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Ventral tegmental area involvement in pair bonding in male prairie voles

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Abstract

Dopamine is known to play a critical role in social attachment in monogamous voles. However, little is known about the neurochemical regulation of central dopamine release during pair bond formation. Here we examine the effects on partner preference formation in male prairie voles of neurochemical manipulations in the ventral tegmental area (VTA), a major source of dopamine to brain regions implicated in pair bonding. Administration of NBQX, an AMPA receptor antagonist, or bicuculline, a GABA receptor antagonist, into the VTA induced partner preferences within 6 h in the absence of mating. We also found that, after unilateral administration of NBQX into the VTA, neuronal activation, as indicated by the expression of the immediate early gene c-*fos*, was decreased in the nucleus accumbens, prefrontal cortex, and medial amygdala, but was unchanged in the lateral septum and in a control region, the arcuate nucleus. These results confirm a role for the VTA in partner preference formation in monogamous voles and extend the list of neurochemicals important in pair bonding to include glutamate and GABA.

Keywords: Mating systems; Glutamate; Monogamy; *Microtus*; Reward

1. Introduction

Prairie voles (*Microtus ochrogaster*) display characteristics associated with a monogamous life strategy including a lack of sexual dimorphism, biparental care of young, and the formation of pair bonds between males and females [19]. In the laboratory, pair bonds between male and female prairie voles are manifested by a robust preference to associate with the familiar partner versus with a conspecific stranger [19]. Importantly, such partner preferences are readily quantifiable and provide a benchmark by which the effects of experimental manipulations can be assessed.

Over the past several years it has become increasingly clear that dopamine plays a central role in pair bond formation in voles. Studies comparing monogamous and non-monogamous species of voles have identified differences in the functioning of dopamine systems [9] and in other neurotransmitter systems that interact with dopamine in the regulation of pair bonding [30,31,46,51]. To date most research into dopamine's role in pair bonding has focused on mesolimbic dopamine "reward" pathways, and in particular, on the ventral striatum/nucleus accumbens (NAcc). The release of dopamine within the NAcc is thought to be rewarding and thus could mediate a positive association with the familiar partner in a pair bond [28]. Indeed, it has been shown that activation of the D2 sub-type of dopamine receptors within the NAcc is critical for pair bond formation in both sexes of prairie voles [1,20]. Further, pair bond formation results in modifications of the NAcc dopamine system that may serve to maintain a monogamous mating system [2].

Together these studies have established dopamine as being critical for pair bonding. However, a thorough understanding of the role of dopamine in pair bonding requires understanding dopamine sources as well as dopamine targets. The ventral tegmental area (VTA) is a major source of dopamine within the brain and several lines of evidence suggest that the VTA may play an important role in pair bonding. First, the VTA has been implicated in reward pathways that may be important in pair bonding. For example, rats and mice will self-administer drugs into the VTA [12–14,27] and drugs administered into the VTA can induce place preferences [6]. Second, mating activates the VTA in both sexes [3,24,25] and is known to facilitate the formation of pair bonds in voles [48,49]. Third, the VTA projects to [43] brain regions that have been implicated in pair bond formation such as the amygdala, lateral septum, prefrontal cortex, and NAcc [1,15,20,32,34,51], while brain regions implicated in pair bonding, in turn, can influence the

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VTA [17,18]. Finally, stress, which affects pair bonding in monogamous voles [16], also alters the activity of dopaminergic cells within the VTA [40]. Thus, the VTA may represent an important point of convergence for processes critical for pair bond formation.

Neurochemical regulation of dopaminergic output from the VTA is complex and the effects of pharmacological manipulations within the VTA vary depending on the targets of the VTA projections. For example, administration of glutamate receptor antagonists into the VTA can decrease dopamine release in the frontal cortex while increasing dopamine release in the NAcc [45]. In areas such as the NAcc where dopamine release is under tonic inhibitory control, the effect of glutamate receptor blockade on dopamine release is thought to involve a reduction of the activity of GABAergic interneurons in the VTA that, in turn, modulate the activity of dopaminergic cells [13]. This idea is supported by evidence that administration of GABA agonists into the VTA can decrease extracellular dopamine levels within the NAcc [22,50] and that rats will self-administer glutamate antagonists or GABA receptor antagonists, but not GABA agonists [27] into the VTA. GABA modulation of dopamine function also is supported by evidence that systemic administration of the dopamine antagonist sulpiride blocks the rewarding effects of GABA receptor blockade [12]. Finally, GABA agonists reduce the rewarding effects of self-stimulation within the ventral pallidum [38], further suggesting that GABAergic modulation of the dopamine system affects reward pathways.

