Pair Bond Formation by Monogamous Prairie Voles in Novel Environments

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Abstract

Background: Social bonds between spouses (pair bond) positively affect health conditions. Recently, we reported that the administration of serotonin-selective reuptake inhibitors to monogamous prairie voles ameliorated the impairment of pair bonding caused by traumatic stresses, suggesting the involvement of the serotonergic system in the formation of pair bonds in this model animal. Before investigating this possibility, we investigated the cohabitation condition which leads to a pair bond using our colony of prairie voles.

Methods: Male prairie voles were cohabited with females for 96 h in a control experiment. Otherwise, subject males were cohabited with an estradiol-primed females for 6 h or 24 h. Subsequently, partner preferences by subject male prairie voles were assessed for 3 h.

Results: All subjects in the 96 h cohabitation group preferred to huddle with their partner than stranger females. Twenty-four hour cohabitation induced partner preference; however a 6-h cohabitation did not, despite accompanying mating bouts.

Conclusions: The formation of pair bonds by prairie voles requires 24 h of cohabitation. Mating bouts in a 6-h cohabitation appear to be insufficient for the formation of a pair bond. Such factors need to be considered when performing experiments using pharmacological methods that enhance or inhibit pair bonds.

Introduction

Social bonds between spouses (pair bonding), parents and children, and peers positively affect human health conditions, whereas the disruption of such bonds can cause negative effects on mental and physical health. Over 95% of mammals have a polygamous mating system while 5% exhibit a monogamous one, where a couple of male and female mates prefer each other than strangers of the opposite sex. Prairie vole (Microtus ochrogaster) is a monogamous rodent found in central North America. Once male and female prairie voles mate, they prefer their partners than strangers until one of a pair dies.¹ Such sociopsychological traits may modulate stress responses. The presence of a partner male prairie vole ameliorates a female’s stress response and enhances her recovery from stress via oxytocin signaling.²³ Further, male prairie voles that bonded with their partners display higher heart variability rates than unbonded males, suggesting a higher basal parasympathetic tone in bonded males.⁴ To understand the positive effects of pair bonding on mental and physical health, it is important to clarify the neuronal circuits and underlying mechanisms of pair bonding. Numerous research have revealed the modulation of pair bonding by neuropeptides including vasopressin, oxytocin, and corticotropin releasing factors.¹²³ The dopaminergic system also modulates pair bonding.⁵ Recently, we reported that traumatic stress inhibits the formation of pair bonds and that the administration of serotonin-selective reuptake inhibitors prevent the impairment of pair
bond formation caused by traumatic stress, suggesting the involvement of the serotonergic system in the formation of pair bonds. In order to identify the neurotransmitters modulating pair bonding, an intracerebroventricular or a site-specific injection of antagonists or agonists has been useful. Empirically, a 24 h cohabitation of a pair of male and female prairie voles is used to investigate the effects of the administration of drugs that inhibit pair bond formation, whereas less than 6 h of cohabitation, without mating bouts, is employed after drug administration to enhance the formation of pair bonds. As mentioned above, we are interested in the relationship between the serotonergic system and pair bonding. It seems necessary to reveal the method used to assess partner preferences in our laboratory, since our colony of prairie voles were imported and have been bred for over a dozen years. Inbred breeding for long durations may reduce genetic diversity. The diversity of the microsatellite sequence of the vasopressin V1a receptor contributes to socio-social traits, including the formation of pair bonds in prairie voles. We used our apparatus to assess the partner preferences of prairie voles. As previously reported, the male prairie voles in our colony displayed partner preferences after 24 h of cohabitation, but not after 6 h. Half of the 6-h cohabitation group preferred to huddle with a stranger female, even though they mated with their partners during cohabitation. This suggests that the reason a 6 h cohabitation is insufficient for the formation of a pair bond is not simply the absence of mating bouts; reconstitution and delivery of neurotransmitter receptors, which is essential for the formation of pair bonds, may take over 24 h.

