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Research report

A single prolonged stress paradigm produces enduring impairments in social bonding in monogamous prairie voles

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HIGHLIGHTS

- Single prolonged stress (SPS) disturbed the formation of pair bond in prairie voles.
- The administration of SSRI prevented the disturbance of pair bonding.
- SPS transiently decreased oxytocin levels in the SON.
- SPS affected the response of oxytocin neurons in the SON to exposure to a partner.
- SPS-treated voles may be suitable to study the effect of trauma on social bonding.

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ABSTRACT

Traumatic events such as natural disasters, violent crimes, tragic accidents, and war, can have devastating impacts on social relationships, including marital partnerships. We developed a single prolonged stress (SPS) paradigm, which consisted of restraint, forced swimming, and ether anesthesia, to establish an animal model relevant to post-traumatic stress disorder. We applied a SPS paradigm to a monogamous rodent, the prairie vole (*Microtus ochrogaster*) in order to determine whether a traumatic event affects the establishment of pair bonds. We did not detect effects of the SPS treatment on anhedonic or anxiety-like behavior. Sham-treated male voles huddled with their partner females, following a 6 day cohabitation, for a longer duration than with a novel female, indicative of a pair bond. In contrast, SPS-treated voles indiscriminately huddled with the novel and partner females. Interestingly, the impairment of pair bonding was rescued by oral administration of paroxetine, a selective serotonin reuptake inhibitor (SSRI), after the SPS treatment. Immunohistochemical analyses revealed that oxytocin immunoreactivity (IR) was significantly decreased in the supraoptic nucleus (SON), but not in the paraventricular nucleus (PVN), 7 days after SPS treatment, and recovered 14 days after SPS treatment. After the presentation of a partner female, oxytocin neurons labeled with Fos IR was significantly increased in SPS-treated voles compared with sham-treated voles regardless of paroxetine administration.

Our results suggest that traumatic events disturb the formation of pair bond possibly through an interaction with the serotonergic system, and that SSRIs are candidates for the treatment of social problems caused by traumatic events. Further, a vole SPS model may be useful for understanding mechanisms underlying the impairment of social bonding by traumatic events.

Abbreviations: GR, glucocorticoid receptor; IR, immunoreactivity immunoreactive; LS, lateral septum; MR, mineralocorticoid receptor; MeA, medial amygdala; NAcc, accumbens nucleus; pBNST, principal bed nucleus of the stria terminalis; PBS, phosphate buffered saline; PBS-T, PBS containing 0.3% Triton X-100; PTSD, post-traumatic stress disorder; PVN, paraventricular nucleus; SON, supraoptic nucleus; SPS, single prolonged stress; SSRI, selective serotonin reuptake inhibitor; TH, tyrosine hydroxylase; V1aR, vasopressin 1a receptor; VP, ventral pallidum.

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1. Introduction

The establishment of social relationships with family members, colleagues, and neighbors is critical for health and wellbeing. However, traumatic events sometimes interfere with the ability to form social relationships. Recent reports indicate significant comorbidity between post-traumatic stress disorder (PTSD) and social anxiety [1,2]. Social attachment between sexual partners is one of the basic social relationships that is important for mental health. The pair bond is a selective and enduring relationship between mating partners in social monogamous animals, including human beings [3–5]. Traumatic events deteriorate not only social relationships with friends but also marital relations [6]. Higher levels of post-traumatic stress symptoms are associated with lower couple functioning [7]. Veterans with PTSD exhibit more frequent displays of hostility and fewer expressions of acceptance and humor in both themselves and their partners [8]. The molecular and cellular mechanisms underlying the deterioration of the ability to maintain social relationships by PTSD are still unknown, although functional neuroimaging studies implicate several brain regions including amygdala, ventral medial prefrontal cortex, dorsal anterior cingulate cortex, and hippocampus, in PTSD [9]. An animal model of single prolonged stress (SPS) is useful for studying neurobiological mechanisms of PTSD, because of their construct, face and predict validity [10]. A SPS paradigm is comprised of three stressors in a single day (restraint, forced swimming, and a loss of consciousness by ether anesthesia) and a 7-day quiescent period. The quiescent period is required for PTSD-like phenotype to develop, rather than only a single stressor day [11]. SPS-treated rats mimic neuroendocrinological impairments observed in PTSD patients such as enhanced negative feedback of the hypothalamo-pituitary-adrenal axis [11]. Neuroendocrinological impairments in SPS-treated rats are associated with an increased level of glucocorticoid receptor (GR) mRNA and down-regulation of mineralocorticoid receptor (MR) mRNA in the hippocampal CA1 and CA2 regions [12]. Further, SPS-treated animals also replicate deficits in the extinction of fear memories, which is one of cardinal symptoms in PTSD patients [13,14]. Elevated fear responses caused by a SPS treatment are ameliorated by chronic administration of paroxetine, which is a selective serotonin reuptake inhibitor (SSRI) and is sometimes administrated to PTSD patients [15]. Although there are many studies using animal models of PTSD including the SPS paradigm, there are no reports describing the influences of traumatic events specifically on social attachment behaviors, especially pair bonding, since traditional laboratory animals such as rat (*Rattus norvegius)* and mouse (*Mus musculus*) do not display a socially monogamous mating strategy. [16–18]

