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Central vasopressin V1a receptor activation is independently necessary for both partner preference formation and expression in socially monogamous male prairie voles

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Abstract

The neuropeptide arginine vasopressin (AVP) modulates a variety of species-specific social behaviors. In socially monogamous male prairie voles, AVP acts centrally via vasopressin V1a receptor (V1aR) to facilitate mating-induced partner preferences. The display of a partner preference requires at least two temporally distinct processes: social bond formation as well as its recall, or expression. Studies to date have not determined in which of these processes V1aR acts to promote partner preferences. Here, male prairie voles were administered intracerebroventricularly a V1aR antagonist (AVPA) at different time points to investigate the role of V1aR in social bond formation and expression. Animals receiving AVPA prior to cohabitation with mating or immediately prior to partner preference testing failed to display a partner preference, while animals receiving AVPA immediately after cohabitation with mating and control animals receiving vehicle at all three time points displayed partner preferences. These results suggest that V1aR signaling is necessary for both the formation and expression of partner preferences, and that these processes are dissociable.

Keywords

pair bond; vasopressin; vasopressin receptor 1a; prairie vole

INTRODUCTION

Arginine vasopressin (AVP) is a nine amino acid peptide that modulates a variety of behaviors and cognitive processes related to social functioning (Caldwell, Lee, Macbeth, & Young III, 2008; Donaldson & Young, 2008). Many of the behavioral effects of AVP are mediated by its

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action on central vasopressin V1a receptor (V1aR) subtype, one of three AVP receptor subtypes. A long history of experimentation on AVP and V1aR has elucidated a critical role for activation of this receptor in social recognition as well as more complex male-typical social behaviors (for instance see Albers, Dean, Karom, Smith, & Huhman, 2006; Bielsky & Young, 2004; Dantzer, Bluthé, Koob, & Le Moal, 1987; Ferguson, Young, & Insel, 2002; Popik & van Ree, 1998; van Wimersma Greidanus & Maigret, 1996; Winslow & Insel, 2004).

Behaviorally, AVP was first studied for its role in learning and memory and later in association with V1aR for its specific role in social memories (De Wied, 1971; Popik & van Ree, 1998; van Wimersma Greidanus, van Ree, & de Wied, 1983). Using various social recognition paradigms (Ferguson et al., 2002), multiple experiments have demonstrated that application of AVP extends the recall of short-term social recognition abilities (Dantzer et al., 1987; Engelmann & Landgraf, 1994; Everts & Koolhaas, 1997; Le Moal, Dantzer, Michaud, & Koob, 1987). In contrast, antiserum-mediated depletion of AVP (van Wimersma Greidanus & Maigret, 1996), pharmacological blockade (Dantzer et al., 1987; Everts & Koolhaas, 1997, 1999), genetic mutation/deletion (Bielsky, Hu, Szegda, Westphal, & Young, 2004; Egashira et al., 2007; Engelmann & Landgraf, 1994), or antisense-mediated decreases (Landgraf et al., 1995) in V1aR all inhibit social recognition. Furthermore, replacement of V1aR specifically in the lateral septum of V1aR knockout mouse restores their social recognition abilities (Bielsky, Hu, Ren, Terwilliger, & Young, 2005).

In other, less commonly studied rodent models, V1aR has been implicated in modulating complex social interactions in males. For instance, pharmacological inhibition of V1aR in Syrian hamsters (*Mesocricetus auratus*) decreases flank marking behavior (Ferris, Delville, Grzonka, Luber-Narod, & Insel, 1993) while AVP administration increases it (Ferris, Albers, Wesolowski, Goldman, & Luman, 1984). In socially monogamous prairie voles (*Microtus ochrogaster*), AVP modulates pair bonding in males as assessed through the use of the partner preference test (Cho, DeVries, Williams, & Carter, 1999; Winslow, Hastings, Carter, Harbaugh, & Insel, 1993). While pair bonding is naturally facilitated by mating, centrally applied AVP is sufficient to facilitate a partner preference in the absence of mating (Cho et al., 1999). Conversely, partner preference is blocked by a selective V1aR antagonist (Winslow et al., 1993). Intriguingly, artificial introduction of V1aR into the ventral pallidum of non-monogamous meadow voles also endows this species with the ability to display partner preferences following mating (Lim et al., 2004).

