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Disruption of social bonds induces behavioral and physiological dysregulation in male and female prairie voles

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Abstract

The social disruption of losing a partner may have particularly strong adverse effects on psychological and physiological functioning. More specifically, social stressors may play a mediating role in the association between mood disorders and cardiovascular dysfunction. This study investigated the hypothesis that the disruption of established social bonds between male and female prairie voles would produce depressive behaviors and cardiac dysregulation, coupled with endocrine and autonomic nervous system dysfunction. In Experiment 1, behaviors related to depression, cardiac function, and autonomic nervous system regulation were monitored in male prairie voles during social bonding with a female partner, social isolation from the bonded partner, and a behavioral stressor. Social isolation produced depressive behaviors, increased heart rate, heart rhythm dysregulation, and autonomic imbalance characterized by increased sympathetic and decreased parasympathetic drive to the heart. In Experiment 2, behaviors related to depression and endocrine function were measured following social bonding and social isolation in both male and female prairie voles. Social isolation produced similar levels of depressive behaviors in both sexes, as well as significant elevations of adrenocorticotropic hormone and corticosterone. These alterations in behavioral and physiological functioning provide insight into the mechanisms by which social stressors negatively influence emotional and cardiovascular health in humans.

Keywords

adrenocorticotropic hormone; autonomic nervous system; behavior; cardiovascular; corticosterone; depression; heart rate variability; microtus; respiratory sinus arrhythmia; social isolation; stress

1. Introduction

Supportive social relationships have a positive influence on mood and emotion as well as physiological functioning. For instance, they help protect against cardiovascular disease (CVD), improve responses to depression, and facilitate adaptive stress coping reactions (Blazer, 1982; Cacioppo, & Cacioppo, 2012; Eisenberger, 2013; Frasure-Smith et al., 2000;

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Kikusui et al., 2006; Norman et al., 2013; Orth-Gomer et al., 1993). Frasure-Smith et al. (2000) assessed baseline depression and social support in patients suffering from myocardial infarction, along with cardiac prognosis and changes in depression symptoms after the infarction. High levels of perceived social support were associated with improvements in depressive symptoms and a reduced impact of depression on mortality over the first year following the infarction.

Conversely, disruption of social bonds, social isolation, and perceived isolation (loneliness) are associated with various forms of dysfunction and mortality both in humans and animal models (Barger, 2013; Cacioppo, & Hawkley, 2003; Grippo et al., 2007c; Grippo et al., 2011; Seeman, & Crimmins, 2001; Uchino, 2006). For example, individuals with low levels of social engagement experience an increased risk of general and CVD-related mortality (Ramsay et al., 2008); and both social isolation and feelings of loneliness are correlated with increased mortality in older men and women (Steptoe et al., 2013). Men and women may respond differently to social and environmental stress. While some studies indicate that women are more likely than men to experience depressive or anxiety disorders, men are more likely to report greater impairment in everyday functioning as a result of these psychological disturbances (Scott, 2011).

The specific neurobiological mechanisms that underlie emotional and cardiovascular dysfunction are not well defined, however, both types of disorders share similar physiological dysfunctions and both appear to be influenced by the social environment. A better understanding of the influence of social experiences on health may lead to improved outcomes for millions of individuals worldwide affected by CVD and/or depressive disorders (American Heart Association, 2011; Murray & Lopez, 1996; National Institute of Mental Health, 2009). Both depression and CVD are characterized by an imbalance of autonomic cardiac regulation, altered heart rate (HR) and heart rate variability (HRV), vascular disturbances, and neurohumoral and immune dysregulation (Burg et al., 2013; Carney, & Freedland, 2003; Hance et al., 1996; Dantzer, 2006; Penninx et al., 2001). Similarly, endocrine dysregulation (such as increased cortisol) is linked with atherosclerosis of carotid arteries (Dekker et al., 2008), and hypothalamic-pituitary-adrenal (HPA) axis dysfunction has been implicated in depression (Hinkelmann et al., 2009; Holsboer et al., 1984). These endocrine and autonomic mechanisms may be disrupted during social stress, which are likely to influence stress reactivity in both depressive and cardiovascular disorders. Social support has been suggested to have a positive influence on health by increasing an individual's valuation of self-esteem, control, and health behaviors; and decrease his/her appraisal of stress (Uchino et al., 1999). This down-regulation of responses to adverse events may in turn decrease the stress placed on the individual's body, resulting in more adaptive responses to stressors and greater overall health (Uchino et al., 1999).

Studies involving animal models provide insight into the neurobiological and biobehavioral mechanisms that underlie the associations among social stress, emotion, and cardiovascular dysfunction. In particular, the prairie vole is a socially monogamous rodent species that provides an excellent tool for studying relationships among the social environment, behavior, and physiology. These rodents form monogamous social bonds between males and females, live in extended families, and engage in bi-parental and allo-parental care of offspring, similar to human social systems (Carter, 2001; Getz et al., 1981; Cushing et al., 2001; Young, & Wang, 2004). Prairie voles have been employed previously to investigate the neurobiological basis of attachment behavior (Aragona et al., 2003; Cushing et al., 2003; DeVries et al., 1995) and several aspects of dysfunction as a result of isolation and the disruption of social bonds (Bosch et al., 2009; Grippo et al., 2012; Lieberwirth et al., 2012; Pournajafi-Nazarloo et al., 2011).

