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Cardiac and behavioral effects of social isolation and experimental manipulation of autonomic balance

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Abstract

Improved understanding of how depression and social isolation interact to increase cardiac morbidity and mortality will improve public health. This experiment evaluated the effect of pharmacological autonomic blockade on cardiac and behavioral reactivity following social isolation in prairie voles. Experiment 1 validated the dose and time course of pharmacological autonomic antagonism of peripheral β -adrenergic (atenolol) and muscarinic cholinergic receptors (atropine methyl nitrate), and Experiment 2 used a novel protocol to investigate behavioral responses in the tail suspension test during pharmacological autonomic blockade as a function of social isolation (vs. paired control). Prairie voles isolated for 4 weeks (vs. paired) displayed significantly elevated heart rate and reduced heart rate variability. Autonomic receptor antagonism by atenolol led to exaggerated reductions in heart rate and standard deviation of normal-to-normal intervals, and lower amplitude of respiratory sinus arrhythmia in the isolated group (vs. paired). Administration of atropine led to an attenuated increase in heart rate in the isolated group (vs. paired), and similar near-zero levels of respiratory sinus arrhythmia amplitude in both groups. During the tail suspension test, isolated animals (vs. paired) displayed significantly greater immobility. In paired animals, atenolol administration did not influence immobility; atropine administration increased the duration of immobility (vs. vehicle). In isolated animals, atenolol administration increased the duration of immobility; atropine did not influence immobility duration (vs. vehicle). The current study contributes to our understanding of differential effects of social isolation and autonomic imbalance on cardiac and behavioral reactivity.

Keywords

autonomic imbalance; depression; negative affect; prairie vole; social isolation; tail suspension test

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⁸Declaration of Interest
None.

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1. Introduction

Depression is one of the most important health concerns currently facing society (Lippi et al., 2009). Not only is it a debilitating disorder in its own right, but depression is also bi-directionally associated with deleterious physiological conditions including cardiovascular disease (CVD) (Glassman, 2007; Lippi et al., 2009; Musselman et al., 1998; Pedersen et al., 2017; Piña et al., 2018). Due to the suffering caused by depression and associated medical morbidity and mortality concerns, the World Health Organization has ranked depression as one of the leading causes of disability worldwide (Üstün et al., 2004). The American Heart Association also recommends routine screening for depression in patients with CVD, in an effort to promote treatment, education, and other necessary support (Lichtman et al., 2008; Lichtman et al., 2014).

Given the public health relevance of depression and related medical conditions, it is prudent to focus on physiological and behavioral mechanisms that underlie this condition. Disrupted autonomic balance may play a significant role in the development of depression and its association with CVD. Specifically, both increased sympathetic drive and reduced contribution from the parasympathetic nervous system have been implicated in the link between depression and CVD (Carney et al., 2001; Hu et al., in press; Nahshoni et al., 2004; Rechlin et al., 1994; Sgoifo et al., 2015; Veith et al., 1994). For example, depressed individuals display higher resting heart rate (HR), altered basal and stressor-associated blood pressure, higher basal levels of circulating norepinephrine, and greater sympathetic reactivity to stressors, compared to non-depressed controls (Kayano et al., 2015; Nahshoni et al., 2004; Sheffield et al., 1998; Siever & Davis, 1985; Veith et al., 1994). Furthermore, a specific reduction in parasympathetic regulation of the heart has been reported, with depressed individuals demonstrating reduced heart rate variability (HRV) and atypical parasympathetic reactivity to stressors (Hu et al., in press; Hughes & Stoney, 2000; Lovallo, 2005; Musselman et al., 1998; Sgoifo et al., 2015). Studies with animal models support the hypothesis that the autonomic nervous system is disrupted in depressive disorders (Carnevali et al., 2017; Gripipo, 2009).

Environmental stress, and more specifically stress from the social environment, also mediates the association of depression with autonomic and cardiovascular disruptions. Individuals who report negative social interactions or smaller social networks are more likely to be depressed, compared to those who are more socially integrated (Cacioppo et al., 2006). Social stressors also contribute to an increased risk of morbidity and mortality from CVD (Eng et al., 2002; Ramsay et al., 2008; Rutledge et al., 2004; Steptoe et al., 2013). Studies using a variety of animal models, including rodents and non-human primates, provide further evidence for an association of social stress, depression, and autonomic dysfunction (Sgoifo et al., 2015; Shively & Day, 2015). Although limited evidence from human and animal models suggests that disruptions of autonomic balance may influence cardiovascular morbidity associated with social stress (Gripipo, 2011; Hawkley & Cacioppo, 2003; Kiecolt-Glaser & Wilson, 2017; Sgoifo et al., 2015) the specific mechanisms and causal pathways underlying these relationships are not fully elucidated.

