ABSTRACT

SOCIAL BONDS, CARDIOVASCULAR FUNCTION, AND SEROTONIN: INSIGHTS FROM AN ANIMAL MODEL

Neal Daniel McNeal, Ph.D.
Department of Psychology
Northern Illinois University, 2015
Angela J. Grippo, Director

Social relationships positively influence psychological and biological function in humans and other mammals. The disruption of an individual's social environment through separation or death often results in social isolation, which can adversely impact mental and cardiovascular health (Frasure-Smith, Lesperance, & Talajic, 1993; Gore, 1978). The general purpose of this experiment is to further investigate neurobiological mechanisms mediating the adverse effects of social stress and focused on the role of serotonin in that relationship. An animal model was used to simulate social stress in humans. Specifically, the goal of this study is to explore the effects of sertraline hydrochloride (Zoloft) on the behavioral and physiological consequences of disrupted social bonds. We hypothesized that treatment with the antidepressant drug sertraline hydrochloride would buffer the deleterious changes in behavioral and cardiac function following social stress. Specifically, animals administered sertraline hydrochloride were expected to display lower heart rates and higher heart rate variability than the control group (i.e., vehicle only) during basal and behavioral assessment periods. To investigate this, male prairie voles were paired with an unrelated female partner for five days.

Following this pairing period, all male prairie voles were isolated for the remainder of the study. After 5 days of isolation, half of the males received sertraline hydrochloride for the remaining 15 days of the experiment. Finally, behavioral assessments were conducted 24 hours apart on the final 2 days of the experiment. This investigation is the first to evaluate cardiac and behavioral responses to antidepressants in the context of social isolation using an animal model.

In general, sertraline hydrochloride was limited in its ability to buffer the negative cardiac and behavioral changes associated with social stress during this experiment. All male prairie voles (regardless of group assignment) displayed a significant increase in heart rate throughout isolation and a decrease in heart rate variability 10 days after isolation, which then recovered to pre-isolation levels. However, isolated male prairie voles administered sertraline hydrochloride did not display a statistically significant reduction in heart rate or increase in heart rate variability as predicted. Further, the sertraline hydrochloride group displayed increased heart rates on days 5 and 10, but not 14, of the drug administration period. The sertraline hydrochloride group's responses during the behavioral tests were mixed. During the tail-suspension test, the sertraline hydrochloride group displayed slightly, but not significantly, lower heart rates and higher heart rate variability than the vehicle group; however, during the forced swim test those results were reversed. Further, in the recovery period immediately following the tail-suspension test, the sertraline hydrochloride group exhibited a longer latency to return heart rate to basal levels, but subsequently displayed a slightly higher heart rate variability, versus the vehicle group.

In conclusion, sertraline hydrochloride treatment was not entirely effective in ameliorating social isolation induced increases in heart rate and depression-relevant behavior. The results of this experiment can help inform our understanding of the mechanisms through which social stress can negatively influence cardiac and behavioral function. Further, these findings contribute new knowledge regarding the utility of the prairie vole model for the study of potential neurobiological mechanisms that underlie the interactions between emotion and cardiovascular function, and how social bond disruption influences that relationship. Future work to extend the present results will improve our understanding of how negative social experiences mediate adverse behavioral and cardiovascular changes that consequently influence quality of life in humans.

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SOCIAL BONDS, CARDIOVASCULAR FUNCTION, AND SEROTONIN: INSIGHTS FROM AN ANIMAL MODEL

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NEAL DANIEL MCNEAL

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Dissertation Director:
Angela J. Grippo

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HPA	Hypothalamic-pituitary-adrenal
SDNN index	Standard deviation of the beat-to-beat intervals
SEM	Standard error of the mean

