Physiology & Behavior 139 (2015) 314–320

Contents lists available at ScienceDirect

Physiology & Behavior

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

Arginine vasotocin reduces levels of cooperative behaviour in a cleaner fish

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HIGHLIGHTS

- In nature, cleaner fish need to invest in unrelated partners to yield current and future benefits.
- We confirm the importance of the AVT/AVP system as an agent affecting levels of cooperation.
- AVT offers a potential mechanistic pathway for the reported flexible service quality.

ARTICLE INFO

Article history:
Received 25 July 2014
Received in revised form 13 November 2014
Accepted 14 November 2014
Available online 20 November 2014

Keywords:
Cleaner fish
Cooperative levels
Labroides dimidiatus
Neuropeptides
Arginine vasotocin
Isotocin
Decision-making

ABSTRACT

Cooperation between unrelated individuals usually involves investments that often mean a decrease in immediate payoffs, but ensure future benefits. Here we investigated the potential role of the neuropeptides Arginine-vasotocin (AVT) and Isotocin (IT) as proximate agents affecting individuals' cooperative levels in the Indo-pacific bluestreak cleaner wrasse *Labroides dimidiatus*. Their 'client' reef fish partners only benefit from interacting if cleaners eat ectoparasites and refrain from gleaning preferred client mucus. Thus, cleaners must control their impulse to eat according to their preference, and eat less preferred items to maintain ongoing interactions and avoid clients' leaving or punishing. We found that solely the experimental transient higher dosage of AVT led to a decrease of cleaners' willingness to feed against their preference, while IT and AVT antagonists had no significant effects. The sole effect of AVT on cleaner's performance may imply a link between AVT's influence and a potential activation of a stress response. Our results confirm the importance of the AVT/AVP system as an agent affecting levels of cooperation, offering a potential mechanistic pathway for the reported flexible service quality that cleaners provide their clients.

1. Introduction

Cooperation between unrelated individuals often involves investments, which means a decrease in immediate payoffs (for the actor), in order to contribute to the enhancement of benefits in another individual [1]. Evolutionary models usually focus on questions related to potential strategies, which may ensure that investments yield future benefits and hence stabilise cooperation [1–3]. However, current models are agnostic about proximal mechanisms that need to be in place to enhance the individuals' ability to decide whether or not to invest.

Knowledge on how changes in an individual's physiological/neurological state affect cooperative and social behaviour is needed [4,5], in order to understand variation within and between individuals as well as between species. The neuropeptides arginine vasopressin (AVP) and oxytocin (OT) are well known modulators of a diverse range of vertebrate social processes and emotions, including that of humans [6–8]. For example, within humans, experimental setups aiming to increase OT levels have demonstrated that these mediate rises in prosociality, which include trust [9,10] generosity [11,12], empathy [12], and social memory [13], while behavioural manifestations of prosociality have now been linked to individual differences in rs53576 genotype of the OT receptor [14]. Partner support is also a good facilitator of increases in OT plasma levels in both men and women [15]. Regarding AVP, studies have now examined its effects (via intranasal administration) on human facial responses linked to social communication, revealing that AVP influences the response to ambiguous social stimuli [16] and that its effects are sex specific with respect to responses towards same-sex faces, i.e. agonistic in men and affiliative in women [17]. Finally, in a recent study, Rilling and colleagues [18], demonstrate that intranasal AVT and IT administration mediate biassed effects in human males and

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http://dx.doi.org/10.1016/j.physbeh.2014.11.052
0031-9384/

females interacting in a Prisioner's Dillemma task. Taken together, the above studies suggest that these systems offer a general mechanistic framework involved in the regulation of complex social processes.

The nonapeptides AVP and OT neural expression and gene regulation appear to be widely conserved across vertebrates and have peripheral (hormonal) as well as central (neuromodulator) actions [19]. Indeed, recent work in non-mammalian vertebrates indicates that the social function of OT may be ancient in terms of its evolutionary framework [20]. However, both systems are highly pleiotropic, affecting a wide range of behaviours across functional contexts (e.g. pair bonding, parental care, anxiety, memory, recognition, communication and aggression; for reviews please see [21,22]). For example, in teleost fish, studies have found a relation between IT and the increase of sociality in goldfish, *Carrasius auratus* [23], and with zebrafish, *Danio rerio* [24], while under the influence of AVT, Thompson and Walton [23] found that exogenous administration of AVT inhibited approach behavior. Regarding cooperative contexts, in meerkats (*Suricata suricata*), individuals treated with OT were observed to increase their investment in communal and cooperative activities [25], while in a cooperative breeding fish (*Neolamprogus pulcher*), IT increased the response to social information, namely in increasing individual sensitivity to differences in opponent size and aggressive feedback [26].

