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Treatment with the glucocorticoid antagonist RU486 reduces cooperative cleaning visits of a common reef fish, the lined bristletooth

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ABSTRACT

Cooperation often involves a conflict of interest. This is particularly true in situations where one individual seeks out a service but cannot properly control the quality of the service given by the partner who would gain from defecting. An example is cleaning mutualism involving the bluestreak cleaner wrasse (*Labroides dimidiatus*) and its reef-fish 'clients'. These cleaners may reduce the stress experienced by their clients by removing parasites; however they occasionally cheat clients (i.e. defect) by eating mucus and other living tissues. Here we present experimental support for the hypothesis that stress responses increase the motivation for clients to seek out such risky asymmetric interactions. We manipulated the stress response by blocking glucocorticoid receptors with the antagonist RU486 in a species that is a regular visitor of cleaner fish, the lined bristletooth (*Ctenochaetus striatus*). Field observations 1 week after treatment with RU486 showed that antagonist treatment led to a reduction in cleaning duration compared to control treatment. This was not explained by a general effect on client behavior as intraspecific social behavior appeared unaffected. We propose that antagonist treatment reduced stress responses to the presence of ectoparasites, which in turn reduced the client's perception of benefits from seeking out cleaning interactions. The results demonstrate a hitherto overlooked variable role of stress and stress responses on cooperative behavior.

Introduction

Cooperation generally involves an exchange of goods and services (Noë, 2001). Cooperative acts are often an investment: they reduce current payoffs of the actor and increase the current payoff of the recipient, as exemplified in a prisoner's dilemma payoff matrix (Axelrod and Hamilton, 1981). Conflicts of interest arise in that individuals would benefit from reducing their investment as long as the partner does not alter its cooperative behavior in response. In the iterated prisoner's dilemma game a defecting individual risks that the partner will defect in return in the next round. However when the partner lacks this option to reciprocate, the temptation to defect should be particularly strong. Such asymmetric games are commonly found in nature (Bshary and Bronstein, 2004; Bshary and Bronstein, 2011). In these cases, individuals with fewer options to control their partner (including via punishment and partner choice) should seek out interactions with this partner only if the costs of defection are relatively low in comparison to the benefits of cooperation (Johnstone and Bshary, 2002).

A related question is how individuals make appropriate decisions about when to interact with a partner. This question can be addressed by investigating the role of cognition in decision making, for example by studying how prior experience affects partner choice. Alternatively, it can be addressed by studying how physiological processes are affected by partners and whether these processes influence subsequent decisions to interact with those partners (Brosnan and Bshary, 2010). Recently the latter approach has been used to study whether hormones play a role in cooperative games in humans. For example trust, which affects the likelihood of individuals to invest in potentially risky cooperative interactions, has been shown to be promoted by oxytocin (Kosfeld et al., 2005; Baumgartner et al., 2008). One mechanism by which oxytocin might exert this effect on trust is by suppressing the standard physiological response to a risky situation, namely the glucocorticoid stress response (Ditzen et al., 2009). Glucocorticoids are released by the adrenals and target a wide range of peripheral and neural tissues to optimize behavioral stress responses when needed (Selye, 1936; Korte et al., 2005; Oliveira and Galhardo, 2009). Consequently, in humans the release of glucocorticoids (cortisol) in response to stress is associated with lowered levels of interpersonal trust (Takahashi et al., 2005). Therefore, stress may reduce an individual's trust and thereby the motivation to invest in cooperative interactions that bear the risk of exploitation by an uncooperative partner.

While human studies have typically investigated dyadic interactions, more complicated interactions exist in multi-player interactions. This is the case in cleaning mutualism involving the bluestreak cleaner wrasse and its reef-fish clients which can be thought of as triadic since it involves the cleaner fish, the client and the client's ectoparasites (Eibl-Eibesfeldt, 1955; Limbaugh, 1961; Bshary and Côté, 2008). The health of clients (player 1) is constantly challenged by ectoparasites

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(player 2). Clients may reduce the impact of ectoparasites (Jones and Grutter, 2005; Grutter, 2008; Jones and Grutter, 2008) by seeking out the service of cleaner fish (player 3) that forage on ectoparasites off the skin of their clients (Grutter, 1996b; Grutter, 1999; Bshary and Grutter, 2002a; Grutter and Lester, 2002). While several studies support the notion that interactions with cleaners are beneficial to clients (Gorlick et al., 1987; Grutter, 1999; Ros et al., 2011; but see Grutter, 1996a), interacting with cleaners is not without risk as the latter prefer to eat the client's protective and nutrient rich mucus over ectoparasites (Grutter, 2001). We refer to cleaners eating mucus as cheating while we refer to cleaners eating ectoparasites as cooperating (Grutter and Bshary, 2003). The risk for clients to be cheated by cleaners is reduced when their ectoparasite load is high (Bshary and Grutter, 2002a; Soares et al., 2008). Therefore, clients must balance the costs of high exposure to ectoparasites with the costs of being cheated by cleaners when deciding whether or not to interact with cleaner fish. Cortisol may play a crucial role in this decision making: higher ectoparasite load in salmonids increases the glucocorticoid stress response (Ruane et al., 2000; Wagner et al., 2008), while decreased ectoparasite load in clients with access to a bluestreak cleaner wrasse is related to a lower glucocorticoid stress response (Bshary et al., 2007).