Substantial evidence indicates that prairie voles are sensitive to disruptions of the social environment. For example, female prairie voles exposed to long-term social isolation from a family member exhibit several deleterious changes in behavioral, endocrine, and autonomic function including depressive and anxiety behaviors, increased HR, decreased HRV, exaggerated cardiac and neuroendocrine reactivity to acute stressors, dysregulated autonomic cardiac control, and endothelial dysfunction (Bales et al., 2006; Grippo et al., 2007c; Grippo et al., 2012; Stowe et al., 2005; Peuler et al., 2012). Further, early life social isolation has been associated with anxiogenic behaviors and altered social interactions, in addition to increased expression of stress hormones in the paraventricular nucleus of the hypothalamus (Pan et al., 2009). Finally, male prairie voles exposed to short-term social isolation from a female social partner exhibit poor stress-coping behaviors and increased circulating hormone levels following separation (Bosch et al., 2009). These characteristics contribute to the utility of the prairie vole for the investigation of neurobiological mechanisms underlying social stress and negative health consequences.

The specific autonomic and endocrine mechanisms underlying the effects of disrupted pair bonds are not well understood. As such, the study of disrupting male-female social bonds in prairie voles, such as that described by Bosch et al. (2009), offers a unique opportunity to investigate the neurobiological mechanisms that may influence physiological and psychological dysfunction following partner loss. The present experiments investigated the disruption of an established social bond between male and female prairie voles. Experiment 1 investigated the specific effects of social bond disruption on depressive behaviors, and autonomic and cardiac function in male prairie voles. Experiment 2 extended the investigation of the deleterious effects of disrupted social bonds on behavior and neuroendocrine function in both male and female prairie voles. These experiments tested the hypothesis that the disruption of established social bonds would result in: (a) adverse changes in autonomic and cardiac regulation during basal and stress periods, including increased HR, decreased HRV, and autonomic imbalance; (b) increased neuroendocrine reactivity following exposure to stress; and (c) behavioral responses to stress that are associated with negative affective states. The investigation of these changes in a translational animal model will help explain the underlying mechanisms by which social stressors deleteriously influence behavior and physiology in humans.

2. Methods and Materials

2.1. Experiment 1

2.1.1. Animals—Seventeen male prairie voles (60–90 days old) were bred in-house at Northern Illinois University. Offspring were removed from breeding pairs at 21 days of age, and housed in same-sex sibling pairs until the commencement of experimentation. Animals were allowed *ad libitum* access to food and tap water, maintained at a room temperature of 20–21°C and a relative humidity of 40–50%, and under a standard 14:10 light/dark cycle (lights on at 0630). All experimental protocols were approved by the Northern Illinois University Institutional Animal Care and Use Committee and followed National Institute of Health guidelines as stated in the *Guide for the Care and Use of Laboratory Animals*.

2.1.2. General Experimental Design—Table 1 depicts the timeline of all procedures in Experiment 1. Briefly, a radiotelemetry transmitter was implanted into each male prairie vole for the recording of continuous electrocardiogram (ECG) and activity variables. Following recovery, animals underwent a baseline period of ECG and activity recordings. Each experimental animal was then removed from its home cage and paired with an unrelated female prairie vole. A social bonding assessment was conducted during this period to determine whether the prairie vole pairs had formed a bond. Five days after pairing, half

of the pairs were housed individually (n = 9), while the other half remained as pair-housed controls (n = 8) for an additional 5 days. A forced swim test (FST) and assessments of autonomic nervous system function were conducted following the social isolation/pairing period (while the experimental group remained isolated). Handling and cage changes were matched between the groups.

2.1.3. Telemetric Transmitter Implantation—Wireless radiofrequency transmitters (model TA10ETA-F10; Data Sciences International, St. Paul, Minnesota) were implanted intraperitoneally into male prairie voles similar to methods used previously (Grippo et al., 2007b). Animals were anesthetized with a mixture of isoflurane (Baxter, IL USA) and oxygen throughout the surgical procedures. Briefly, the body of the transmitter was implanted into the intraperitoneal space, and wire leads were sutured (subcutaneously) to the muscle on the left and right of the heart. Following transmitter implantation, animals were housed for 5 days in custom designed divided cages that permitted adequate healing of suture wounds (see Grippo et al., 2007b). All animals were then returned to standard cages (with the respective sibling) to recover for an additional 5–6 days. Animals were assessed for the following characteristics of recovery: (a) visible signs of eating and drinking, (b) adequate urination and defecation, (c) adequate activity level (approximately 2 counts per minute or higher), (d) adequate body temperature (approximately 37.5° C), and (e) stabilization of HR.

2.1.4. Electrocardiographic Recordings—ECG signals were collected via radiotelemetric recordings (sampling rate 5 kHz, 12-bit precision digitizing), either continuously or at hourly intervals throughout all experimental protocols. Multiple segments of 1–5 minutes of stable, continuous data were used to evaluate HR, HRV and activity level. Because activity levels are high in prairie voles and occur in approximately 2–3 hour ultradian rhythms throughout the light and dark periods (Grippo et al., 2007b), resting cardiac parameters were derived from ECG data sampled during a period of minimal activity (5 counts per minute or lower; 256 Hz sampling rate), during which time the animal may have been sitting, resting quietly, or sleeping.

