General and Comparative Endocrinology 212 (2015) 106–113

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Arginine vasotocin regulates social ascent in the African cichlid fish *Astatotilapia burtoni*

Lin S. Huffman [a], Flora I. Hinz [a], Sophie Wojcik [b], Nadia Aubin-Horth [b,c], Hans A. Hofmann [a,*]

[a] The University of Texas at Austin, Department of Integrative Biology, Institute for Cellular and Molecular Biology, Center for Brain, Behavior, and Evolution, Austin, TX 78712, USA
[b] Université de Montréal, Département de Sciences Biologiques, Montréal, Québec H2V 2S9, Canada
[c] Université Laval, Département de Biologie & Institut de Biologie Intégrative et des Systèmes, Québec, Québec G1V 0A6, Canada

ARTICLE INFO

Article history:
Available online 21 March 2014

Keywords:
Nonapeptides
Manning compound
Aggression
Social dominance
Plasticity

ABSTRACT

Neuropeptides modulate many aspects of behavior and physiology in a broad range of animals. Arginine vasotocin (AVT) is implicated in mediating social behavior in teleost fish, although its specific role varies between species, sexes, life stages, and social context. To investigate whether the effects of AVT on behavior depend on social context, we used the African cichlid fish *Astatotilapia burtoni*, which is well-known for its remarkable behavioral plasticity. We pharmacologically manipulated the AVT system in established socially dominant and subordinate *A. burtoni* males, as well as in males ascending to dominance status in a socially unstable environment. Our results show that exogenous AVT causes a stress response, as evidenced by reduced behavioral activity and increased circulating levels of cortisol in established dominant and subordinate males. Administration of the AVT antagonist Manning compound, on the other hand, did not affect established subordinate or dominant males. However, AVT antagonist-treated males ascending from subordinate to dominant status exhibited reduced aggressive and increased courtship behavior compared to vehicle-treated animals. Finally, we measured circulating cortisol levels and brain gene expression levels of AVT and its behaviorally relevant V1a2 receptor in all three social phenotypes and found that plasma cortisol and mRNA levels of both genes were increased in ascending males compared to dominant and subordinate males. Our results provide a more detailed understanding of the role of the AVT system in the regulation of complex behavior in a dynamically changing social environment.

1. Introduction

Neuropeptide systems such as the nonapeptide arginine vasotocin (AVT) and its mammalian homolog arginine vasopressin (AVP) are present in diverse taxa, but their functional roles in behavior can vary widely. In addition to being involved in the regulation of a variety of social behaviors in all vertebrate species studied thus far (reproduction, Salek et al., 2002; pair-bonding, Winslow et al., 1993; Oldfield and Hofmann, 2011; parental care, Wang et al., 1994; Kleszczyńska et al., 2012; affiliation, Landgraf et al., 2003; Young and Wang, 2004; social approach, Thompson and Walton, 2004; Braida et al., 2012), the AVT/AVP system also regulates central and peripheral stress responses (Engelmann et al., 2004), although its specific role appears to differ between species, sexes, life stages, and social contexts (for review, see Goodson (2008), Godwin (2010)). This neuropeptide system has also been associated with social status (Ferris et al., 1989; Godwin et al., 2000; Goodson and Bass, 2001; Aubin-Horth et al., 2007; Greenwood et al., 2008; Almeida et al., 2011; Lema et al., 2012) and with aggressive behavior in males (Ferris et al., 1997; Goodson, 1998; Delville et al., 2000). However, the involvement of AVT in male aggression can depend on social context (Semsar et al., 2001; Greenwood et al., 2008; Filby et al., 2010), although a detailed understanding is still lacking.

Even though the role of AVT in behavioral regulation appears to be conserved in some manner across vertebrates, it is clear that AVT might play different roles in different social contexts, and it has been suggested that this may be observed even within a single species (Goodson, 2008; Ophir, 2011). To gain a better understanding of these processes we need a model system in which differences in social context are associated with differences in behaviors and AVT levels, and in which we can experimentally test the behavioral role of AVT in these different social contexts.

The African cichlid fish *Astatotilapia burtoni* is an established model system in social neuroscience and is well-suited to examine AVT function due to its remarkable behavioral plasticity and ease of experimentation (Hofmann, 2003; Fernald and Maruska,

* Corresponding author. Address: The University of Texas at Austin, Department of Integrative Biology, 2415 Speedway – C0990, Austin, TX 78712, USA.
E-mail address: hans@utexas.edu (H.A. Hofmann).

