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Research report

# Breaking bonds in male prairie vole: Long-term effects on emotional and social behavior, physiology, and neurochemistry

P. Sun[a,b,1], A.S. Smith[b,1,2], K. Lei[b], Y. Liu[b], Z. Wang[b,*]

[a] Animal Academy of Scientific and Technology, Henan University of Science and Technology, Luoyang 471003, China
[b] Department of Psychology and Program in Neuroscience, Florida State University, Tallahassee, FL 32306, USA

## HIGHLIGHTS

- Male prairie voles express bond loss via increased stress behavior and physiology.
- Partner loss disrupt bond-related behavior in a time-dependent manner in male voles.
- Partner loss alter neuropeptide systems involved in vole pair bonding.
- We review the distinct effects of social isolation and bond loss in voles.

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## ABSTRACT

Social relationships are essential for many fundamental aspects of life while bond disruption can be detrimental to mental and physical health. Male prairie voles form enduring social bonds with their female partners, allowing the evaluation of partner loss on behavior, physiology, and neurochemistry. Males were evaluated for partner preference formation induced by 24 h of mating, and half were separated from their partner for 4 wk. In Experiment 1, partner loss significantly increased anxiety-like behaviors in the elevated plus maze and light–dark box tests and marginally increased depressive-like behaviors in the forced swim test. In addition, while intruder-directed aggression is common in pair bonded prairie voles, separated males were affiliative and lacked aggression toward an unfamiliar female and an intruding male conspecific. Partner loss increased the density of oxytocin-immunoreactivity (-ir), vasopressin-ir, and corticotrophin-releasing hormone-ir cells in the paraventricular nucleus of the hypothalamus and oxytocin-ir cells in the supraoptic nucleus. Tyrosine hydroxylase-ir was not affected. In Experiment 2, partner preference was observed after 2 wk of partner loss but eliminated after 4 wk partner loss. Body weight gain and plasma corticosterone concentrations were elevated throughout the 4 wk. No effects were observed for plasma oxytocin or vasopressin. Together, partner loss elicits anxiety-like and depression-like behaviors, disrupts bond-related behaviors, and alters neuropeptide systems that regulate such behaviors. Thus, partner loss in male prairie voles may provide a model to better understand the behavior, pathology, and neurobiology underlying partner loss and grief.



## 1. Introduction

Social living is beneficial for many species, resulting in increased individual survival and fitness. One factor that contributes to such benefits is the anxiolytic effects of social contact with a bonded partner, referred to as social buffering [1–6]. While healthy, committed social relationships provide salubrious effects, social separation or loss of bonded partners is a major risk factor to mental and physical health. With over 800,000 new widows and widowers in the United States annually, spousal bereavement is a significant cause of psychiatric and medical morbidity, and includes psychiatric sequelae such as depression, anxiety, substance abuse, and complicated grief[1] [9–11]. For example, after the death of a spouse, there is a greater prevalence of major depressive

* Corresponding author at: Department of Psychology and Program in Neuroscience, Florida State University, 1107 W. Call Street, Tallahassee, FL 32306, USA. Tel.: +1 850 644 5057.
*E-mail address:* zwang@psy.fsu.edu (Z. Wang).
[1] These authors contributed equally to this work.
[2] Present address: Section on Neural Gene Expression, NIMH, NIH, DHHS, Bethesda, MD 20892, USA.

[1] It is considered normal for individuals who lost a loved one to experience intrusive thoughts of the deceased, sadness, and yearning for reunion, all common symptoms of grief [7]. However, individuals who fail to dampen symptoms within 18 months may be diagnosed with complicated grief [8].

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disorder, post-traumatic stress, panic disorders, and general anxiety disorder [12–16], the odds of a new or worsened physical illness can increase by 1:40 times [17,18], and mortality rates double for the surviving spouse in the first year [19–21], with bereavement effects strongest immediately after social loss [21,22]. Even after counseling, this population is at a greater risk for mental disorders [23,24]. These negative effects may be due, in part, to the stress of losing a loved one, challenges of adapting to widowhood, loss of psychological, social, and economic resources, and social isolation and loneliness that can follow spousal loss. Thus, understanding the behavioral pathologies and neuroendocrine mechanisms that underlie the challenges to mental health and normal behavioral routines associated with social loss are important to improve treatment of subsequent mental disorders.

The socially monogamous prairie vole (*Microtus ochrogaster*) is a highly social rodent species that has been used to study the neurobiological mechanisms that govern social behaviors [25–28] and consequences of social isolation [29]. Pair bonding in prairie voles reinforce social behavior through activation of the brain reward centers and stress buffering effects of close social contact [6,25,30–32]. These driving forces employ a number of neurochemical systems including oxytocin (Oxt), vasopressin (AVP), dopamine (DA), and corticotrophin-releasing hormone (CRH). In addition, the absence of social contact in prairie voles can promote a disruption to normal behavioral routines and biological functions that mimic symptomatology of depression and anxiety disorders in humans [33,34], though there are gender differences. Female prairie voles isolated from social contact from a same-sex sibling display behaviors relevant to depression and anxiety, observable after 4 days and up to 6 wk of isolation [33–39]. Interestingly, male prairie voles do not display robust behavioral abnormalities in result to isolation from a familiar same-sex conspecific [37,40]; however, separation from a female bonded partner can be rather distressing, affecting normal behavioral routines and biological function [40,41]. Thus, bond loss in male prairie voles provide a model to characterize the impact that bereavement has on normal behavioral routines and function of neuronal systems.