2.1.5. Quantification of Cardiac Variables—All ECG signals were exported into a data file and examined manually with custom-designed software to ensure all R-waves were detected (Brain-Body Center, University of Illinois at Chicago, Chicago, IL; Porges, 1985; Porges, & Bohrer, 1990). HR was evaluated using the number of beats per unit time (beats per minute, bpm). HRV was evaluated using the standard deviation of normal-to-normal intervals (SDNN index) and amplitude of respiratory sinus arrhythmia (RSA). The SDNN index was calculated from the standard deviation of all R-R intervals from each data segment (Task Force of the European Society of Cardiology, 1996).

The amplitude of RSA has been hypothesized to represent the functional impact of myelinated vagal efferent pathways originating in the nucleus ambiguus on the sinoatrial node (see Porges, 2007). RSA was assessed with previously-published time-frequency procedures that have been validated in humans (Porges, 1985; Porges, & Bohrer, 1990), applied to small mammals (Yongue, 1982), modified for the prairie vole (Grippo et al., 2007b), and are appropriate for use during period of both low activity and exercise (Byrne et al., 1996; Houtveen et al., 2002; Porges et al., 2007).

2.1.6. Baseline Measurement Period—Following recovery from the implantation of the radiotelemetry transmitter, the baseline period consisted of 3 days during which time the animals were housed in sibling pairs.

2.1.7. Social Bonding Period—After baseline measurements, all male animals were separated from their respective siblings and paired with an unrelated female of approximately the same age and body weight in a new, clean cage, for 5 days. To determine whether male and female pairs formed a social bond, a 3-hour assessment of behavior was conducted 48 hours following initial pairing. Based on previous research involving the study of pair bonding in prairie voles (Williams et al., 1992), male-female pairs typically bond within 48 hours of introduction. This has been observed in a 3-hour partner preference test in which the experimental animal spent the majority of its time in side-by-side contact with a familiar animal versus an unfamiliar animal, when allowed to choose freely between these 2 animals (Williams et al., 1992). Video data were scored manually by an experimentally-blind investigator for affiliative behaviors, individual behaviors of each animal in the pair, and to determine the amount of time the animals spent in side-by-side contact, an operational index of attachment (Williams et al., 1992).

2.1.8. Isolation Period—Following 5 days of social pairing, the male-female pairs were randomly assigned to isolated (n = 9 males and 9 females) or paired (control, n = 8 males and 8 females) conditions, similar to the methods described by Bosch et al. (2009). Male prairie voles in the isolated group were separated from the females for the remainder of the experiment and housed individually without auditory, olfactory, or visual cues. Paired animals were continually housed with the female partners.

2.1.9. Learned Helplessness Assessment—Following the period of isolation or continued pairing (while the experimental group remained isolated), the FST was used as an index of learned helplessness (e.g., “behavioral despair”). This task consisted of a training period (15-minute swim period), followed by a test period (5 minutes), separated by 24 hours (Cryan et al., 2005). The swim tank was a clear Plexiglas cylinder (height 46 cm, diameter 20 cm) filled with 18 cm of 25 ± 1° C clean tap water. Following the FST, animals were returned to the home cage and allowed access to a heat lamp for 10 minutes.

Behaviors were digitally video recorded and then imported into analysis software (Noldus Observer XT 8.0, Noldus Information Technology, Wageningen, Netherlands). Behaviors during the FST were categorized manually by 2 trained observers who were blind to the experimental conditions, according to the following criteria: (a) *struggling*, movements during which the forelimbs break the water surface; (b) *climbing*, movements during which the forelimbs break the water surface and are in direct contact with the wall of the apparatus; (c) *swimming*, movements of the fore and hind limbs resulting in purposeful motion without breaking the water surface; and (d) *immobility*, idle floating or treading water during which time the animal uses limb movement to maintain its equilibrium without any directed movement of the limbs or trunk. Struggling, climbing and swimming were summed to provide one index of active coping behaviors; immobility was used as the operational index of learned helplessness (Cryan et al., 2005).

2.1.10. Autonomic Nervous System Function Assessment—Forty-eight hours following the FST (while the experimental group remained isolated), HR was measured under the following pharmacological conditions: (a) sympathetic receptor antagonism (β1-adrenergic receptor blockade; atenolol, 8 mg/kg IP; Sigma-Aldrich, St. Louis, MO); (b) parasympathetic receptor antagonism (cholinergic receptor blockade; atropine methyl nitrate, 4 mg/kg IP; Sigma-Aldrich); and (c) dual blockade (both drugs). Drugs were administered in a counterbalanced manner, during a 6-day period, with 48 hours between injections. The data were manually examined to determine the peak HR response following each drug injection, during a period of stable ECG recording that was not confounded by animal movement.

2.1.11. Statistical Analyses—The data are presented as means ± standard error of the mean (SEM) for all analyses and figures. A probability value of $p < 0.05$ was considered to be statistically significant. Any periods of ECG involving animal movement artifact were excluded from the analyses. The data were analyzed with 2-factor mixed-design analyses of variance (ANOVA) and Student's *t*-tests.

2.2. Experiment 2

2.2.1. Animals—Twenty adult male (60–90 days old) and 20 adult female (60–90-days old) prairie voles were used for the experimental procedures in Experiment 2. All breeding, housing, and handling conditions were identical to those described in Experiment 1.

2.2.2. General Experimental Design—Table 2 shows the timeline for all procedures employed in Experiment 2. Briefly, each male and female experimental animal was removed from its home cage and paired with an unrelated animal of the opposite sex for a total of 5 days. Half of the pairs (n = 10) were then housed individually, while the other half remained as pair-housed controls (n = 10), identical to the methods described in Experiment 1. Each animal was exposed to the FST (while the experimental group remained isolated), and plasma was collected 10 minutes following the 5-minute FST.

