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Isotocin neuronal phenotypes differ among social systems in cichlid fishes

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Social living has evolved numerous times across a diverse array of animal taxa. An open question is how the transition to a social lifestyle has shaped, and been shaped by, the underlying neurohormonal machinery of social behaviour. The nonapeptide neurohormones, implicated in the regulation of social behaviours, are prime candidates for the neuroendocrine substrates of social evolution. Here, we examined the brains of eight cichlid fish species with divergent social systems, comparing the number and size of preoptic neurons that express the nonapeptides isotocin and vasotocin. While controlling for the influence of phylogeny and body size, we found that the highly social cooperatively breeding species ($n = 4$) had fewer parvocellular isotocin neurons than the less social independently breeding species ($n = 4$), suggesting that the evolutionary transition to group living and cooperative breeding was associated

with a reduction in the number of these neurons. In a complementary analysis, we found that the size and number of isotocin neurons significantly differentiated the cooperatively breeding from the independently breeding species. Our results suggest that isotocin is related to sociality in cichlids and may provide a mechanistic substrate for the evolution of sociality.

1. Introduction

The evolutionary transition from a solitary to a social lifestyle has occurred many times throughout the animal kingdom. An important and open question is whether or not evolution acts on conserved mechanistic pathways (e.g. neural circuits) during these transitions or if there are many possible proximate routes to sociality [1,2]. Across species, the underlying mechanisms regulating social behaviour may be shared and, therefore, we may find similar changes in the neurohormonal machinery controlling the relevant social adaptations among independent social lineages [3]. Thus, it may be possible to detect a consistent mechanistic signature of sociality when comparing highly social species to their less social counterparts. Uncovering such a relationship between sociality and neuronal phenotype would suggest parallelism in the mechanistic basis of social system evolution, helping us to better understand the transition to a social lifestyle.

In order to address this issue, we examined the mechanistic correlates of cooperative breeding as an example of a complex social lifestyle. Cooperative breeding is a social system in which non-breeders belong to social groups and assist in the reproductive efforts of the dominant individuals in their group [4–6]. Cooperative breeders must identify, remember and differentially respond to multiple group members who vary in social status and have distinct individual relationships within the social group [7,8]. Therefore, the transition from independent to cooperative breeding requires behavioural and cognitive adaptations for this heightened level of sociality [9]. For example, cooperative breeders, like other highly social species, must tolerate adult conspecifics other than their mate and offspring [10], and be able to minimize the costs of social conflict [11]. In fishes, cooperative breeding is found only in the lamprologine cichlids of Lake Tanganyika, Africa, where it has arisen several times [12–16]. Multiple closely related cooperative and independently breeding lamprologines live sympatrically, sharing similar diets, biotic and abiotic habitat requirements, and predators [17–20]. Hence these fishes offer an excellent opportunity for comparative analyses of the behavioural and mechanistic underpinnings of complex social lifestyles [14,15,21–23].

In a wide diversity of taxa, the regulation of social behaviour is influenced by the nonapeptides oxytocin and vasopressin (and their non-mammalian homologues), and the impact of these nonapeptides on social behaviour has been well documented [24–28]. In birds and mammals, these nonapeptides have been shown to influence an array of social behaviours and cognitive propensities, including affiliation, bonding, social recognition, social memory, cooperation and aggression [3,29–32]. Many of these behavioural and cognitive characteristics differ between highly social and less social species, including between cooperative and independent breeders [21,33], and thus these nonapeptides provide promising candidates for the proximate substrate of social system evolution [34]. Indeed, nonapeptide circuits have been shown to correlate with social systems in both birds and mammals [10,35–38].

Among teleost fishes, the nonapeptides homologous to oxytocin and vasopressin are known as isotocin and vasotocin, respectively [39]. Extensive evidence has accumulated showing that vasotocin also plays a key role in modulating social behaviour in fishes [40–49]. By contrast, the research on the behavioural role of isotocin is relatively limited [24,50]. However, a small but growing body of work confirms that as with oxytocin in mammals and mesotocin in birds, isotocin is also an important modulator of social behaviour in fishes [22,51–56]. Therefore, both isotocin and vasotocin may be prime proximate targets of social evolution in fishes.

Isotocin and vasotocin are produced in three neuronal groups located in the preoptic area [50], a key brain region for the regulation of social behaviour [34,57]. These areas, known as the parvocellular, magnocellular and gigantocellular populations, can be differentiated by their cell sizes (gigantocellular > magnocellular > parvocellular), by their cytoarchitecture and by their spatial location [58]. Each of these three cell groups projects to the posterior pituitary where nonapeptides are released into the periphery, as well as to diverse targets throughout the brain [59,60], including forebrain regions that have been linked to social behaviour (e.g. the ventral telencephalon [34,57]). Parvocellular, magnocellular and gigantocellular cells appear to serve different functions in the regulation of social behaviour [60,61]. For example, in the African cichlid *Astatotilapia burtoni*, parvocellular vasotocin cells

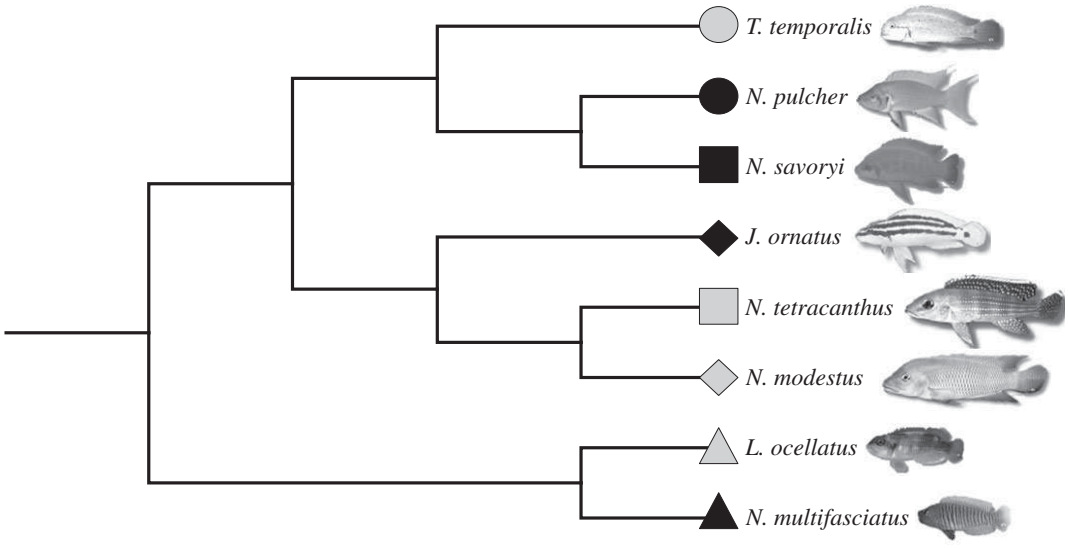

Figure 1. The phylogenetic relationships among the eight species of cichlid fishes included in the current study. Black symbols represent cooperatively breeding species; grey symbols represent independently breeding species. Each shape–colour combination represents a different species.

are associated with submissive behaviour in subordinate males while magnocellular cells are associated with aggression in dominant males [45]. Godwin & Thompson [24] hypothesized that this role for magnocellular vasotocin cells in regulating approach and aggression and for parvocellular cells in regulating social withdrawal and submission may be a more general pattern in fishes.

