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Cortisol mediates cleaner wrasse switch from cooperation to cheating and tactical deception

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ABSTRACT

Recent empirical research, mostly done on humans, recognizes that individuals' physiological state affects levels of cooperation. An individual's internal state may affect the payoffs of behavioural alternatives, which in turn could influence the decision to either cooperate or to defect. However, little is known about the physiology underlying condition dependent cooperation. Here, we demonstrate that shifts in cortisol levels affect levels of cooperation in wild cleaner wrasse *Labroides dimidiatus*. These cleaners cooperate by removing ectoparasites from visiting 'client' reef fishes but prefer to eat client mucus, which constitutes cheating. We exogenously administrated one of three different compounds to adults, that is, (a) cortisol, (b) glucocorticoid receptor antagonist mifepristone RU486 or (c) sham (saline), and observed their cleaning behaviour during the following 45 min. The effects of cortisol match an earlier observational study that first described the existence of "cheating" cleaners: such cleaners provide small clients with more tactile stimulation with their pectoral and pelvic fins, a behaviour that attracts larger clients that are then bitten to obtain mucus. Blocking glucocorticoid receptors led to more tactile stimulation to large clients. As energy demands and associated cortisol concentration level shifts affect cleaner wrasse behavioural patterns, cortisol potentially offers a general mechanism for condition dependent cooperation in vertebrates.

Introduction

What conditions make an individual help another unrelated individual, i.e. increase the recipient's direct fitness? Today, a large variety of functional concepts describe conditions and strategies that explain how the helper gains direct fitness benefits as well (Bshary and Bronstein, 2011; Lehmann and Keller, 2006; Leimar and Hammerstein, 2010; Sachs et al., 2004; West et al., 2007). In contrast, explicit studies on the link between physiology and helping behaviour among unrelated individuals are currently rare and are largely restricted to humans. For example, Kosfeld et al. (2005) showed that the application of oxytocin increases trust in humans and hence their tendency to cooperate in situations where cheating by the partner is an obvious risk. Also in humans, lower levels of the neurotransmitter serotonin reduce cooperative play during an Iterated Prisoners Dilemma Game (Wood et al., 2006) while its enhancement seems to contribute to the increased cooperative communication and play during Mixed-Motive Games (Tse and Bond, 2002a, 2002b). Recently, the neuropeptide arginine vasotocin was implicated in the regulation of cooperative behaviour in a fish cleaning mutualism (Soares et al., 2012), which was a strong indication of the potential role of cortisol as another candidate modulator of cooperative levels and defection. For example, in meerkats, the level of investment of helpers when raising offspring depended on cortisol levels, with higher levels associated with a greater investment (Carlson et al., 2006).

Glucocorticoids (GCs) are a key component of the stress response, which modulates a variety of biological processes that prepare animals for novel, and sometimes extremely challenging, social and environmental shifts (Dallman, 2005; Lupien et al., 2009). GCs coordinate multiple modes of actions, some of which are fast enough (seconds to minutes) to contribute to rapid behavioural adaptation (Tasker et al., 2005, 2006). In humans, rapid central effects of GCs are related to fear detection and consolidation of memories that are linked to strong emotional contexts, which can be negative or positive (Lupien et al., 2007). In non-human models, fast, non-genomic GC actions are known to mediate an increase in locomotion, food intake, ingestion of carbohydrates, vocalization, and aggressive behaviour, while contributing to a decrease in sexual clasping, memory, and adrenocorticotropic hormone (ACTH) secretion (Dallman, 2005). Furthermore, changes in baseline glucocorticoids (i.e. cortisol) levels are also known to affect

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attention levels and alertness (Chapotot et al., 1998). However, much remains to be discovered when it comes to the contribution of GCs to social decision-making processing in non-human animals.

An ideal model animal to study the effects of manipulating circulating levels of cortisol on cooperative behaviour is the cleaner wrasse *Labroides dimidiatus*. These cleaners provide a service to so-called client fish by removing ectoparasites, but also mucus and scales (Bshary and Côté, 2008; Côté, 2000; Randall, 1958). Male cleaner wrasses are harem holders and most frequently live and clean in pairs, usually with the largest female of his harem, although the other females are regularly visited (Robertson, 1972). A conflict of interest exists between cleaners and clients because the cleaners prefer mucus over ectoparasites, where eating mucus constitutes cheating (Grutter and Bshary, 2003). Clients use various partner control mechanisms to keep cleaner service quality high, including the threat of reciprocity by predatory clients, partner switching, punishment, and even image scoring when acting as bystanders in an interaction (Bshary, 2011; Pinto et al., 2011). As a consequence of clients exerting partner control, cleaners have to decide in each interaction how frequently they dare to eat their client fish's mucus, despite the risk of negative client responses. Interestingly, cleaners may vary in how they respond to this trade-off. Bshary (2002a) provided a first description of two very different cleaner behavioural strategies, which are not fixed (Bshary and D'Souza, 2005): the majority of 'normal' cleaners show low interest in small clients and rarely cheat larger clients, while a small minority of 'biting' cleaners cheat large non-predatory clients with approximately six times higher frequency (Bshary, 2002a). Interestingly, such biting cleaners seek small clients and mainly provide them with tactile stimulation (with their pectoral and pelvic fins) (Bshary, 2002a). Tactile stimulation lowers basal and acute cortisol levels in clients (Soares et al., 2011), and cleaners normally use it to build relationships with new clients, to reconcile after having cheated and also as a pre-conflict management strategy with predators (Bshary, 2002b; Bshary and Würth, 2001; Grutter, 2004). Because clients arriving at a cleaning station are most likely to invite inspection if they witness an ongoing interaction without conflict (Bshary, 2002a; Bshary and Grutter, 2006; Pinto et al., 2011), providing regularly tactile stimulation to small clients will attract any potential observer (Bshary, 2002a). Thus, large clients that happen to observe such an interaction are deceived by biting cleaners due to a signal out of its typical context (Bshary, 2002a): clients rely on false information to invite for inspection and are then cheated. 'Biting' individuals were invariably females, and biting was typically documented during the spawning season (Bshary and D'Souza, 2005).