Despite extensive experimentation on V1aR's role in various social behaviors, relatively little is known about how V1aR may modulate different phases of these behaviors. Complex social behaviors involving learning and memory, such as pair bonding, require a coordination of many neural systems and are thought to involve two temporally distinct phases, formation and maintenance/expression. With regard to pair bonding, it remains unclear whether V1aR activation influences one or both of these phases. Much of the pharmacological evidence for the role of V1aR activation in pair bonding has resulted from work with a V1aR antagonist known as the Manning compound ($d(CH_2)_5[Tyr(Me)]AVP$) (Cho et al., 1999; Lim & Young, 2004; Manning et al., 2008). *Ex vivo* studies following central injection of $d(CH_2)_5[Tyr(Me)]AVP$ have shown that V1aR remains blocked for at least 18 hours after administration in the vole brain (Winslow et al., 1993). Given that this antagonist may be acting over relatively long periods of time, it is unclear whether its effects on partner preference are due to its action on bond formation during the initial mating and cohabitation period and/or whether it is blocking the expression of the bond during the partner preference test itself. Therefore, we designed a modified behavioral assay that allows us to differentiate the effects of V1aR antagonist administration during different phases of social bonding. Using this approach, we demonstrate that V1aR activation is independently required for both bond formation and its expression/maintenance.

METHODS

Subjects

Subjects were sexually naive male prairie voles 70-100 days of age from our outbred breeding colony originally derived from wild populations in Illinois. Individuals were housed in same sex trios following weaning at 21 days of age. Animals were maintained on a 14:10 light:dark cycle and given access to food and water *ad libitum*. Stimulus animals consisted of equivalent age non-related females primed with 2 µg estradiol benzoate daily for three days to induce sexual receptivity. All experiments were approved by the institutional guidelines set forth by the Animal Care and Use Committee of Emory University and conformed to the guidelines of the National Institutes of Health.

Experimental Design

Male prairie voles were randomly assigned to four experimental groups named to indicate the time of AVPA administration, and received three intracerebroventricular (i.c.v.) injections over the course of the experiment. The “pre-mating” treatment group received AVPA prior to cohabitation and mating, the “post-mating” group received AVPA following cohabitation and mating, and the “pre-testing” group received AVPA prior to testing. Vehicle was injected at time points when AVPA was not administered to these groups, and control animals received AVPA at all three injection time points (Figure 1A).

All subject animals underwent the following experimental timecourse. On day one, male prairie voles were cannulated intracerebroventricularly (i.c.v.) and singly housed to recover from surgery for three days. On day four, following i.c.v. injection, they were paired with an estrogen-primed female for a 24 hr period of cohabitation and mating. Pairs were introduced between 1100 and 1300 hrs. After cohabitation/mating, males received a second i.c.v. injection and were returned to their home cages for 72 hr. On day eight, following a third and final injection, they were then tested in the partner preference paradigm (Figure 1A). Testing was initiated between 1100 and 1300 hrs. Subjects were sacrificed to investigate cannula placement upon completion of testing.

Cannulation

Adult male prairie voles were anaesthetized with isoflourane and placed in a stereotaxic apparatus with blunt earbars. An incision was made in the scalp to reveal the dorsal surface of the skull. A small hole was drilled in the skull at the cannula placement site. A single guide cannula (26 gauge) was slowly lowered through the hole and affixed to the skull with Superglue and dental cement. Coordinates for i.c.v. cannulation are as follows: anteroposterior +0.06 mm; mediolateral +0.10 mm; dorsoventral +3.0 mm (guide cannula = 2.8 mm, internal cannula = 3.0 mm). Guide cannula were covered with dummy cannula between injections. All cannulae were ordered from Plastics One, Roanoke, VA.

The experiment required cannulae to remain in place for eight days. Prairie voles lack calcified skulls, and a few of the cannulae dislodged themselves prior to the end of the experiment. Because of this, four animals were excluded from the study - two from the pre-mating group, one from the post-mating group, and one from the pre-testing group.

