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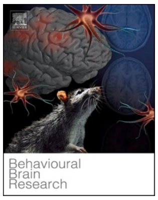

Research article

Central administration of oxytocin increases social interaction and shoaling behaviour in guppies

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ABSTRACT

The nonapeptides vasotocin, oxytocin and their homologues regulate a wide range of social behaviours such as mating, aggression, social recognition and parental care across vertebrates. These varied influences across diverse taxa suggest a highly-conserved, ancestral role for nonapeptides in animal social behaviour. Here, we address the role of nonapeptides in a foundational social behaviour, the tendency of individuals to group with conspecifics. We investigated the effects of administration of nonapeptides on shoaling behaviour in the guppy (*Poecilia reticulata*), a small freshwater fish that is a model system for studying the evolution of social behaviour in the wild. We conducted two experiments using intracerebroventricular administration in wild-origin guppies to investigate the effects of nonapeptides and their antagonists on grouping behaviour, focusing first on oxytocin, and then on vasotocin. We monitored shoaling behaviour for 2.5 h after each administration and found that after 90 min, oxytocin significantly increased social interaction, with a similar effect on shoaling behaviour. Vasotocin did not produce significant changes in social interaction or shoaling preferences, and putative receptor antagonists for oxytocin and vasotocin did not have clear behavioural effects. These findings show that central administration of oxytocin increases shoaling tendencies in guppies, suggesting it influences this fundamental social behaviour. We also found that effects were time-dependent, highlighting the importance of studying the temporal dynamics of nonapeptide actions on behaviour. Our work also demonstrates the feasibility of intracerebroventricular injections for central pharmacological manipulations in small fish, opening new potential avenues for behavioural neuroscience in non-model species.

1. Introduction

Nonapeptides are a family of nine amino acid neuropeptides that are highly conserved across animal taxa, with little variation in molecular structure and with common roles in the regulation of both physiological and behavioural processes. There are two nonapeptide lineages in vertebrates, vasotocin (includes vasopressin in mammals) and oxytocin (includes isotocin in teleost fish and mesotocin in birds), which arose from an ancient gene duplication event in early fish of an ancestral single nonapeptide, as is still found in invertebrates [1,2]. The nonapeptides in the oxytocin and vasotocin lineages play critical roles in regulating social behaviour across vertebrates, influencing processes including sexual behaviour, pair bonding, parental care, aggression and cooperation [3,4]. These behavioural effects of nonapeptides are primarily mediated through their actions in brain regions involved in emotional processing and social interactions, such as the limbic system and hypothalamus [5,6], and many researchers have investigated how nonapeptide effects in these brain areas influence affiliative behaviours and social bonding. For example, in the monogamous prairie vole (*Microtus ochrogaster*), central infusion of vasotocin in males increases affiliative behaviour towards females and aggression toward rival males, while central infusion of oxytocin in females stimulates the formation of partner preferences. These effects are absent in non-monogamous vole species [7–9], and comparative studies of social and non-social rodent species have consistently shown associations between nonapeptides and sociality [10].

While nonapeptides have established roles in the broad regulation of social behaviours, the specific behaviours they influence and the

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direction of effects vary considerably between species. Goodson [3] suggested that different forms of social behaviours may have evolved independently in various species and may be differentially regulated by nonapeptides in each case. This hypothesis highlights the need for studies that explore nonapeptide function across a broad range of vertebrates, particularly in non-mammalian species, to further elucidate how these peptides may have contributed to the evolution of the variety of social behaviour seen in vertebrates [11]. Although the functions of nonapeptides in the regulation of more complex social behaviours have been well studied in mammals, birds and even some amphibians, their effects on one of the simpler, but most fundamental aspects of social living, grouping behaviour, remain relatively understudied. Grouping behaviour is a widespread phenomenon observed across animal species, and it has been the focus of extensive research in behavioural, theoretical and evolutionary biology [12]. Although there has been comparatively little research into the role of nonapeptides in grouping behaviour, the relatively few studies to date suggest this simple social behaviour may be modulated by nonapeptides in different vertebrate taxa.