In this study, we examined the number and size of the parvocellular, magnocellular and gigantocellular isotocin and vasotocin neurons in the preoptic area of each of eight species of lamprologine cichlids using animals collected from the wild (figure 1). We selected four highly social cooperative breeders (*Neolamprologus pulcher*, *N. multifasciatus*, *N. savoryi* and *Julidochromis ornatus*) and four species that are less social independent breeders (*N. tetracanthus*, *N. modestus*, *Telmatochromis temporalis* and *Lamprologus ocellatus*), representing three independent transitions to cooperative breeding [16]. These species live in similar habitats, characterized by a mix of sandy and rocky substrate at depths of 5–15 m, and are exposed to similar environmental conditions in Lake Tanganyika. We compared the number and size of each cell type in each cell group, controlling for body size and phylogenetic relatedness, to look for consistent differences between the cooperatively and independently breeding species. Using a complementary approach, we used discriminant function analyses to determine whether individual fish could be correctly classified into cooperatively or independently breeding social systems based on the size and number of their isotocin or vasotocin cells. A consistent pattern of nonapeptide cell size or number between cooperatively and independently breeding species would suggest that these cell populations were modified in parallel during the emergence of cooperative breeding in the lamprologine cichlids, the only group of fishes to have evolved true cooperative breeding.

2. Material and methods

2.1. Study site and field methods

All fish were sexually mature males captured from the southern basin of Lake Tanganyika near Mpulungu, Zambia (8°46′52″ S, 31°5′18″ E) in February–March, 2013. Ten adult males from each of the eight cichlid species were located using SCUBA at depths of 6–12 m and captured using fence- and hand nets. Each fish was slowly brought to the surface, measured for standard body length (the distance from the tip of the snout to the end of the caudal peduncle) with callipers (to 0.1 mm; see the electronic supplementary material, table S1 for the average length of each species), anaesthetized by immersion in a benzocaine solution, and swiftly decapitated. Sex was confirmed by post-mortem examination of the gonads. Whole brains were carefully extracted, and preserved in 4% phosphate-buffered paraformaldehyde prior to transport back to the University of Alberta, Canada.

2.2. Histological methods

Prior to immunohistochemistry, brains were cryoprotected in 30% sucrose in 0.1 M phosphate-buffered saline (PBS) for 24 h, embedded in gelatin, and sectioned on the coronal plane at a thickness of 40 µm. Twelve of the 80 brains (six cooperative breeders: two *N. multifasciatus*, two *N. pulcher*, two *N. savoryi*; and six independent breeders: two *L. ocellatus*, three *N. modestus*, one *T. temporalis*) were damaged during extraction from the skull or sectioning and therefore were not used, reducing our final sample size to 68 fish (34 from each social system). Free floating sections were incubated in blocking serum (1 : 10 normal donkey serum, Jackson Immunoresearch Laboratories) with 0.1 M PBS and 0.04% Triton X for 1 h. Tissue was then double-labelled with polyclonal anti-oxytocin (Peninsula Laboratories International; catalogue number T-5021) and anti-vasopressin (Peninsula Laboratories International; catalogue number T-4563) antibodies raised in guinea pigs and rabbits, respectively, against the mammalian forms of oxytocin and vasopressin (1 : 5000, Peninsula Laboratories, San Carlos, CA) with 0.1 M PBS and 10% normal donkey serum for 24 h (−4°C). After rinsing with PBS, immunoreactive isotocin and vasotocin cells were stained by incubating for 2 h in fluorescent secondary antibodies (1 : 200 Alexafluora 594 donkey anti-guinea pig; 1 : 200 Alexafluora 488 donkey anti-rabbit; Jackson Immunoresearch Laboratories; catalogue numbers: 706-005-148 and 711-005-152, respectively). The tissue was then rinsed in PBS again and mounted onto gelatinized slides.

Oxytocin and vasopressin positive neuron cell bodies were visualized with a confocal microscope (Leica TCS SP5) using 488 nm argon ion and 543 nm green HeNe lasers and a 63× water immersion lens. Z-step sizes were adjusted according to the size and density of cell groups in individual brains. The mammalian oxytocin antibody that we used stains both isotocin and vasotocin cells in fishes, while the vasopressin antibody is specific to vasotocin expressing cells (J. Goodson, personal communication). Using a double labelling technique, vasotocin cells were identified as those that were immunoreactive to both the oxytocin and vasopressin antibodies, while the isotocin cells were those stained only by the oxytocin antibody (figure 2). In the fishes studied to date, isotocin and vasotocin cells are intermingled, but each individual cell produces only isotocin or vasotocin (reviewed in [50]). Fiji software (ImageJ version 2.0.0) was used to measure the area of isotocin and vasotocin cells by tracing the circumference of the fluorescently labelled cell body, and cell counts were obtained using the Cell Counter plugin.

Images were scored blind to the social system of each species and the body length of each individual. Vasotocin and isotocin expressing cell bodies were found exclusively in the preoptic area. Each nonapeptide cell group (parvocellular, magnocellular and gigantocellular) was distinguished within each individual fish using a combination of cell size, morphology and location criteria (following [58]; figure 2). We counted all cells of each type in each cell group that showed a clearly discernable perimeter and a visible neurite. We randomly selected cells from which to measure cell body area by assigning a unique integer to each cell and selecting 5% of the cells (minimum 10) of each type, in each group, in each fish using a random number generator.

2.3. Phylogenetic tree

Phylogenetic relationships among lamprologine cichlids are complicated by introgressive hybridization, which makes phylogenies constructed solely from mitochondrial DNA unreliable for some species [62–64]. Therefore, we used a recent tree for the lamprologines [16] that estimates the phylogenetic relationships for 69 species of lamprologine cichlids based on three mitochondrial and six nuclear nucleotide sequences, using a Bayesian Markov chain Monte Carlo model. For the purposes of the current study, the consensus tree from Dey *et al*. [16] was trimmed to include only the eight species of interest and visualized using MESQUITE v. 3.10 [65] (figure 1).

2.4. Statistical analyses

We used Bayesian phylogenetically controlled statistical analyses to test for associations between social system and isotocin cell count, isotocin cell area, vasotocin cell count and vasotocin cell area. We included cell group (parvocellular, magnocellular and gigantocellular), along with fish identity as a random effect. Because body size has been shown to correlate with nonapeptide cell size and number in other fish species [61,66], we also included body length as a covariate. When our model revealed a significant effect of cell group, we conducted post-hoc tests of the association between social system and nonapeptide cell count or cell area separately for each cell group, including body length as a covariate, and fish identity as a random effect.