Injections and use of the d(CH₂)₅[Tyr(Me)]AVP

Isoflourane anaesthetized subjects (n = 10/grp) received injections in a 2 µl bolus through an internal cannula (33 gauge) that extended 0.2 mm past the end of the guide cannula. Vehicle control injections consisted of 2 µl of lactated Ringer's solution; antagonist injections contained 5 ng of V1aR antagonist, d(CH₂)₅[Tyr(Me)]AVP (Bachem # H-5350, Torrance, CA) dissolved in 2 µl of lactated Ringer's solution. Following the first and third injections, animals were

returned to their home cages for 2 hr before cohabitation with a female and 2 hr prior to partner preference testing to allow diffusion of the antagonist throughout the brain.

As with any pharmacological antagonist, the specificity of the agent is an important consideration. We chose $d(CH_2)_5[Tyr(Me)]AVP$ because it has been routinely used in previous experiments investigating the role of V1aR in pair bonding, and we sought to be able to interpret our results in the context of previous findings. However, social behavior is known to be influenced by the activation of a variety of neurohormonal receptors including the related oxytocin and vasopressin V1b receptors. While the ability of $d(CH_2)_5[Tyr(Me)]AVP$ to bind the vasopressin V1b receptor has not been directly tested, physiological evidence suggests that it elicits its effects primarily via action at the V1a receptor in rodents (Lee, Yang, Chen, al-Azawi, & Hsu, 1995). In contrast, it is known that $d(CH_2)_5[Tyr(Me)]AVP$ can bind the oxytocin receptor but selectivity for V1aR can be achieved via use of appropriate concentrations of the compound (Manning et al., 2008). We administered the same dosage as used in previous prairie vole studies (Winslow et al., 1993).

Behavioral testing

The cohabitation period was filmed for all animals and mating was scored during the first four hours. Three males (one from the control group and two from the post-mating group) were excluded from the study because they failed to mate during this time. Partner preference testing was performed in a three chambered apparatus as described previously (Williams, Catania, & Carter, 1992; Winslow *et al.*, 1993). The chambers (each 20cm deep X 50 cm long X 40 cm wide) were connected by plastic tunnels. The cohabitation partner and a novel female, or stranger, were tethered in chambers at opposite ends of the apparatus with a neutral, empty chamber in between. Males were introduced to the neutral chamber and allowed to move freely within the apparatus. Time spent in contact with the partner and stranger stimulus animals was recorded for 3 hours. Videos were scored at 16X speed by an experimenter blind to the treatment groups. A two-way ANOVA (treatment X stimulus) followed by Tukey's post-hoc test was used to assess partner preference. The number of tunnel crossings was also scored for the 3 hour test as a measure of general locomotion. Both tunnel crossings and mating behavior were analyzed with a one-way ANOVA. Following testing, 2 μ l of 10% India ink was injected through the cannula and animals were rapidly euthanized with CO₂ asphyxiation. Dye spread was immediately determined via brain slicing and animals were excluded if dye was not observed within both sides of the ventricle. Three animals (two from AVPA pre-testing and one from AVPA pre-mating groups) were excluded following this analysis.

RESULTS

Our analysis identified a significant interaction between treatment group and stimulus animal (two-way ANOVA, $F[3, 52] = 3.014$; $p = 0.038$). Central administration of AVPA prior to mating/cohabitation ($n = 7$) or prior to partner preference testing ($n = 7$) is sufficient to block the display of a partner preference (Figure 1B; Tukey's post-hoc test for partner versus stranger, $p > 0.5$ for both groups). In contrast, the control group displayed partner preferences ($n = 9$; Tukey's post-hoc test for partner versus stranger, $p = 0.001$), and AVPA administered immediately after the 24 cohabitation/mating period did not disrupt partner preference ($n = 7$; Tukey's post-hoc test for partner versus stranger, $p = 0.008$). This latter finding indicates that the effects of AVPA administration before mating and before testing are independent and that AVPA administered at either of the first two time points is no longer biologically active by the time partner preference testing was performed.

Because partner preference is facilitated by mating, we analyzed mating bouts across the treatment groups (Figure 1C). Mating bouts did not differ significantly across groups (one way ANOVA, $F[3,26] = 0.881$; $p > 0.4$). Likewise, we did not observe differences in locomotion

among groups based on the number of tunnel crossings in the partner preference test (Figure 1D, one way ANOVA, ($F[3,26] = 0.976$; $p > 0.4$).

