Hormones and Behavior 63 (2013) 437–446

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

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

Aggression- and sex-induced neural activity across vasotocin populations in the brown anole

David Kabelik *, Veronica C. Alix, Emily R. Burford, Leah J. Singh

Department of Biology, Rhodes College, 2000N Parkway, Memphis, TN, 38112, USA

ARTICLE INFO

Article history:
Received 3 September 2012
Revised 12 November 2012
Accepted 21 November 2012
Available online 30 November 2012

Keywords:
Anolis
Aggression
Agonistic
Courtship
Lizard
Fos
Sexual behavior
Vasopressin
Vasotocin

ABSTRACT

Activity within the social behavior neural network is modulated by the neuropeptide arginine vasotocin (AVT) and its mammalian homologue arginine vasopressin (AVP). However, central AVT/AVP release causes different behavioral effects across species and social environments. These differences may be due to the activation of different neuronal AVT/AVP populations or to similar activity patterns causing different behavioral outputs. We examined neural activity (assessed as Fos induction) within AVT neurons in male brown anole lizards (*Anolis sagrei*) participating in aggressive or sexual encounters. Lizards possess simple amniote nervous systems, and their examination provides a comparative framework to complement avian and mammalian studies. In accordance with findings in other species, AVT neurons in the anole paraventricular nucleus (PVN) were activated during aggressive encounters; but unlike in other species, a positive correlation was found between aggression levels and activation. Activation of AVT neurons within the supraoptic nucleus (SON) occurred nonspecifically with participation in either aggressive or sexual encounters. Activation of AVT neurons in the preoptic area (POA) and bed nucleus of the stria terminalis (BNST) was associated with engagement in sexual behaviors. The above findings are congruent with neural activation patterns observed in other species, even when the behavioral outputs (i.e., aggression level) differed. However, aggressive encounters also increased activation of AVT neurons in the BNST, which is incongruous with findings in other species. Thus, some species differences involve the encoding of social stimuli as different neural activation patterns within the AVT/AVP network, whereas other behavioral differences arise downstream of this system.

Introduction

Social behaviors are differentially regulated by varying activity levels across a social behavior neural network consisting of various brain regions and neural chemicals (Albers, 2012; Goodson, 2005; Goodson and Kabelik, 2009; Newman, 1999; O'Connell and Hofmann, 2011, 2012; Sakata et al., 2000; Yang and Wilczynski, 2007). The neuropeptide arginine vasotocin (AVT), known in mammals as arginine vasopressin (AVP) due to a single amino acid substitution, is a neuromodulator known to regulate social behaviors across a wide variety of vertebrate taxa (Albers, 2012; De Vries and Panzica, 2006; Godwin and Thompson, 2012; Goodson, 2008; Goodson et al., 2012; Moore, 1992). However, this neuropeptide's role in the regulation of social behaviors is unclear, likely due both to species differences in AVT/AVP distributions (Bester-Meredith et al., 1999; Dewan et al., 2011; Moore and Lowry, 1998; O'Connell and Hofmann, 2012) and the propensity for different AVT/AVP populations to become activated under different experimental/social conditions (Goodson and Bass, 2001; Goodson and Kabelik, 2009; Goodson et al., 2009b; Kabelik et al., 2009; Veenema et al., 2010). These different populations can then modulate different target sites and elicit different behavioral responses. For instance, Veenema et al. (2010) demonstrated that AVP release in the lateral septum of Male Wistar rats correlates positively with intermale aggression, whereas release in the BNST correlates negatively with such aggression. Furthermore, each AVT/AVP population may itself play multiple roles, with involvements in the regulation of multiple social behaviors and neuroendocrine functions (Caldwell et al., 2008; Goodson and Kabelik, 2009; Landgraf and Neumann, 2004; Marler et al., 2003). Hence, it is important to examine distinct patterns of AVT/AVP activity across multiple nodes of its own network in order to better understand how this AVT/AVP network modulates activity in the greater social behavior neural network.

