Hormones and Behavior 80 (2016) 30–38

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Species-specific patterns of nonapeptide brain gene expression relative to pair-bonding behavior in grouping and non-grouping cichlids

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A R T I C L E I N F O

Article history:
Received 30 June 2015
Revised 18 October 2015
Accepted 24 October 2015
Available online 28 October 2015

Keywords:
Selective attachment bonding
Pair bonding
Social evolution
Lamprologine
Teleost

A B S T R A C T

Comparative studies have revealed that vasopressin–oxytocin pathways are associated with both pair bonding and grouping behavior. However, the relationship between pair bonding and grouping behavior remains unclear. In this study, our aim was to identify whether two species that differ in grouping behavior display a corresponding difference in their pair bonds, and in the underlying vasopressin–oxytocin hormonal pathways. Using two species of cichlid fishes, the highly social *Neolamprologus pulcher* and the non-social *Telmatochromis temporalis*, we measured proximity of pairs during pair bond formation, and then measured social behaviors (proximity, aggression, submission, affiliation) and brain gene expression of isotocin and arginine vasotocin (the teleost homologues of oxytocin and vasopressin, respectively), as well as their receptors, after a temporary separation and subsequent reunion of the bonded pairs. Pairs of the social species spent more time in close proximity relative to the non-social species. Rates of aggression increased in both species following the separation and reunion treatment, relative to controls that were not separated. Overall, whole brain expression of isotocin was higher in the social species relative to the non-social species, and correlated with proximity, submission, and affiliation, but only in the social species. Our results suggest that both a social and a non-social cichlid species have similar behavioral responses to a temporary separation from a mate, and we found no difference in the brain gene expression of measured hormones and receptors based on our separation–reunion treatment. However, our results highlight the importance of isotocin in mediating submissive and affiliative behaviors in cichlid fishes, and demonstrate that isotocin has species-specific correlations with socially relevant behaviors.

Introduction

Pair bonding, or the preferential interaction of individuals to the exclusion of other potential partners, forms the basis for many social interactions, and therefore represents an interesting subset of social behavior. The processes that underlie the formation and maintenance of pair bonds are complex, and have been studied from both a behavioral and a mechanistic point of view (see review by Lim and Young, 2006). At the behavioral level, pair bonding can be broken down into three underlying components, all of which appear to be mediated at the mechanistic level by the vasopressin–oxytocin family of nonapeptide hormones (see review by Lim and Young, 2006). At the first stage of pair bonding, the individual must be motivated to approach a conspecific. Both oxytocin and vasopressin appear to modulate the motivation to approach other conspecifics. Rats (*Rattus norvegicus*) and mice (*Mus musculus*) treated with an oxytocin receptor antagonist reduced social approach, while socially defeated rats given oxytocin showed less social avoidance relative to controls (Lukas et al., 2011). In goldfish (*Carassius auratus*), treatment with isotocin (IT, the teleost fish homologue of oxytocin; Hoyle, 1999) increased social approach, while treatment with arginine vasotocin (AVT, the fish homologue of vasopressin; Hoyle, 1999) decreased social approach (Thompson and Walton, 2004). At the second stage of pair bonding, where individuals must be able to differentiate familiar and unfamiliar conspecifics, these two nonapeptides again appear to have important influence. For example, transgenic mice that lack the oxytocin gene are unable to recognize familiar individuals despite repeated exposure (Choleris et al., 2003; Ferguson et al., 2000), but their ability to recognize a familiar individual can be restored by treatment with oxytocin (Ferguson et al., 2001). Finally, at the third stage of social bonding, the individual must form a pair bond with the familiar conspecific such that the individual preferentially interacts with that conspecific, to the exclusion of other potential social partners (Lim and Young, 2006). Comparisons of closely related *Microtus* vole species suggest that differences in the distribution of oxytocin and vasopressin receptors are related to differences in the degree of social bonding (Insel and Shapiro, 1992; Insel et al., 1994; Young and Wang, 2004). In non-mammalian vertebrates, a general AVT/IT receptor antagonist delayed the formation of new pair bonds in the monogamous convict cichlid (*Amatitlania nigrofasciata*) but did not disrupt bonds in established pairs (Oldfield and Hofmann, 2011).

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http://dx.doi.org/10.1016/j.yhbeh.2015.10.015
0018-506X/© 2015 Published by Elsevier Inc.

The relationship between the capacity for pair bonding and grouping behavior remains unclear, at both the behavioral and mechanistic level (Goodson, 2013). For group-living species with individual recognition, pair bonds form the basis for all subsequent within-group interactions. Therefore, it could be predicted that group-living animals will show stronger pair bonds relative to non-grouping animals, since they form multiple bonds with many individuals. Alternatively, it is possible that group-living animals have weakened pair bonds relative to non-grouping animals, since they routinely interact with many different individuals, and may be less selective in the choice of their social partners than more solitary animals that interact with only their mates and offspring. At the mechanistic level, the vasopressin–oxytocin family of nonapeptide hormones, as well as being linked to pair bonding behavior, has also been implicated as the mechanistic substrate for grouping behavior. For example, species-typical group size is related to nonapeptide receptor distribution in estrilid finches, and treating normally gregarious zebra finch (*Taeniopygia guttata*) with an oxytocin receptor antagonist reduced their preference for associating with large social groups (Goodson et al., 2009). Our aim in this study was therefore to identify whether species that vary in their grouping behavior display a corresponding difference in the strength and resilience of their pair bonds, and to understand the relationships among grouping, pair bonding, and vasopressin–oxytocin nonapeptide hormonal pathways.