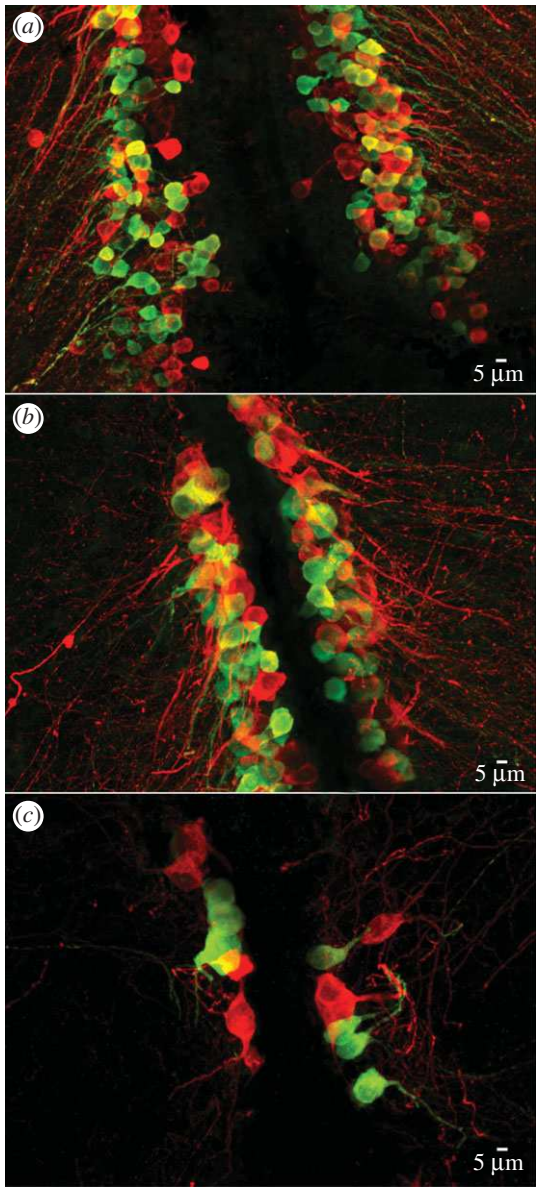

Figure 2. Confocal photomicrographs showing immunohistochemical labelling of nonapeptides in the preoptic area of a wild caught male *Neolamprologus pulcher*. Green cells are vasotocin positive and red cells are isotocin positive. (*a*) Parvocellular, (*b*) magnocellular, (*c*) gigantocellular cell groups. The top of each panel corresponds to the dorsal aspect.

For all models and post-hoc tests, we used the package 'MCMCglmm' [67] to perform generalized linear mixed models based on a Markov chain Monte Carlo algorithm. Within the MCMCglmm package, the phylogenetically controlled analysis is implemented by including the phylogenetic tree as a random factor in the model (ç). Following examples from de Villemereuil & Nakagawa [68], we defined our priors for the model as $V = 1$ and $\nu = 0.02$ for both random effects and the residual variance, which correspond to an inverse-Gamma distribution with shape and scale parameters equal to 0.01, which is canonical [69]. We ran each model for 5 million iterations, with a burnin of 1000, and a thinning interval of 500. With these priors and settings, there was no autocorrelation between successive stored iterations for any of the models [70]. Because Bayesian statistics are based on iterative processes, the outcomes can vary slightly between runs. Therefore, we repeated the analyses three times, and report mean values for the 95% highest posterior density interval (HPD), as well as the P_{MCMC}, which are the Bayesian equivalents of 95% confidence intervals and *p*-values, respectively. Associations were considered significant when the 95% HPD excluded zero, and P_{MCMC} was less than 0.05. The Bayesian phylogenetically controlled analyses were conducted using R v. 3.2.1 within R STUDIO.

In order to further examine whether the isotocin or vasotocin neuronal phenotypes differed predictably between cooperatively and independently breeding species, we conducted a discriminant

Table 1. Results of Bayesian phylogenetically controlled generalized linear mixed models to test for associations between social system and: isotocin cell count, isotocin cell area, vasotocin cell count and vasotocin cell area. We included cell group (parvocellular, magnocellular and gigantocellular) and body length as covariates. For cell group, magnocellular and gigantocellular were assessed relative to parvocellular. Fish identity was included as a random effect. Because Bayesian statistics are based on iterative processes, the outcomes can vary slightly between runs. Therefore, we repeated the analyses three times, and report mean values for the 95% highest posterior density interval (HPD), as well as the P_{MCMC}, which are the Bayesian equivalents of 95% confidence intervals and *p*-values, respectively. Fixed effects in italics are considered statistically significant (i.e. the 95% HPD excludes zero, and P_{MCMC} is less than 0.05). For full statistical details, see Material and methods.

independent variable	fixed effects	95% HPD		P_{MCMC}
isotocin cell count	*social system*	*2.7*	*62.4*	*0.03*
	body size (SL)	2.6	99.4	0.07
	brain area (*magnocellular*)	*36.1*	*77.7*	*<0.001*
	brain region (*gigantocellular*)	*151.9*	*192.8*	*<0.001*
vasotocin cell count	social system	−8.4	48.1	0.12
	body size (SL)	−41.3	42.4	0.77
	brain area (*magnocellular*)	*12.3*	*33.6*	*<0.001*
	brain region (*gigantocellular*)	*72.4*	*92.5*	*<0.001*
isotocin cell area	social system	−29.4	92.2	0.24
	body size (*SL*)	*44.1*	*223.1*	*0.008*
	brain area (*magnocellular*)	*−180.2*	*−140.5*	*<0.001*
	brain region (*gigantocellular*)	*−294.5*	*−254.4*	*<0.001*
vasotocin cell area	social system	−21.1	16.4	0.79
	body size (*SL*)	*33.7*	*93.5*	*<0.001*
	brain area (*magnocellular*)	*−68.7*	*−53.3*	*<0.001*
	brain region (*gigantocellular*)	*−126.1*	*−109.6*	*<0.001*

function analysis for each nonapeptide. We included this supplementary analysis because discriminant function analysis is a sensitive method for studying group differences among several variables simultaneously [71]. To control for body size, we used the residuals of the linear regression of body length on each variable in our analyses. To test for the predictive ability of the resultant discriminant functions, we used a leave-one-out cross-validation process, wherein each animal is classified based on the discriminant function computed while excluding that individual, resulting in a conservative test of predictive power [72]. The discriminant function analyses were done using IBM SPSS Statistics version 23.

3. Results

3.1. Phylogenetically controlled analyses

Cooperatively breeding species had fewer isotocin cells in their preoptic area than the independently breeding species (table 1). More specifically, the cooperative breeders had fewer parvocellular isotocin cells than the independent breeders (table 2 and figure 3*a*). There was no association between magnocellular or gigantocellular isotocin cell counts and social system (table 2 and figure 3*b*,*c*). We did not detect any relationship between social system and vasotocin cell count (table 1 and figure 3*d*–*f*). Also there was no relationship between social system and isotocin (table 1 and figure 4*a*–*c*) or vasotocin cell area (table 1 and figure 4*d*–*f*).

3.2. Discriminant function analyses

Using both the number and size of the isotocin neurons in each of the parvocellular, magnocellular and gigantocellular areas as predictors, the discriminant function analysis was able to correctly classify

Table 2. All of our initial models revealed a significant effect of cell group (see the electronic supplementary material, table S1). Therefore, we conducted post-hoc tests of the association between social system and nonapeptide cell count or cell area separately for each cell group. As above, these results were generated using Bayesian phylogenetically controlled generalized linear mixed models, including body length as a covariate, and fish identity as a random effect. Mean values for the 95% highest posterior density interval (HPD) and P_{MCMC} are presented. Fixed effects in italics are considered statistically significant (i.e. the 95% HPD excludes zero, and P_{MCMC} is less than 0.05). For full statistical details, see Material and methods.

