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Stress axis regulation during social ascension in a group-living cichlid fish

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ABSTRACT

Animals living in groups often form social hierarchies, with characteristic behaviours and physiologies associated with rank. However, when social opportunities arise and a subordinate ascends into a dominant position, quick adjustments are necessary to secure this position. Such periods of social transition are typically associated with elevated glucocorticoid production, but the precise regulation of the stress axis during these occasions is not well understood. Using the group-living cichlid, *Neolamprologus pulcher*, the effects of social ascension on the stress axis were assessed. Ascenders rapidly filled experimentally created vacancies, adopting a dominant behavioural phenotype within 72 h—elevating aggression, activity, and workload, while receiving high rates of affiliative behaviours from their group members. Despite assuming behavioural dominance within their groups, ascenders displayed higher cortisol levels than dominants three days post-ascension. Additionally, compared to subordinates, ascenders had increased transcript abundance of steroidogenic acute regulatory protein (*star*) and cytochrome *p*450 side-chain cleavage enzyme (*p450scc*) in the head kidney, indicating activation of the stress axis. Cortisol levels were lowest in ascenders that displayed low rates of aggression, potentially reflecting the reestablishment of social stability in these groups. Increased transcript abundance of both glucocorticoid receptors (*gr1* and *gr2*) in the brain's preoptic area (POA) of ascenders compared to dominants suggested an enhanced capacity for cortisol regulation via negative feedback. Our results reveal a regulatory cascade of behavioural and physiological interactions and highlight the importance of investigating the underlying mechanisms regulating the stress axis.

1. Introduction

Living in a social group can provide a number of advantages, such as increased vigilance (Evans et al., 2016; Roberts, 1996), improved food acquisition (Evans et al., 2016; Ward and Zahavi, 1973) and workload sharing and load lightening (Ausband et al., 2016; Balshine et al., 2001; Dornhaus et al., 2008). Thus, by living in a group, individuals can save time and energy. However, social life also has costs. Conflicts within groups often arise over access to food, shelter, and reproductive opportunities (Milinski and Parker, 1991; Stockley and Bro-Jørgensen, 2011). Hierarchy formation, a common phenomenon in social groups, is thought to have evolved to reduce conflict over such limited resources. The most competitive individuals typically attain dominant positions, and secure primary access to resources, while less competitive individuals are subordinate and typically have less access to resources. Consequently, dominants and subordinates often differ considerably in terms of behaviour, physiology, health, and fitness (Sapolsky, 2004, 2005; Silk et al., 2003).

Levels of stress frequently vary with social rank and these rank-related physiological phenotypes have been well investigated (Creel, 2001; Creel et al., 2013; Goymann and Wingfield, 2004). However, the majority of studies only measure glucocorticoid levels, and not the mechanisms regulating glucocorticoid production. Moreover, the relationship between social rank and stress is often complex, influenced by a variety of factors. For example, during periods of social instability, challenges in rank order often are associated with elevated glucocorticoid levels (Sapolsky, 1992) but as rank is established glucocorticoids levels usually decrease (Engh et al., 2006; Van Meter et al., 2009). To date, few studies have focused on the mechanisms regulating stress axis dynamics during periods of social instability, but such knowledge would strongly enhance our understanding of the factors influencing social stress.

To study the mechanisms regulating stress during social transitions, we used *Neolamprologus pulcher*, a cooperatively breeding cichlid fish that lives in permanent social groups. Groups consist of a dominant breeding pair and 1–20 subordinate helpers within a hierarchy (Wong and Balshine, 2011). Dominants are more aggressive (Fitzpatrick et al., 2008), more active (Sopinka et al., 2009), and perform more territory

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defense (Desjardins et al., 2008) compared to subordinates. When a dominant position becomes available, a subordinate can ascend to the dominant position (Balshine-Earn et al., 1998; Bergmüller et al., 2005; Fitzpatrick et al., 2008). The effects of social transition on cortisol production as yet have not been assessed in *N. pulcher*, although in *Astatotilapia burtoni*—a closely related cichlid, where transitions between territorial and non-territorial status occur repeatedly and reversibly—social ascension is associated with a rapid increase in circulating cortisol levels (< 30 min; Maruska, 2015) that can persist for 3 or more days (Huffman et al., 2015). However, the mechanisms underlying these changes in glucocorticoid production remain poorly understood.

We tested the hypothesis that periods of social ascension activate the entire stress axis, from the preoptic area (POA) of the brain where stress responses are initiated via release of corticotropin-releasing factor (CRF) to the head kidney, where cortisol is produced by interrenal cells. We removed dominant males from social groups, creating an opportunity for subordinate males to ascend in social rank and assume the dominant position. We predicted that ascending males would rapidly adopt a dominant behavioural phenotype, and that this period would be associated with increased activation of the stress axis. Specifically, we measured and compared circulating cortisol levels as well as transcript abundance of stress axis genes in stable dominant, stable subordinate, and ascending males. In the POA, we targeted CRF (*crf*), which initiates activation of the stress axis following a stressor (Aguilera, 1998). We also measured transcript levels of the glucocorticoid receptors, which contribute to the regulation of cortisol production via negative feedback (Dallman et al., 1994). In most teleost fish, including *N. pulcher* (O'Connor et al., 2013), two isoforms of the glucocorticoid receptor exist (GR1 and GR2; Stolte et al., 2006), and therefore we measured both *gr1* and *gr2*. In the head kidney (analogous to the adrenal in mammals and birds), we measured transcript abundance of melanocortin 2 receptor (*mc2r*), steroidogenic acute regulatory protein (*star*), and cytochrome P450 side-chain cleavage enzyme (*p450scc*). We chose these genes because cortisol synthesis in steroidogenic cells is initiated when MC2R is activated by adrenocorticotropic hormone (Fridmanis et al., 2017), and is rate-limited by the conversion of cholesterol to pregnenolone, involving transfer of cholesterol across the mitochondrial membrane, which is regulated by StAR (Tokarz et al., 2015), and its cleavage to pregnenolone, which is catalyzed by P450scc (Payne and Hales, 2004).