CHAPTER 1

INTRODUCTION

The goal of this study is to explore the effects of sertraline hydrochloride (Zoloft) on the behavioral and physiological consequences of disrupted social bonds. This study will help elucidate the underlying neurobiological mechanisms that facilitate the interaction between social environmental stress and health. Supportive social relationships positively influence psychological and biological function in humans and other mammals. The disruption of social relationships can adversely influence both emotional and cardiovascular health (Cacioppo, Hawkley, & Thisted, 2010; Gore, 1978; Shankar, McMunn, Banks, & Steptoe, 2011). For example, individuals with decreased social engagement or who feel lonely experience an increased risk for depressive disorders, as well as general and cardiovascular disease related mortality (Cacioppo et al., 2010; Ramsay et al., 2008). Similarly, animal models of social isolation demonstrate depressive behaviors, deleteriously altered cardiac function, and disrupted autonomic function during social stress (Grippo, Lamb, Carter, & Porges, 2007d; Grippo, Sgoifo, Mastorci, McNeal, & Trahanas, 2010). Understanding how these negative behavioral and cardiac changes occur may provide novel treatment strategies for individuals that are experiencing social stress, isolation, and loneliness. As such, the current experiment used

prairie voles (*Microtus ochrogaster*) as a laboratory model for the investigation of neurobiological mechanisms involved in the adverse behavioral and cardiac function consequences of social isolation. This unique rodent model has been previously used to investigate the neurobiological basis of attachment behavior (Aragona, Liu, Curtis, Stephan, & Wang, 2003; Cushing, Mogekwu, Le, Hoffman, & Carter, 2003; DeVries, DeVries, Taymans, & Carter, 1995), and the dysfunction that results when those bonds are disrupted (Bosch, Nair, Ahern, Neumann, & Young, 2009; Grippo, Cushing, & Carter, 2007a; Grippo et al., 2007c; Grippo Lamb, Carter, & Porges, 2007d; Grippo, Trahanas, Zimmerman, Porges, & Carter, 2009).

Investigations with this animal model indicate that the prairie vole is a useful translational model for the study of the social environment. These rodents engage in several social behaviors that mirror those of humans, including, living in family groups, cooperatively raising offspring, and developing long-term bonds between males and females (Carter & Getz, 1993; Hofman & Getz, 1988). Moreover, when exposed to social stress (such as short- or long-term social isolation), these animals display many biomarker changes that are consistent with depressive disorders and cardiovascular disease, including behavioral changes, heart rate and rhythm abnormalities, and autonomic imbalance (Bales, Kramer, Lewis-Reese, & Carter, 2006; Bosch, Kromer, Brunton, & Neumann, 2004; Grippo et al., 2011; Grippo et al., 2007d; Grippo et al., 2010; Pan, Liu, Young, Zhang, & Wang, 2009; Ruscio, Sweeny, Hazelton, Suppatkul, & Carter, 2007).

Importantly, socially isolated prairie voles display many of the same behavioral and cardiovascular disruptions as depressed humans, thus making them an ideal research model for investigating the link between psychological and cardiovascular dysfunction. Therefore, the present experiment used these animals as a laboratory model for exploring the negative effects of social stress. This experiment investigated whether pharmacological intervention with sertraline hydrochloride can protect against deleterious changes in behavioral and cardiac function following social stress. These findings will improve our understanding of the associations among negative social experiences, behavioral health, and physiological health.

Social bonds influence psychological and cardiovascular health

Social bonds influence health in humans

Social support and physical health are two important factors that influence the overall well-being of an individual. It has been generally accepted that social support can influence the outcome of physical and mental health (Frasure-Smith et al., 1993; Gore, 1978). For example, social support was shown to buffer an individual's perception of stress and economic opportunities in a study of unemployed men, during the time it took to gain new employment (Gore, 1978). Unemployed participants with less social support experienced increases in cholesterol, illness symptoms, and negative affect compared to those who were more socially connected after job termination.

The degree of social engagement also influences the interactions between psychological and physical health. Schwerdtfeger and Friedrich-Mai (2009) investigated these interactions by assessing whether cardiac autonomic control in healthy individuals with depressive symptoms could be altered by social interaction. Participants with a high trait depression score (assessed via State-Trait Depression Scales) (Spielberger, 1995) displayed higher heart rate and elevated negative affect (e.g., questionnaire measures of depressed mood) during the day (Schwerdtfeger & Friedrich-Mai, 2009). Importantly, this relationship was found to be moderated by the participants' social context. When depressive participants were alone, they displayed higher negative affect and lower heart rate variability. However, when participants were engaged in a positive social environment (e.g., with a partner, family members, or friends), they reported lower negative affect scores and exhibited higher heart rate variability.

