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Forebrain neuropeptide regulation of pair association and behavior in cooperating cleaner fish

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HIGHLIGHTS

- Animals establish privileged relationships which contribute to behavioral variation.
- We asked if intra-pair association index is linked with brain AVT and IT changes.
- And whether these mechanisms relate to changes in interspecific service quality.
- Variation in pair relationship was found to influence male and female cleaner fish differently.
- Variation in brain neuropeptide levels is linked to conditional cooperative outcomes.

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ABSTRACT

Animals establish privileged relationships with specific partners, which are treated differently from other conspecifics, and contribute to behavioral variation. However, there is limited information on the underlying physiological mechanisms involved in the establishment of these privileged ties and their relationship to individual cooperation levels. The Indo-Pacific bluestreak cleaner wrasse *Labroides dimidiatus* often forages in mixed-sex pairs when cleaning fish clients. Intra-couple conflicts often arise during a joint client inspection, which may alter the overall quality of cleaning service provided. Here we tested two hypotheses: a) whether intra-pair association (i.e. association index), measured with joint interspecific cleaning and intraspecific behavior, is correlated with neuroendocrine mechanisms involving forebrain neuropeptides arginine vasotocin (AVT) and isotocin (IT) and b) whether these neuropeptide level shifts relate to an individual's interspecific service quality. We found that partner support (number of cleaning interactions and tactile stimulation) received by male cleaners increased with association index. When cleaners inspected clients alone, cleaners' cheating decreased with association index for females but not males. AVT levels did not differ according to sex or association level. Forebrain IT levels increased with association index for males, whereas no relationship was found for females. Finally, cleaner cheating varied between sex and forebrain IT levels. Findings indicate that variation in pairs' relationships influences male and female cleaner fish differently and contributes to the variation of brain neuropeptide levels, which is linked to distinct cooperative outcomes.

1. Introduction

Behavioral variation between individuals is recognized as a fundamental force shaping social interactions and the evolution of complex social behavior that includes cooperation [1,2]. These behavioral differences prompt individuals to learn more about others (before and during interactions), which may lead to distinct cooperative outcomes. For instance, they may influence individuals to invest more when dealing with partners that reciprocate or to abandon uncooperative partners for more cooperative ones [2]. One of the greatest contributors to individual behavioral variation is the existence of social ties or familiarity, which encourages the establishment of individuals' relationships with specific partners (e.g. pairbonding, alliances, and friendships [3]). However, there is limited information on the underlying physiological mechanisms that involve the establishment of social ties and their direct consequences to individual behavioral variation (see [4]).

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The formation and maintenance of stable male–female relationships are sustained by selective socio-sexual behavior between the pair, and by preference for one partner over other potential ones (e.g. pair bonds; [5,6]). But the maintenance of long lasting social attachments should also be sustained by specific neurohormonal frameworks, which should be linked to the behavioral responses of any paired individual, on two different levels: a) at the expression of a series of "bilateral" behaviors between pair partners and b) on "multilateral" interactions between the pair and the social environment [4]. Two neuropeptides are considered to be critical mediators of partner-preference formation and social attachment: oxytocin (OT) and arginine vasopressin (AVP) [7]. The neuropeptide OT is well known for its links to social bonds, which includes affectionate behavior between partners [8], displays of romantic love, and increases of prosociality within the context of cooperative behavior. For example, exogenous administration of OT seems to be responsible for an increase of investment in communal and cooperative activities in meerkats [9], the facilitation of partner-directed behavior in marmosets [10], and the promotion of humans' trust and reciprocity [11,12]. The role of AVP in partner recognition and bonding, mostly derived from rodent studies, shows that both AVP and OT are involved but they have sex specific roles; for example, males and females are more sensitive to AVP and OT, respectively, which may be due to different brain receptor distributions [13,14].

The converse may also occur, with neuropeptide levels changing in response to behavioral variations in socio-sexual and affiliative behavior. For instance, partner support modulates the rise of OT plasma levels in both men and women [15] and physical contact (such as massaging, hugging and/or grooming) promotes the elevation of OT levels in humans and other primates and also in rodents [8,16,17]. In pair-bonded tamarins, OT levels relate to the amount of grooming and mutual contact in females and sexual behavior of males [18]. Measures of relationship distress correlate with OT levels in women and with AVP levels in men [19]. This suggests that shifts in levels of neuropeptide are directly linked with social environment and partnership quality. However, to be able to broadly understand these findings, we must look at vertebrate species other than primates and other mammals.