DISCUSSION

Social bond formation is a complex phenomenon that involves several cognitive processes, including social information processing, social reward and reinforcement, and learning and memory. The neural mechanisms underlying the display of a social bond may also be temporally divided into those involved in the formation of the bond and those involved in the expression of behaviors indicative of the bond, e.g. partner preference. While previous work has shown that V1aR activation is involved in the pair bonding process in prairie voles, no studies have attempted to differentiate between its role in pair bond formation versus expression. By developing a modified behavioral assay that differentiates between these phases, we demonstrate that V1aR activation is independently necessary for bond formation and expression. These findings suggest that V1aR is involved in at least two temporally dissociable processes required for social bond formation and contributes to our understanding of the molecular neurocircuitry underlying social bonding.

Proposed preliminary model for V1aR modulation of pair bonding

V1aR is expressed in a wide array of brain regions, and the behavioral relevance of many of these populations has not been thoroughly investigated. However, investigation of the role of V1aR activation within the lateral septum and ventral pallidum point to a preliminary model for V1aR modulation of pair bonding. V1aR in these regions has been examined in relation to both social recognition in rats and mice, and the display of a partner preference in prairie voles. For social recognition processes, V1aR in the lateral septum appears to play an important role. Infusion of a selective V1aR antagonist or antisense oligonucleotide targeting V1aR in the lateral septum blocks social recognition while restoring V1aR selectively within the lateral septum in V1aR knockout mice restores social recognition abilities (Bielsky & Young, 2004; Ferguson et al., 2002; Landgraf et al., 1995; Popik & van Ree, 1998; van Wimersma Greidanus & Maigret, 1996; Winslow & Insel, 2004). AVP administration either before or after the initial social encounter extends the duration of social recognition, suggesting a potential role for this peptide in the consolidation or recall of social memories (Ferguson et al., 2002; Popik & van Ree, 1998).

Social learning and memory is only one set of cognitive processes required for partner preference formation in monogamous species (Young & Wang, 2004). It is therefore not surprising that multiple brain V1aR populations are necessary for this behavior. In particular, AVPA infused into either the lateral septum or the ventral pallidum blocks the display of partner preferences (Lim & Young, 2004; Liu, Curtis, & Wang, 2001). In the lateral septum, V1aR may modulate social cognition as it does in other rodents. The ventral pallidum, however, is thought to be involved in reinforcement and motivation (for instance see Smith, Tindell, Aldridge, & Berridge, 2009). Given what is known about the function of the lateral septum and ventral pallidum, as well as the role of V1aR in these regions, we hypothesize that V1aR activation within these areas has different roles during bond formation and expression. During pair bond formation, V1aR activation in the ventral pallidum may facilitate the coupling of the olfactory signature of a mate with the reinforcing aspects of mating. Correspondingly, within the lateral septum, V1aR activation may be responsible for the formation and/or the recall of a social memory during expression of behaviors associated with the social bond, e.g. the display of a partner preference. Future studies are needed in order to experimentally test this model. In addition, future studies may also establish the importance of other V1aR populations that have not been investigated with as much depth as those within the septum and pallidum.

The field of learning and memory has placed a great emphasis on dissecting the different phases of acquiring, consolidating, and recalling information. The investigation of each of these phases independently has informed our understanding of the molecular and neuroanatomical regulation of these processes. Less specificity has been applied to understanding the different components and phases of other complex behaviors involving learning and memory, such as pair bonding. Experiments such as those presented here are a valuable first step to achieve the level of sophistication in the field of social attachment that is common in the broader field of learning and memory.

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Figure 1.

AVPA administration blocks partner preference formation and expression. A) Experimental timecourse for AVPA administration. All males were cannulated and received three injections, one prior to mating, one after mating, and one prior to partner preference testing. Controls received three vehicle injections while all other subjects received one injection of AVPA and two vehicle injections. B) Graph shows time spent huddling with partner (dark bars) or stranger (open bars) for each treatment group. Subjects that received AVPA prior to cohabitation/mating or prior to testing failed to show a partner preference ($p > 0.5$) while the control groups, including post-mating injection of AVPA, did show a preference ($p < 0.01$). C) There were no group differences in the number of mating bouts during the first four hours of cohabitation (p

> 0.4). D) There were also no group differences in the number of tunnel crossings during the partner preference test, a measure of locomotion ($p > 0.4$). Values represent mean + SEM; * indicates $p < 0.01$.