The measure of heart rate variability used here is important, because it can demonstrate that changes in social situation impact the neurobiological mechanisms regulating physiological (specifically cardiac) function. Briefly, heart rate variability is a representation of the variance of the heart’s beat-to-beat intervals, with greater beat-to-beat variability reflected by increased variance. This metric is often transformed to the standard deviation of the beat-to-beat intervals, also called SDNN index (Allen, Chambers, & Towers, 2007), and has been recommended as a measure for overall variability (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). As such, the SDNN index is used as a heart rate variability measure that is representative of both sympathetic and

parasympathetic input to the heart. This is important, as heart rate variability can fluctuate based on changes in social context and mood (Allen et al., 2007). Decreased heart rate variability reflects a reduced capacity to vary the intervals between successive heartbeats and is indicative of autonomic nervous system dysregulation (Carney et al., 1995). Social stress from negative environmental situations --such as partner loss-- can negatively influence health, including measures of cardiovascular health (such as heart rate variability and related variables).

The disruption of social bonds has been linked with cardiovascular dysfunction (Cacioppo, Hawkley, & Berntson, 2003; Uchino, 2006). These changes can increase the risk of developing emotion-related disorders (such as depression) and cardiovascular disease (Seeman et al., 2004). For example, a large prospective study performed by Kaplan et al. (1988) found a stepwise increase in men's isolation scores was linked with death from all causes (simply more likely to die) and cardiovascular disease related deaths. Their findings are derived from a five-year study assessing (via questionnaire and state death records) mortality as a function of social isolation. The authors found that, the more a subject reported isolation, the more likely they were to die (Kaplan et al., 1988). What made their results noteworthy is that the significant findings in men were independent of traditional risk factors (via multivariate modeling with adjustment for the following factors) of: age, cohort, province, cholesterol, mean weighted blood pressure, body mass index, smoking, education level, urban/rural residence, and a family history of cardiovascular disease (Kaplan et al., 1988).

More recent corroborative evidence has also been observed in the context of physical health. For example, a large cohort study of elderly men performed by Ramsay et al. (2008) examined the effect of social engagement on cardiovascular disease mortality risk. Results showed that participants with an increased social engagement score experienced decreased general mortality risk and cardiovascular disease mortality risk. Similar to the Kaplan et al. (1988) study, their results were also independent of other common comorbidity factors such as: smoking, alcohol use, physical activity, body weight, disability, and socioeconomic status (Ramsay et al., 2008). Negative social environment changes have also been observed to deleteriously alter measures of health in animal models.

Social bonds influence health in animal models

In order to better understand the link between social stress and negative health consequences, investigations using laboratory animals are essential tools for understanding common and causal mechanisms. The disruption of social bonds and social isolation are associated with psychological and cardiovascular dysfunction in animal models (Grippo et al., 2007d; Manuck, Clarkson, Lusso, Taub, & Miller, 1983; Shively et al., 2005). For example, adult female cynomolgus monkeys (*Macaca fascicularis*) living as socially stressed subordinates (e.g., animals at the bottom of the dominance hierarchy) display low levels of activity, high heart rate, hormone disturbances, and increased

mortality (Shively et al., 2005). Similarly, socially stressed adult male cynomolgus monkeys also display more extensive coronary artery atherosclerosis than unstressed controls (Manuck et al., 1983). Importantly, both of the groups were fed a diet low in fat and cholesterol and did not differ in serum lipids, serum glucose, or weight. Together, these results show psychosocial factors that negatively influence health biomarkers in humans also do so in non-human primates (Manuck et al., 1983). Social stress has also been observed to negatively influence behavioral and biological function in other animal models.