2.2.3. Social Bonding Period—All male and female prairie voles were removed from their respective siblings and paired with an unrelated opposite-sex animal of approximately the same age and body weight, in a new, clean cage, for 5 days, as described in Experiment 1.

2.2.4. Isolation Period—Male-female prairie vole pairs were randomly assigned to isolated (n = 10 males and 10 females) or paired (control, n = 10 males and 10 females) conditions for the remainder of the experiment, as specified in Experiment 1.

2.2.5. Learned Helplessness Assessment—The FST was used as an assessment of behavioral response to an inescapable stressor, as described in Experiment 1.

2.2.6. Collection of Plasma—Ten minutes following the end of the 5-minute FST, all animals were anesthetized with a mixture of ketamine (67 mg/kg, sc; NLS Animal Health, Owings Mills, MD) and xylazine (13.33 mg/kg, sc; NLS Animal Health). Blood was sampled within 2 minutes of the anesthetic injection, from the periorbital sinus via a heparanized capillary tube, and was collected during a period not exceeding 1.5 minutes. The blood was placed immediately on ice, and then centrifuged at 4° C, at 3500 rpm, for 15 minutes to obtain plasma. Plasma aliquots were stored at −80° C until assayed for circulating ACTH and corticosterone.

2.2.7. Circulating Hormone Analyses—Plasma levels of ACTH and corticosterone were determined using commercially available enzyme-linked immunosorbent assay kits (ACTH, EK-001-21, Phoenix Pharmaceuticals, Burlingame, CA; corticosterone, ADI-900-097, Enzo Life Sciences, Farmingdale, NY). Plasma samples were diluted according to the kit instructions to give results reliably within the linear portion of the standard curve (ACTH, 1:7; corticosterone, 1:500). The sensitivity of the kit for ACTH is 0.08 ng/ml (range 0–25 ng/ml) and for corticosterone is 27.0 pg/ml (range 32–20,000 pg/ml).

2.2.8. Data Analyses—Data were analyzed as specified in Experiment 1.

3. Results

3.1. Experiment 1

3.1.1. Social Bonding Assessment—All male-female pairs displayed similar side-by-side contact scores during the social bonding assessment, and additional measures of pair behaviors yielded no significant group differences. During the 3-hour assessment, all animals engaged in side-by-side contact, and all pairs engaged in side-by-side contact within two standard deviations of the mean of the pairs. The amount of time the male-female pairs spent in side-by-side contact was normally distributed. However, the animals assigned to the isolated group spent slightly more time in side-by-side contact than those assigned to the paired control group [paired, 5357 ± 199 seconds; isolated: 6164 ± 291 seconds; $t(15) = 2.234$, $p = 0.041$].

Additional behaviors during the social bonding assessment were collapsed into two general categories, affiliative and individual behaviors. Observed behaviors that appeared pro-social were categorized as affiliation behaviors, and included: (a) sniffing; (b) one animal grooming itself while in side-by-side contact; (c) one animal grooming its partner while in side-by-side contact; and (d) mating. There were no group differences in affiliation behavior scores between the male-female prairie vole pairs ($p > 0.05$ for all comparisons; data not shown).

Total individual behaviors represented a collection of behaviors exhibited by the male-female pairs during which time the animals were separated from one another. These behaviors were defined by one or both of the animals' activity around an observed resting location for the male-female prairie vole pair. This location was operationalized as a “home base” for behavioral scoring purposes. The activities that comprised total individual behaviors included: (a) one animal away from the home base; (b) both animals eating or drinking; (c) one animal eating or drinking and one animal away from the home base; (d) both animals away from the home base; and (e) one animal eating or drinking while the other animal was in the home base. Similar to total affiliative behaviors, there were no between-group differences in individual behaviors ($p > 0.05$ for all comparisons; data not shown).

3.1.2. Resting Cardiac Parameters—Social bond disruption significantly increased HR in isolated male prairie voles compared to pair-housed animals, and increased HR and reduced HRV when compare to pre-isolation values (Figure 1). The ANOVA for HR yielded a non-significant main effect of time [$F(2,45) = 3.060$, $p = 0.057$], but a significant main effect of group [$F(1,45) = 4.147$, $p = 0.048$], and a group by time interaction [$F(2,45) = 5.941$, $p = 0.005$]. The HR of the two groups did not differ during the baseline ($p > 0.05$) or social bonding period ($p > 0.05$). However, during the isolation period, HR in the isolated group was higher than its respective HR during social bonding with the female partner [$t(8) = 5.901$, $p = 0.000$] and that of the paired group [$t(15) = 3.392$, $p = 0.004$].

The ANOVA for SDNN index yielded no significant main effects of group ($p > 0.05$), time ($p > 0.05$), or group by time interaction ($p > 0.05$). The ANOVA for RSA amplitude yielded no significant main effects of group ($p > 0.05$) time ($p > 0.05$), or group by time interaction ($p > 0.05$). No follow-up comparisons were conducted (data not shown).