In birds, comparisons of group sizes in different finch species indicate that more gregarious species also have higher levels of nonapeptide receptor expression [13]. Sex-specific effects of nonapeptides on grouping behaviour are also reported in birds, as preferences for larger groups are increased in female zebra finches (*Taeniopygia guttata*) by central infusions of oxytocin and reduced by oxytocin receptor antagonists, but in male zebra finches it is disruption of vasotocin signalling that reduces these preferences [13,14]. Studies of shoaling behaviour in fish have shown inconsistent effects of nonapeptides, with variation both between and within species. In zebrafish (*Danio rerio*), peripheral administrations of oxytocin or an oxytocin receptor antagonist had no effect on shoaling or social interaction, while both vasotocin and a vasotocin receptor antagonist decreased shoaling and social interactions [15]. However, in the East African daffodil cichlid (*Neolamprologus pulcher*), peripheral administration of oxytocin reduced grouping behaviour and an oxytocin receptor antagonist promoted it in males only [16], while in guppies (*Poecilia reticulata*), shoaling behaviour was not reported to change after peripheral administration of oxytocin [17].

While the variable effects seen in these fish administration studies may be due to species-specific differences in nonapeptide mechanisms, experimental methods may also play a role. When administered intraperitoneally, nonapeptides may act on peripheral receptors, potentially leading to changes in peripheral physiology that indirectly affect behaviour, and so masking direct effects due to the action of nonapeptides on central receptors. Peripherally-injected nonapeptides and their antagonists may also not fully reach central receptors as the teleost blood-brain barrier, while cytologically different from that of mammals [18], is functionally similar [19,20] and so may limit effects when nonapeptides are not directly administered to the brain. Although intracerebral administration is a common behavioural neuroscience technique in rodents, it is relatively rarely used in fish, likely due to the practical challenges associated with performing targeted brain injections in small aquatic animals. However, one of the few studies to investigate fish grouping behaviours after central administration found clearer effects of nonapeptides on social approach. In male goldfish (*Carassius auratus*), central administration of vasotocin reduced the time that highly social subjects spent near another male, while oxytocin significantly increased this time in less social subjects [21]. We therefore sought to use a similar approach to investigate the effects of centrally-administered nonapeptides on shoaling behaviour in guppies, small shoaling fish whose ecology, evolution, and behaviour have been extensively researched [22,23]. In their natural freshwater habitat in Trinidad, the shoaling tendencies of guppy populations are generally positively associated with local predation pressure [24–26]. This variation in grouping behaviour, and the wider utility of fish for neuroscience research into social behaviour mechanisms [27], made guppies an ideal study species for this study into the central effects of nonapeptides on grouping behaviour.

Our objective was to investigate the effects on shoaling behaviour of intracerebroventricular (ICV) administration of nonapeptides and putative nonapeptide antagonists in wild-type guppies, using central administration to avoid potential confounding peripheral effects. ICV administration has been successfully implemented in studies with fish species of similar size to guppies [28–32], and we based our approach on these previously published methods. We carried out two experiments: the first involved the administration of saline, oxytocin (specifically the teleost homologue isotocin) or an oxytocin receptor antagonist, and the second involved the administration of saline, vasotocin or a vasotocin receptor antagonist. In both studies we measured shoaling and social interactions repeatedly over a 2.5-hour period following administration to determine the timing of peak effects, and to assess whether the effects of nonapeptide administration on grouping behaviour persist over time.

2. Material and methods

2.1. Animals and housing

Subjects were 121 adult female guppies selected at random from a laboratory breeding population of mixed-origin wild Trinidadian guppies, housed in mixed-sex 110 L housing tanks. Thirty unfamiliar female guppies were housed in a separate 110 L tank, with four male guppies, and were used as the source for female stimulus shoal fish. We used females due to their typically stronger shoaling tendencies [33–35] and to focus on the effects of nonapeptides on social rather than mating behaviour [36]. All housing tanks had gravel, plants and a shelter, as well as a filter and a heater, and were kept at 26 ± 1 °C with a 12-hour light cycle (lights on at 07:00 h with a 30-minute dawn/dusk period). Fish were fed flake food daily (TetraMin Tropical Flakes, Germany) and supplementary decapsulated brine shrimp eggs (*Artemia sp.*, Brine Shrimp Direct, Ogden UT, USA) three times a week.

