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Vasopressin cell groups exhibit strongly divergent responses to copulation and male–male interactions in mice

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ABSTRACT

Arginine vasopressin (AVP) and its nonmammalian homolog arginine vasotocin influence social behaviors ranging from affiliation to resident–intruder aggression. Although numerous sites of action have been established for these behavioral effects, the involvement of specific AVP cell groups in the brain is poorly understood, and socially elicited Fos responses have not been quantified for many of the AVP cell groups found in rodents. Surprisingly, this includes the AVP population in the posterior part of the medial bed nucleus of the stria terminalis (BSTMP), which has been extensively implicated, albeit indirectly, in various aspects of affiliation and other social behaviors. We examined the Fos responses of eight hypothalamic and three extra-hypothalamic AVP-immunoreactive (-ir) cell groups to copulation, nonaggressive male–male interaction, and aggressive male–male interaction in both dominant and subordinate C57BL/6J mice. The BSTMP cells exhibited a response profile that was unlike all other cell groups: from a control baseline of ~5% of AVP-ir neurons colocalizing with Fos, colocalization increased significantly to ~12% following nonaggressive male–male interaction, and to ~70% following copulation. Aggressive interactions did not increase colocalization beyond the level observed in nonaggressive male mice. These results suggest that BSTMP neurons in mice may increase AVP-Fos colocalization selectively in response to affiliation-related stimuli, similar to findings in finches. In contrast, virtually all other cell groups were responsive to negative aspects of interaction, either through elevated AVP-Fos colocalization in subordinate animals, positive correlations of AVP-Fos colocalization with bites received, and/or negative correlations of AVP-Fos colocalization with dominance. These findings greatly expand what is known of the contributions of specific brain AVP cell groups to social behavior.

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Introduction

The neuropeptide arginine vasopressin (AVP) and its avian homolog arginine vasotocin (AVT) influence a variety of social behaviors, including pair bonding in voles (Winslow et al., 1993; Insel and Hulihan, 1995; Lim et al., 2004); social recognition in male mice and rats (Engelmann et al., 1994; Everts and Koolhaas, 1997; Bielsky et al., 2004, 2005; Choleris et al., 2009); maternal behaviors in rats (Bosch and Neumann, 2008; Nephew and Bridges, 2008); and social communication in fishes, birds and rodents (Albers et al., 1986; Maney et al., 1997; Goodson, 1998a; Goodson and Bass, 2000). Modulation of aggression by AVT/AVP is complex and can vary across contexts and phenotypes (Goodson et al., 2009a; Kabelik et al., 2009; also see Beiderbeck et al., 2007), and although numerous neural loci are likely involved, resident–intruder aggression is potently facilitated by activation of V_{1a} receptors in the anterior hypothalamus (AH), as shown in male Syrian hamsters (*Mesocricetus auratus*; Ferris et al., 1997) and prairie voles (*Microtus ochrogaster*; Gobrogge et al., 2009).

Sites of action have been established for many of AVP's behavioral effects, but surprisingly little is known about the social stimulus properties that elicit responses from discrete AVP cell populations in the brain. For instance, AVP strongly promotes mating-induced behaviors in monogamous voles (Young and Wang, 2004; Lim and Young, 2006), but the source(s) of mating-induced AVP release has not been identified. Similarly, little or no functional data are available for most of the smaller populations of AVP cells in the extended amygdala, preoptic area (POA), and hypothalamus. These data are critical to understanding AVP-mediated effects as it is difficult to attribute site-specific effects to a particular AVP cell group, given that those effects may occur at sites distal from terminal distributions, and volumetric peptide release from dendrites may effectively bathe large amounts of the brain in AVP (Landgraf and Neumann, 2004; Ludwig and Leng, 2006). Adding to this complexity is that projections from multiple cell groups often appear to overlap. Indeed, functionally opposed AVT cell groups exhibit overlapping projection fields in songbirds, suggesting that the behavioral properties of a given cell

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group depend upon distributed patterns of neuromodulation across the brain, not simply site-specific actions (Goodson and Kabelik, 2009).

All of these observations highlight a strong need for behaviorally relevant data on the AVP cells themselves, and on the kinds of environmental stimuli to which AVP cell groups respond. Thus, a major goal of the present experiments was to address that need. A second goal was to test the hypothesis that AVP cell groups in mice exhibit opposing response profiles, as recently shown in songbirds for the AVT populations in the medial bed nucleus of the stria terminalis (BSTM) and paraventricular nucleus of the hypothalamus (PVN), which exhibit increased Fos activity to positive and negative social stimuli, respectively (Goodson and Wang, 2006; Goodson and Kabelik, 2009).