Given the critical role for central dopamine in pair bonding, and the importance of processes within the VTA in regulating dopamine release, the goal of the present study was to examine a potential role for the VTA in pair bond formation in monogamous voles. We first examined the effects on pair bond formation of blockade of glutamate and GABA receptors in the VTA. We then used immunocytochemical visualization of the protein product of the immediate early gene c-*fos* to assess patterns of neuronal activation after glutamate receptor blockade.

2. Methods

Subjects were male offspring of the F4 generation of a laboratory colony of prairie voles originating from Illinois. After weaning at about 21 days of age, pups were kept in same-sex sibling pairs until used in experiments. All animals were housed in plastic shoebox style cages ($29 \times 19 \times 13$ cm) under a 14 : 10 light : dark cycle with ad libitum food and water. All subjects were about 70 days of age at the time of the experiments.

2.1. Brain cannulation

Males were outfitted with bilateral 26 gauge guide cannulae (Plastics One, Roanoke, VA, USA) placed 1 mm dorsal to the VTA (stereotaxic coordinates from Bregma: 3.5 mm caudal, 0.6 mm bilateral, 5.1 mm ventral, Incisor Bar at −2.5 mm) under sodium pentibarbitol (1 mg/10 g body weight) anesthesia. Males were allowed three days of post-surgical recovery in the presence of the sibling.

2.2. Behavioral testing

On the test day, animals were randomly assigned to groups that received VTA administration of vehicle (100 nl/side of a salt solution isotonic for Na^+, Mg^{++}, Ca^{++}, and K^+ [42], $n=8$), or of vehicle containing 10 ng of the AMPA receptor antagonist NBQX (tetrahydro-6-nitro-2,3-dioxo-benzol[f]quinoxaline-7-sulfonamide disodium salt, Sigma, $n=8$), or 5 ng of the $GABA_A$ receptor antagonist bicuculline (Sigma, $n=7$). Drug dosages were based on reported effective doses in other models employing site-specific central administration of these compounds [12,26]. Drug administration was via injection cannulae that extended 1 mm beyond the end of the guide cannulae. Drug solutions were administered bilaterally at a rate of 50 nl/min for 2 min using a syringe pump. Immediately following treatment, each male was paired with an ovariectomized female for 6 h in a clean cage containing food and water. We and others have previously shown that 6 h of non-sexual cohabitation does not induce partner preferences in male prairie voles [20,29,47,48]. Throughout the cohabitation period, the animals' interactions were videotaped (Panasonic 12 : 1 compression time-lapse video recorder and low-light camera) and the tapes subsequently were examined to verify the absence of mating.

At the end of the 6 h cohabitation period, each male was tested for a partner preference. The apparatus for the partner preference test consisted of a central cage ($20 \times 25 \times 45$ cm) joined by hollow tubes (7.5×16 cm) to two identical parallel cages. One of these latter cages contained the familiar female partner, and the other contained an ovariectomized unfamiliar female. The females were tethered to restrict their movements to their respective cages and thus had no direct contact with each other. The male was released into the central cage and had free access to all cages. All cages contained food and water. A customized computer program (R. Henderson, Florida State University) using a series of light beams across the connecting tubes was used to monitor movement of the male among the cages. The computer program recorded the amount of time the male spent in each cage and the number of transits between cages. Throughout the test, the animals again were videotaped for behavioral analysis. Variables included the time spent by the male in each female's cage, number of transits between cages (measures of activity to ensure that treatments did not affect locomotor behavior), and the frequency and amount of time the male spent in direct contact with each female. The amount of time males spent in the neutral cage was assessed as a measure of time spent in isolation. Each test lasted for 3 h. For each group, comparisons of time spent in direct contact with the partner vs. that with the stranger (partner preference) were made using paired *t*-tests. Between groups treatment effects on other behavioral measures were evaluated using one-way ANOVA. After all behavioral testing was completed, subjects were overanesthetized, and the brains removed. Cannula