2.2. Experimental treatments

In our first experiment, subjects received ICV injections of either i) saline, ii) oxytocin (isotocin, Bachem Americas, Torrance CA, USA) in saline (200 ng/μL) or iii) the oxytocin receptor antagonist desGly-NH2,d(CH2)5[D-Tyr2,Thr4]OVT [37] in saline (150 ng/μL). In our second experiment, subjects received ICV injections of either i) saline, ii) vasotocin (Bachem Americas) in saline (200 ng/μL) or iii) the vasotocin receptor antagonist d(CH2)5[Tyr(Me)2,Dab5]AVP [38] in saline (150 ng/μL). Concentrations were determined from previous similar studies involving central administration of nonapeptides in fish [21,39,40]. All treatments included coadministration of 0.5% Evans blue dye [28,30,41,42] so that the accuracy of injections into the ventricular system could be confirmed at the conclusion of the experiment. In pilot work we evaluated three dyes (Alcian blue, fluorescein, and Evans blue) at different concentrations and found Evans blue was the most suitable and that a concentration of 0.5% was sufficient to determine injection accuracy.

2.3. ICV injection procedure

We used the technique developed by Barbosa et al. [43] with some modifications. We manufactured glass capillary needles for injections and individually calibrated each before use (supplementary material). Subjects were anaesthetized with 40 ppm eugenol (MP Biomedicals LLC, OH, USA), immobilized and a small (<200 μm) hole was drilled in the skull using a microdrill (WPI, Sarasota FL, USA) while observing with a dissection microscope to ensure the microdrill did not contact the brain. A pre-filled glass capillary needle was lowered 300 μm below the surface of the head into the third ventricle using a Leitz micromanipulator (Leica Microsystems, Concord ON, Canada), and a Picospritzer III (Parker Hannifin, Milton ON, Canada) was used to inject 300 nL of treatment.

The ICV procedure took 117 ± 26.95 (mean±SD) seconds per fish, after which each subject was weighed (mean±SD subject mass: 98.4 ± 41.1 mg), and then transferred to a transparent container (9.5 ×4.5 ×19 cm) in the centre of the testing tank to recover from anaesthesia. Subjects were determined to have recovered when they were observed to be mobile and swimming normally in the recovery chamber with no disturbance to equilibrium. In pilot studies, we found that individuals recovered within seven minutes, so we began behavioural tests seven minutes after placing each subject in the recovery container.

2.4. *Behavioural testing*

Behavioural tests were performed in a 75 L 76x31x31 cm tank divided with two silicone-sealed transparent plastic partitions into a central 60cm-wide compartment and two 8cm-wide end compartments (Fig. 1). The tank contained sand substrate and 10 cm depth of water, with thermometers and a heater in each compartment and water temperature maintained at 26 ± 1°C. Lighting conditions were the same as for the housing tanks and tests were performed during the light phase of the day between 0900 and 1600 h. The central compartment held the subject, while one end compartment held a shoal of 5 female guppies and the other was empty to provide an equivalent visual stimulus, but without the shoal. The end compartment containing the shoal was pseudorandomised across tests. In order to maintain a consistent level of engagement from the shoal, a new set of five female guppies was randomly selected from the stimulus fish tank for each test, and then given a 20-minute acclimation period to the testing arena. Removable, opaque white partitions blocked the subject's view of the end compartments before the beginning of the test, and vertical lines drawn on the front of the tank visually divided the central compartment into ten 6cm-wide zones. At the start of each test, the subject was released from the recovery container and the opaque partitions were removed by the experimenter.

Each test was 160 min long and subjects’ behaviour was measured for 10 min at three timepoints: 30 min, 90 min and 150 min after the start. Subjects’ position and behaviour was scored live using the software JWatcher V1.0 [44] by an experimenter who was blind to the treatments and observed tests from an opaque black hide so they could not be seen by the fish. We recorded the time each subject spent in the two end zones of the central compartment and used this to calculate a "shoaling preference" based on subjects' time in the shoal end zone minus time in the no-shoal end zone, following standard definitions of shoaling as two body lengths from a shoal [45,46]. We also recorded time spent swimming headfirst against each end partition, and calculated a "social interaction preference" based on the time subjects spent swimming headfirst at the shoal partition minus time spent swimming headfirst at the no-shoal partition. We have previously found this measure to be an effective indicator of motivation to join a social group in shoaling fish [15]. Ten subjects did not recover or died after surgery. A further ten tests were not analysed due to subjects displaying abnormal swimming behaviour such as constant circular movement or an inability to swim straight (5 saline, 3 oxytocin, 2 oxytocin antagonist), and one due to interruption by a fire alarm (oxytocin antagonist).