Finally, we sought to clarify the relationship of AVP neurons to male–male aggression. Although AVP acts within the AH to promote resident–intruder aggression in Syrian hamsters and prairie voles (Ferris et al., 1997; Gobrogge et al., 2009), findings in rats and songbirds suggest that AVT/AVP relates to aggression in complex ways that reflect social context, anxiety phenotype, and constitutive aggressiveness (Beiderbeck et al., 2007; Veenema and Neumann, 2007; Goodson et al., 2009a; Kabelik et al., 2009). In order to address these experimental goals, we quantified the immunocolocalization of Fos and AVP in male C57BL/6J mice following (1) copulation, (2) nonaggressive male–male interaction, and (3) aggressive male–male interactions. In the male–male aggressive interactions, both dominant and subordinate mice were examined, and data were collected for 11 AVP-immunoreactive (-ir) cell groups, representing virtually all definable groups in the mouse brain.

Methods

Animals and housing

Adult C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were individually housed and maintained on either a 12:12 light/dark cycle (resident–intruder tests) or a 14:10 cycle (copulation tests). Food and water were available *ad libitum* except during testing. All subjects were adults (>12 weeks old) at time of testing. All experiments were performed in accordance with the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals (1996) and were approved by the Institutional Animal Care and Use Committee at Indiana University.

Ovariectomies and estrus induction

At >10 weeks of age, female partners for copulation tests were bilaterally ovariectomized. Animals were deeply anesthetized for this procedure with an intraperitoneal (i.p.) injection of a cocktail comprised of ketamine (20 mg/ml; Henry Schein, Melville, NY), xylazine (4 mg/ml; Henry Schein), and 0.9% saline, or sodium pentobarbital (50 mg/ml; Henry Schein). Animals were returned to their home cages and dosed orally with meloxicam (Metacam, 1 mg/kg; Henry Schein) for 3 days following surgeries. Behavioral receptivity was later induced with subcutaneous injections of estradiol benzoate (10 μg/0.05 ml peanut oil; Sigma, St. Louis, MO) and progesterone (500 μg/0.1 ml peanut oil; Sigma) at 48 and 6 h, respectively, prior to testing. Steroid solutions were first dissolved in acetone and allowed ample time for evaporation prior to the addition of peanut oil.

Receptivity screening

To determine whether females were sexually receptive and to ensure that subject males were sexually motivated, animals were screened for copulatory behavior ~1 week prior to testing. Within the first hour after lights-off, females were placed in the male's cage and males were allowed two bouts of intromission (i.e., periods that consist of a mount and subsequent thrusting), or until 30 min had elapsed. Only males that successfully intromitted twice within 30 min were included in the study.

Copulation testing

Males were habituated to the test room for 1 h after lights-off for two days prior to testing. On the day of testing and perfusion, animals were habituated to the test room 3 h prior to the start of testing. At lights-off, a receptive stimulus female was placed in the subject male's cage. The number and latency to mounts, intromissions, and ejaculation were quantified by direct observation under red light. Tests were terminated at ejaculation, after which the male was placed in a dark, quiet area until perfusion. Control males received the same treatment, with handling at 0 and 45 min, except that no female was introduced.

Resident–intruder testing

To reduce the level of nonspecific Fos activation, resident–intruder pairs were acclimated to cage-swapping in the test room once per day for 7 days prior to testing. These acclimations occurred in the first hr after lights-off. Animals were placed in each other's cage for 15 min, after which they were returned to their home cage. Control males were placed in a clean cage or handled and left in their own cage for 15 min.

On the day of testing and perfusion, subjects were moved to the test room for habituation 3 h prior to the start time. At lights-off, an intruder male was placed into a resident's cage for 15 min. Interactions were observed under red light to determine dominant-subordinate status and were also recorded with a digital video camcorder (Canon ZR40) for quantification of latency to aggression, the number of bites, the number of aggressive bouts, and duration of aggressive bouts. In these tests, bites were always given by the dominant male, though residency did not determine social status (i.e., dominant males were not necessarily residents). After the 15 min test, the intruder was returned to his cage. Animals that chemoinvestigated but did not engage in aggressive interactions were designated as “nonaggressive” and were also euthanized for Fos analyses. Both resident and intruder males were handled at the start and finish of testing. All animals were kept in a dark, quiet area until perfusion.

Perfusion

Subjects in the resident–intruder experiment were perfused 75 min following the start of testing. The timing of perfusions in the copulation experiment varied from 75 to 90 min after the start of testing because subjects exhibited different latencies to copulation (i.e., subjects that ejaculated in 30 min or less were perfused at 75 min and those that exhibited longer latencies were perfused 45 min after ejaculation, with a maximum of 90 min allowed after the start of testing). Handled controls were perfused at 75 min. Animals were deeply anesthetized with an i.p. injection of a ketamine cocktail comprised of ketamine (20 mg/ml), xylazine (4 mg/ml), and 0.9% saline, or sodium pentobarbital and perfused transcardially with 0.1 M phosphate buffered saline (PBS) for exsanguination followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). Extracted brains were post-fixed overnight then transferred to a 30% sucrose solution for 2 days.

Immunocytochemistry

Brain tissue was cut into three series of 40 μm free-floating coronal sections using a cryostat and stored at −80 °C in cryoprotectant prior to immunocytochemical labeling. Immunocytochemistry was performed as follows: tissue was rinsed 6×10 min in 0.1 M PBS; followed by 20 min in 10 mM sodium citrate (pH 9.5, ceramic well plates placed into