3.1.3. Learned Helplessness—Social bond disruption altered depressive behaviors, and resulted in a significant increase in HR in isolated male prairie voles during the FST (Figure 2). Isolated animals exhibited relatively higher immobility levels (92.7 ± 21.0 seconds) than paired animals (40.6 ± 15.3 seconds; $t(14) = 2.069$, $p = 0.058$]. The ANOVA for HR during the FST yielded significant main effects of group [$F(1,49) = 42.702$, $p = 0.000$] and time

[$F(4,49) = 20.544$, $p = 0.000$], but no significant group by time interaction ($p > 0.05$). Between-group minute-by-minute comparison indicated that the isolated group exhibited a higher HR than the paired group across all 5 time points: (a) minute 1 [$t(1,9) = 2.890$, $p = 0.018$]; (b) minute 2 [$t(1,10) = 3.659$, $p = 0.004$]; (c) minute 3 [$t(1,9) = 2.593$, $p = 0.029$]; (d) minute 4 [$t(1,11) = 3.347$, $p = 0.007$]; and (e) minute 5 [$t(1,10) = 2.799$, $p = 0.019$]. The ANOVA for SDNN index yielded non-significant main effects of group ($p > 0.05$) and time ($p > 0.05$), and no group by time interaction ($p > 0.05$; data not shown). Comparisons of cardiac data 3 hours following the FST indicated no differences in HR ($p > 0.05$) or SDNN index ($p > 0.05$) between the paired and isolated groups (data not shown).

3.1.4. Autonomic Nervous System Function—Social bond disruption resulted in autonomic imbalance characterized by increased sympathetic and decreased parasympathetic drive to the heart, compared to paired animals (Figure 3). Prior to administration of the autonomic nervous system receptor antagonists, the isolated group displayed a significantly elevated HR relative to the paired group [t(15) = 3.392, p = 0.004].

The ANOVA for absolute HR values, relative to pre-drug HR values, yielded a main effect of drug treatment [F(3,53) = 87.734, p = 0.000] and a drug treatment by group interaction [F(3,53) = 4.408, p = 0.008]. Following atenolol administration, absolute HR was significantly greater in the isolated group versus the paired group [$t(14) = 2.169$, $p = 0.048$]. However, isolated prairie voles displayed a greater reduction in HR (from pre-drug values) versus paired animals [$t(1,14) = 2.292$, $p = 0.038$]. Following atropine administration, absolute HR did not differ between paired and isolated groups ($p > 0.05$). However, isolated prairie voles exhibited an attenuated increase in HR (from pre-drug values) versus paired animals [$t(1,13) = 3.190$, $p = 0.007$]. There were no significant differences in absolute HR or change from pre-drug values between paired and isolated groups following dual autonomic blockade (p > 0.05 for both comparisons).

3.2. Experiment 2

3.2.1. Learned Helplessness—Social bond disruption resulted in an increase in depressive behaviors in both male and female isolated groups, compared to paired males and females; however no sex difference was observed (Figure 4). The ANOVA for immobility yielded a main effect of group [F(1,36) = 43.069, p = 0.000], but no significant main effect of sex ($p > 0.05$) or group by sex interaction ($p > 0.05$). Given the lack of a significant interaction, males and females were collapsed for the purposes of pairwise comparisons. Isolated animals displayed significantly more immobility during the FST versus paired animals [t(38) = 6.354, p = 0.000].

3.2.2. Circulating Hormones—Social bond disruption resulted in elevated levels of both ACTH and corticosterone in males and females, versus paired animals (Figure 5). A sex difference was not observed in either ACTH or corticosterone levels. The ANOVA for ACTH levels yielded a main effect of group [F(1,36) = 5.801, p = 0.024], but no significant main effect of sex ($p > 0.05$) or group by sex interaction ($p > 0.05$). Males and females were collapsed for the purposes of pairwise comparisons. Isolated animals displayed significantly greater levels of ACTH than paired animals [t(28) = 2.65, p = 0.006].

The ANOVA for corticosterone levels yielded a main effect of group [F(1,36) = 10.192, p = 0.004], but no significant main effect of sex ($p > 0.05$) or group by sex interaction ($p > 0.05$). Males and females were collapsed for the purposes of pairwise comparisons. Isolated animals displayed significantly greater levels of corticosterone than paired animals [t(28) = 3.11, p = 0.002].

4. Discussion

The disruption of social bonds can have significant effects on psychological and cardiovascular health. The current series of experiments used the prairie vole model to investigate the behavioral and physiological consequences resulting from the disruption of established social bonds between mated pairs. The present experiments focused on prairie voles because these animals display a number of analogous social behaviors to humans and are an excellent translational model for investigating the influence of social experiences on health (Getz, & Hofmann, 1986; Williams et al., 1992; Grippo et al., 2007c; Roberts et al., 1998). The present studies demonstrate that the disruption of a pair bond produces behavioral disturbances associated with depression and cardiac dysfunction, coupled with indices of both neuroendocrine and autonomic dysregulation.

The findings from these experiments provide insight into negative emotional consequences of disrupted social bonds. The assessment of learned helplessness during the FST was investigated due to its relevance to the relationship between depression and ineffective coping styles (Lazarus, 2006). Both male and female prairie voles displayed an increase in passive behavioral responding during the FST, which has been described as a valid operational measure of learned helplessness or behavioral despair (Cryan et al., 2005; Bielajew et al., 2003). The behavioral results from both males and females show the same pattern; isolated animals displayed increased helpless behaviors (immobility) compared to paired animals. The disruption of established social bonds in prairie voles adversely influences behavioral reactivity to a stressor, predisposing them to depressive behaviors. These results are consistent with previous findings from both long-term (Grippo et al., 2007c) and short-term (Bosch et al., 2009) social isolation in prairie voles, and studies of social stressors in both men and women (see Kiecolt-Glaser, & Newton, 2001; Phillipson, 1997; Rehman et al., 2008).