The prairie vole is an ideal animal model in which to explore the hypothesis that social environmental disruptions are associated with autonomic imbalance, thereby precipitating depression. The prairie vole is a rodent species that exhibits autonomic regulation of the heart similar to both human and non-human primates, including a high level of resting parasympathetic tone and a strong vagal brake on HR. Prairie voles also display social behaviors that are similar to humans, such as forming opposite-sex bonds, exhibiting bi-parental care of offspring, and living in family groups (Carter & Keverne, 2002; Young et al., 2011). Social stressors, including social isolation and the disruption of established social bonds, produce a variety of behavioral, neuroendocrine, and physiological disturbances in this species. For example, long-term social isolation induces behavioral and neuroendocrine responses relevant to mood disorders, including learned helplessness, anhedonia, altered exploration, and dysregulation of the hypothalamic-pituitary-adrenal axis (Bosch et al., 2009; Carter et al., 2009; Grippe et al., 2007b; Osako et al., 2018; Sun et al., 2014). Isolated prairie voles also exhibit increased resting HR, reduced HRV, and increased arrhythmias during a stressor compared to paired control animals, mediated in part by increased sympathetic cardiac tone and reduced vagal control of the heart (Grippe et al., 2007b; Grippe et al., 2012).

Given the value of the prairie vole model for understanding interactions among the social environment, behavior, and autonomic function, the aim of the current study was to specifically investigate the influence of autonomic imbalance on depressive behaviors as a function of social stress. These questions were addressed through experimental manipulation of autonomic balance following social isolation. Experiment 1 first validated the dose and time course of pharmacological agents to effectively block peripheral autonomic nervous system receptors on the heart to ensure that the cardiac responses are consistent with previous reports (Grippe et al., 2007a; Ishii et al., 1996); and also tested the novel hypothesis that alterations in autonomic balance mediate both cardiac rate and rhythm responses to social isolation. Experiment 2 tested the novel hypothesis that alterations in autonomic balance mediate depression-relevant behavioral responses to social isolation.

2. Materials and Methods

2.1 Animals

136 adult (60–90 days of age), reproductively naïve, male prairie voles (30–50 g) that had been housed with a same sex sibling since weaning, were used for the experimental procedures ($n = 26$ prairie voles in Experiment 1 and $n = 110$ prairie voles in Experiment 2). For the experiments described here, only one animal from each sibling pair was studied. Animals were descendants of a wild stock originally captured near Champaign, IL. Animals were maintained on a 14/10h light/dark cycle (lights on at 6:30 am), with a temperature of $25 \pm 1^\circ \text{C}$ and a relative humidity of $24 \pm 1 \text{ g/m}^3$. All animals were allowed *ad libitum* access to food (Purina rabbit chow) and water. All procedures were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Northern Illinois University Institutional Care and Use Committee.

2.2 General Experimental Design and Sample Sizes

The general design of Experiments 1 and 2 is described here, with specific methodological details in the following sections. Experiment 1 was designed to validate the dose and time course of selective pharmacological autonomic blockade to ensure peripheral antagonism of β -adrenergic and muscarinic cholinergic receptors with atenolol and atropine methyl nitrate, respectively; and to investigate the resultant changes in HR and HRV following selective pharmacological autonomic blockade as a function of social isolation (vs. paired control conditions). Prairie voles were implanted with a radiotelemetry transmitter, followed by a recovery period of 10–14 days. Animals were then assigned to either the paired control condition (remained paired with a male sibling) or social isolation condition (isolated from the sibling) for 4 weeks. Following this period, animals in both groups were subjected to selective pharmacological autonomic blockade, involving administration of (a) distilled water vehicle, (b) atenolol, and (c) atropine methyl nitrate, in counterbalanced fashion over 6 days, with 48 hours between each injection. Electrocardiogram (ECG) and activity variables were recorded continuously following each injection for analysis of HR and HRV reactivity to autonomic blockade.

Experiment 2 was designed to investigate the resultant changes in behavior during an operation test of depression, the tail suspension test (TST), following selective pharmacological autonomic blockade. Prairie voles were assigned to either the paired control condition (remained paired with a male sibling) or social isolation condition (isolated from the sibling) for 4 weeks. Following this period, animals in each group were assigned to receive one selective pharmacological autonomic blockade injection, involving administration of: (a) distilled water vehicle, (b) atenolol, or (c) atropine methyl nitrate. One hour after the injection, each animal was exposed to the TST for analysis of behavioral responses to autonomic blockade.