After testing, subject fish were euthanized by immersion in ice water [47–49], brains were dissected and snap frozen for subsequent sectioning. Accuracy of injections was confirmed by checking for Evans Blue diffusion during sectioning, and fifteen fish were removed from the dataset at this point as treatments had not diffused through the ventricular system (5 saline, 5 oxytocin, 1 oxytocin antagonist, 3 vasotocin, 1 vasotocin antagonist). The final sample sizes were thus 43 subjects in the oxytocin experiment (saline: n = 15; oxytocin: n = 12; oxytocin antagonist: n = 16) and 42 subjects in the vasotocin experiment (saline: n = 13; vasotocin: n = 14; vasotocin antagonist: n = 15).

2.5. *Statistical analyses*

Data from the oxytocin and vasotocin experiments were analysed separately using R v.4.3.2 [50] and RStudio v.2023.12.1 [51]. We used the package 'lme4' [52] to fit separate linear mixed models to the data on social interaction preference and shoaling preference from each experiment, with treatment, observation timepoint, subject mass, and the interaction between treatment and time as factors in the models. Subject identity was included as a random factor to account for the repeated observations of each subject. Model fit was checked using the 'performance' R package [53], as a result of which the social interaction preference scores for both experiments were square root transformed to meet assumptions. Likelihood ratio tests were used to evaluate interactions and factors in all models. We then ran planned contrasts to compare saline controls with the other administered treatments at each timepoint using the 'emmeans' package [54] and the 'mvt' adjustment for multiple comparisons. We examined the relationship between social

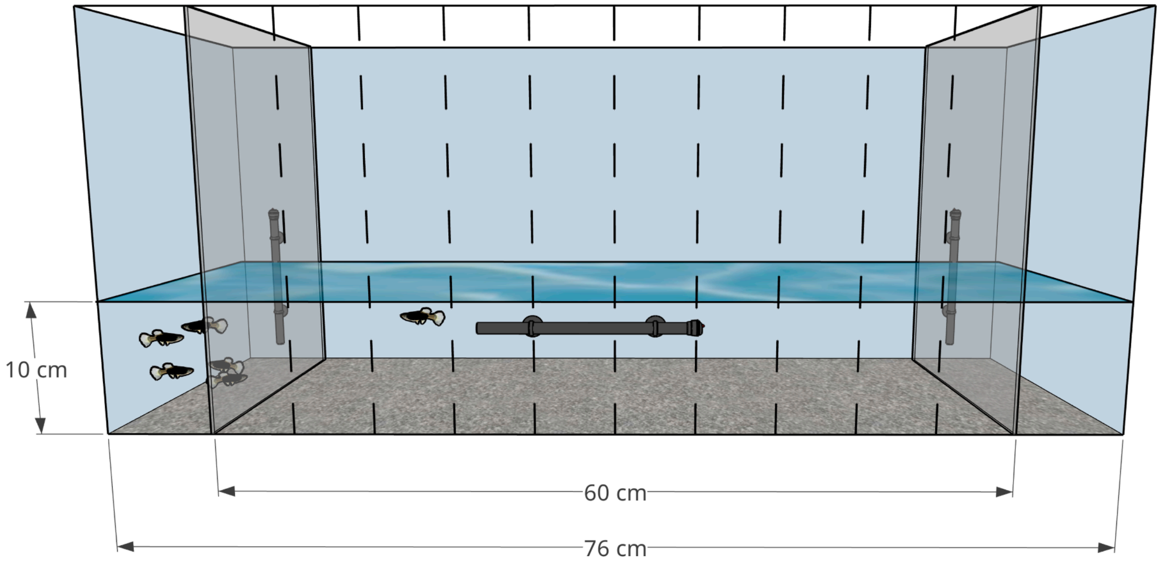

Fig. 1. Behavioural testing tank. This schematic shows the testing tank, divided into three sealed sections with acrylic partitions. The central section contained the subject, and the end sections contained either five female guppies or no fish. The central section had vertical lines drawn on the glass 6 cm apart, so that the position of the subject could be readily tracked and the time spent in the two end zones adjacent to the transparent partitions could be measured.