In addition to inducing depression-relevant behaviors, the disruption of an established social bond produces changes in both resting and stressor-induced HR. In Experiment 1, male prairie voles displayed a significantly higher resting HR after the loss of a female partner, compared to pre-isolation values and paired control animals. These findings are in line with previous studies of the cardiovascular consequences of long-term social isolation in prairie voles (Grippo et al., 2007c; Grippo et al., 2011). Interestingly, in the present study, the disruption of a social bond between mated prairie vole pairs produced a significant elevation in HR much earlier (after 5 days) than in previous studies that have investigated the effects of disrupting family bonds in this species (after 2–4 weeks; Grippo et al., 2007c). However, in contrast to HR alterations, HRV was not significantly affected by 5 days of social bond disruption in the present study. This is consistent with previous data indicating that 2–4 weeks of disruption of a family bond was necessary to produce a reduction in HRV (Grippo et al., 2007c). Five days of social isolation from either family members or mated partners is not sufficient to induce significant HRV alterations.

In addition to an elevation of HR during resting periods, HR also was increased in isolated male prairie voles during the FST. Compared to pair-housed control animals, the isolated animals displayed a significantly higher HR across the 5-minute swim period. Our laboratory was the first to characterize cardiac variables during the FST in prairie voles, showing that long-term social isolation in females is associated with increased HR, reduced HRV, and arrhythmias during this behavioral stressor (Grippo et al., 2012). The present findings shed further light on the integration of behavioral and physiological reactions to stress in this species. Isolated prairie voles are more reactive to the stress of the FST, and are unable to regulate their physiological state as well as pair-housed animals. In particular, it is notable that, while isolated prairie voles were less active than their paired counterparts

during the FST (i.e., displaying higher rates of immobility), this group exhibited an exaggerated cardiac response to the stressor. These findings therefore suggest that an inability to maintain appropriate cardiovascular control during periods of stress may influence cardiac morbidity and mortality in depressed individuals.

The resting and stressor-associated cardiac dysfunction may be mediated by autonomic dysregulation. Compared to pair-housed control animals, isolated male prairie voles displayed autonomic nervous system disturbances, characterized by increased sympathetic and decreased parasympathetic innervation to the heart. This imbalance of autonomic regulation contributed to the increased HR observed during the isolation period and during the FST, but did not appear to influence HRV. An imbalance of autonomic cardiac control has been reported in both CVD and depression in humans (Carney et al., 2005; Udupa et al., 2007), and in animal models (Grippo et al., 2002; Grippo et al., 2007c). An increase in sympathetic drive coupled with a decrease in parasympathetic drive contributes to morbidity and mortality from CVD (Carney et al., 2001; Carney et al., 2008; Cheng et al., 2003). The present findings suggest that social support from a partner can be protective against autonomic imbalance and associated HR dysfunction.

The disruption of social bonds also influences neuroendocrine regulation. In Experiment 2, both male and female isolated groups exhibited increased circulating concentrations of ACTH and corticosterone following the FST, when compared to their respective pair-housed controls. These findings are in line with Bosch et al. (2009), who reported increased corticosterone and depression-relevant behaviors following male-female social bond disruption. The lack of a sex difference in HPA axis reactivity to a short-term stressor in the present study indicates that both males and females are sensitive to the loss of a socially-bonded partner. Similarly, studies with humans suggest that various forms of morbidity and mortality are influenced by social stress in both sexes (for instance, Kiecolt-Glaser, & Newton, 2001; Steptoe et al., 2013).

When the findings from Experiments 1 and 2 are considered together, they suggest that the loss of a socially-bonded partner has significant negative consequences for behavior, stress reactivity, neuroendocrine function, and autonomic regulation of the heart. These consequences might represent an evolutionary adaption favoring monogamy through adverse responses to separation, encouraging an organism to remain with a social partner or reconnect with its missing partner. Two parents remaining together - and thus providing for the offspring - would be adaptive, and increase the survival of the progeny (Carter, 1998).

The findings from the present experiments inform our understanding of mechanisms by which social stress deleteriously influences behavioral and physiological processes. Indeed, an inability to adequately cope with stressors has been shown to adversely affect several behavioral and physiological functions, and is observed in humans suffering from depression and CVD (Cryan et al., 2005; Sapolsky, 1996; Porges, 2009; Thayer, & Brosschot, 2005). Alterations in central nervous system functioning may explain the current findings and additional negative responses to stress, biasing an organism toward more passive behavioral strategies and visceral regulation (Bielajew et al., 2003; Cryan et al., 2005). Behavior and biological function are theorized to form a circuit involving cortical direction of brainstem nuclei responsible for control of autonomic efferent projections (Thayer, & Brosschot, 2005). When this cortical-subcortical circuit is disrupted - for instance via social stress - it can produce a corresponding withdrawal of parasympathetic regulation of the heart (Porges, 2009). The resulting change in autonomic regulation is associated with increased basal HR, heart rhythm dysfunction, an excess of sympathetic drive, and behavioral despair (Grippo et al., 2010; Grippo, & Johnson, 2009; Pieper, & Brosschot, 2005).

