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Corticosteroid receptor gene expression is related to sex and social behaviour in a social fish

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ABSTRACT

Circulating corticosteroids have been related to social status in a variety of species. However, our understanding of corticosteroid receptor expression and its relationship with sociality is still in its infancy. Knowledge of variation in receptor expression is critical to understand the physiological relevance of differences in circulating corticosteroid concentrations. In this study, we examined corticosteroid receptor gene expression in relation to dominance rank, sex, and social behaviour in the highly social cichlid fish, *Neolamprologus pulcher*. We examined the relative gene expression of the three known teleost corticosteroid receptors: glucocorticoid receptor 1 (GR1), glucocorticoid receptor 2 (GR2), and the mineralocorticoid receptor (MR) in liver and brain tissue of dominant and subordinate *N. pulcher* males and females. Phylogenetic analysis revealed the *N. pulcher* gene originally described as GR2, clustered with other teleost GR1 genes, while the originally-described *N. pulcher* GR1 gene clustered with the GR2 genes of other teleosts. Therefore we propose a change in the original nomenclature of the *N. pulcher* GRs: GR1 (formerly GR2) and GR2 (formerly GR1) and adopt this new nomenclature throughout this manuscript. Liver MR transcript levels were higher in males than females, and positively related to submissive behaviour. Liver GR2 (formerly GR1) transcript levels were also higher in males than females. Collectively, the results demonstrate sex differences in corticosteroid receptor abundance, and suggest tissue- and receptor-specific roles for corticosteroid receptors in mediating aspects of social behaviour.

1. Introduction

Corticosteroids are involved in a suite of physiological maintenance functions including circadian rhythms, osmotic balance, and energy storage and mobilisation (see reviews by Mommsen et al., 1999; Sapolsky et al., 2000; Romero, 2004; Bonier et al., 2009). When an individual is faced with an acute challenge, circulating corticosteroid levels increase several-fold, and initiate many physiological and behavioural changes that are collectively termed a stress response. These responses promote individual survival during and after exposure to the challenging event (see reviews by Wingfield et al., 1998; Breuner et al., 2008). The corticosteroid response is controlled by negative feedback loops, and circulating corticosteroid levels return to baseline after the challenge subsides (see reviews by Sapolsky et al., 2000; Romero, 2004). However, if challenges are persistent or repeated, circulating corticosteroid levels can become chronically elevated, and are often associated with muscle catabolism, and suppression of immune function and reproduction (see reviews by Greenberg and Wingfield, 1987; Barton, 2002; Moore and Jessop, 2003). The negative feedback loops controlling corticosteroid secretion mean that individuals with chronically elevated corticosteroid levels are likely to have a reduced capacity to mount an acute stress response when faced with an immediate challenge (Sapolsky et al., 2000; Romero, 2004). Thus, circulating corticosteroids can have very different physiological effects depending on circulating concentration (i.e., baseline or stress-induced) and on the duration that a particular circulating level is maintained.

The general patterns of circulating corticosteroids in a social context have recently become more clear (see reviews by Creel, 2001; Goymann and Wingfield, 2004; Young et al., 2006; Schoech et al., 2007; Rubenstein and Shen, 2009). In dyadic aggressive encounters, losers typically display elevated corticosteroid levels relative to winners (see reviews by Gilmour et al., 2005; Sapolsky, 2005; although see Correa et al., 2003; Øverli et al., 1999; Buchner et al., 2004; Earley et al., 2006; Earley and Hsu, 2008 for exceptions). However, in stable social groups, dominance hierarchies readily emerge and within these established hierarchies the patterns of corticosteroid levels among individuals depend on a variety of social factors. In particular, the costs of group life shouldered by different individuals within the group are a reliable predictor of the variation in baseline corticosteroid secretion

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(Creel, 2001; Abbot et al., 2003; Goymann and Wingfield, 2004; Rubenstein and Shen, 2009). Costs of group living include behaviours such as maintaining the group territory, defending the group's territory against conspecific intruders and predators, caring for offspring (by parents and by non-reproductive subordinates), policing or reproductively suppressing other individuals within the group, and/or participating in agonistic interactions that maintain a dominance hierarchy with other group members. The sum of all such costly behaviours contributes to the overall physiological burden for the individual. The physiological cost associated with the adaptation to adverse social and physical situations is termed 'allostatic load' (McEwen and Wingfield, 2003; Goymann and Wingfield, 2004). Within a social group, individuals that bear a high allostatic load tend to display relatively higher baseline circulating corticosteroid levels compared with individuals with a lower allostatic load (Goymann and Wingfield, 2004; Rubenstein and Shen, 2009). Furthermore, subordinate individuals in highly social groups will often have low circulating corticosteroid levels compared to dominants, and this may be because they are able to effectively avoid direct aggression through the use of appeasing submissive displays (Bergmüller and Taborsky, 2005).