interaction and shoaling preferences using repeated measures correlations to control for the non-independence of the repeated measurements of each subject, using the 'rmcorr' R package [55]. We ran separate analyses for each experiment, and interaction preference scores in both analyses were square root transformed to meet assumptions.

Data and analysis code will be available on publication via the Zenodo repository (doi:10.5281/zenodo.14336672).

2.6. Ethical note

All tests and procedures were approved by the by the Animal Care Committee of McGill University (Protocol #7133) and were carried out in accordance with the guidelines of the Canadian Council on Animal Care, the Association for the Study of Animal Behaviour, and the US National Research Council (8th edition). Stimulus shoal fish were returned to our breeding populations at the conclusion of the study.

3. Results

3.1. Experiment 1: oxytocin

There was a significant interaction between treatment and timepoint on subjects' social interaction preference (χ^2=11.966, p = 0.018) in the oxytocin experiment (Fig. 2A), as well as significant main effects of treatment (χ^2=16.905, p = 0.010) and timepoint (χ^2=18.105, p = 0.006), but no significant effect of subject body mass (χ^2=0.092, p = 0.762). Contrasts showed that oxytocin-treated subjects had significantly higher social interaction preference scores than the saline subjects at 90 min (p = 0.017) but not at other timepoints, nor were there any differences between the oxytocin receptor antagonist subjects and saline subjects (all p < 0.1, see supplementary material table S1A). The interaction between treatment and timepoint on shoaling preferences in the oxytocin experiment (Fig. 2B) was a non-significant trend (χ^2=7.841, p = 0.098), and there was a significant main effect of treatment (χ^2=12.943, p = 0.044), but no significant effect of timepoint (χ^2=10.006, p = 0.124) or of subject mass (χ^2=0.547, p = 0.460). Contrasts showed that oxytocin-treated subjects had stronger shoaling preferences than saline subjects at 90 min (p = 0.015) but not at other time points, and that there were no differences between subjects treated with oxytocin receptor antagonist or saline at any timepoints (all p > 0.1, see supplementary material table S1B). There was a significant positive correlation between social interaction and shoaling preference scores for subjects in the oxytocin experiment (R_{rm}=0.821, df=85, p < 0.001).

3.2. Experiment 2: vasotocin

There was a non-significant trend (χ^2=9.440, p = 0.051) for the interaction between treatment and timepoint on social interaction preferences in the vasotocin experiment (Fig. 3A), as well as for the main effect of treatment (χ^2=11.755, p = 0.068). The main effect of timepoint on social interaction preferences was significant (χ^2=20.307, p = 0.002), but there was no significant effect of subject mass (χ^2=0.287, p = 0.592). Planned contrasts found no significant differences in social interaction preferences between control subjects and either the vasotocin or anti-vasotocin subjects at any time points (all p > 0.2, see supplementary material table S2A). There was no interaction (χ^2=4.968, p = 0.291) between treatment and timepoint on shoaling preferences in the vasotocin experiment (Fig. 3B), nor were there significant main effects of treatment (χ^2=5.739, p = 0.453), of timepoint (χ^2=8.773, p = 0.187), or of subject mass (χ^2=0.322, p = 0.571). Contrasts found no significant differences in shoaling preferences between control subjects and either the vasotocin or anti-vasotocin subjects at any time points (all p > 0.7, see supplementary material table S2B). There was a significant positive correlation between social interaction and shoaling preference scores for subjects in the vasotocin experiment (R_{rm}=0.662, df=83, p < 0.001).