The prairie vole (*Microtus ochrogaster*) is a socially monogamous rodent, which forms a pair bond with its mate, and has contributed significantly to our understanding of the neurobiological mechanisms underlying the formation and maintenance of the pair bond and other prosocial behaviors [4,19,reviewed in 20,21]. Cohabitation with an opposite sex animal for 24 h is enough to produce an enduring pair bond, which is indicated by showing a partner preference and aggression toward conspecific strangers [22]. Two related neuropeptides, oxytocin and vasopressin, are crucial for the formation and maintenance of pair bond. These neuropeptides are mainly produced in neurons in paraventricular and supraoptic nuclei (PVN and SON, respectively) of hypothalamus, although other brain regions also contain neurons producing them [23–25]. Oxytocin neurons in the PVN and SON modulate behavioral responses associated with pair bonding by releasing oxytocin in the accumbens nucleus (NAcc) [26]. In addition to the NAcc, oxytocin neurons projecting to some brain regions such as the amygdala and olfactory bulb from the PVN are proposed to modulate pair bonding formation [27,28]. In females, and perhaps males as well, oxytocin facilitates pair bond formation through oxytocin receptors at the NAcc and prefrontal cortex, since the injection of oxytocin receptor antagonist during cohabitation prevents pair bond formation [29]. Recently, oxytocin receptor signaling has been reported to be crucial for pair bond formation in male prairie voles, since male prairie voles intracerebroventricularly administrated oxytocin receptor antagonist do not show partner preference and show reduced sexual behavior [30]. Furthermore, males with a genetic polymorphism leading to reduced oxytocin receptor expression in the NAcc display impairments in pair bond formation [31]. In males, the fact that the administration of vasopressin 1a receptor antagonist at the lateral septum (LS) and ventral pallidum (VP) disrupts pair bonding indicates that vasopressin 1a receptor at these brain regions is also necessary for pair bond formation and perhaps expression in males [32,33]. Overexpression of vasopressin 1a receptor at the VP using viral vector gene transfer enhances partner preference in promiscuous meadow voles [34]. Involvement of oxytocin receptor at the LS in the pair bond formation is also suggested in male prairie voles [35]. In male prairie voles, oxytocin enhances the correlated connectivity of brain regions involved in social information processing and reward [30].

In addition to neuropeptides, the dopamine system modulates pair bond formation. The activation of dopamine D1 receptor at the NAcc prevents pair bond formation, whereas the activation of D2 receptor facilitates it [36]. Besides the ventral tegmental area, various brain regions contain neuronal cells expressing tyrosine hydroxylase (TH), which is the late limiting enzyme for synthesis of dopamine and other catecholamines. TH-immnoreactive (IR) cells in the principal bed nucleus of the stria terminalis (pBNST) and medial amygdala (MeA) are suggested to be related to social behaviors across the reproductive cycles [37].

Here we applied a SPS paradigm to male prairie voles in order to deteremine whether traumatic events impair pair bond formation in this model organism or not. SPS-treated voles did not show a partner preference, the laboratory proxy for a pair bond, although anhedonia and enhancement of anxiety-like behavior was not affected by the SPS paradigm. OxytocinIR in the SON was transiently reduced after the SPS treatment. Interestingly, administration of an SSRI rescued the impairment in the display of partner preference following SPS. It is possible that traumatic events disturb pair bond formation through the perturbation of the coordinated functions of oxytocin and serotonin systems.

2. Materials and methods

2.1. Animals

Prairie voles were housed in a polycarbonate standard cage ($32 \times 21 \times 12$ cm) under standard laboratory conditions (a 12 h light/dark cycle, 23° C, bedding with wood shavings (white flake, Oriental Yeast Co., Ltd., Tokyo, Japan), free access to food (standard rabbit chow RC4, Oriental Yeast Co., Ltd.) and water ad libitum). Animals were housed 4–6 per cage in same sex groups after weaning at 4–5 week-old. Sexually naïve male prairie voles (21 ± 3.0 week-old) were used as subjects. Subjects were individually housed for 4 days before the SPS or sham treatment. All animal 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.