Power analyses were conducted utilizing preliminary data to determine sample sizes for the current study. For the analyses, Cohen's *d* was calculated, and a desired statistical power of 0.8 was set to minimize chances of a type II error, with a probability level of $p < 0.05$. Sample sizes of 8–14 per group were deemed appropriate. This information – coupled with results from previous studies within our lab examining multiple dependent measurements in the same animal, accounting for minimal sample size attrition due to drug injection or behavioral test issues, and attempting to ensure enough statistical power especially in paired control groups – led to the following sample sizes: (a) $n = 18$ paired and 8 isolated animals in Experiment 1; (b) $n = 23$ paired and 14 isolated animals in the β -adrenergic blockade group in Experiment 2; (c) $n = 23$ paired and 12 isolated animals in the muscarinic cholinergic receptor blockade group in Experiment 2; and (d) $n = 25$ paired and 13 isolated in the vehicle group in Experiment 2.

2.3 Experiment 1, Specific Methods

2.3.1 Implantation of Radiotelemetry Transmitters—Wireless radio transmitters (model TA10ETA-F20; Data Sciences International, St. Paul, Minnesota) were implanted intraperitoneally (ip) under aseptic conditions, during the light period, for continuous recording of ECG and activity variables. Animals were anesthetized with ketamine and

xylazine (67 mg/kg and 13.33 mg/kg, respectively, sc; NLS Animal Health, Owings Mills, Maryland). Transmitter implantation was a modification of procedures described in detail elsewhere (Sgoifo et al., 1996). Briefly, the body of the transmitter was implanted into the abdominal cavity, and the positive and negative leads were tunneled subcutaneously and sutured to the muscle on either side of the heart using DII positioning. Following immediate recovery from anesthesia, animals were housed for 5 days in custom-designed divided cages (Grippe et al., 2007a) with ad libitum access to 2% sucrose (in addition to ad libitum food and water). This housing situation allowed the siblings visual, olfactory, and limited tactile interactions through small holes in a clear cage divider, while permitting adequate healing of suture wounds in the instrumented animal. A heat lamp was positioned to cover approximately 1/3 of the cage housing the instrumented animal for the first 2 nights following surgery, allowing the animal to selfregulate body temperature. Following 5 days of recovery in the divided cages, all animals were returned to the standard home cages (with their respective siblings) to recover for an additional 5–9 days.

2.3.2 Social Isolation—Following surgical recovery, prairie voles were randomly assigned to paired (control; $n = 18$) or isolated ($n = 8$) conditions for 4 weeks. Paired animals were continually housed with their respective siblings during this period; isolated animals were separated from the respective sibling and housed individually (in a separate room, without visual, olfactory, or auditory cues). Handling and cage changing were matched between the two groups.

2.3.3. Selective Pharmacological Autonomic Blockade—Following the 4-week period of social isolation or pairing, all animals were assessed for HR and HRV responses: (a) during β -adrenergic blockade with atenolol (8mg/kg ip; Sigma Aldrich, St. Louis, MO), (b) during muscarinic cholinergic receptor blockade with atropine methyl nitrate, (4mg/kg ip; Sigma Aldrich, St. Louis, MO), or (c) following an injection of distilled water (vehicle). All injections took place during the light period, between 10:00 am and 2:00 pm, followed by several hours of continuous ECG recordings. Substances were administered in a repeated, counterbalanced fashion over a 6 day period, with 48 hours between each administration. Therefore, on the first day of injections, 1/3 of the animals in each group received atenolol, 1/3 received atropine methyl nitrate, and 1/3 received distilled water; followed by one of the other two substances on the second day, and the final substance on the third day. These drugs, doses, and time course were chosen for their ability to effectively and completely block the respective inputs to the heart without significantly altering general activity level and without causing residual effects at the time of the next drug injection, based on preliminary experiments and previously published procedures (Grippe et al., 2007a; Ishii et al., 1996).

2.3.4. Recording and Quantification of Telemetric Variables—ECG signals were recorded with a radiotelemetry receiver (sampling rate 5 kHz, 12-bit precision digitizing; Data Sciences International). Activity level was monitored via the receiver (sampling rate 256 Hz). Quantification of telemetric variables was conducted according to procedures described previously (Porges, 1985; Porges & Bohrer, 1990). Stable, continuous ECG data from the vendor software (Data Sciences International) were used to evaluate cardiac rate

and rhythms, and general activity level. R-wave detections were manually verified with custom-designed software [Brain Body Center, University of Illinois at Chicago, Chicago, IL (Porges, 1985; Porges & Bohrer, 1990). Activity (counts per minutes, cpm) was calculated using the vendor software (Data Sciences International).