In this study we asked whether antagonizing the stress response might reduce the client's tendency to seek out cleaning interactions. Hence this would create the opposite pattern to what has previously been described in humans. We predicted that the modulation of cortisol levels as a function of parasite exposure would play a causal role in the motivation of clients to visit cleaning stations and that this modulation would help to minimize the costs of cheating by bluestreak cleaner wrasses. We tested this hypothesis by measuring the effects of RU486 (Mifepristone) on cleaning visits. In teleosts RU486 has been regularly used as a glucocorticoid receptor antagonist (Bernier et al., 1999) and it has been shown to suppress cortisol mediated effects on a wide range of physiological traits (metabolism: Vijayan and Leatherland, 1992; Bernier and Peter, 2000; reproduction: Goos and Consten, 2002; osmotic stress: McDonald et al., 2004; Shaw et al., 2007), and behavioral traits (social status: DiBattista et al., 2005; vocalizations in fish: Remage-Healey and Bass, 2004; aggression: Schjolden et al., 2009). RU486 has also been shown to have high affinity to progesterone receptor complexes in mammals (Baulieu, 1991; Cadepond et al., 1997; Bury et al., 2003), and recently Chen et al. (2010) reported that RU486 blocked the effects of progesterone on androgen production in zebrafish testes. Thus, RU486 may cause behavioral effects via two pathways: one related with stress, the other with reproduction.

The effect of RU486 was measured on the behavior of free living lined bristletooth, *Ctenochaetus striatus* Quoy and Gaimard 1825. The lined bristletooth is a common surgeonfish with a small home range on shallow reefs (Krone et al., 2008). It has a relatively high ectoparasite load (Soares et al., 2008) and is a common client of the bluestreak cleaner wrasse in the study area (Bshary, 2001). We predicted that if increased stress responses are associated with higher parasite exposure (Bshary et al., 2007)—and hence with increased tendency to seek out cleaners (Grutter, 2001)—that blocking these stress responses with RU486 treatment should lead to a reduction in cleaning interactions in surgeonfish.

Methods

Field site, capture procedures and re-sighting probability

The study was carried out in the gulf of Aquaba in Egypt from October to September 2009 at Mersa Bareika in Ras Mohammed National Park, and from April to Mai 2010 at Rick's reef and Suleiman's reef in Dahab. Both locations are rich in reef patches that are interspaced with areas of sandy substrate. Lined bristletooth were intercepted in their home range by blocking their possible escape routes using a 4 m by 1.5 m barrier net (mesh size 2 cm). In total 48 fish were captured. After each single capture the fish was anesthetized and randomly assigned to one of the two experimental groups. In Mersa Bareika reef patches were at 5 to 9 m deep and fish were brought to the shore for anesthesia, surgery and marking of fish. Close to Dahab reef patches were 7 to 15 m deep and less accessible from the shore than in Mersa Bareika. Therefore all procedures at Dahab were carried out while scuba diving. The number of fish that were re-sighted 1 week after release was higher after handling fish underwater than after handling fish at the shore (Table 1: 81% vs 44%).

Experimental treatment

Silastic implants (Degania Silicone Europe GmbH, Regensburg, Germany) measured 1.3 cm length, with inner diameter of 1.47 mm and outer diameter of 1.96 mm. These were filled with either 20 µl castor oil only (Rectapur grade, VWR), or 20 µl of the glucocorticoid receptor antagonist Mifepristone (RU486, Sigma) dissolved in castor oil (200 mg/ml castor oil). The ends of the implants were sealed with silicon glue.

Of the 48 individuals we captured (Table 1; Mersa Bareika: n = 27, Dahab: n = 21; Mersa Bareika only: fork length = 14.8 cm, S.E. = 0.3; bodyweight = 87 g; S.E. = 3), 23 individuals were assigned as control: C group; and 25 individuals received a Mifepristone filled implant: RU486 group. The implant contained about 50 mg RU486/kg fish bodyweight. This dosage was at the lower end of the range in comparison to other studies on teleosts: (100–50 mg/kg in the goldfish, *Carassius auratus* Bernier and Peter, 2000; 50 mg/kg in the common carp, *Cyprinus carpio* Goos and Consten, 2002; 1100 mg/kg in the rainbow trout, *Oncorhynchus mykiss* DiBattista et al., 2005). In the goldfish 50 mg/kg effectively blocked glucocorticoid receptors in the hypothalamic–pituitary–interrenal axis as it increased corticotropin-releasing hormone and cortisol production (Bernier and Peter, 2000). In the common carp this dosage prevented the effects of cold stress on spermatogenesis (Goos and Consten, 2002).

All surgery was carried out at the field sites and under anesthesia with phenoxyethanol (Koi Med Sleep, Fishmed GmbH, Switzerland). Underwater (while scuba diving) this was accomplished by adding the anesthesia to a plastic transparent zip-lock bag in which the fish was kept during all handling. A 1.5 mm incision was made close to the pelvic fin using the sharp point of a 21 gauge needle through which the silastic tube was inserted into the peritoneal cavity. After surgery, fish were temporarily marked with a unique combination of color beads attached to the dorsal fin. After handling, the fish were allowed to recover from anesthesia for 15 to 30 min and subsequently they were released at the site of capture.

Behavioral observations

Behavior was recorded by means of focal sampling (Martin and Bateson, 1993), between days six and eight after capture. Focal individuals were approached to approximately 4 m and allowed to habituate to the observer's presence, as was mostly evident by returning to normal feeding activities (~5 min). Observation periods were between 30 and 60 min depending on searching times for the focal

Table 1
Overview of treatments and of achieved behavioral records of the lined bristletooth.

Place of capture	Treatment	Marked and released	Behavior recorded (+only sighted)
Mersa Bareika:	Control	13	6
handling at shore	RU486	14	5 (+1)
Dahab: handling	Control	10	7
while scuba diving	RU486	11	8 (±2)
Total n-values		48	26 (+3)