The present study used male prairie voles to evaluate potential effects of partner loss on behavior, physiology, and neurochemistry. Previous studies have shown that 24 h mating reliably induces partner preference formation in most male prairie voles [42–45]. In the current study, we used this paradigm to pre-screen our male subjects. Subsequently, these pair-bonded males were randomly assigned into one of two experimental groups that were either separated from or continuously housed with their partner for 4 wk to evaluate the impact that this form of social loss on anxiety-like, depression-like, and social behaviors. In addition, we investigated the changes that manifest in body weight, circulating hormones (Oxt, AVP, and corticosterone), and neurochemical systems sensitive to disruptions to the social environment (i.e., Oxt, AVP, DA, and CRH) in brain regions that synthesize these neurochemicals, including the paraventricular and supraoptic nucleus of the hypothalamus (PVN and SON, respectively), ventral tegmental area (VTA), and rostral zona incerta (ZIR). As 4 wk of partner separation led to disruptions in behaviors associated with pair-bonding, we established a second cohort of males that were paired or separated from their female partner for 2 or 4 wk[2] to examine the effect of separation durations on partner preference expression. We predict that partner loss will induce a state of distress in male prairie voles that should manifest in increased anxiety-like and depression-like behaviors and physiology without impacting bond-related behaviors.

## 2. Materials and methods

### 2.1. Subjects

Male prairie voles (*M. ochrogaster*) were descended from populations in southern Illinois and captive-bred at Florida State University. Voles were weaned on postnatal Day 21 and housed with a same-sex conspecific in plexiglass cages (29 cm $L \times 18$ cm $W \times 13$ cm $H$) containing cedar chip bedding with food and water ad libitum. Colony rooms were maintained on a 14L:10D photoperiod (lights on at 07:00 h) and at a temperature range of $21 \pm 1\,^{\circ}C$. All experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee at Florida State University.

### 2.2. Experimental procedure

#### 2.2.1. Experiment 1: behavior and neurochemistry after 4 wk partner loss

Sexually naïve males were weighted and paired with an ovariectomized, estrogen-primed female for 24 h then tested in a 3-h partner preference test (PPT), see details below. Males that displayed a partner preference were randomly divided into paired ($n = 6$) or separated ($n = 7$) group. Separation involved removing the male from the home cage and female partner and housing it in a new cage, identical to the size of the home cage, alone for 4 wk. In contrast, paired males were moved along with their female partner to a novel cage, similar to the separated males, and housed with their partner for the duration of the experiment. After 4 wk, an array of behavioral testing was conducted on males from both housing conditions to evaluate the impact of partner loss on anxiety-like, depression-like, and social behaviors (see below). Subjects received two behavioral tests each day with the morning test starting at 09:00 h and the afternoon at 15:00 h. Tested were conducted in the following order: Day 1 – open field (OF), light–dark box (LDB); Day 2 – affiliation test (AFF), resident-intruder test (RIT); Day 3 – elevated plus maze (EPM), and forced swim (FS). All tests were performed in an isolated behavior room maintained under similar temperature- and light-controlled environmental conditions as the colony rooms. To adapt to the environment, animals were brought to the testing room an hour before testing. Behaviors were videotaped and scored later by a single trained observer blind to condition using a computer-assisted data acquisition system (J-watcher, http://www.jwatcher.ucla.edu). For all tests, the light in the room measured ~300 lx, except LDB test during which the light in the room measured 850 lx. Subjects were returned to their home cages immediately after each behavioral test. The day after the last behavioral test (i.e. FS), subjects were perfused, and brains were harvested and stored at $-80\,^{\circ}C$ until processed.

#### 2.2.2. Experiment 2: partner preference and physiology during partner loss

A second cohort of males were established using the same paradigm as Experiment 1 in which males cohabitated with a female for 24 h then were tested in a 3-h PPT. Thereafter, they were randomly divided into 2 wk paired ($n = 6$), 2 wk separated ($n = 6$),

[2] Previous literature has demonstrated that after 24 h cohabitation, female prairie voles maintain a partner preference over an opposite-sex stranger for at least 2 wk, even in the absence of further exposure to the male mate [46]. Bosch and colleagues [38] demonstrated that 5-day paired males display a partner preference even after 5 days of separation from their female partner. Our pilot experiments confirmed that male prairie voles sustain a selective partner preference for 2 wk following the initial 24 h cohabitation, either with or without further exposure to their female mate (data not shown). Therefore, we utilized this 2 wk cohabitation/separation paradigm as a temporal control in the current study.