independent variable		fixed effects	95% HPD		P_{MCMC}
isotocin cell count	parvocellular	*social system*	*5.8*	*143.4*	*0.03*
		body size (SL)	−21.4	236.2	0.13
	magnocellular	social system	−51.2	54.7	0.78
		body size (SL)	*10.9*	*147.7*	*0.03*
	gigantocellular	social system	−1.0	14.0	0.06
		body size (SL)	−9.9	18.5	0.55
vasotocin cell count	parvocellular	social system	−11.9	106.9	0.10
		body size (SL)	−105.7	85.6	0.93
	magnocellular	social system	−18.5	30.6	0.52
		body size (SL)	−21.5	47.5	0.32
	gigantocellular	social system	−2.3	11.3	0.28
		body size (SL)	−7.9	12.9	0.56
isotocin cell area	parvocellular	social system	−30.4	35.1	0.96
		body size (SL)	−19.0	50.9	0.34
	magnocellular	social system	−32.4	112.4	0.20
		body size (SL)	−7.7	146.2	0.08
	gigantocellular	social system	−14.4	164.2	0.07
		body size (SL)	*106.3*	*386.2*	*<0.001*
vasotocin cell area	parvocellular	social system	−18.6	10.4	0.62
		body size (SL)	−5.9	34.2	0.10
	magnocellular	social system	−27.0	10.3	0.38
		body size (SL)	*24.9*	*84.4*	*0.004*
	gigantocellular	social system	−18.1	31.6	0.56
		body size (SL)	*72.8*	*159.0*	*<0.001*

individuals into cooperatively or independently breeding systems (Wilks $\lambda = 0.71$, $\chi^2 = 21.49$, d.f. = 6, $p = 0.001$; figure 5*a*). The isotocin analysis correctly classified 72% of individuals into cross-validated social systems. The size and number of vasotocin cells; however, did not result in the accurate classification of fish into social system (Wilks $\lambda = 0.83$, $\chi^2 = 11.48$, d.f. = 6, $p = 0.08$; figure 5*b*), correctly classifying only 57% of individuals as cooperative or independent breeders.

4. Discussion

We detected an association between the number of isotocin expressing cells in the preoptic area and social system in cichlid fishes. Specifically, in our sample of eight species of lamprologine cichlids, all collected in the wild, we found that males from highly social cooperatively breeding species had fewer parvocellular isotocin cells in their preoptic area than did males from less social independently breeding species, even after accounting for body size and phylogenetic relatedness. Furthermore, the fishes in our sample could be classified successfully into their social system on the basis of their isotocin neurons alone, suggesting that isotocin neuronal phenotypes differ systematically between cooperatively and independently breeding lamprologine cichlids. We did not detect any such differences between

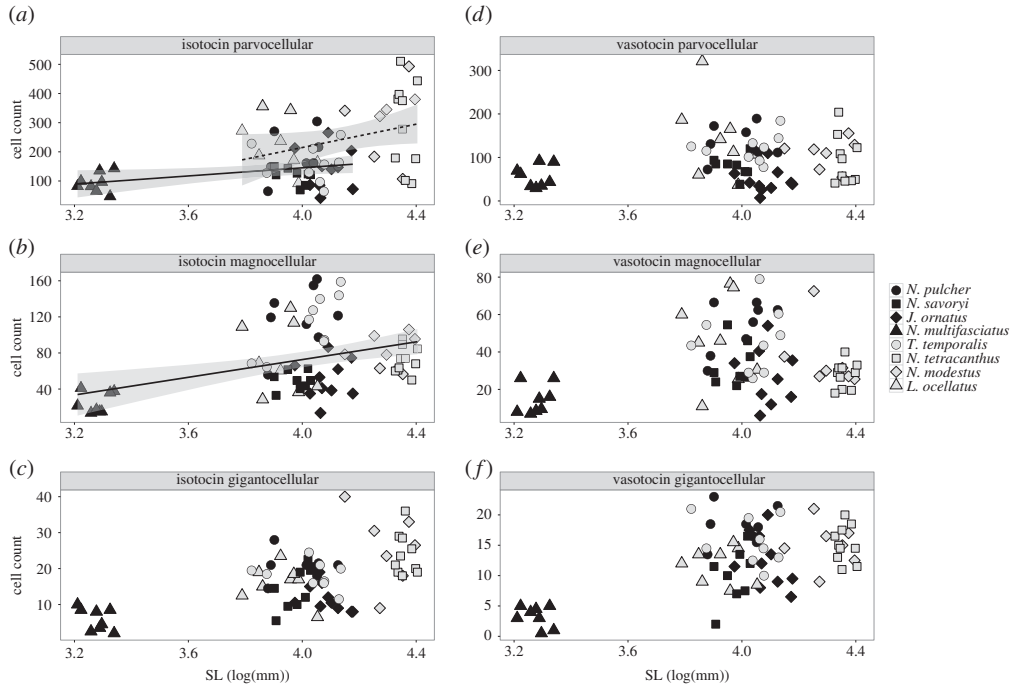

Figure 3. Isotocin (*a–c*) and vasotocin (*d–f*) cell counts plotted against standard length (SL) for each of eight species of lamprologine cichlid fishes. Black symbols represent cooperatively breeding species; grey symbols represent independently breeding species. Fit lines indicate a significant relationship between body length and cell count ($P_{MCMC} < 0.05$) while separate fit lines for cooperatively (solid line) and independently (dashed line) breeding species indicate a social system difference in cell count ($P_{MCMC} < 0.05$).

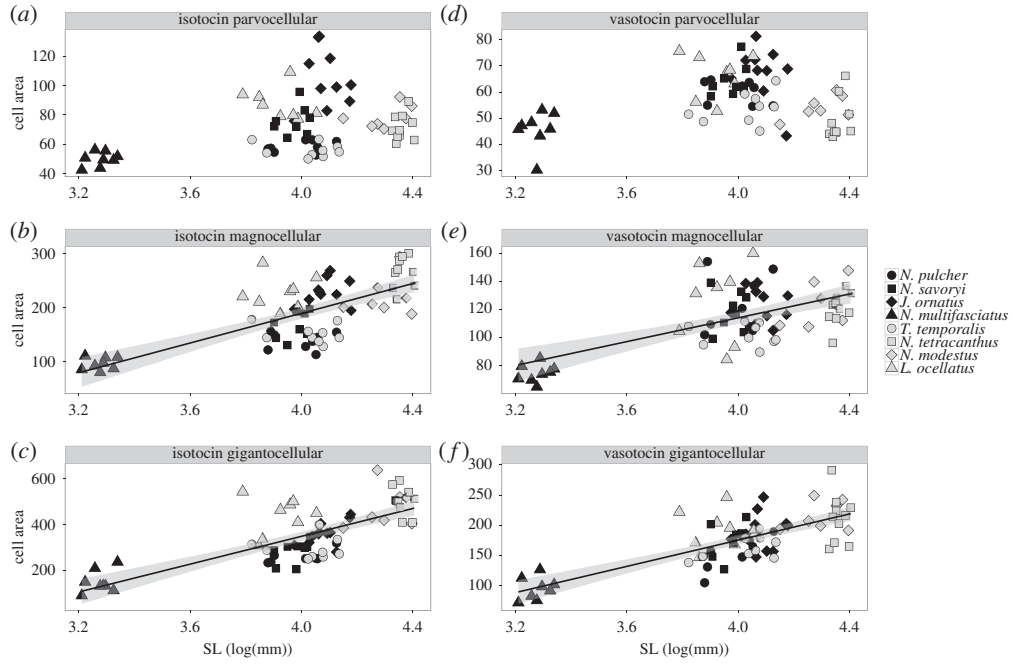

Figure 4. Average isotocin (*a–c*) and vasotocin (*d–f*) cell areas plotted against standard length (SL) for each of eight species of lamprologine cichlid fishes. Black symbols represent cooperatively breeding species; grey symbols represent independently breeding species. Fit lines indicate a significant relationship between standard length and cell area ($P_{MCMC} < 0.05$).

cooperative and independent breeders in vasotocin cell size or number, and fish could not be classified into their social system based on their vasotocin cells.