In addition to altering autonomic nuclei, chronic stress has been shown to differentially affect key neuronal structures involved in the stress response (e.g., decreased dendritic arborization in the hippocampus and increased dendritic arborization in the amygdala; Vyas et al., 2002). Likewise, high levels of circulating stress hormones such as corticosterone are linked with adverse behavioral and health consequences (McEwen, 2003; Sapolsky, Romero, et al., 2000). HPA axis hyperactivity to stressors and latency in returning to basal levels of HPA functioning may be facilitated by morphological or functional changes in neuronal structures that regulate the stress response. The social stress-induced changes observed in the present experiments highlight the interrelatedness of physiological and psychological states with an organism's social environment. The disruption of established social bonds may therefore adversely affect processes responsible for maintaining appropriate regulatory activity in the brain.

The social disruption of losing a partner negatively influences physiological and behavioral functioning in socially monogamous prairie voles. This research is important in the context of humans suffering from social isolation and other forms of social stress which often result in negative consequences such as depression, poor stress coping, HR and rhythm dysfunction, and autonomic imbalance (Sapolsky, 1996; Sapolsky et al., 2000; Ramsay et al., 2008; Uchino et al., 1999). Future studies will benefit from focusing on long-term structural and functional changes in brain regions that regulate social behavior, stress reactivity, and autonomic and endocrine function. Additional studies with socially monogamous rodents will inform new treatment strategies to improve the quality of life for individuals who experience negative health consequences from partner loss or loneliness.

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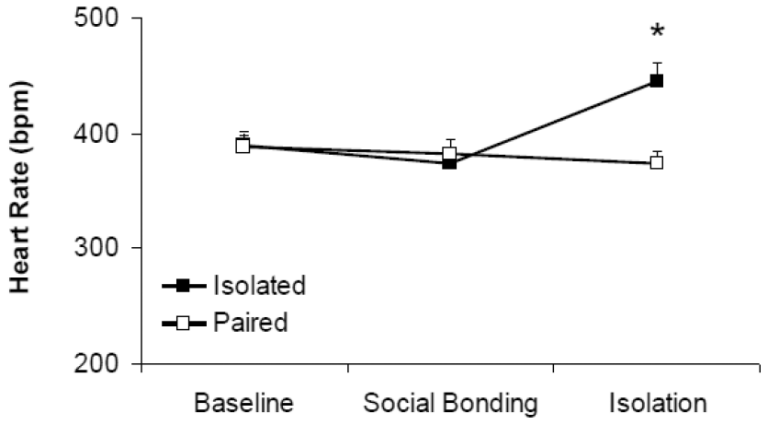

Figure 1.

Mean (+ SEM) HR in prairie voles at baseline, following 5 days of social bonding, and following 5 days of social isolation or continued pairing. *$P < 0.05$ versus respective paired value; $^{\#}p < 0.05$ versus respective baseline value.

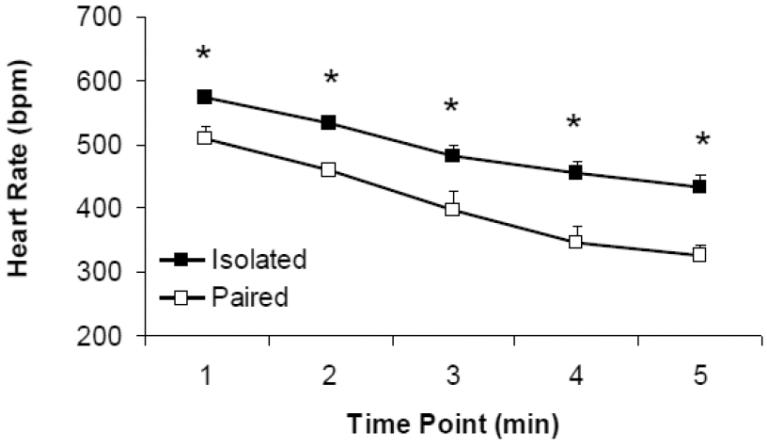

Figure 2.

Mean (+ SEM) HR in paired and isolated prairie voles during the 5-minute FST. *$P < 0.05$ versus respective paired value. Note: the HR decrease in both groups corresponds to a decrease in body temperature across the 5-minute swim period.

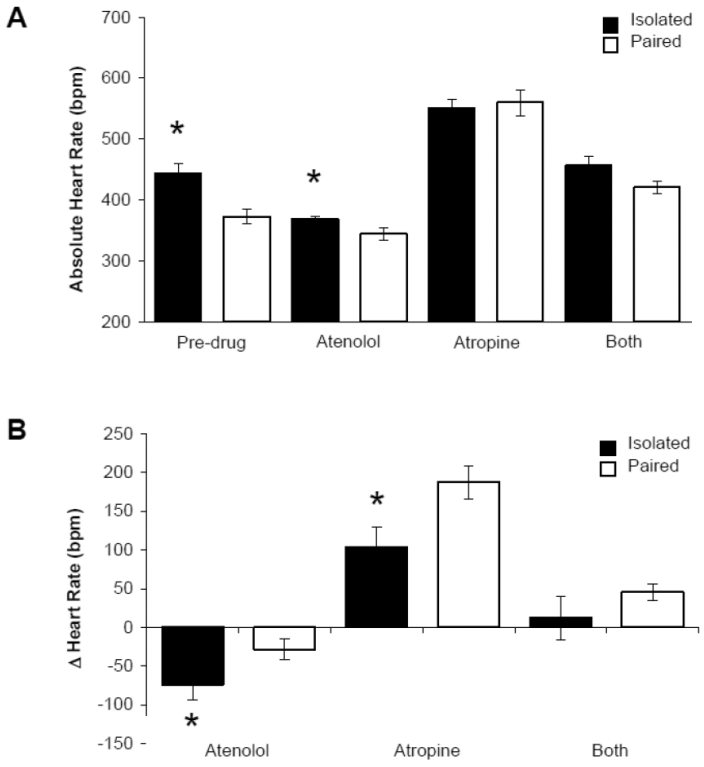

Figure 3.
Mean (+ SEM) absolute HR (Panel A) and change in HR from pre-drug values (Panel B) in paired and isolated prairie voles prior to and during autonomic receptor antagonism with atenolol, atropine methyl nitrate, and a combination of both drugs. *$P < 0.05$ versus respective paired value.

