwood 2009).

The results of our social group experiment suggest that isotocin may join steroid hormones (Bender et al. 2006; Fitzpatrick et al. 2008; Taves et al. 2009) as an important proximate modulator of the social dominance hierarchy in *N. pulcher*. Increased submission given in response to within-group aggression should increase the linearity and stability of the dominance hierarchy by attenuating conflict and reducing the likelihood of eviction (Bergmüller & Taborsky 2005). If so, then the dominance hierarchy in groups where some or all members have experimentally increased levels of IT ought to be more stable and well defined. Conversely, the experimental reduction of IT levels using an antagonist should destabilize hierarchies, increasing conflict and the likelihood of group member eviction. Such follow-up experiments would provide important insights into the role of the oxytocin system in regulating the structure of hierarchical animal societies.

Together, our results support the hypothesis that the oxytocin system modulates responses to social information. Oxytocin may act in the brain to divert limited attention towards social interactions and away from other nonsocial activities (Ross & Young 2009), and therefore, is germane to the neurobiology of social decision making (O'Connell & Hofmann 2011). If so, then selection acting on the oxytocin system may be a crucial component of the evolution of social complexity, and an increased understanding of the sociobiological functions of oxytocin will lead to a fuller understanding of social evolution (Goodson 2008; Goodson & Thompson 2010). Our results contribute to an expanding literature that demonstrates the highly conserved basic behavioural functions of the oxytocin system throughout the vertebrate taxon.

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