In this study, we have shown for the first time that isotocin neuronal phenotypes differ among closely related species of fishes and that these differences in neurons map on to the variation observed in

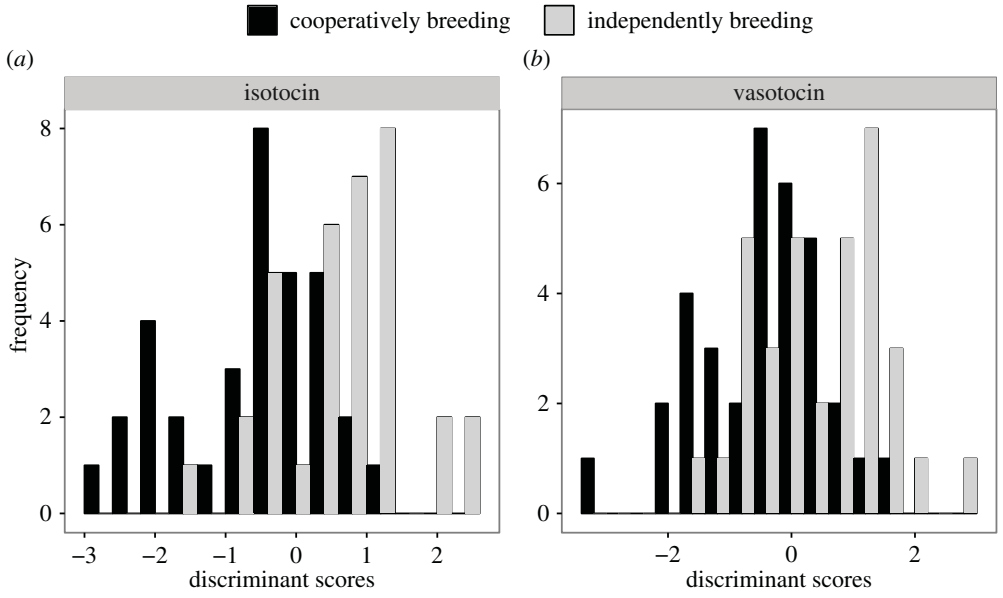

Figure 5. Discriminant function scores for (*a*) isotocin neuronal phenotypes and (*b*) vasotocin neuronal phenotypes. Individual fish were significantly classified into their cross-validated social system using their isotocin ($p < 0.05$) but not their vasotocin neuronal phenotype ($p > 0.05$).

social systems. Previous work in one of our sampled species, the cooperatively breeding *N. pulcher*, suggests that isotocin regulates social behaviour. For example, exogenous administrations of isotocin reduced shoaling motivation among unfamiliar individuals, while blocking isotocin had the opposite effect [53]. *Neolamprologus pulcher* with higher levels of free isotocin in their brains showed lower rates of affiliative behaviour than did individuals with lower levels of this peptide [54]. Collectively, these results suggest that isotocin may inhibit affiliative tendency or social tolerance. In mammals, oxytocin is typically characterized as stimulating pro-social behaviour; however, oxytocin may also reduce social tendencies and promote anti-social behaviours in some species and/or social contexts [73]. Because isotocin is likely to have different functions depending on where in the brain it is released and with which receptors it interacts [27], more information about the precise role of each of the three isotocin cell populations, their projections throughout the brain and their patterns of receptor binding will be essential to unravel the complete role of isotocin in modulating social behaviour in the lamprologines. Our current data suggest that the parvocellular region is potentially important in generating differences in social behaviour among closely related cichlid species. Future work should endeavour to examine the effects of isotocin release on different parvocellular targets and to correlate parvocellular isotocin levels with observations of social behaviour within individuals.

Previous molecular studies have found that cooperatively breeding and independently breeding lamprologine cichlids do not show a consistent pattern of isotocin brain gene expression, with some cooperatively breeding species showing higher expression of isotocin than their independently breeding relatives while other cooperative breeders show lower expression or no difference [14,22]. There are several possible reasons why our isotocin cell count data contrast with the previous data on isotocin brain gene expression. First, measures of whole brain gene expression capture isotocin transcription occurring in all three cell groups, and thus could have obscured the pattern we observed in the parvocellular region. However, it is worth noting that the parvocellular difference that we observed was strong enough to drive an overall difference in isotocin cell number across cell populations. Second, cell size or number data may contrast with gene expression data (e.g. [74]) and higher isotocin gene expression could indicate greater production of isotocin, while a greater number or larger size of isotocin cells may indicate greater storage [75]. It is possible that cooperative breeders store less of the peptide and instead turn it over more rapidly, through either central signalling or release into the periphery [48]. For example, dominant *N. pulcher* have higher vasotocin gene expression in their brains than do subordinate group members [76], but subordinates have higher levels of free vasotocin in their brains [54], suggesting a possible production versus storage discrepancy. Our study highlights the need for multiple complementary approaches in order to understand the role of the nonapeptides in regulating social behaviour within and across species.

Social and mating system differences between species of birds and mammals tend to be mediated by differences in the number and location of nonapeptide receptors in the brain rather than differences in the nonapeptide producing cells themselves, which tend to be highly consistent in the species studied thus far [10,73,77–79]. Mammals in particular show dramatic species differences in nonapeptide receptor distribution; however, the links to sociality vary in direction and magnitude across species [73]. The current data on nonapeptide receptors in fish brains is limited, particularly for isotocin [24,50,60,80], and this would be a fertile area for future work. A key implication of our results is that, in contrast to mammals, nonapeptide production or storage in the preoptic area can differ among related species in relation to their social behaviour.

We did not find any consistent association between cooperative breeding and the vasotocin neurons in our sample of eight lamprologine cichlid fishes. Our results contrast with Dewan *et al*. [81], who found that a shoaling species of butterfly fish (*Chaetodon miliaris*) had larger vasotocin cells in their preoptic area when compared with a closely related solitary species (*Chaetodon multicinctus*). Although it is not possible to conclusively attribute neural differences to differences in social system by comparing only a single pair of species, their findings do suggest that vasotocin neurons can differ between closely related fish species that differ in social system. Our null result with respect to vasotocin suggests that vasotocin neurons are less consistently associated with social system in the lamprologine cichlids than are isotocin neurons, although further work on individuals of both sexes from a greater variety of species coupled with data on social status and individual behaviour will be required to fully understand the relationship between social system and vasotocin across cichlids.

The cause and effect relationship between neuronal phenotype and social system is not necessarily straightforward. Because social context can affect nonapeptide neuronal phenotypes (e.g. [82]), differences between cooperative and independent breeders may be a consequence rather than a cause of the different social organizations that these fishes live within. Early life experience in a social group could also have organizational effects on nonapeptide neuronal phenotypes and therefore developmental conditions rather than evolved diversity may partially explain distinctions between cooperatively and independently breeding lamprologines in their isotocin neuronal phenotype. Controlled developmental experiments in the laboratory (e.g. [83–85]) will be required to disentangle these possibilities and conclusively rule out plastic differences in nonapeptide cells between cooperative and independent breeders. The species that we examined here would be amenable to such controlled experimentation.

We found that cooperatively breeding lamprologine cichlids differ from their closest independently breeding relatives in isotocin but not vasotocin neuronal phenotypes. Controlling for phylogeny and body size, cooperative breeders had fewer parvocellular isotocin cells than did the independent breeders. Future studies should aim to examine isotocin circuits in more detail, and in particular a comparison of receptor distributions between cooperatively and independently breeding species would be valuable. Additionally, controlled laboratory experiments aimed at mapping individual variation in social behaviour onto nonapeptide producing cells or establishing the effects of developmental environment and social context on nonapeptide neuronal phenotype in these cichlids would likely be illuminating. Future work should also examine both males and females, as sex differences in nonapeptide circuits and function have been observed in other fish species (e.g. [61,66,74,86]). Our study highlights isotocin as a potential mechanistic substrate of social system evolution in the most speciose group of vertebrates, the teleost fishes.