The Indo-Pacific bluestreak cleaner wrasse *Labroides dimidiatus* is often found in mixed-sex pairs. Male cleaner wrasses are harem holders and most frequently live and clean in pairs, usually with the largest female of his harem although the male also visits other females regularly [20]. Cleaners provide a service by removing ectoparasites, dead or damaged tissue of other visiting reef fish (hereafter referred as 'clients') [21]. However, cleaners prefer to feed on client mucus, which is detrimental to the client and constitutes cheating [22]. Instances of cheating contribute to a conflict of interests between clients and cleaners [22]. Clients need cleaners to eat against their preference in order to gain a good cleaning service which they achieve by: a) refraining from visiting a cleaner that provided a poor service in the past, b) avoiding cleaners they observe cheating other clients and/or c) by aggressively punishing cheating cleaners with chases [23].

Because cleaners may inspect clients alone or simultaneously with a partner, the quality of cleaning service provided to the clients may also be a source of intra-couple conflict when cleaning together [24]. Intra-couple conflicts arise because the benefits of cheating can be gained by only one cleaner during a joint inspection (e.g. the first to cheat will induce the client to leave). However, the service provided to clients by paired inspections is of better quality mainly because females behave more cooperatively in joint inspections than during solitary ones [24]. This happens because the larger male cleaner punishes (i.e. aggressively chases) the females that cheat whereas females never chase the male [25]. Moreover, in laboratory conditions, male to female punishment seems to vary according to circumstances, with males punishing their female partners more severely when high value client models are at stake or when partners are similar in size [26]. Males tend to behave more aggressively with unfamiliar females, with such females responding by behaving more cooperatively [27]. However, compared with laboratory environments involving pairs of cleaners confined to a limited aquarium space, and consequently continuously paired, in natural conditions the situation is not always as extreme. Instead, in naturally behaving pairs, pair association may decrease with increasing harem size, because male visitation rate to other females should also vary. This should result in pairs with a wide range of different relationships, varying in association strength.

Here, we examine possible correlates of variation in forebrain neuropeptide levels of arginine vasotocin (AVT) and isotocin (IT), fish homologs of mammalian AVP and OT respectively, by simultaneously measuring pair association (i.e. at the intraspecific level), and how these neuropeptides correlate with individual interspecific cooperative levels in naturally coupled pairs. The preoptic area (i.e. anterior hypothalamus), which is located in the forebrain, contains a high density of AVP/AVT-OT/IT elements and is a primary site of behavioral integration of vertebrates [28]. We tested two hypotheses: 1) whether the intra-pair relationship, measured by the rates of joint interspecific cleaning and intraspecific behavior, is correlated with brain levels of AVT and IT and 2) whether these mechanisms are also associated with individuals' interspecific service quality. The method we used measures the concentration of free forebrain nonapeptides AVT and IT after their dissociation from non-covalent complexes. This provides information based solely on the biologically active fraction of peptides, which is engaged in conversion of environmental signals into specific reaction of individuals (e.g. behavioral expression [29,30]). Moreover, our study provides a novel approach to the neuroendocrine mechanisms of behavioral variation aiming at a tropical reef fish living in natural conditions.

2. Methods

2.1. Field methods

This study was conducted on two reefs around Lizard Island (Lizard Island Research Station, Australia, 14° 40′ S, 145° 289′ E) between September and October 2012. All observations and collections were made by two SCUBA divers, between 10:00 and 15:00 h. Twenty cleaner fish (10 naturally-coupled male–female pairs) were selected randomly from cleaning stations that varied in depth between 3 and 10 m. Males are always larger than and dominant to their female partner. Each cleaner (male and female) was then randomly assigned to one of the two divers in place. Both cleaners (male and female) were then observed and videotaped (during the same session) for the next 45 min using video cameras in waterproof cases (Sony HDR-XR155) from a distance of between 2 and 3 m. At the end of the observation, the pair was captured using hand and barrier nets. Total length (TL) and total weight (TW) of females ranged from 6.3 to 8.5 cm (mean ± SD: 7.25 ± 0.65 cm) and 2.5 to 6.2 g (3.82 ± 1.14 g) and for males from 7.5 to 9 cm (8.35 ± 0.45 cm) and 4.3 to 7.3 g (5.73 ± 0.99 g), respectively. The sex of the individuals was confirmed by direct inspection of the gonads. Only a maximum of three couples were observed per day. After capture, fish were immediately brought to the field station where they were anesthetized (overdose of MS-222, Sigma) until muscular and opercular movements completely ceased, after which they were killed by decapitation. The forebrain, composed of olfactory bulbs, telencephalon and diencephalon, was extracted and placed in a cryo-Eppendorf tube, immediately frozen, and stored at −80 °C in a liquid nitrogen container. The container was then taken by air to the mainland (resulting in samples being in liquid nitrogen for 10 to 15 days), and from there transported by air to Poland, in dry ice (an additional 2 days), for subsequent analysis.

2.2. Quantification of nonapeptides by high performance liquid chromatography with fluorescence detection (HPLC-FL)

Brain samples were weighed, for further calculation of nonapeptides' levels (peptide content was expressed per milligram of brain tissue).