HR was quantified using the number of beats per minute (bpm). The R-R intervals were analyzed for variations using custom-designed software and included standard deviation of all R-R [normal-to-normal (N-N)] intervals (SDNN index) and respiratory sinus arrhythmia (RSA) amplitude. RSA amplitude was assessed with a modification of procedures described elsewhere, which have been validated in prairie voles and other species, including validation during periods of both low and high activity (Byrne et al., 1996; Houtveen et al., 2002; Porges, 2007; Yongue et al., 1982). The amplitude of RSA is hypothesized to represent the impact of myelinated vagal efferent pathways originating in the nucleus ambiguus (Porges, 2007). The ECG signal was exported into a data file and examined to ensure all R waves were detected. Preliminary spectral analyses identified spectral peaks within the approximate frequency band in which breathing is observed in mammals of similar size, 1.0–4.0 Hz (Grippo et al., 2007a). The R-R intervals were resampled at 20 Hz and, to comply with the assumption of stationarity, detrended with a 21-point cubic moving polynomial to remove low-frequency (trend) components below 0.5 Hz. The residuals of this procedure were free of aperiodic and slow periodic processes that may have violated the assumption of stationarity. A bandpass filter was applied to define RSA by extracting only the variance in the HR spectrum between the frequencies of 1.0–4.0 Hz.

The peak response in each cardiac variable, beginning 30 minutes following each drug injection, was evaluated using continuous ECG data. The data were manually inspected to determine the peak HR, SDNN, and RSA amplitude response during 1 hour window that included a stable ECG recording not confounded by movement artifact (which included 5–10 minutes of ECG data from each animal).

2.4 Experiment 2, Specific Methods

2.4.1 Social Isolation—Prairie voles were randomly assigned to paired (control; $n = 71$) or isolated ($n = 39$) conditions for 4 weeks, using method identical to those described in Experiment 1.

2.4.2 Selective Pharmacological Autonomic Blockade—Following the 4-week period of social isolation or paired housing, animals in each group were assessed for behavioral responses to one of the 3 drug doses described in Experiment 1, using the same dose and time course: (a) β -adrenergic blockade with atenolol, 8mg/kg ip ($n = 23$ paired and 14 isolated); (b) muscarinic cholinergic receptor blockade with atropine methyl nitrate, 4mg/kg ip ($n = 23$ paired and 12 isolated); or (c) distilled water vehicle ($n = 25$ paired and 13 isolated). All injections took place during the light period, between 10:00 am and 2:00 pm.

2.4.3 Tail Suspension Test—The TST was used to evaluate behavior in each animal. The procedure was adapted from previously published methods (Steru et al., 1985) and has been used to measure depression-relevant and stress-coping behaviors in prairie voles

(Bosch et al., 2009; McNeal et al., 2017). One hour following the drug injection, each prairie vole (paired or isolated) was suspended by its tail using adhesive tape to a metal bar (5 mm) and hung in the middle of an opaque plastic box ($32 \times 28 \times 29 \text{ cm}^3$) approximately 25 cm above the apparatus floor, for 5 minutes. Each 5-minute trial was digitally video recorded and later scored via the Observer XT 8.0 program (Noldus; Wageningen, Netherlands), by trained observers who were naïve to the drug treatment. Trials were scored for the duration of immobility exhibited by each animal (i.e., no movement besides those required for respiration; operational index of depressive behavior), versus the duration of active coping behaviors (i.e., active movements characterized by either contortions of the body and/or flailing of the limbs) (Steru et al., 1985).

2.5 Statistical Analysis

For Experiment 1, mixed-design analyses of variance (ANOVA) were conducted to investigate cardiac and activity variables as a function of social isolation and drug administration, with housing (paired vs. isolated housing) as the independent factor and drug administration (vehicle, atenolol, and atropine) as the repeated factor. Student's t-tests were conducted for pairwise comparisons of absolute cardiac variables, change in variables relative to vehicle administration, and activity levels.

For Experiment 2, single-factor ANOVAs were conducted to determine differences in behavior during the TST among drug administration (vehicle, atenolol, or atropine) in paired and isolated groups separately. Student's t-tests were conducted for a priori, hypothesis-driven comparisons, using a Bonferroni correction for multiple comparisons.

For all analyses and figures, data are shown as means $\pm$ (or +) standard error of the mean (SEM). A probability value of $p < 0.05$ was considered to be statistically significant for all ANOVAs and Student's t-tests. For multiple t-tests, the probability value was adjusted with a Bonferroni correction; results were considered to be statistically significant at $P < 0.05$ when the adjusted probability value was exceeded.