4. Discussion

We investigated the effects of oxytocin and vasotocin on grouping behaviour in guppies by analysing how ICV administration of these nonapeptides modulated shoaling and social interaction preferences. We found that administration of oxytocin significantly increased grouping behaviour in guppies, with the most pronounced effects occurring 90 min after treatment when oxytocin-treated fish had higher social

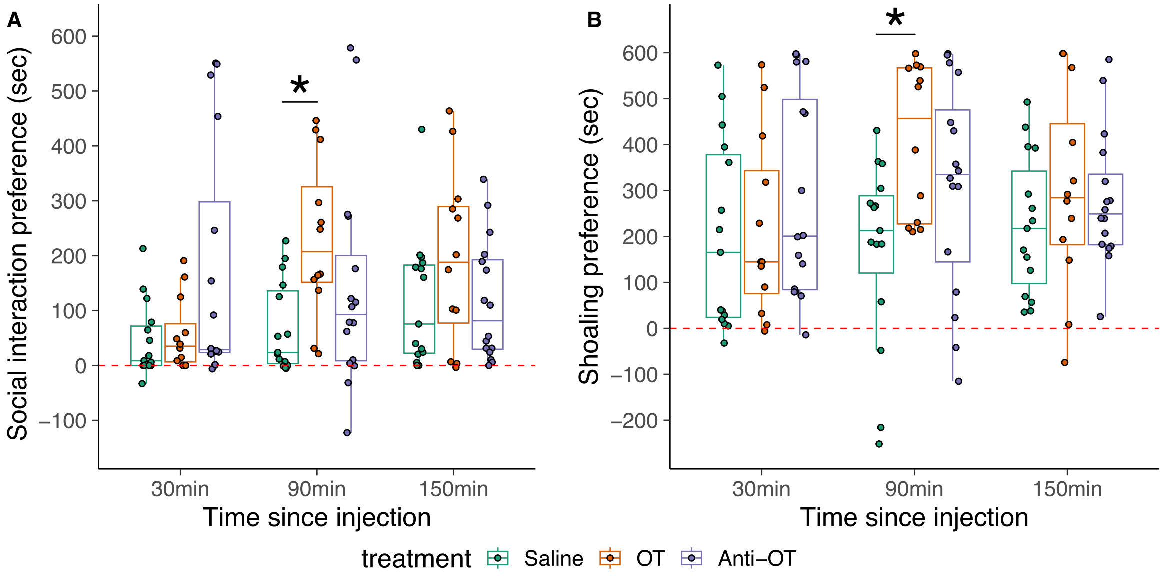

Fig. 2. Oxytocin administration effects. Plots show boxplots with overlaid raw data summarising behaviour of guppy subjects treated with saline (n = 15), oxytocin (n = 12) and oxytocin receptor antagonist (anti-oxytocin, n = 16) in 160-minute long tests after ICV administration. A: Social interaction preference scores, calculated based on the time subjects spent swimming headfirst at the shoal partition minus time spent swimming headfirst at the no-shoal partition. B: Shoaling preference scores, calculated based on the time subjects spent in the 6 cm zone adjacent to the shoal partition minus time in the 6 cm zone adjacent to the no-shoal partition. Positive values indicate a preference for the shoal. *: p < 0.05.

