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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.

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 and 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

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reactivity to stressors (Hu et al., in press; Hughes and 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; Grippo, 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 and Day, 2015). Although limited evidence from human and animal models suggests that disruptions of autonomic balance may influence cardiovascular morbidity associated with social stress (Grippo, 2011; Hawkley and Cacioppo, 2003; Kiecolt-Glaser and 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 (Grippo et al., 2007a). 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 and 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; Grippo 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 (Grippo et al., 2007b; Grippo 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 (Grippo 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 14/10 h light/dark cycle (lights on at 6:30 am), with a temperature of 25 ± 1 °C and a relative humidity of 24 ± 1 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 h 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