A change in reproductive status is typically accompanied by a rise in GC concentration, which implies an increase in the costs of maintaining homeostasis, e.g. an increase in allostatic load (Goymann and Wingfield, 2004). Shifts in social status are also known to have a profound influence on animals' allostatic load (Abbott et al., 2003; Creel, 2001; Goymann and Wingfield, 2004). Cleaner wrasses are protogynous hermaphrodites, i.e. individuals first reproduce as females and eventually change sex into males that control a harem of females (Robertson, 1972). It is thus conceivable that female cleaner wrasse, first, should experience a rise in energetic demands during reproduction, and second, this may be enhanced by selection pressure on fast growth in order to become a male and achieve a relatively higher reproductive output (Robertson, 1972; Sakai et al., 2001). This rise in allostatic load should be related to an elevation of female GCs levels, which might play a role in the decision to switch (even if only temporarily) to become a 'biting' cleaner. This would occur under the assumption that the biting strategy increases current energy uptake via the ingestion of higher amounts of client energy-rich mucus (which cleaners prefer when compared with ectoparasites; Grutter and Bshary, 2003). In addition, it would occur at the expense of future benefits because visiting clients are known to respond to a poor service by switching to different stations for their next inspection (Bshary and Schäffer, 2002; Soares et al., 2013).

The role of stress-related mechanisms on the modulation of cleaner fish levels of cooperation remains little understood. Cleaner wrasses' ability to switch between behavioural tactics revealed the existence of a conditional strategy (Bshary, 2002a); however, the underlying physiological mechanisms are unclear. Here, we aimed to discover the potential role of changes in cortisol levels and the ability of cortisol signalling pathways to operate on the social decision-making process of the cleaner wrasse. We conducted our study in natural conditions to determine whether exogenous administration of GCs (cortisol and the GC receptor antagonist mifepristone RU486) would produce variations in their degree of cooperation (tactile stimulation and cheating, the latter measured as client "jolts" in response to a feeding bite; Bshary and Grutter, 2002; Soares et al., 2008) when dealing with interspecific partners. Because the data from Bshary (2001) suggest that changes in service quality may vary according to a client's value as a food patch, we recorded client size as a correlate of this value (Bshary, 2001; Grutter, 1994).

Methods

Field methods

Field experiments were carried out on 10 different reefs around Lizard Island (Lizard Island Research Station, Australia, 14° 40′S, 145° 28′E) between August and September 2011, in which 24 female cleaner fish were tested. Larval settlement of *L. dimidiatus* at these reefs mostly occurs in November and December (larvae settle about 3 weeks after hatching, Brothers et al., 1983), which indicates that cleaner spawning occurs between October and December (Grutter, 2012; Waldie et al., 2011) and that our field experiments therefore occurred in a "non-spawning" season. We thus assumed that all sampled females were "normal" cleaners. All manipulations and observations were made by two SCUBA divers, between 10:00 and 16:00 h. Cleaner fish were selected haphazardly across the reefs and cleaning stations varied in depth between 1.5 and 12 m. Individuals were captured using a barrier net and measured to the nearest mm (TL—total length). TL of the fish ranged from 6.0 to 8.7 cm. Body weight was then estimated from a length–weight regression (Soares et al., 2012). We then gave the focal female an intra-muscular injection of one of three compounds: (a) hydrocortisone ("cortisol"), dosage 1 μg per gram of body mass (gbm, Sigma, H4001), (b) GC receptor antagonist Mifepristone RU486, dosage 3 μg per gbm (Sigma, M8046) and (c) saline (0.9 NaCl). The steroids were first dissolved in 50 μl of ethanol and only then were the solutions made with saline (and left overnight to complete ethanol evaporation). The control solution used (saline) was also prepared with an equivalent amount of ethanol as the treatment groups. Injection volumes ranged from 20 to 50 μl. Fish handling never exceeded 3 min. Once an individual was released, it was then videotaped for the next 45 min, using video cameras in waterproof cases (Sony HDR-XR155). The order of the treatments was randomized for each dive and all treatments used different cleaner fish. Because this study was done exclusively in field conditions with limitations of time and number of fish used (collecting permit allowance), and because the removal of blood would equate to animal death, dosages chosen were based on previous studies (DiBattista et al., 2005; Remage-Healey and Bass, 2004) and not through dosage effect tests.

Behavioural data collection

Video recordings were made from a distance of 2–3 m. During each video analysis, we recorded the following measures: (a) family identification and TL of each client (estimated visually to the nearest cm, using the focal cleaner fish's size estimation as proxy) visiting the cleaning station; (b) the number of tactile stimulations provided