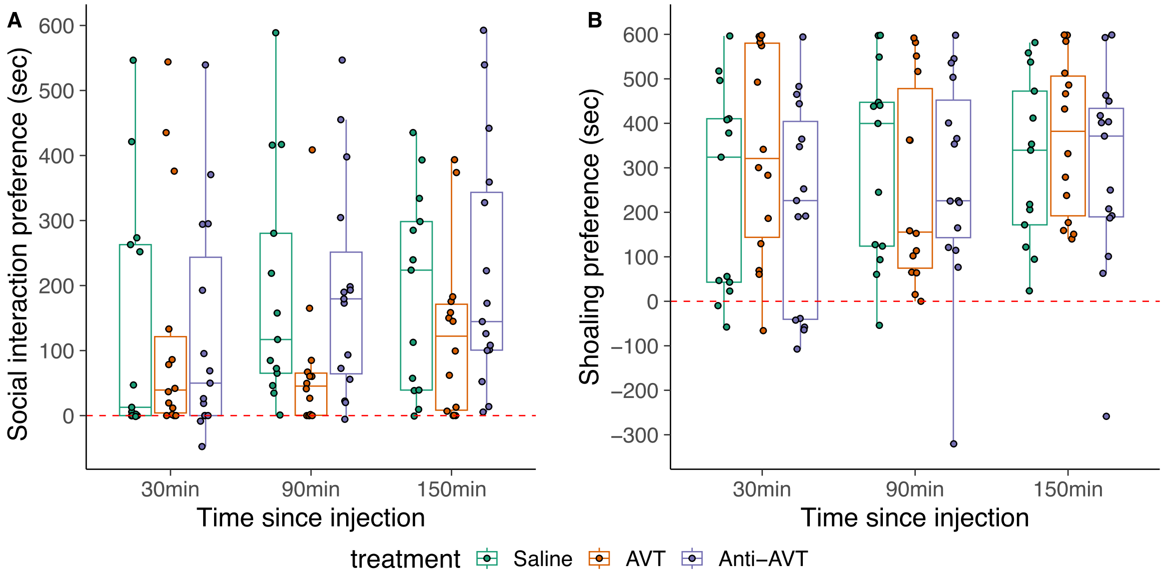

Fig. 3. Vasotocin administration effects. Plots show boxplots with overlaid raw data summarising behaviour of subjects treated with saline (n = 13), vasotocin (n = 14) and vasotocin receptor antagonist (anti-vasotocin, n = 15) in 160-minute long tests after ICV administration. A: Social interaction preference score, calculated based on the time subjects spent swimming headfirst at the shoal partition minus time spent swimming headfirst at the no-shoal partition. B: Shoaling preference scores, calculated based on the time subjects spent in the 6 cm zone adjacent to the shoal partition minus time in the 6 cm zone adjacent to the no-shoal partition.

interaction preferences compared to saline-treated subjects, with weaker but consistent evidence for increased shoaling preference scores. These findings align with previous research in fish showing that central administration of oxytocin in male goldfish increased time spent close to conspecifics [21], and that blocking oxytocin signalling through either receptor gene knockout [56] or targeted antagonism of the oxytocin receptors [57] reduced social preferences in larval zebrafish. Oxytocin is involved in the regulation of complex social behaviours and social cognition in many species [11,58,59], however these results add to growing evidence that oxytocin plays important roles in foundational social behaviours, including the tendency to group with conspecifics.

We found delayed effects of oxytocin administration, with increases in social interaction and shoaling behaviour reaching a peak 90-minutes after administration. In mammals, the nonapeptides have half-lives in plasma of less than two minutes [60,61], however half-lives in the brain are ten-fold higher [62] and the behavioural and physiological effects of nonapeptides in different vertebrate species can last for several hours [63,64]. Peripheral injections of oxytocin have been shown to increase sociality in mice up to 24 h after adminstration [65], while ICV administration of oxytocin in mice strengthens social preferences 65 min after injection, but not before this timepoint [66]. Most ICV nonapeptide administration studies in teleosts have focused on immediate behavioural responses to oxytocin and vasotocin [21,39,67–69], however peripheral administration of vasotocin has been shown to dramatically increase aggression in beaugregory damselfish (*Stegastes leucostictus*) for at least two hours after injection [70]. The time-dependent increase in guppy grouping behaviour that we observed may be due to slower downstream effects mediated by interactions with other neurotransmitter systems [71], however there is also some intriguing evidence in mammals that oxytocin metabolites can have direct, persistent effects on social behaviour. Oxytocin is metabolised in the brain into distinct peptide fragments [72] which persist for longer and have themselves been shown to increase social preferences in mice [73]. Our results suggest that the delayed effects of nonapeptides on social behaviour reported in some mammalian studies may also occur in other vertebrates, and reinforce the importance of studying responses over time. In both experiments, we found that social interaction and shoaling preferences were positively correlated, with higher shoaling tendencies associated with increased social interaction. The consistent effects of oxytocin on both measures suggest that they are biologically similar, however shoaling scores may reflect the influence of social attraction on space use, while social interaction scores may represent active motivation to join the social group [15].