We here examine behavioral induction of the immediate early gene (IEG) product Fos, a marker of neural activity (Herdegen and Leah, 1998; Hoffman et al., 1993), within a number of neural AVT populations that are relatively conserved among tetrapod vertebrates and that have been shown to be relevant to the regulation of social behaviors (De Vries and Panzica, 2006; Goodson and Kabelik, 2009; Moore and Lowry, 1998). We examine these AVT populations within the very small and simple brain of the brown anole lizard (*Anolis sagrei*). Reptilian brains are considered evolutionarily primitive and similar to ancestral mammals (MacLean, 1978), and their examination can shed light on the evolution and functionality of more highly derived mammalian and avian circuitry. Even though lizards serve as excellent models of neural connectivity

* Corresponding author. Fax: +1 901 843 3565.
E-mail address: kabelikd@rhodes.edu (D. Kabelik).

0018-506X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yhbeh.2012.11.016

among nuclei in the social behavior neural network (Sakata et al., 2000; Yang and Wilczynski, 2007), few investigations of AVT within reptilian brains have been conducted and information about the involvement of AVT in the regulation of social behaviors is greatly lacking. That is, although reptilian studies exist demonstrating the effects of AVT on parturition and oviposition (Figler et al., 1989; Guillette and Jones, 1980, 1982; Jones and Guillette, 1982; Propper et al., 1992a), we know of no studies demonstrating direct associations between AVT and the performance of aggressive or sexual behaviors. Hence, we here examine Fos induction (a measure of neural activity) within AVT neurons of male brown anoles following exposure to same-sex versus opposite sex individuals. Furthermore, we examine the individual variation within each of these social interaction treatment groups to determine associations between AVT activity and behavioral intensity, frequency, and latency to perform aggressive or sexual behaviors. Finally, we also compare the stimulus animals' behaviors with AVT-Fos colocalization rates within focal males' brains.

The AVT populations that we here examine include the preoptic area (POA), the bed nucleus of the stria terminalis (BNST), the paraventricular nucleus (PVN), and the supraoptic nucleus (SON). While the PVN and SON consist primarily of large magnocellular AVT/AVP neurons known primarily for their role in the regulation of osmotic homeostasis via release into the periphery, some AVT/AVP neurons in the PVN are also known to be involved in stress-reactivity, especially when the stressors include emotionally salient stimuli (Caldwell et al., 2008; Engelmann et al., 2004; Goodson and Kabelik, 2009; Herman et al., 2003; Landgraf and Neumann, 2004). Furthermore, studies have demonstrated the occurrence of central release from the soma and dendrites of these neurons (Landgraf and Neumann, 2004; Ludwig and Leng, 2006). In some species, including anoles (Propper et al., 1992b), a strip of parvocellular AVT neurons also extends medially from the BNST to the preoptic area (POA); the latter is the ancestral vertebrate source for all AVT/AVP projections (Goodson and Bass, 2001; Huffman et al., 2012; Moore and Lowry, 1998). The BNST neurons are part of the extended amygdala, a region that also includes several amygdalar nuclei (Newman, 1999), though few AVT neurons are present in the brown anole amygdala. These parvocellular AVT neurons project centrally (De Vries and Panzica, 2006; Goodson and Kabelik, 2009).

Previous research in a variety of species has demonstrated the activation of AVT/AVP neurons within the extended amygdala following positive social interactions, while negative social interactions have sometimes been shown to suppressed such activity (Goodson and Kabelik, 2009; Ho et al., 2010). The AVT/AVP neurons of the PVN, on the other hand, primarily show increased activity during stressful situations (Goodson and Kabelik, 2009; Ho et al., 2010). Fewer studies have demonstrated a role of AVT/AVP neurons within the SON in the regulation of social behaviors, although this AVP population has been shown to exhibit changes in IEG induction in golden hamsters or mice participating in aggressive encounters (Delville et al., 2000; Ho et al., 2010) and some involvement of AVP in the SON has been shown on social memory (Landgraf and Neumann, 2004). Based on these published findings, we predicted that in brown anoles aggressive interactions would suppress activity in AVT neurons of the BNST and increase activity of AVT neurons in the PVN and SON, whereas sexual interactions would increase activity in the POA and BNST, and possibly SON, without affecting the PVN population. Our results only partially support these predictions.