3. Results

3.1 Experiment 1: Cardiac and Activity Responses to Selective Pharmacological Autonomic Blockade

Socially isolated prairie voles administered vehicle displayed a higher HR vs. paired prairie voles. Administration of atenolol led to an exaggerated reduction in HR in the isolated group (vs. the paired group); whereas administration of atropine led to an attenuated increase in HR in the isolated group (vs. the paired group; Figure 1). The ANOVA for absolute HR values yielded a main effect of housing [$F(1,23) = 4.38$, $P < 0.04$], a main effect of drug administration [$F(2,46) = 282.84$, $P < 0.0001$], and a housing by drug interaction [$F(2,46) = 57.76$, $P < 0.0001$]. The isolated group displayed a higher absolute HR following vehicle administration vs. the paired group [$t(24) = 8.49$, $P < 0.0001$]. Absolute HR did not differ between isolated and paired groups following atenolol administration ($P > 0.05$); however the reduction in HR (relative to vehicle administration) was significantly exaggerated in the isolated group vs. the paired group [$t(24) = 7.66$, $P < 0.0001$]. The isolated group displayed

a lower absolute HR than the paired group [$t(24) = 4.42$, $P < 0.0002$], and an attenuated increase in HR (relative to vehicle administration), following atropine administration [$t(24) = 10.59$, $P < 0.0001$].

Socially isolated prairie voles administered vehicle displayed a lower SDNN index vs. paired prairie voles. Administration of atenolol led to an exaggerated reduction in SDNN index in the isolated group (vs. the paired group); but administration of atropine did not significantly influence SDNN index in either group (Figure 2). The ANOVA for absolute SDNN index values yielded a main effect of housing [$F(1,23) = 13.90$, $P < 0.001$], a main effect of drug administration [$F(2,46) = 4.27$, $P < 0.02$], and a housing by drug interaction [$F(2,46) = 8.65$, $P < 0.0006$]. The isolated group displayed a lower SDNN index vs. the paired group following vehicle administration [$t(24) = 5.81$, $P < 0.0001$]. Administration of atenolol led to a significantly lower absolute SDNN index in the isolated group vs. the paired group [$t(24) = 2.96$, $P < 0.007$], but no difference in change in SDNN index (relative to vehicle administration; $P > 0.05$). Absolute SDNN index did not differ between isolated and paired groups following atropine administration ($P > 0.05$), however the change in SDNN index (relative to vehicle administration) was significantly different [$t(24) = 4.00$, $P < 0.0005$], characterized by an increase in SDNN index in the isolated group, but a decrease in SDNN index in the paired group.

Socially isolated prairie voles displayed lower RSA amplitude values vs. paired prairie voles following vehicle administration. Administration of atenolol was associated with lower RSA amplitude in the isolated group vs. the paired group; whereas administration of atropine was associated with similar – near zero – levels of RSA amplitude in both groups (Figure 3). The ANOVA for absolute RSA amplitude yielded a main effect of housing [$F(1,23) = 7.70$, $P < 0.01$], a main effect of drug administration [$F(2,46) = 32.59$, $P < 0.0001$], and a marginal housing by drug interaction [$F(2,46) = 5.39$, $P = 0.05$]. Administration of vehicle was associated with a significantly lower absolute RSA amplitude in the isolated group vs. the paired group [$t(24) = 5.36$, $P < 0.0001$]. Administration of atenolol was associated with a lower absolute RSA amplitude in the isolated group vs. the paired group [$t(24) = 2.04$, $P < 0.05$], and an attenuated reduction in RSA amplitude (relative to vehicle administration) in the isolated group vs. the paired group [$t(24) = 2.33$, $P < 0.03$]. Administration of atropine led to similar absolute RSA amplitude in both isolated and paired groups ($P > 0.05$); and the reduction in RSA amplitude following atropine administration (relative to vehicle administration) was significantly attenuated in the isolated group vs. the paired group [$t(24) = 2.17$, $P < 0.04$].

Neither social isolation nor administration of autonomic blockade drugs influenced activity level during the peak cardiac responses (paired, vehicle = 0.64 ± 0.24 cpm; paired, atenolol = 0.66 ± 0.32 cpm; paired, atropine = 0.53 ± 0.26 cpm; isolated, vehicle = 0.38 ± 0.15 cpm; isolated, atenolol = 0.58 ± 0.31 cpm; isolated, atropine = 0.3 ± 0.19 cpm; $P > 0.05$ for both main effects and interaction effect; no followup tests were conducted).

3.2 Experiment 2: Behavioral Responses to Selective Pharmacological Autonomic Blockade

Immobility duration in the TST was significantly higher in the socially isolated group vs. the paired group. In the paired group, administration of atenolol did not influence immobility duration relative to vehicle; whereas administration of atropine was associated with greater duration of immobility relative to administration of vehicle. In contrast, in the isolated group, administration of atenolol was associated with greater duration of immobility relative to administration of vehicle; whereas administration of atropine did not influence immobility duration relative to administration of vehicle (Figure 4).