Ethics. Handling time and stress were minimized for all study animals. None of the included species are endangered or threatened and all are highly abundant in the studied area of Lake Tanganyika. The methods described for animal capture and euthanasia were approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol No. 10-11-71) and adhered to both Canadian and Zambian laws, as well as the guidelines of the Canadian Council for Animal Care.

Data accessibility. The full dataset is available as the electronic supplementary material.

Authors' contributions. A.R.R., C.M.O., D.R.W., P.L.H. and S.B. conceived and designed the study. A.R.R., C.M.O., J.K.H., I.Y.L. and S.M.R. conducted the field sampling. E.N. and J.C. performed the immunohistochemistry and microscopy. E.N. quantified the cells. C.M.O. and A.R.R. analysed the data. C.M.O. made the figures. A.R.R. wrote the first draft of the manuscript. All coauthors contributed to the final version of the manuscript. P.L.H. and S.B. should jointly be considered the senior author on this work.

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References

1. Hofmann HA *et al.* 2014 An evolutionary framework for studying mechanisms of social behavior. *Trends Ecol. Evol.* **29**, 581–589. (doi:10.1016/j.tree.2014.07.008)
2. Rubenstein DR, Hofmann HA. 2015 Proximate pathways underlying social behavior. *Curr. Opt. Behav. Sci.* **6**, 154–159. (doi:10.1016/j.cobeha.2015.11.007)
3. Goodson JL. 2013 Deconstructing sociality, social evolution and relevant nonapeptide functions. *Psychoneuroendocrinology* **38**, 465–478. (doi:10.1016/j.psyneuen.2012.12.005)
4. Gaston A. 1978 The evolution of group territorial behavior and cooperative breeding. *Am. Nat.* **112**, 1091–1100. (doi:10.1086/283348)
5. Hatchwell BJ, Komdeur J. 2000 Ecological constraints, life history traits and the evolution of cooperative breeding. *Anim. Behav.* **59**, 1079–1086. (doi:10.1006/anbe.2000.1394)
6. Hatchwell BJ. 2009 The evolution of cooperative breeding in birds: kinship, dispersal and life history. *Phil. Trans. R. Soc. B* **364**, 3217–3227. (doi:10.1098/rstb.2009.0109)
7. Freeberg TM, Dunbar RIM, Ord TJ. 2012 Social complexity as a proximate and ultimate factor in communicative complexity. *Phil. Trans. R. Soc. B* **367**, 1785–1801. (doi:10.1098/rstb.2011.0213)
8. Groenewoud F, Frommen JG, Josi D, Tanaka H, Jungwirth A, Taborsky M. 2016 Predation risk drives social complexity in cooperative breeders. *Proc. Natl Acad. Sci. USA* **113**, 4104–4109. (doi:10.1073/pnas.1524178113)
9. Burkart JM, van Schaik CP. 2009 Cognitive consequences of cooperative breeding in primates? *Anim. Cogn.* **13**, 1–19. (doi:10.1007/s10071-009-0263-7)
10. Beery AK, Lacey EA, Francis DD. 2008 Oxytocin and vasopressin receptor distributions in a solitary and a social species of tuco-tuco (*Ctenomys haigi* and *Ctenomys sociabilis*). *J. Comp. Neurol.* **507**, 1847–1859. (doi:10.1002/cne.21638)
11. Aureli F, Cords M, van Schaik CP. 2002 Conflict resolution following aggression in gregarious animals: a predictive framework. *Anim. Behav.* **64**, 325–343. (doi:10.1006/anbe.2002.3071)
12. Taborsky M. 1994 Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. *Adv. Study Behav.* **23**, 1–100. (doi:10.1016/S0065-3454(08)60351-4)
13. Heg D, Bachar Z. 2006 Cooperative breeding in the Lake Tanganyika cichlid *Julidochromis ornatus*. *Environ. Biol. Fishes* **76**, 265–281. (doi:10.1007/s10641-006-9032-5)
14. O'Connor CM, Marsh-Rollo SE, Ghio SC, Balshine S, Aubin-Horth N. 2015 Is there convergence in the molecular pathways underlying the repeated evolution of sociality in African cichlids? *Horm. Behav.* **75**, 160–168. (doi:10.1016/j.yhbeh.2015.07.008)
15. Reddon AR, O'Connor CM, Ligocki IY, Hellmann JK, Marsh-Rollo SE, Hamilton IM, Balshine S. 2016 No evidence for larger brains in cooperatively breeding cichlid fishes. *Can. J. Zool.* **94**, 373–378. (doi:10.1139/cjz-2015–0118)
16. Dey CJ, O'Connor CM, Wilkinson H, Shultz S, Balshine S, Fitzpatrick JL. 2017 Direct benefits and evolutionary transitions to complex societies. *Nat. Ecol. Evol.* **1**, 0137. (doi:10.1038/s41559-017-0137)
17. Kuwamura T. 1997 The evolution of parental care and mating systems among Tanganyikan cichlids. In *Fish communities in Lake Tanganyika* (eds M Hori, M Nogoshi), pp. 59–86. Kyoto, Japan: Kyoto University Press.
18. Kuwamura T. 1986 Parental care and mating systems of cichlid fishes in Lake Tanganyika: a preliminary field survey. *J. Ethol.* **4**, 129–146. (doi:10.1007/BF02348115)
19. Brichard P. 1989 *Book of cichlids and all the fishes of Lake Tanganyika*. Neptune, NJ: TFH Publications.
20. Konings A. 2005 *Back to nature guide to Tanganyika cichlids*, 2nd edn. El Paso, TX: Cichlid Press.
21. Hick K, Reddon AR, O'Connor CM, Balshine S. 2014 Strategic and tactical fighting decisions in cichlid fishes with divergent social systems. *Behaviour* **151**, 47–71. (doi:10.1163/1568539X-00003122)
22. O'Connor CM, Marsh-Rollo SE, Aubin-Horth N, Balshine S. 2016 Species-specific patterns of nonapeptide brain gene expression relative to pair-bonding behavior in grouping and non-grouping cichlids. *Horm. Behav.* **80**, 30–38. (doi:10.1016/j.yhbeh.2015.10.015)
23. Balshine S, Wong MYL, Reddon AR. In press. Social motivation and conflict resolution tactics as potential building blocks of sociality in cichlid fishes. *Behav. Proc.* (doi:10.1016/j.beproc.2017.01.001)
24. Godwin J, Thompson R. 2012 Nonapeptides and social behavior in fishes. *Horm. Behav.* **61**, 230–238. (doi:10.1016/j.yhbeh.2011.12.016)
25. Goodson JL, Kelly AM, Kingsbury MA. 2012 Evolving nonapeptide mechanisms of gregariousness and social diversity in birds. *Horm. Behav.* **61**, 239–250. (doi:10.1016/j.yhbeh.2012.01.005)
26. Boyd SK. 2013 Vasotocin modulation of social behaviors in amphibians. In *Oxytocin, vasopressin and related peptides in the regulation of behavior* (eds E Choleris, DW Pfaff, M Kavaliers), pp. 97–109. Cambridge, UK: Cambridge University Press.
27. Albers HE. 2015 Species, sex and individual differences in the vasotocin/vasopressin system: relationship to neurochemical signaling in the social behavior neural network. *Front. Neuroendocrinol.* **36**, 49–71. (doi:10.1016/j.yfrne.2014.07.001)
28. Ferguson JN, Young LJ, Hearn EF, Matzuk MM, Insel TR, Winslow JT. 2000 Social amnesia in mice lacking the oxytocin gene. *Nat. Genet.* **25**, 284–288. (doi:10.1038/77040)
29. Lim MM, Young LJ. 2006 Neuropeptidergic regulation of affiliative behavior and social bonding in animals. *Horm. Behav.* **50**, 506–517. (doi:10.1016/j.yhbeh.2006.06.028)
30. Madden JR, Clutton-Brock TH. 2011 Experimental peripheral administration of oxytocin elevates a suite of cooperative behaviours in a wild social mammal. *Proc. R. Soc. B* **278**, 1189–1194. (doi:10.1098/rspb.2010.1675)
31. Wilson LC, Goodson JL, Kingsbury MA. 2016 Seasonal variation in group size is related to seasonal variation in neuropeptide receptor density. *Brain Behav. Evol.* **88**, 111–126. (doi:10.1159/000448372)
32. Ondrasek NR. 2016 Emerging frontiers in social neuroendocrinology and the study of nonapeptides. *Ethology* **122**, 443–455. (doi:10.1111/eth.12493)
33. Soares MC, Bshary R, Fusani L, Goymann W, Hau M, Hirschenhauser K, Oliveira RF. 2010 Hormonal mechanisms of cooperative behaviour. *Phil. Trans. R. Soc. B* **365**, 2737–2750. (doi:10.1098/rstb.2010.0151)
34. Goodson JL. 2008 Nonapeptides and the evolutionary patterning of sociality. *Prog. Brain Res.* **170**, 3–15. (doi:10.1016/S0079-6123(08)00401-9)
35. Goodson JL, Evans AK, Wang Y. 2006 Neuropeptide binding reflects convergent and divergent evolution in species-typical group sizes. *Horm. Behav.* **50**, 223–236. (doi:10.1016/j.yhbeh.2006.03.005)
36. Goodson JL, Schrock SE, Klatt JD, Kabelik D, Kingsbury MA. 2009 Mesotocin and nonapeptide receptors promote estrildid flocking behavior. *Science* **325**, 862–866. (doi:10.1126/science.1174929)
37. Kalamatianos T, Faulkes CG, Oosthuizen MK, Poorun R, Bennett NC, Coen CW. 2010 Telencephalic binding