Methods

Subjects and treatment groups

The focal subjects of this study were fifty-six adult male brown anole (*A. sagrei*) lizards, obtained from a commercial supplier, and weighing an average of 4.8 ± 0.13 g ($\bar{x} \pm$ S.E.). Twelve focal males were assigned to a control group, while 22 were assigned to a male–male aggressive encounter group and another 22 to a male–female sexual encounter group. Additional male and female brown anoles served as stimulus animals for the behavioral encounters; some stimulus animals were used repeatedly, but only following a week off between behavioral trials. All procedures involving live animals were approved by the Institutional Animal Care and Use Committee.

Behavioral trials

The behavioral trials were carried out from mid-January to mid-March, 2011. Animals were housed under long-day (14 L:10D) conditions. The terrarium was illuminated by a 40 W full-spectrum fluorescent light (Colortone 50, Philips, Amsterdam, Netherlands) suspended 20 cm above a wire-mesh terrarium lid. Heat was provided beyond ambient room temperature by means of a 60-W incandescent white light bulb suspended 5 cm above the terrarium lid. Reproductive status was verified at the time of sacrifice; all testes were determined to be enlarged from winter recrudescence (enlargement begins in December in wild populations; mean combined testes mass $\pm$ S.E. $= 85 \pm 5$ mg; range = 22–157 mg), and this entire range of testes sizes corresponds to periods of high testosterone production in this species (Lee et al., 1989; Tokarz et al., 1998).

Prior to the onset of behavioral trials, the experimental subjects were housed for at least 72 h in one half of a (30.5 cm H $\times$ 26 cm W $\times$ 51 cm L) terrarium, divided into two equal halves (measured along the long edge) by an opaque partition. A focal male was always housed in one terrarium half; the other half was either empty (control treatment), contained a conspecific male that was smaller by one to two mm snout-vent length (male–male aggressive encounter group), or contained a female (male–female sexual encounter group). All animals were kept in visual isolation from others. At the time of a trial, the opaque divider was removed and a 14-min behavioral trial was run. Behaviors of the focal male and (when present) the stimulus animal were recorded from behind a blind. An ethogram of the scored behaviors is included as Table 1. At the end of the trial, the animals were gently separated using the divider and a wooden dowel and maintained undisturbed until they were sacrificed 90-min from the start time of the behavior trial. This time period was chosen because it is both optimal for peak Fos induction, and also sufficient to allow for Fos degradation in instances of reduced Fos induction (Herdegen and Leah, 1998; Hoffman et al., 1993).

Tissue preparation and immunohistochemistry

Subjects were sacrificed by means of rapid decapitation and each brain was immediately dissected and sunk in 4% paraformaldehyde (in 0.1 M phosphate buffer) at 4 °C for 1 h. Perfusion was not used because of the small heart size of these lizards. The average brain weight was 52 ± 6.8 mg ($\bar{x} \pm$ S.E.), thus immersion in paraformaldehyde sufficed for rapid fixation of this tissue. Each brain was then embedded in an 8% gelatin block and placed back into 4% paraformaldehyde for overnight postfixation at 4 °C. The encased brain was then transferred to 30% sucrose in 0.1 M phosphate buffered saline (PBS) and left for 24 h at 4 °C. Brains were then stored at −80 °C until sectioning on a cryostat, at which point two 50-μm series of alternating coronal sections were kept. One series underwent immunohistochemical processing for this study; multiple immunohistochemistry runs were conducted, each containing brains from a mixture of treatment groups. We attempted to distribute treatment groups evenly across these runs. After rinsing sections for 30 min in PBS, they were placed into 1% sodium borohydride (Sigma-Aldrich, St. Louis, MO, USA) for 20 min to reduce autofluorescence. After another 30-min PBS rinse, sections were transferred to a blocking solution consisting of 2.5% donkey serum (Sigma-Aldrich) and 0.03% Triton-X-100 (Fisher Scientific, Pittsburgh, PA, USA) in PBS for 1 h. Next, the sections were placed for 40 h at 4 °C in blocking solution containing 0.1 μg/ml rabbit anti-Fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), 0.2 μg/ml of guinea pig anti-vasopressin antibody (Bachem, Torrance, CA, USA), and 0.5 μg/ml sheep anti-tyrosine