A single-factor ANOVA for immobility duration in the paired group yielded a main effect of drug administration [$F(2,68) = 3.52$, $P < 0.04$]. T-tests with a Bonferroni correction indicated that the duration of immobility was significantly higher following atropine vs. both vehicle [$t(46) = 1.99$, $P < 0.03$] and atenolol administration [$t(44) = 2.49$, $P < 0.008$]. Administration of vehicle did not significantly influence immobility duration vs. administration of atenolol ($P > 0.05$).

A single-factor ANOVA for immobility duration in the isolated group yielded a main effect of drug administration [$F(2,36) = 8.82$, $P < 0.0008$]. T-tests with a Bonferroni correction indicated that the duration of immobility was significantly higher following atenolol vs. both vehicle [$t(25) = 3.51$, $P < 0.0009$] and atropine administration [$t(24) = 2.97$, $P < 0.003$]. Administration of vehicle did not significantly influence immobility duration vs. administration of atropine ($P > 0.05$).

4. Conclusions

Given interactions of the autonomic nervous system, behavior, and emotion (Carnevali et al., 2017; Carney et al., 2007; Sgoifo et al., 2015), the present study was designed to achieve several goals. Experiment 1 validated the dose and time course of selective autonomic blockade agents on cardiac function to ensure consistency of cardiac responsiveness to pharmacological autonomic blockade with previously published data (Grippio et al., 2007a; Ishii et al., 1996); and characterized for the first time both cardiac rate and rhythm responses to selective autonomic blockade in prairie voles following social isolation (vs. paired control conditions). Experiment 2 investigated the effects of experimental manipulation of autonomic inputs to the heart on behavioral responses in an operational measure of depression (TST) for the first time following social isolation (vs. paired control conditions) in the prairie vole model. These data indicate that autonomic regulation of both cardiac function and depressive behaviors are differentially altered in prairie voles as a function of social isolation.

As demonstrated in Experiment 1, selective autonomic blockade with pharmacological agents alters HR and HRV in prairie voles. Atenolol and atropine have been demonstrated in previous studies of rodents – including prairie voles – to specifically antagonize β -adrenergic and cholinergic receptors on the heart, respectively, consequently altering cardiac function (Grippio et al., 2007a; Ishii et al., 1996). Consistent with these previous studies, the present findings confirm that the dose of each drug is sufficient to antagonize receptors on

the heart, with a peak response occurring approximately 1 hour following drug administration, in turn influencing both HR and HRV in paired and isolated prairie voles. For example, as expected, cholinergic receptor antagonism with atropine was associated with a reduction in RSA amplitude to near zero in both paired and isolated groups, consistent with the hypothesis that RSA amplitude represents a specific and sensitive index of parasympathetic regulation of the heart (Porges, 2007).

In addition to validating the specific drugs, dose, and time course for altering cardiac function following selective pharmacological autonomic blockade, Experiment 1 characterized both cardiac rate and rhythm responses to autonomic blockade as a function of social isolation (vs. paired control conditions). Consistent with previous research describing the cardiac consequences of long-term social isolation in female prairie voles (Grippo et al., 2007b; Grippo et al., 2012), the present findings indicate that 4 weeks of social isolation in male prairie voles produces increased HR, reduced SDNN index, and reduced RSA amplitude following vehicle administration (i.e., under conditions of undisrupted autonomic tone). These data provide further evidence that social isolation disrupts neural control of cardiac function and increases the workload of the heart.

The present study also offers novel insight regarding the role of autonomic imbalance in mediating HR and HRV dysfunction following social isolation. Relative to paired control conditions, β -adrenergic receptor antagonism with atenolol in the isolated group was associated primarily with an exaggerated reduction in HR (vs. vehicle administration), whereas cholinergic receptor antagonism with atropine was associated with an attenuated increase in HR and SDNN, and an attenuated reduction in RSA. This pattern of cardiac reactivity suggests that cardiac sympathetic drive is exaggerated, while cardiac parasympathetic tone is attenuated, following long-term social isolation. These findings are consistent with a pattern of reactivity associated with depression, social stress, and cardiac morbidity and mortality in humans (Barton et al., 2007; Carney & Freedland, 2003; Pitzalis et al., 2001; Rozanski et al., 2005), and may therefore aid in new strategies for the treatment of cardiovascular consequences of social stress.