The effects of corticosteroids are mediated through intracellular glucocorticoid (GR) and mineralocorticoid receptors (MR) that act as ligand-dependent transcription factors (Mommsen et al., 1999; Prunet et al., 2006; Stolte et al., 2006; Bury and Sturm, 2007). Teleost fish express both GRs and MRs (see reviews by Prunet et al., 2006; Bury and Sturm, 2007) and most fish species examined to date possess two GR isoforms (GR1 and GR2, Greenwood et al., 2003; Bury et al., 2003), with one known exception in zebrafish (*Danio rerio*), that possess only a single GR (Alsop and Vijayan, 2008). Accumulating evidence indicates that the gene expression of these receptors is influenced by circulating corticosteroid levels. For example, Johansen et al. (2011) recently demonstrated that rainbow trout (*Oncorhynchus mykiss*) bred for a low post-stress cortisol response exhibited higher brain MR mRNA transcript levels compared to fish bred for high cortisol responsiveness to a stressor. Similar patterns of GR mRNA abundance between trout with different cortisol responsiveness were attributed to an autoregulatory pathway that involves negative feedback signalling from circulating corticosteroids (Sathiyaa and Vijayan, 2003). Given this information and the effects of social status and behaviour on corticosteroid concentrations, both social status and social interactions are likely to influence corticosteroid receptor expression. This possibility has yet to be explored and quantified in a species where individuals that live in groups with stable, long-term, social hierarchies.

In the current study, we investigated patterns of corticosteroid receptor gene expression as a function of dominance rank, sex, and social behaviour in *Neolamprologus pulcher*, a highly social cooperatively breeding cichlid endemic to Lake Tanganyika, Africa. *N. pulcher* live in stable social groups consisting of a dominant breeding male and female, and 1–20 non-breeding subordinates of either sex (Taborsky and Limberger, 1981; Balshine et al., 2001; Heg et al., 2005). Dominant individuals display higher circulating cortisol concentrations (cortisol being the primary corticosteroid in fish, Mommsen et al., 1999) than subordinate non-reproductive individuals (Mileva et al., 2009), and dominant individuals also have a higher allostatic load (i.e., higher physiological costs) than subordinate individuals (Mileva et al., 2009). Although Mileva et al. (2009) failed to detect a significant relationship between specific social behaviours and cortisol concentrations, Bender et al. (2006) found that the most submissive individuals displayed the lowest circulating cortisol levels. No sex differences in circulating cortisol have been documented in *N. pulcher* (Bender et al., 2008; Mileva et al., 2009), but females display more costly maintenance behaviours (i.e., provide more parental care and more territory defence) than do males within the social groups (Balshine et al., 2001; Desjardins et al., 2008; Mileva et al., 2009).

In this study, we also used the available sequences from the three documented corticosteroid receptors in *N. pulcher* to perform a phylogenetic analysis and place the *N. pulcher* sequences within the broader context of other known fish corticosteroid receptors. We then examined the relative gene expression of these three corticosteroid receptors in brain and liver tissues of individual *N. pulcher*. The brain was chosen as a target organ because it is integral to social function and controls behavioural interactions while the liver was selected because of its key role in growth and metabolic responses to stress, which vary between dominant and subordinate *N. pulcher* (Taborsky, 1984; Mileva et al., 2009; Sopinka et al., 2009). We predicted a general pattern of reduced corticosteroid gene expression in those individuals typically associated with having high circulating corticosteroid levels. As dominant individuals display higher corticosteroid levels than subordinates (Mileva et al., 2009), we predicted that dominant individuals would display lower corticosteroid receptor gene expression than subordinates. Since females typically display more costly maintenance behaviours than males (Balshine et al., 2001; Mileva et al., 2009), we predicted that females would display lower corticosteroid receptor gene expression compared to males. We predicted a negative relationship between corticosteroid gene expression and maintenance behaviours (i.e., parental care and policing behaviours). Since submissive behaviour is related to lower circulating corticosteroid levels (Bender et al., 2006), we predicted a positive relationship between submissive behaviour and corticosteroid receptor gene expression.

2. Materials and methods

2.1. Experimental animals

All fish used in this experiment were adults from a breeding colony of *N. pulcher* held at McMaster University, Hamilton, Ontario, Canada. Fish were descendants of male and female breeding pairs caught in Lake Tanganyika, Zambia, in 2001 and 2002. Animals were housed in social groups consisting of a male and female dominant breeding pair with 1–20 subordinate helpers. Each social group inhabited a 189 L freshwater tank outfitted with a heater, thermometer, 2 foam filters, ~2 cm of coral sand substrate, a mirror placed at each end of the tank, and two inverted flowerpot halves for use as shelter and a spawning substrate. The light:dark cycle was kept constant at 13:11 h and water temperature was maintained at 26 ± 2 °C. Fish were fed 6 days per week with Nutrafin Basix commercial flake cichlid food.

2.2. Experimental protocol

The social groups used in this study represented a subset of the social groups used in Mileva et al. (2009). In total, $n = 30$ social groups were observed for Mileva et al. (2009), and $n = 7$ of these groups were randomly selected for the current study. The dominant breeding pair and the two largest subordinate helpers from each social group (mean group size 10.1 ± 1.5 standard error of the mean [SEM]) were identified through the use of ethograms available for this species (e.g., Buchner et al., 2004; Sopinka et al., 2009). To accurately track individuals during detailed behavioural observations, all focal fish were netted from their home tanks, sexed by examination of external features, weighed, measured (standard length and mass), and uniquely fin-clipped before being returned to their home tank. Fin clipping does not adversely affect behaviour (Stiver et al., 2004). Detailed behavioural observations were carried out 3–7 days following the measurement and fin clipping of focal individuals. Each individual was observed three times, in 10 min intervals between 8:00 and 13:00 h. All behaviours were recorded for each focal individual following the ethogram outlined in Sopinka et al. (2009). Briefly, the behaviours scored were aggressive behaviours, including aggressive displays (head-up posture or frontal displays with the operculum flared), chasing, biting, and ramming (making contact with another