Despite the clear effects of oxytocin administration on behaviour, we saw no response to the oxytocin receptor antagonist and the grouping behaviour of fish treated with it did not differ from that of saline-treated fish. As such, our results do not necessarily show that oxytocin's effects on grouping behaviour in guppies are specifically mediated via oxytocin receptors. We used highly selective antagonists of the mammalian nonapeptide receptors [37,38] and although these have been successfully used in other fish species [21,74], this does not assure receptor selectivity or effective receptor antagonism in guppies. While nonapeptides themselves are highly conserved in vertebrates, their receptors have undergone far more evolutionary divergence [2], with multiple homologous forms in teleosts [75]. Given the very similar molecular structures of oxytocin and vasotocin, variation in receptor sequence or structure may also result in non-specific binding of the antagonists to nonapeptide receptors, potentially resulting in mixed responses to the antagonists if they bind to both receptor types. A potential alternative to our chosen oxytocin antagonist could be the non-peptidergic antagonist L−368,899 which has been validated and shown to be a selective and effective antagonist of oxytocin receptors in zebrafish [57], however similar validation work would be required to confirm suitability in guppies or other fish.

Our results are also notable as they differ from those reported in some studies that used peripheral injections and did not find clear effects of oxytocin on shoaling behaviour, for example in guppies [17] and in zebrafish [15]. ICV injection ensures administered substances circulate throughout the brain via the ventricular system [43], confirmed here by co-administration with Evans blue dye which enabled us to only analyse the behaviour of subjects that received successful ICV injections. While intraperitoneal injections are simpler to carry out, peripherally administered substances may not cross the blood-brain barrier [20] and so may not elicit central effects in a consistent or predictable manner [76,77]. Although there are technical challenges to this approach, ICV administration is feasible in small fish and is a powerful addition to the behavioural neuroscience toolkit for understanding the underlying mechanisms of behaviour in fish [78], including commonly studied smaller species such as guppies, sticklebacks and zebrafish.

In contrast to the effects of oxytocin, we did not find that vasotocin affected grouping behaviour in guppies. Vasotocin administration did not affect shoaling preferences, and while social interaction preferences were slightly lower in fish that received vasotocin injections, this was not a statistically significant difference. The literature indicates that vasotocin has mixed influence on social behaviour in fish, with extensive variation across social contexts, between species, and depending on sex or dominance status [75,78–80]. Although vasotocin appears to inhibit social behaviour in some fish species [15,21,81], it is associated with increases in social behaviour in others [82,83]. Perhaps most surprising is that we did not see an effect of vasotocin in our guppy subjects despite wild guppy populations that are known for increased sociality and shoaling behaviour exhibiting elevated levels of vasotocin expression [84]. However, similarly complex relationships between social behaviour and endogenous versus exogenous vasotocin are seen in the cichlid fish *Astatotilapia burtoni* in which more social territorial males have higher vasotocin levels [85], and yet ICV administration of vasotocin does not produce clear effects on individual behaviour [68]. There were also no clear behavioural effects of the mammalian vasotocin receptor antagonist in our study, and as with oxytocin, validation of specific antagonists for the teleost nonapeptide receptors would help to disentangle the precise roles that vasotocin plays in shoaling and other social behaviour in fish. While vasotocin appears to influence social behaviour in teleosts, our findings and those of others highlight extensive within- and between-species differences in how vasotocin modulates social behaviour [3], and further research will be needed to elucidate the complex and variable roles that vasotocin plays in social behaviour across fish species.

Our study builds on previous research into simple social behaviour in fish and indicates that oxytocin modulates grouping behaviour in guppies, a key study species for understanding the evolution of social behaviour. Our results show that centrally-administered oxytocin increases grouping behaviour, a foundational form of social behaviour, and complement previous work showing that nonapeptides also influence more complex social behaviours in teleosts, including social hierarchy position [80,85,86], parental care [87,88] and cooperative behaviour [89,90]. Nonapeptides thus influence a wide range of both simple and complex social behaviours in teleosts, as well as in other vertebrate taxa, suggesting ancient evolutionary origins for the role of nonapeptides in animal social interactions.

CRediT authorship contribution statement

Simon M Reader: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **María J Cabrera-Álvarez:** Writing – original draft, Methodology, Investigation. **William T Swaney:** Writing – original draft, Visualization, Methodology, Formal analysis.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2026.116363.

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