Materials and Methods

Animals

Prairie voles that were bred for over 10 generations were kindly gifted to us by Dr. Larry Young, Department of Psychiatry, Emory University. They were kept under a standard condition (a 14:10 h light/dark cycle, 23°C, bedding with wood shavings, free access to food and water ad libitum) in the Biosource Center of Gunma University Graduate School of Medicine. After weaning at 28 days old, 4–6 subjects of the same sex were housed together. Sexually naïve prairie voles at 16.9 ± 0.53 weeks old were used in the current experiments.

All experiments were performed in accordance with the Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology, Japan, and approved by the Animal Experiment Committee at Gunma University (No. 13-010, 18-021).

Cohabitation

Before cohabitation, subject voles were individually housed for 5 days to reduce social effects from cage mates. For natural cohabitation, a male prairie vole was cohorted with an unrelated female in a new cage for 96 h. For short-term (6 or 24 h) cohabitation, female prairie voles were ovariectomized and primed by estradiol injections as previously described. In brief, female prairie voles were ovariectomized under anesthesia with isoflurane, and recovered for 7 days. Females were subcutaneously injected with 2.0 µg of β-estradiol 3-benzoate (Sigma, St. Louis, MO, E8515) in sesame oil (Sigma 85067) for a consecutive period of 3 days. A primed female and subject male vole were cohabited in a new cage for 6 or 24 h. Mating bouts were monitored by a video camera (GZ-MG575, JVC Kenwood Co., Yokohama, Japan) in the 6 h cohabitation. After 6 h of cohabitation, subject and female voles were transferred to new cages, and a partner preference test was performed the following day. For the 24 h cohabitation group, cohabitation was immediately followed by a partner preference test.

Partner preference test

Partner preference was assessed according to our previous report. The test apparatus consisted of three transparent acrylic chambers (20 × 25 × 20 cm), whose floors were covered with wood shavings. A neutral chamber was located behind two stimulus chambers which were connected to the neutral chamber by acrylic hollow tubes (6 cm diameter × 12 cm). The partner female vole, which was cohabited with the subject male vole, was tethered to one stimulus chamber and a stranger female, which had never encountered the subject vole, was tethered to another stimulus chamber. The location of stimulus females was counterbalanced. Each stimulus female vole was used twice, once as the partner and the other time as a stranger, so that stimulator voles had equivalent social and sexual experiences. A subject male vole was placed in the neutral chamber and allowed to move freely into any of the three chambers for 3 h (Fig. 1A). Behaviors were video recorded from the front side. The time spent on huddling behavior (side-by-side contact) with each female was measured. Partner preference was defined as subjects spending significantly more time huddling with their partners than with strangers, which was assessed by a paired t-test. Data are presented as means ± standard error. p < 0.05 was considered significant.

Results

All male prairie voles who were cohabited with a female for 96 h spent more time huddling with their partner than with stranger females. The duration of huddling with their partner female prairie voles ranged from 9 min to 89 min, while that with a stranger female was 0 min in seven of the eight subjects. One subject that displayed the longest huddling time with the partner spent only 1 min huddling with a stranger female. Statistical analysis indicated that subject males spent significantly longer durations huddling with their partner females than with stranger females (t = 5.678, p = 0.001, Fig. 1B).
Fig. 1  Partner preference was measured by an apparatus composed of three chambers. (A) Photo (upper panel) and schematic (lower panel) images of an apparatus used for testing partner preference. A neutral chamber was placed behind stimulus chambers and connected to them via hollow tubes. The partner and stranger females were tethered to separate stimulus chambers. A subject male prairie vole was placed in the neutral chamber and allowed to move freely into any chamber for 3 h. (B) Partner preference after 96 h of cohabitation. All male prairie voles spent more time huddling with their partners than with strangers. *, p < 0.05.