In addition to characterizing cardiac reactivity to selective pharmacological autonomic blockade following social isolation in prairie voles, the present study used a novel procedure to experimentally manipulate autonomic balance in prairie voles to investigate depressive behaviors in a validated operational measure in rodents (Cryan et al., 2005; Steru et al., 1985). Given that social stress and depression may be associated with exaggerated sympathetic tone and reduced parasympathetic tone, we hypothesized that disrupting autonomic balance would result in an increase in depressive behavior in the TST. The data generally support our hypothesis; however the specific pattern of behavioral disruptions in the TST was mediated by social housing condition. First, to understand the influence of social isolation on behavioral reactivity in the TST, we compared behavioral responses of paired and isolated groups following vehicle administration (i.e., under conditions of undisrupted autonomic tone). Consistent with previous research on the behavioral consequences of social stress in the prairie vole model (Bosch et al., 2009), isolated prairie voles displayed greater levels of immobility in the TST, relative to paired prairie voles. This behavioral pattern is indicative of a maladaptive coping strategy during the stressor, which is

hypothesized to represent an operational index of depression (Cryan et al., 2005). The differential behavioral reactivity following social isolation observed here contributes to a body of literature from both animal and human studies suggesting that long-term social stress negatively influences mood and emotion (Cacioppo et al., 2015; Hawkley & Capitanio, 2015).

In addition to providing further evidence that long-term social stress contributes to mood dysfunction, the present study demonstrated for the first time that social isolation in prairie voles interacts with autonomic regulation of the heart to increase depression-relevant behaviors. In Experiment 2, paired and isolated prairie voles displayed different behavioral patterns following selective pharmacological autonomic blockade. Following paired control housing – which may be considered to mimic a “natural” or “ideal” social environmental condition in prairie voles – cholinergic receptor antagonism with atropine produced an increase in immobility (depression-like behavior) in the TST, whereas β -adrenergic receptor antagonism with atenolol did not significantly alter behavior relative to vehicle administration. This behavioral pattern indicates that autonomic imbalance, characterized specifically by an attenuation of parasympathetic drive, produces a depression-like state in paired prairie voles, mimicking the behavioral pattern observed following social isolation. In other words, both social isolation and experimentally reducing parasympathetic drive in prairie voles produce a depression-like state in the TST.

In contrast with the behavioral reactivity of paired prairie voles, isolated prairie voles displayed the opposite response to selective pharmacological autonomic blockade. Following social isolation, β -adrenergic receptor antagonism with atenolol produced an increase in immobility (depression-like behavior) in the TST; while cholinergic receptor antagonism with atropine did not significantly alter behavior relative to vehicle administration. Coupled with the cardiac responsiveness to autonomic blockade from Experiment 1 and findings from previous research using the prairie vole model (Grippo et al., 2007b), this behavioral pattern may indicate a causal pathway through which a reduction in parasympathetic drive precipitates excess sympathetic drive to induce a depression-like state in isolated animals.

Taken together, the data from the present study lend additional support to the hypothesis that autonomic imbalance – characterized by both exaggerated sympathetic tone and reduced parasympathetic tone – is one of the mechanisms mediating the development of depression. Furthermore, these results indicate that the social environment interacts with autonomic regulation of the heart, which may be important for understanding the development of depression. However, although the current findings are consistent with previous studies focused on social isolation, stress, and CVD in humans and other animal models (Cacioppo et al., 2015; Carnevali et al., 2017; Steptoe et al., 2013), they are not completely in line with some previous studies that investigated the effects of atropine sulfate administration on behaviors in the TST. Previous studies in other rodents, such as mice and gerbils, found that atropine sulfate treatment significantly decreased immobility time in the TST, and also led to hyper-locomotion (Pradhan & Roth, 1968; Steru et al., 1985; Varty et al., 2003). The difference between our findings and these previous studies may be a function of the drugs used. In contrast to atropine methyl nitrate, which is hypothesized to act peripherally

(Raboin et al., 2006), atropine sulfate crosses the blood-brain barrier (Fountain et al., 2013); therefore a central mechanism of action may have influenced behavior and locomotion in previous studies. But although atropine methyl nitrate is purported to act peripherally, it is possible that some proportion of the drug can alter central functions through actions in areas of vulnerable blood-brain barrier permeability (e.g., circumventricular organs) (Ferguson, 2014), and hence the influence of central process on the behavioral changes observed here should not be discounted. However, the lack of systematic activity changes following both atropine methyl nitrate and atenolol in Experiment 1 may provide some evidence that actions at central structures do not influence general activity (at least in prairie voles at the doses used here); by contrast, these drugs may have specific effects on stress-coping behaviors via specific sympathetic and parasympathetic pathways.