sites for oxytocin and social organization: a comparative study of eusocial naked mole-rats and solitary cape mole-rats. *J. Comp. Neurol.* **518**, 1792–1813. (doi:10.1002/cne.22302)

38. Goodson JL, Wilson LC, Schrock SE. 2012 To flock or fight: neurochemical signatures of divergent life histories in sparrows. *Proc. Natl Acad. Sci. USA* **109**, 10 685–10 692. (doi:10.1073/pnas.1203394109)
39. Hoyle CH. 1999 Neuropeptide families and their receptors: evolutionary perspectives. *Brain Res.* **848**, 1–25. (doi:10.1016/S0006-8993(99)01975-7)
40. Thompson RR, Walton JC. 2004 Peptide effects on social behavior: effects of vasotocin and isotocin on social approach behavior in male goldfish (*Carassius auratus*). *Behav. Neurosci.* **118**, 620–626. (doi:10.1037/0735–7044.118.3.620)
41. Larson ET, O'Malley DM, Melloni Jr RH. 2006 Aggression and vasotocin are associated with dominant–subordinate relationships in zebrafish. *Behav. Brain Res.* **167**, 94–102. (doi:10.1016/j.bbr.2005.08.020)
42. Lema SC, Nevitt GA. 2004 Variation in vasotocin immunoreactivity in the brain of recently isolated populations of a death valley pupfish, *Cyprinodon nevadensis*. *Gen Comp. Endocrinol.* **135**, 300–309. (doi:10.1016/j.ygcen.2003.10.006)
43. Santangelo N, Bass AH. 2006 New insights into neuropeptide modulation of aggression: field studies of arginine vasotocin in a territorial tropical damselfish. *Proc. R. Soc. B* **273**, 3085–3092. (doi:10.1098/rspb.2006.3683)
44. Santangelo N, Bass AH. 2010 Individual behavioral and neuronal phenotypes for arginine vasotocin mediated courtship and aggression in a territorial teleost. *Brain Behav. Evol.* **75**, 282–291. (doi:10.1159/000316867)
45. Greenwood AK, Wark AR, Fernald RD, Hofmann HA. 2008 Expression of arginine vasotocin in distinct preoptic regions is associated with dominant and subordinate behaviour in an African cichlid fish. *Proc. R. Soc. B* **275**, 2393–2402. (doi:10.1098/rspb.2008.0622)
46. Filby A, Paull G, Hickmore T, Tyler C. 2010 Unravelling the neurophysiological basis of aggression in a fish model. *BMC Genomics* **11**, 498. (doi:10.1186/1471-2164-11-498)
47. Dewan AK, Tricas TC. 2011 Arginine vasotocin neuronal phenotypes and their relationship to aggressive behavior in the territorial monogamous multiband butterflyfish, *Chaetodon multicinctus*. *Brain Res.* **1401**, 74–84. (doi:10.1016/j.brainres.2011.05.029)
48. Almeida O, Gozdowska M, Kulczykowska E, Oliveira RF. 2012 Brain levels of arginine–vasotocin and isotocin in dominant and subordinate males of a cichlid fish. *Horm. Behav.* **61**, 212–217. (doi:10.1016/j.yhbeh.2011.12.008)
49. Kagawa N, Nishiyama Y, Kato K, Takahashi H, Kobayashi Y, Sakamoto H, Sakamoto T. 2013 Potential roles of arginine-vasotocin in the regulation of aggressive behavior in the mudskipper (*Periophthalmus modestus*). *Gen. Comp. Endocrinol.* **194**, 257–263. (doi:10.1016/j.ygcen.2013.09.023)
50. Thompson RR, Walton JC. 2013 Social regulatory functions of vasotocin and isotocin in fish. In *Oxytocin, vasopressin and related peptides in the regulation of behavior* (eds E Choleris, DW Pfaff, M Kavaliers), pp. 75–96. Cambridge, UK: Cambridge University Press.
51. O'Connell LA, Matthews BJ, Hofmann HA. 2012 Isotocin regulates paternal care in a monogamous cichlid fish. *Horm. Behav.* **61**, 725–733. (doi:10.1016/j.yhbeh.2012.03.009)
52. Reddon AR, O'Connor CM, Marsh-Rollo SE, Balshine S. 2012 Effects of isotocin on social responses in a cooperatively breeding fish. *Anim. Behav.* **84**, 753–760. (doi:10.1016/j.anbehav.2012.07.021)
53. Reddon AR, Voisin MR, O'Connor CM, Balshine S. 2014 Isotocin and sociality in the cooperatively breeding cichlid fish, *Neolamprologus pulcher*. *Behaviour* **151**, 1389–1411. (doi:10.1163/1568539X-00003190)
54. Reddon AR, O'Connor CM, Marsh-Rollo SE, Balshine S, Gozdowska M, Kulczykowska E. 2015 Brain nonapeptide levels are related to social status and affiliative behaviour in a cooperatively breeding cichlid fish. *R. Soc. open sci.* **2**, 140072. (doi:10.1098/rsos.140072)
55. Hellmann JK, Reddon AR, Ligocki IY, O'Connor CM, Garvy KA, Marsh-Rollo SE, Hamilton IM, Balshine S. 2015 Group response to social perturbation: impacts of isotocin and the social landscape. *Anim. Behav.* **105**, 55–62. (doi:10.1016/j.anbehav.2015.03.029)
56. Lema SC, Sanders KE, Walti KA. 2015 Arginine vasotocin, isotocin and nonapeptide receptor gene expression link to social status and aggression in sex-dependent patterns. *J. Neuroendocrinol.* **27**, 142–157. (doi:10.1111/jne.12239)
57. Goodson JL. 2005 The vertebrate social behavior network: evolutionary themes and variations. *Horm. Behav.* **48**, 11–22. (doi:10.1016/j.yhbeh.2005.02.003)
58. Bradford MRJ, Northcutt RG. 1983 Organization of the diencephalon and pretectum of the ray-finned fishes. In *Fish neurobiology*, vol. 2 (eds RE Davis, RG Northcutt), pp. 117–164. Ann Arbor, MI: University of Michigan Press.
59. Saito D, Komatsuda M, Urano A. 2004 Functional organization of preoptic vasotocin and isotocin neurons in the brain of rainbow trout: central and neurohypophysial projections of single neurons. *Neuroscience* **124**, 973–984. (doi:10.1016/j.neuroscience.2003.12.038)
60. Huffman LS, O'Connell LA, Kenkel CD, Kline RJ, Khan IA, Hofmann HA. 2012 Distribution of nonapeptide systems in the forebrain of an African cichlid fish, *Astatotilapia burtoni*. *J. Chem. Neuroanat.* **44**, 86–97. (doi:10.1016/j.jchemneu.2012.05.002)