Fig. 2  Partner preference was established after 24 h of cohabitation, but not after 6 h of cohabitation. (A) After 6 h of cohabitation. Three males spent more time with stranger females than with their partners, although 4 subjects displayed partner preference. (B) After 24 h of cohabitation. Five out of 7 subjects spent longer durations with their partners than with strangers. Two subjects spent no time huddling with any females. *, p < 0.05.
Because mating bouts are believed to be necessary for pair bonding, we only analyzed subjects displaying mating bouts during the 6 h cohabitation. Eight male prairie voles out of 11 subjects displayed mating bouts. The duration of huddling with a partner female ranged between 0 and 132.5 min, while that with a stranger female was between 0 and 49.5 min. A paired t-test indicated no significant difference in huddling duration between partner and stranger females (t(7) = 0.708, p = 0.502, Fig. 2A), although 4 prairie voles that displayed mating bouts during cohabitation preferred to huddle with their partner rather than with a stranger female.

In contrast, cohabitation with an estradiol-primed female for 24 h appeared to be enough to form pair bonding. A paired t test revealed that subjects indicated significantly longer durations huddling with their partners than with stranger females (t(7) = 3.296, p = 0.016, Fig. 2B). Five subjects spent less than 6 min huddling with stranger females, while they huddled with their partners for more than 40 min. However, two male prairie voles out of 7 subjects displayed no huddling behaviors with neither their partner nor a stranger female.

Discussion

All subjects in the 96-h cohabitation group spent longer times huddling with their partner females than with stranger females, suggesting that partner preference was appropriately assessed by our apparatus. The partner preference test utilized a neutral chamber connected to two stimulus chambers. Two kinds of positional relationships existed among them. One is as three chambers connected in a series, another is that a neutral chamber was connected to two stimulus chambers as a V shape like we used in the current study. Both systems appear to be useful, although auditory and olfactory cues from stimulus animals may directly interfere with the subjects’ behaviors in the former. Further, the fact that the duration of huddling with a partner was around 10 to 90 min suggests that the genetic variation of genes related to pair bonding is still maintained in spite of over 15 years of inbred breeding.

In this study, we indicated that more than 24 h of cohabitation is necessary for partner preference by male prairie voles. This fact appears to be consistent with previous reports. Cohabitation for 6 h without mating bouts does not induce partner preference in male prairie voles, whereas female prairie voles display partner preferences even after 3 h of cohabitation in the absence of mating bouts. Unlike traditional experimental rodents, such as mice and rats, mature female prairie voles do not display the estrus cycle unless they encounter an unrelated male prairie vole. It takes more than 24 h for the exposure to a male prairie vole to induce estrus in females. Hence, female prairie voles are ovariec tomized and estradiol-primed in many experiments, including the current study, when short term cohabitation conditions (6-24 h) are required. Even though female prairie voles are receptive of copulation under such artificial conditions, the absence of mating bouts during 6 h of cohabitation does not induce partner preference in male voles. These observations lead to a speculation that mating is important in facilitating the formation of partner preference in male prairie voles. Indeed, mating bouts within 30 min after the beginning of cohabitation induce cFos expression in tyrosine hydroxylase positive neurons at the principal nucleus of the bed nucleus of the stria terminalis and the posterodorsal medial amygdala. The activation of dopaminergic neurons in these brain regions is presumed to be involved in pair bonding. However, in the analysis of the current study, we restricted the subjects to those displaying mating bouts. Nonetheless, a 6-h cohabitation did not induce partner preference, suggesting that mating bouts are not sufficient in the formation of partner preference in male prairie voles. Recently, a neuronal mechanism underlying the formation of partner preference has been proposed. In this mode, mating bouts enhance the density of vasopressin V1a receptor in the anterior hypothalamus, and oxytocin and dopamine D1-type receptors in the nucleus accumbens. These neuroplastic changes, based on molecular events including the transcription, translation, post-translational modification, and intracellular trafficking of receptors, surely last tens of hours.

In this study, we indicated that 6 h of cohabitation was insufficient for the formation of a pair bond despite mating bouts, and that 24 h of cohabitation resulted in the formation of pair bonds amongst prairie voles. These facts should be considered when neurobiological mechanisms underlying pair bonds are investigated using pharmacological techniques that enhance or prevent the formation of pair bonds.

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Disclosure statement

The authors declare no conflicts of interest associated with this work.

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