http://dx.doi.org/10.1016/j.ygcen.2014.03.004
0016-6480/

2012). In this species, males are either dominant (DOM) or subordinate (SUB). DOM males establish and aggressively defend display territories, are brightly colored, and attract females for reproduction. Non-territorial SUB males are non-reproductive and non-aggressive, cryptically colored, and shoal with females and juveniles. Males transition between social states every 4–7 weeks on average depending on social and environmental conditions, likely as a consequence of the high energetic cost associated with being dominant (Hofmann et al., 1999): DOMs grow at a slower rate than SUBs, which allows the latter to eventually gain a size advantage and overtake occupied territories (Hofmann et al., 1999; Hofmann, 2003). This remarkable plasticity has been well characterized at the behavioral, hormonal, and genomic levels for the established DOM and SUB phenotypes (Hofmann et al., 1999; Hofmann and Fernald, 2000; Parikh et al., 2006; Trainor and Hofmann, 2006, 2007; Renn et al., 2008; O'Connell and Hofmann, 2012), including detailed examinations of the phenotypes of males ascending from SUB to DOM (Hofmann and Fernald, 2000; Burmeister et al., 2007; Maruska and Fernald, 2010, 2011; Maruska et al., 2011, 2012; Huffman et al., 2012a). These studies suggest that social status and experience greatly influence how animals respond to the social environment. Interestingly, AVT expression in the brain varies with social status, with DOMs having higher brain expression levels than SUBs (Renn et al., 2008). More specifically, *A. burtoni* DOM males have higher expression in the gigantocellular nucleus of the POA, and SUB males have higher expression in the parvocellular nucleus of the POA, while expression in the magnocellular nucleus does not differ between phenotypes (Greenwood et al., 2008). The expression of AVT has not yet been examined in males ascending from SUB to DOM. Furthermore, brain expression levels of the V1a2 receptor are not known for any of the male social phenotypes. This receptor is the behaviorally relevant AVT receptor based on studies that have shown expression in brain regions associated with behavior and reproduction (Kline, 2010). While these studies clearly suggest that AVT plays a role in the regulation of social status and aggression in *A. burtoni*, no functional tests have been carried out to dissect the role of AVT, yet this species provides an exceptional opportunity to do so in males of the same species that experience dramatically different social environments.

Here, we examined the functional role of the AVT system in *A. burtoni* males depending on social context. Specifically, we measured neural mRNA levels of AVT and its receptor in the DOM and SUB males that live in a stable social context and ascending males that face an unstable context. We also tested whether the effects of AVT on behavior depend on social context by pharmacologically manipulating the AVT system using an agonist (AVT) and an antagonist to the V1a receptor (Manning compound, MC). We predicted that AVT and V1a2 gene expression would be highest in DOMs and ascending males. Furthermore, since ascending males are exposed to social instability compared to established DOM and SUB males, we predicted that ascending males would be more sensitive to AVT manipulation.

2. Materials and methods

2.1. Animals

All animals used in this study were adult *A. burtoni* males from a laboratory stock originally derived from a wild population in Lake Tanganyika, Africa (Fernald and Hirata, 1977). Fish were maintained at 28 °C on a 12:12 h light/dark cycle with 10 min dawn and dusk periods to mimic their native tropical environment in 110 L aquaria that were integrated into a re-circulating life support system. All tanks contained gravel substrate to facilitate digging behavior and terra cotta pot shards, which served as territorial shelters. Communities were allowed to settle for approximately 1 week before experiments began. All procedures were in accordance with and approved by Institutional Animal Care and Use Committees at The University of Texas and Harvard University.

2.2. Pharmacological manipulations in established DOM and SUB males

To investigate the role of AVT in established males, we tested the effects of AVT and a V1a receptor antagonist (Manning compound, MC) on social status and behavior. We set up communities of ten males and ten females with five terra cotta pots as shelters and allowed them to settle for 5–7 days. One DOM male per community was chosen as the focal male, and after being weighed and measured for standard length on Day 1, he was observed for 10 min on Days 1–3 between the hours of 11:00 and 13:00 to establish a baseline of behavior. Aggressive, reproductive, and neutral behaviors were recorded as described previously (Fernald and Hirata, 1977) as well as any changes in social status. For each observation, aggressive behaviors (chasing, lateral threat displays, border threats) were summed to comprise an aggression score; reproductive behaviors (courting, quivering, digging) were summed to comprise a reproduction score. On Days 4–6, all males from the tank were removed to standardize netting stress and each focal male received an intraperitoneal saline injection ca. 60 min prior to observation, to establish any injection effect on behavior. On Days 7–9, again 60 min prior to observation, each focal male received an injection of either saline ($n = 11$) or AVT (Sigma, 1 μg/gbw; $n = 10$) or MC (Sigma, 3.2 μg/gbw; $n = 6$) dissolved in saline such that each male received only one treatment, for three consecutive days. Doses were based on previous work in bluehead wrasse (Semsar and Godwin, 2004). We also tested a range of doses below those previously used (0.5–0.008 μg/gbw, $n = 6$–14). Following observation on Day 9, their plasma was collected from the dorsal aorta using heparinized 26G butterfly infusion sets (Surflo) and kept on ice until processing. Following blood collection, the animals were killed, and the brains and testes were collected for analysis (see next section).

2.3. Pharmacological manipulations in ascending males

To test the role of AVT in SUBs and ascending males, we set up communities as described previously and chose one SUB male per tank as the focal male. After being weighed and measured for standard length on Day 1, the focal male was observed for 10 min on Days 1–3 at 10:00 h to establish a baseline of subordinate behavior. On Day 4, we again netted all of the males in the tank to inject 60 min before observations. For the ascending males, we did not return the DOM males to their tanks when we removed all of the males, to provide SUB males with an opportunity to compete for dominance. This provided open territories for the SUB males to compete for on Days 4–6. Focal males were injected with either saline ($n = 10$ SUB, $n = 24$ ascending), AVT ($n = 10$ SUB, $n = 8$ ascending), or MC ($n = 9$ SUB, $n = 10$ ascending), 60 min prior to observation at 10:00 h, such that each male received one treatment, for three consecutive days. Following observation on Day 6, males were weighed, their blood was drawn for cortisol measurement, and they were killed for tissue collection.

2.4. Hormone measurements and tissue processing

To separate the plasma from the serum, blood samples were centrifuged at 4000 rpm for 10 min, and the plasma was stored at −80 °C until analysis. Cortisol was measured from plasma samples using ELISA (Assay Designs). Plasma samples were thawed on