The differential outcomes between the current study and previous work also may hinge on the fact that prairie voles – unlike most rodents (and more in line with humans and larger mammals) – have a basal autonomic balance that is characterized by high parasympathetic tone (Grippo et al., 2007a), and both β -adrenergic and cholinergic receptor antagonism influence cardiac function in this species (Grippo et al., 2007b). By contrast, in un-manipulated rats and mice, only treatment with a β -adrenergic antagonist was shown to significantly influence HR and HRV (Grippo et al., 2002; Ishii et al., 1996). Similarly, following a series of chronic stressors, rats have been shown to display depressive-like behaviors and exaggerated sympathetic tone without a corresponding withdrawal of parasympathetic tone (Grippo et al., 2002). Therefore, the antagonism of parasympathetic inputs to the heart, which facilitates greater sympathetic influence, may model negative affective behaviors in the prairie vole system differently than what has been observed previously in other rodents systems.

The increased depression-relevant behaviors following a disruption of autonomic balance can, perhaps, also be explained by the Polyvagal Theory – which suggests that mammals use a three-tiered hierarchical response strategy during states of stress (Porges, 2001). The early response would be to regulate cardiac responsiveness appropriately by decreasing parasympathetic tone to the heart (tier 1). This response is mediated by a reduced tone from the ventral vagal complex, which provides inhibitory input to heart's pacemaker via myelinated fibers originating from the nucleus ambiguus. If this adjustment is not sufficient or not available, as would be the case when an animal is treated with atropine, the sympathetic nervous system may be recruited to deal with the environmental challenge (tier 2). If increased mobilization is not effective, as in the case of an inescapable stressor such as the TST, the animal may move to prolonged immobilization (tier 3). This response would be mediated by an increase in tone from the dorsal vagal complex, which provides inhibitory input to the sinoatrial node of the heart via unmyelinated fibers originating from the dorsal motor nucleus (Porges, 2001). In support of this hypothesis, previous studies have reported that non-laboratory rodents (e.g. wild caught rats, ground squirrels) were more likely to show immobility in response to a laboratory stressor than domesticated rodents (Hofer, 1970). The prairie voles used here and in other studies are recent descendants from wild caught animals, and also may use this three-tiered stress-coping strategy. The strong reliance on the surrounding social environment displayed in prairie voles may therefore have

implications for understanding the mechanisms through which the brain regulates both behavior and the autonomic nervous system in humans.

The present data provide novel evidence that autonomic imbalance is an underlying mechanism in the expression of depressive behaviors. Given the similarities in social structure and autonomic regulation of the heart between prairie voles and humans, these findings can provide insight into the development and/or maintenance of depression in humans. Continued use of animal models to investigate interactions of the social environment, behavior, and autonomic function will inform our understanding of mechanisms underlying depressive disorders and comorbid cardiovascular conditions.

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Highlights

- Depression and social isolation interact to influence cardiac function
- Animal models are a useful tool to study these interactions
- Autonomic function and behavior were studied in a novel paradigm in prairie voles
- Both social isolation and autonomic imbalance contribute to depression

Figure 1:

Mean (+ SEM) absolute HR in paired and isolated prairie voles 1 hour following sympathetic receptor antagonism with atenolol (8 mg/kg, ip), parasympathetic receptor antagonism with atropine methyl nitrate (atropine; 4 mg/kg, ip), and distilled water vehicle (top panel); and change in HR relative to vehicle administration (bottom panel). * $P < 0.05$ vs. paired with the same drug administration. Note the scale difference between the two panels.

Figure 2:

Mean (+ SEM) absolute SDNN index in paired and isolated prairie voles 1 hour following sympathetic receptor antagonism with atenolol (8 mg/kg, ip), parasympathetic receptor antagonism with atropine methyl nitrate (atropine; 4 mg/kg, ip), and distilled water vehicle (top panel); and change in SDNN index relative to vehicle administration (bottom panel). * $P < 0.05$ vs. paired with the same drug administration. Note the scale difference between the two panels.

Figure 3:

Mean (+ SEM) absolute RSA amplitude in paired and isolated prairie voles 1 hour following sympathetic receptor antagonism with atenolol (8 mg/kg, ip), parasympathetic receptor antagonism with atropine methyl nitrate (atropine; 4 mg/kg, ip), and distilled water vehicle (top panel); and change in RSA amplitude relative to vehicle administration (bottom panel). * $P < 0.05$ vs. paired with the same drug administration. Note the scale difference between the two panels.

Figure 4:

Mean (+ SEM) duration of immobility in paired and isolated prairie voles during the TST 1 hour following sympathetic receptor antagonism with atenolol (8 mg/kg, ip), parasympathetic receptor antagonism with atropine methyl nitrate (atropine; 4 mg/kg, ip), and distilled water vehicle. * $P < 0.05$ vs. paired with the same drug administration; ^ $P < 0.05$ vs. atropine administration in the same housing group; # $P < 0.05$ vs. atenolol administration in the same housing group.