Studies utilizing the prairie vole (Microtus ochrogaster) have indicated that this species is also sensitive to negative social environmental changes (Bosch et al., 2009; Grippo et al., 2007d; Ruscio et al., 2007). In their natural environment, prairie voles live in family groups, with the male and female cooperatively raising the young (Roberts, Williams, Wang, & Carter, 1998). Adult prairie voles form robust social bonds, including both family bonds and male-female bonds. Typically prairie voles are socially monogamous and show strong partner preference both in the laboratory and the natural environment (Getz & Hofmann, 1986; Williams, Catania, & Carter, 1992). Furthermore, autonomic regulation of cardiac function in this species includes a high degree of parasympathetic nervous system innervation to the heart, with relatively less influence from the sympathetic nervous system, similar to humans (Grippo et al., 2007b). Taken together, these characteristics indicate that prairie voles are sensitive --both behaviorally and physiologically-- to changes in their social environment.

These features make the prairie vole model useful for investigating the relationships among mood, the social environment, and cardiovascular dysfunction. For instance, Ruscio et al. (2007) found when newly weaned, juvenile prairie voles are exposed to isolation, they exhibit higher plasma stress hormones and increased activity markers in cells producing those hormones. This occurs in the region of the brain responsible for stress hormone control (i.e., paraventricular nucleus of the hypothalamus). These findings are indicative of an increased physiological response to a social stressor (Ruscio et al., 2007).

In addition to studies with juvenile prairie voles, similar physiological responses to social environment disruption have also been observed in adult prairie voles (Grippo et al., 2007a,c,d; Grippo et al., 2010; Grippo et al., 2009). Following four weeks of social isolation from a same-sex sibling, adult female prairie voles displayed multiple analogs to human responses (Grippo, et al., 2007c). Physiologically, negative changes included higher circulating stress hormones and a greater proportion of cells double-labeled for c-fos (a transcription factor associated with neuron activity), when compared to normally housed paired controls. Chronic isolation in these animals also resulted in anhedonia-like behavior as assessed by decreased sucrose consumption (a natural reward in rodents) and depression-relevant behavioral (e.g., decreased behavioral responses) changes, versus paired animals. Further, isolated subjects displayed a significantly higher heart rate during a social stressor (i.e., resident-intruder task) and longer latency to return to basal levels following the test, indicating the possibility of dysfunctional regulatory control of the autonomic nervous system.

The autonomic nervous system mediates neuronal control of many bodily functions, including cardiovascular activity. Briefly, the autonomic nervous system is divided into two limbs: the sympathetic nervous system, responsible for increased cardiac function, and the parasympathetic nervous system, responsible for decreased cardiac function. Interestingly, Grippo et al. (2007) found --similar to a human experiencing a social environmental disruption-- isolated female prairie voles displayed increased sympathetic and decreased parasympathetic input to the heart (Grippo, et al., 2007d). This demonstrates female prairie voles experiencing social isolation displayed a detrimental imbalance in autonomic regulation of cardiac function.

Together, the findings discussed in this section indicated that when social animals experience social environment disturbances they display increased stress response and are unable to regulate their physiological responses with similar efficiency to socially intact controls. These findings demonstrate an association among social stress, negative affective states, deleterious cardiac function, and disrupted regulatory autonomic and hormonal control of physiological function. Importantly, cardiovascular disease and depression share similar pathologies, such as altered behaviors, increased heart rate, and dysfunctional heart rhythms; and both conditions are significantly affected by social stress (Burker, Evon, Loiselle, Finkel, & Mill, 2005; Carney & Freedland, 2003; Kitzlerova & Anders, 2007). Thus, understanding the similar pathologies shared by behavioral and cardiovascular disorders will improve our care and treatment for patients suffering the adverse consequences of social stress.

Social environmental stress is linked with psychological and cardiovascular dysfunction, and those disorders share similar pathologies

There is growing evidence linking social stress to the development of behavioral and physiological dysfunction (Cacioppo et al., 2003; Grippo, et al., 2012a; Ramsay et al., 2008; Seeman et al., 2004; Uchino, 2006). Social isolation and depression are closely linked with negative outcomes such as adverse cardiac function and increased likelihood of death following myocardial infarction (Hance, Carney, Freedland, & Skala, 1996; Penninx et al., 2001). Indeed, social isolation has been associated with depression, and depression is associated with increased social isolation (Gilmartin, Grota, & Sousa, 2013; Pearlin & Johnson, 1977). Similarly, both depression and social isolation independently predict mortality in patients with chronic heart failure (Friedmann et al., 2006). Differentiating between depression and social isolation is a difficult task, and information in this area remains limited (House, 2001).