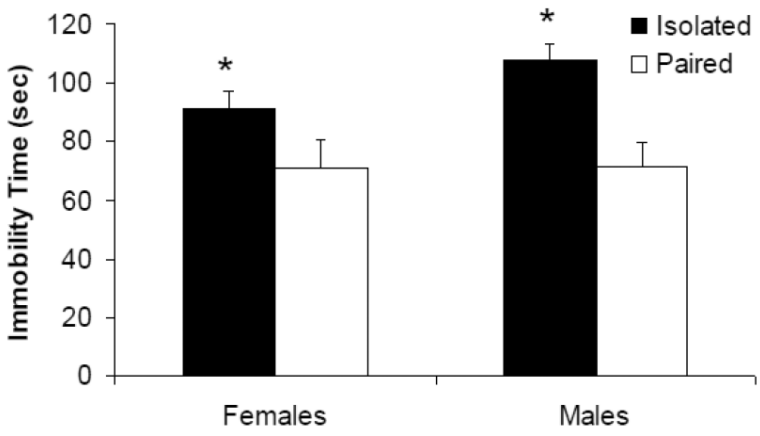

Figure 4.
Mean (+ SEM) immobility time in male and female paired and isolated prairie voles during the 5-minute FST. *$P < 0.05$ versus respective paired value.

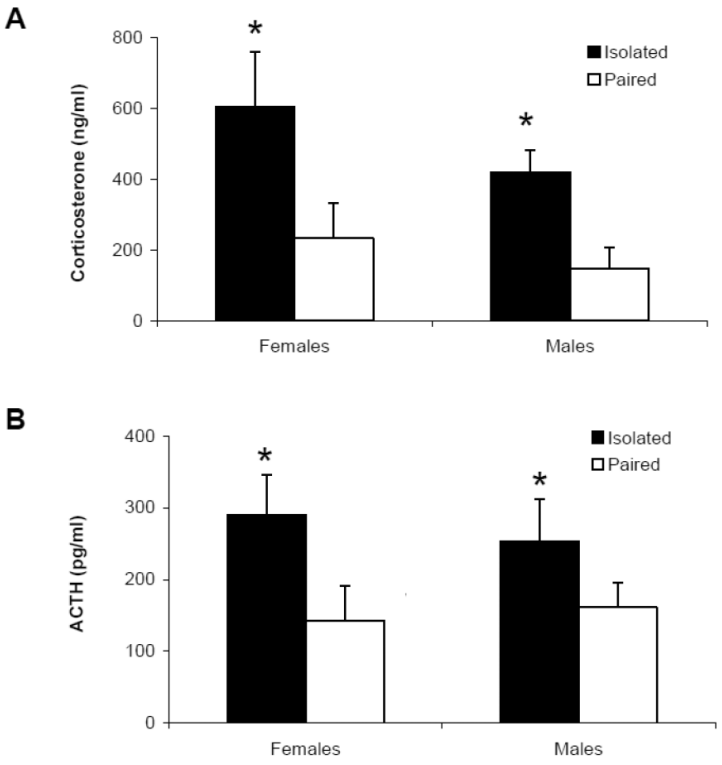

Figure 5.
Mean (+ SEM) ACTH (Panel A) and corticosterone (Panel B) levels in male and female paired and isolated prairie voles 10 minutes following the 5-minute FST. **P* < 0.05 versus respective paired value.

Table 1

Experimental Timeline for Experiment 1

Procedure	Schedule
Telemetric Transmitter Implantation	Days 1–2
Recovery in Divided Cages	Days 2–6 (depending on date of transmitter implantation)
- ECG and activity measurements	
Recovery in Standard Cages	Days 6–12 (depending on date of transmitter implantation)
- ECG and activity measurements	
Baseline Period	Days 12–15
- ECG and activity measurements	
5 Day Social Bonding Period	Days 15–20
- ECG and activity measurements	
Social Bond Assessment	Day 17
- Digital video recording of behavior	
5 Day Isolation Period	Days 20–25
- ECG and activity measurements	
Forced Swim Test	Days 25–26
- With continued isolation	
- Digital video recording of behavior	
- ECG and activity measurements	
Assessment of Autonomic Nervous System Function	Days 28–34
- With continued isolation	
- ECG and activity measurements	

Table 2

Experimental Timeline for Experiment 2

Procedure	Schedule
5-Day Pairing Period	Days 1–5
5-Day Isolation Period	Days 6–10
Forced Swim Test	Days 10–11
- With continued isolation	
- Digital video recording of behavior	
Assessment of Endocrine Function	Day 11
- With continued isolation	
- Plasma collected 10 minutes after Forced Swim Test	