61. Almeida O, Oliveira RF. 2015 Social status and arginine vasotocin neuronal phenotypes in a cichlid fish. *Brain Behav. Evol.* **85**, 203–213. (doi:10.1159/000381251)
62. Schelly R, Salzburger W, Koblmüller S, Duftner N, Sturmbauer C. 2006 Phylogenetic relationships of the lamprologine cichlid genus *Lepidiolamprologus* (Teleostei: Perciformes) based on mitochondrial and nuclear sequences, suggesting introgressive hybridization. *Mol. Phylogen. Evol.* **38**, 426–438. (doi:10.1016/j.ympev.2005.04.023)
63. Day JJ, Santini S, Garcia-Moreno J. 2007 Phylogenetic relationships of the Lake Tanganyika cichlid tribe Lamprologini: the story from mitochondrial DNA. *Mol. Phylogen. Evol.* **45**, 629–642. (doi:10.1016/j.ympev.2007.02.025)
64. Sturmbauer C, Salzburger W, Duftner N, Schelly R, Koblmüller S. 2010 Evolutionary history of the Lake Tanganyika cichlid tribe Lamprologini (Teleostei: Perciformes) derived from mitochondrial and nuclear DNA data. *Mol. Phylogen. Evol.* **57**, 266–284. (doi:10.1016/j.ympev.2010.06.018)
65. Maddison WP, Maddison DR. 2016 Mesquite: a modular system for evolutionary analysis. Version 3.10. See http://mesquiteproject.org.
66. Foran CM, Bass AH. 1998 Preoptic AVT immunoreactive neurons of a teleost fish with alternative reproductive tactics. *Gen. Comp. Endocrinol.* **111**, 271–282. (doi:10.1006/gcen.1998.7113)
67. Hadfield JD. 2010 MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R Package. *J. Stat. Softw.* **33**, 1–22. (doi:10.18637/jss.v033.i02)
68. de Villemereuil P, Nakagawa S. 2014 General quantitative genetic methods for comparative biology. In *Modern phylogenetic comparative methods and their application in evolutionary biology: concepts and practice* (ed. LZ Garamszegi), pp. 287–303. Berlin, Germany: Springer.
69. Gelman A. 2006 Prior distributions for variance parameters in hierarchical models. *Bayesian Anal.* **1**, 515–534. (doi:10.1214/06-BA117A)
70. Hadfield JD. 2015 MCMCglmm: Course notes. See https://cran.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf.
71. Robinson BW, Wilson DS, Margosian AS. 2000 A pluralistic analysis of character release in pumpkinseed sunfish (*Lepomis gibbosus*). *Ecology* **81**, 2799–2812. (doi:10.2307/177342)
72. Quinn G, Keough M. 2002 *Experimental design and data analysis for biologists*. Cambridge, UK: Cambridge University Press.
73. Beery AK. 2015 Antisocial oxytocin: complex effects on social behavior. *Curr. Opin. Behav. Sci.* **6**, 174–182. (doi:10.1016/j.cobeha.2015.11.006)
74. Grober MS, George AA, Watkins KK, Carneiro LA, Oliveira RF. 2002 Forebrain AVT and courtship in a fish with male alternative reproductive tactics. *Brain Res. Bull.* **57**, 423–425. (doi:10.1016/S0361-9230(01)00704-3)
75. Ota Y, Ando H, Ueda H, Urano A. 1999 Seasonal changes in expression of neurohypophysial hormone genes in the preoptic nucleus of immature female masu salmon. *Gen. Comp. Endocrinol.* **116**, 33–39. (doi:10.1006/gcen.1999.7343)
76. Aubin-Horth N, Desjardins JK, Martei YM, Balshine S, Hofmann HA. 2007 Masculinized dominant females in a cooperatively breeding species. *Mol. Ecol.* **16**, 1349–1358. (doi:10.1111/j.1365-294X.2007.03249.x)
77. Insel TR, Shapiro LE. 1992 Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc. Natl Acad. Sci. USA* **89**, 5981–5985. (doi:10.1073/pnas.89.13.5981)
78. Insel TR, Wang ZX, Ferris CF. 1994 Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. *J. Neurosci.* **14**, 5381–5392.
79. Wang, ZX, Zhou L, Hulihan TJ, Insel TR. 1996 Immunoreactivity of central vasopressin and oxytocin pathways in microtine rodents: a quantitative comparative study. *J. Comp. Neurol.* **366**, 726–737. (doi:10.1002/(SICI)1096-9861(19960318)366:4<726::AID-CNE11>3.0.CO;2-D)

80. Loveland JL, Fernald RD. 2017 Differential activation of vasotocin neurons in contexts that elicit aggression and courtship. *Behav. Brain Res.* **317**, 188–203. (doi:10.1016/j.bbr.2016.09.008)
81. Dewan AK, Maruska KP, Tricas TC. 2008 Arginine vasotocin neuronal phenotypes among congeneric territorial and shoaling reef butterflyfishes: species, sex and reproductive season comparisons. *J. Neuroendocrinol.* **20**, 1382–1394. (doi:10.1111/j.1365–2826.2008.01798.x)
82. Semsar K, Godwin J. 2003 Social influences on the arginine vasotocin system are independent of gonads in a sex-changing fish. *J. Neurosci.* **23**, 4386–4393.
83. Arnold C, Taborsky B. 2010 Social experience in early ontogeny has lasting effects on social skills in cooperatively breeding cichlids. *Anim. Behav.* **79**, 621–630. (doi:10.1016/j.anbehav.2009.12.008)
84. Taborsky B, Arnold C, Junker J, Tschopp A. 2012 The early social environment affects social competence in a cooperative breeder. *Anim. Behav.* **83**, 1067–1074. (doi:10.1016/j.anbehav.2012.01.037)
85. Taborsky B, Tschirren L, Meunier C, Aubin-Horth N. 2012 Stable reprogramming of brain transcription profiles by the early social environment in a cooperatively breeding fish. *Proc. R Soc. B* **280**, 20122605. (doi:10.1098/rspb.2012.2605)
86. Goodson JL, Bass AH. 2001 Social behavior functions and related anatomical characteristics of vasotocin/vasopressin systems in vertebrates. *Brain Res. Rev.* **35**, 246–265. (doi:10.1016/S0165-0173(01)00043-1)