In this study, we investigated the relationships among social system, the strength and resilience of pair bonds, and brain gene expression of the oxytocin–vasopressin family of nonapeptide hormones in two closely related species of Lamprologine cichlids, *Neolamprologus pulcher* and *Telmatochromis temporalis* (Day et al., 2007). *N. pulcher* is a group-living, cooperatively breeding cichlid that lives in permanent social groups comprised of a dominant breeding pair, and up to 20 subordinate conspecifics who jointly maintain and defend the territory (see review by Wong and Balshine, 2011). *N. pulcher* form pair bonds between the dominant male and female in each group, have social bonds among group members, and are strongly aggressive to non-group members. In contrast, *T. temporalis* is a non-grouping species that does not show any cooperative behaviors (Mboko and Kohda, 1999; Katoh et al., 2005; Heg and Bachar, 2006). *T. temporalis* forms pair bonds only between mates, and bonded pairs are aggressive to all other conspecifics, tolerating only their mate and very young offspring. However, the two species are otherwise similar. Both species are endemic to the rocky littoral zone of Lake Tanganyika, East Africa (Kuwamura, 1986; Brichard, 1989; Konings, 1998), approximately 4–6 cm long when mature, spawn under rocky shelters, and provide biparental care (Kuwamura, 1986; Brichard, 1989; Konings, 1998; Sefc, 2011). Both species can be monogamous (Kuwamura, 1986; Sefc, 2011), with opportunistic polygyny (Limberger, 1983; Mboko and Kohda, 1999; Desjardins et al., 2008; Wong et al., 2012). Females are socially monogamous, although there is genetic evidence of extra-pair paternity in both species (Katoh et al., 2005; Dierkes et al., 2008; Hellmann et al., 2015a, 2015b).

We tested the hypotheses that a highly social, group-living species would form pair bonds more quickly, re-establish pair bonds better following a perturbation, and show larger differences in brain nonapeptide hormone gene expression after re-establishing a pair bond compared to more solitary, non-grouping species. To do so, we performed a laboratory study in which we measured behavior first during pair bond formation, and then following a temporary separation and subsequent reunion between the bonded mates. We then measured whole brain gene expression in the same individuals. We predicted that pairs of the social species would spend more time in close proximity during the initial pair bond formation, and would attempt to re-establish a pair bond more quickly following a temporary separation relative to the non-social species. Since IT and AVT pathways have been related to social behavior such as aggression, submission, and affiliation across many species, including *N. pulcher* (Aubin-Horth et al., 2007; Reddon et al., 2012, 2014, 2015; Hellmann et al., 2015a, 2015b), we predicted that whole brain expression of these genes would show a more pronounced change following the temporary separation and reunion in the social species relative to the non-social species (i.e., that there would be variation in genomic reaction norms; Aubin-Horth and Renn, 2009). Finally, beyond these predicted differences at the species level, we predicted that we would see correlations between social behavior and brain nonapeptide gene expression at the individual level.

Methods

Study animals and experimental design

The experiment was conducted January–February 2014 at McMaster University in Hamilton, ON, Canada. Fish were sexually mature, laboratory-reared descendants of wild-caught fish from Lake Tanganyika. All fish were measured for body size (standard length, SL) using calipers, and body mass using an electronic scale, and sexed by examination of the external genital papillae. Each fish was given a unique dorsal fin clip for identification, which does not adversely affect behavior and grows back within two weeks (Stiver et al., 2004). To form pairs, one male and one female of the same species that were previously unfamiliar with one another were placed together in a 200 L aquarium containing 3 cm of coral sand substrate, a water filter, heater, a thermometer, and 2 flowerpot halves as shelters. Pairs were formed such that the male was always 5–15% larger than the female, which is the range of sexual dimorphism observed in wild pairs (Balshine et al., 2001). Since *N. pulcher* live and breed in social groups, each *N. pulcher* pair was also housed with 2–4 small (SL < 20 mm) sexually immature individuals. The water temperature of all aquaria was held at 26 ± 2 °C, and all fish were fed dried prepared cichlid food ad libitum six times per week, and kept on a 13:11 light:dark cycle.

Bonding score

To assess how rapidly each species forms pair bonds, each pair was observed during the early phase of pair bond formation. For 3 days after the pairs were first introduced, each pair was observed once per day for 2 min, and scored as either ‘together’ (within a body length of each other, using the body length of female as the reference; Dey et al., 2013) or ‘apart’ (more than a body length apart from each other) for the majority of the observation period.

Social bond disruption

After a 7–9 day pair bond formation phase, the pairs were randomly assigned to either a ‘separation’ or a ‘control’ treatment. In the ‘separation’ treatment, pairs were separated for 60 min by an opaque barrier. The barrier was then removed and the fish observed for 10 min by an experienced observer. In the ‘control’ treatment, the fish remained together with no disruption, and then were similarly observed for 10 min. For the observation, the fish were scored as either ‘together’ or ‘apart’ using the criteria described above. The behaviors were also scored throughout the 10 min observation period based on an ethogram (Supplementary Table 1). Briefly, behaviors recorded included aggressive, submissive, and affiliative behaviors. Aggressive behaviors were displays such as aggressive head-down postures and frontal displays, as well as overt aggressive acts with physical contact, such as chases, rams, bites, or mouth wrestles. Submissive behaviors are produced by these cichlids in response to aggression from another individual, and consist of head-up submissive postures, quivering submissive displays, as well as fleeing from the aggressor. Affiliative behaviors are spontaneously produced towards another individual, and include behaviors such as swimming closely in parallel, and soft touches. Both submissive and affiliative behaviors represent appeasement gestures, and function to reduce aggression between group members (Bergmüller and Taborsky, 2005; Dey et al., 2013).