Here we use one of the best studied cooperative models, the Indo-pacific bluestreak cleaner wrasse *Labroides dimidiatus*, to investigate how changes in individuals' neuropeptide levels (IT and AVT systems) may be implicated in the mechanisms underlying the adjustment of individuals to the existence of partner control mechanisms in cooperative interactions between unrelated individuals. The cleaners are visited by the other reef fish species (so called clients) for ectoparasite removal [27,28]. A conflict of interest occurs because cleaners prefer to eat client mucus, which constitutes cheating [29]. As clients respond to non-cooperative cleaners with attacking (punishing), leaving or avoidance [30–32], cleaners need to adjust their feeding behaviour to feeding on clients' ectoparasites (against their preference). The problem can easily be abstracted in laboratory experiments involving plates and two types of food, where cleaner wrasses but not closely related species can learn to eat against their preference if that allows them to continue to forage [33,34]. This experimental paradigm has been used successfully in the last few years, having resulted in over a dozen published studies focusing on cleaner wrasses [29,30,33–44] and captures the essence of cleaning interactions as demonstrations of key results have been reproduced in experiments with real cleaner–client interactions [32]. We made use of the experimental design to test how the AVT and IT systems influence the cleaners' ability to feed against preference in order to prolong their foraging interactions.

Nonapeptides seem to be good candidates to modulate cleaner wrasses' decision-making, related to cleaning behaviour. In a first study concerning this system, Soares and colleagues [45] found that AVT administration caused a decrease on interspecific cleaning interactions, while its V1a receptor antagonist (Manning compound) had opposite effects in mediating a rise in cleaners' dishonesty via central effects on the V1a-type receptors. More recently, further support for the involvement of AVT on cleaning behaviour was provided by a comparative neuroanatomical study, where an association between AVT gigantocellular preoptic area (gPOA) neurons and the expression of cleaning behaviour in cleaning wrasses was found [46]. However, given AVT's overall effects regarding our system [45], it was still unclear how it would directly influence cleaners' predisposition to eat against preference and hence how it may contribute to conditional cooperative outcomes. Thus, we expect to find differences in the extent of neuropeptide influence to affect cleaner wrasses foraging decisions, namely that the blocking of AVT effects (via the V1a receptor antagonist Manning compound) should promote a decrease in cooperative levels (more eating according to preference, as it was mentioned in [45]) while the opposite should be observed by the agonist (AVT injection). Regarding IT, we predict that by exogenously increasing its levels, we may observe an enhancement of cleaners' ability to identify and properly respond to social stimuli, which should have a direct influence on their levels of feeding against preference. Nevertheless, concerning IT, few relevant results have been found so far, during previous manipulations in the wild [45].

2. Methods

2.1. Experiments

Experiments were conducted at the fish housing facilities of the Oceanário de Lisboa (Lisbon, Portugal). We used 9 wild caught cleaner wrasses that originated in Maldives and were directly imported to Portugal by a local distributor. The fish were kept in individual aquaria (100 × 40 × 40 cm) combined in a flow through system that pumped water from a larger cleaning tank (150 × 50 × 40 cm) that served as a natural filter. Each tank contained an air supply and a commercial aquarium heater (125 W, Eheim, Jäger). Small PVC pipes (10–15 cm long; 2.5 cm diameter) served as shelter for the fish. Nitrite concentration was kept to a minimum (always below 0.3 mg/l). Fishes were fed daily with mashed prawn flesh or a mixture of mashed prawn flesh and fish flakes spread on plastic (Plexiglas) plates [47].

2.2. Learning against preference task

We followed Bshary and Gruter [33] protocol, with some minor modifications. Cleaners learned to feed from the plates within 1–3 days of exposure. The plates had a variety of patterns (Fig. 1) and each cleaner was exposed to all different protocol steps (plaque pattern) as to become accustomed to the presentation of unfamiliar stimuli (to avoid potentially neophobic cleaners). The experiments began after the fish had been in captivity for at least 15 days. The "learning against preference task" consisted of three phases, namely: (a) an initial preference test; (b) learning phase; and (c) foraging test without any hormonal treatment. The plates used in the experiment were attached to a 40 cm long lever that allowed the experimenter to simulate the behaviour of the client fishes (fleeing, or just calmly leaving after the cleaner finished foraging).

In the initial preference test we offered the cleaners an unfamiliar plate with three prawn items and three flake items (Fig. 1). The sequence of the 6 items (prawn or flake) placed in the grid cells was determined by using tables of random sequences of 0 and 1, where 0 represented prawn and 1 represented flake. The cleaners could eat all items but plates were removed once a cleaner stopped feeding with items still remaining. After three trials that allowed cleaners to become familiar with the plates, we conducted the initial preference test. We then offered the plate three times to each cleaner and scored the first three items eaten. This meant that we could possibly find a 100% preference for either prawn or flakes.

In the learning phase each cleaner was subjected to six learning trials. Cleaners were trained such that eating the less preferred food items (fish flakes) had no consequences, while eating a preferred item led to the immediate removal of the plate ('fleeing'). In each trial, the plate was offered to the fish again after 60 s until the cleaner ate a second preferred food item. There were two parts in this phase: the first where we used a plate with 12 flakes and 2 prawns; and a second where we used a plate with 3 flakes and 3 prawns (equal number of the 2 different items).

In the foraging experiment each cleaner was allowed to interact once with the plate that did not respond to the cleaner's foraging behaviour. In other words, eating a preferred food item had no negative consequences. We scored the first 3 items eaten, allowing the possibility of a 100% bias for either food.

2.3. Neuropeptide treatment

This part consisted of the final foraging experiment but this time each cleaner was sequentially and haphazardly treated (intramuscularly) with