2. Materials and methods

2.1. Experimental animals

The experiment was conducted from November 2016 – April 2017 at McMaster University in Hamilton, Ontario, Canada. Fish were laboratory-reared descendants of wild-caught *Neolamprologus pulcher* from Lake Tanganyika, Africa. Social groups consisting of a dominant breeding male-female pair, 1–3 large helpers (standard length (SL) > 4.5 cm), and 1–4 small helpers (SL < 4 cm) were held within 189 L aquaria. All social groups (n = 20) had been together for at least a month and had produced young prior to any experimental manipulation. Each fish was given a unique dorsal fin clip for identification, which does not adversely affect behaviour (Stiver et al., 2004). Each aquarium contained two large sponge filters, a heater, 3 cm of coral sand for substrate, two terracotta flowerpot halves, two mirrors, and two PVC tubes as shelter. Water was kept at 27 °C and a 13 L:11D photoperiod was maintained throughout the experiment. Fish were fed 1% combined group body weight daily with NorthFin floating cichlid pellets (1 mm; Canadian Aquatic Feed Inc., Toronto, ON, Canada). All experimental protocols were approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol No. 14-02-05), and were in compliance with the guidelines of the Canadian Council on Animal Care (CCAC) regarding the use of animals in research and teaching.

2.2. Experimental protocols

Thirty-two focal fish were targeted in this experiment. At the beginning of the experiment (Day 0), all individuals within a group were weighed and measured, and each group was randomly designated as control (N = 8; average of 6.75 ± 0.45 group members) or treatment (N = 12; average of 6.83 ± 0.42 group members). Behavioural observations (see Section 2.3) were recorded using a video camera (Canon VIXIA HF S200) on Days 10, 11, 13, and 14, and later scored. In treatment groups, dominant males were removed and sampled (mass = 7.45 ± 0.35 g, SL = 6.73 ± 0.18 cm, mean ± SEM; N = 12) on the morning of Day 11—providing an opportunity for subordinate males within these groups to ascend to the dominant position. On the morning of Day 14, ascending males were removed and sampled (mass = 5.21 ± 0.38 g, SL = 5.86 ± 0.17 cm; N = 8). In four of the twelve treatment groups, a clear dominant male had not emerged by Day 14 and therefore target ascending males were not collected from these groups. In control groups, subordinate males were removed and sampled on the morning of Day 11 (mass = 3.79 ± 0.31 g, SL = 5.23 ± 0.12 cm; N = 12). Note that in four control groups, two stable subordinate males were sampled.

2.3. Behavioural analyses

Fish were given a 5 min acclimation period following placement of the camera in front of their tank. Focal fish were then continuously recorded for 10 min and all behaviours performed or received were scored (see Sopinka et al., 2009 for a detailed species-specific ethogram). A dominance index (see Fig. 1A) was determined for each focal fish by subtracting the combined number of aggressive acts (chases, bites, rams, opercular flares, aggressive postures, and lateral displays) received and submissive acts (flees, and submissive postures and displays) given from the total number of aggressive acts given and submissive acts received ($DI = (Agg_{Given} + Sub_{Rec}) - (Agg_{Rec} + Sub_{Given})$, see Fitzpatrick et al., 2008). The total number of affiliative acts (follows, parallel swims, and soft touches) received from all group members was also determined (see Fig. 1B). A workload index (see Balshine et al., 2001) for each fish was assigned by combining the number of visits to the brood chamber, the number of territory maintenance behaviours (digs and carries—the act of picking up and moving substrate with their mouths; see Fig. 1C), and defensive aggressive acts performed towards a mirror and neighbours. To assess locomotor activity, tanks were visually split into 12 quadrats using a grid and the number of times each fish crossed between quadrats was counted (see Fig. 1D). Behaviours are reported as averages of the two observation periods (i.e. Days 10/11 or Days 13/14). Following the initial behavioural recordings on Days 10 and 13, an unfamiliar male conspecific (SL = 5.82 ± 0.14 cm) was placed in the centre of the tank within a clear perforated plastic tube (see Fig. 1E) and the number of aggressive defense acts performed towards the intruding conspecific was recorded over a 10 min period.

2.4. Tissue sampling

Fish were rapidly netted and killed via terminal anaesthesia (0.5 g L^{-1} ethyl-*p*-aminobenzoate; Sigma-Aldrich, Oakville, ON, Canada), and mass and standard length were recorded. All fish were sampled between 0800 h and 1100 h to avoid diurnal fluctuations in