One contribution to this difficulty may be the relationship depression and isolation share with one another. Depression and social isolation (i.e., loneliness) are positively correlated (Cacioppo et al., 2006; Segrin, 1998). Perhaps because of this, depression categorizations often include items for feelings of isolation and vice versa. However, recent work has demonstrated the two constructs are phenotypically different from one another (Cacioppo et al., 2006). Specifically, in a comparison of measures of isolation (i.e., R-UCLA Loneliness Scale) and depression (i.e., Beck Depression

Inventory), Cacioppo et al. (2006) found scale items that assessed measures for one disorder were associated with other measures of the same condition, but the opposite relationships were not observed. For example, the loneliness measure "I feel left out" was associated with other measures of loneliness, while the depression measure "I hate myself" was associated with other measures of depression. Interestingly, no measures exhibited a significant association with both disorders, nor did another measure of depression (i.e., CES-D) differ from the findings of the Beck Depression Inventory. Together, this indicates that depression and social isolation may be different constructs based on the behavioral responding of human subjects (Cacioppo et al., 2006).

However, what remains unclear is whether one disorder is antecedent to the other. This is still an open question, as many reports link social isolation with worsening psychological and physiological health over time (Barnett & Gotlib, 1988; Joiner, 1997; Shankar et al., 2011). Conversely, depression has been associated with adverse behavioral response styles (e.g., rumination) that result in a decrease of social support (Nolen-Hoeksema & Davis, 1999). In line with this, animal model research from our laboratory (using prairie voles) has demonstrated that social isolation results in increased depression-relevant behavior (e.g., anhedonia and inactive behavioral responding), cardiovascular system impairments (e.g., arrhythmias and impaired vascular relaxation), and adverse neuronal regulation (e.g., hypothalamic-pituitary-adrenal (HPA) axis impairment and autonomic imbalance) (Grippo, et al., 2007a,c,d; Grippo et al., 2011; Grippo, et al., 2012a; Peuler, Scotti, Phelps, McNeal, & Grippo, 2012). This work with

prairie voles demonstrates that social isolation precedes behavioral indices of depression and adverse biomarker changes associated with depression.

While the behavioral differences between depression and social isolation are currently being elucidated, their effect on health is difficult to separate as they share many of the same biomarkers. Importantly, this may contribute to observed increases in mortality when a patient experiences these conditions. Regardless of the causal relationship between social isolation and depression, there is a clear relationship between negative mood states and cardiovascular disease. Specifically, the relationship between depression and cardiovascular disease has generated a lot of interest because of the adverse health outcomes exhibited by patients experiencing those disorders.

The bi-directional association between depression and cardiovascular dysfunction

There is a bidirectional association between depression and cardiovascular dysfunction, such that the presence of one condition increases the probability of developing the other condition. This influence is independent of other traditional cardiovascular risk factors such as: (a) hypertension, (b) high cholesterol, (c) increased body mass index, (d) poor physical activity, and (e) a family history of cardiovascular disease (Anda et al., 1993). Depression and cardiovascular disease exhibit similar

symptomatology. For example, both disorders are characterized by an increase in sympathetic activity, a decrease in parasympathetic activity, and an increase in heart rate, as well as a concurrent decrease in heart rate variability. Epidemiological studies clearly highlight the link between depression and cardiovascular disease (Carney & Freedland, 2003; Hance et al., 1996; Penninx et al., 2001).

Numerous epidemiological studies have found depression to be a significant risk factor for mortality and/or cardiac morbidity (Carney, Freedland, Miller, & Jaffe, 2002; Hellstrom, Rozanski, Blumenthal, & Kaplan, 2000; Rozanski, Blumenthal, & Kaplan, 1999). In addition, depression is a significant risk factor for heart disease, both in medically healthy individuals and in patients with cardiovascular disease (Carney & Freedland, 2003). For example, a longitudinal study performed by Penninx et al. (2001) found that depression increases the risk for cardiac mortality in subjects with or without heart disease at baseline. Furthermore, the authors assessed major and minor depression and found cardiac mortality risk was more than twice as high for major depression than for minor depression (Penninx et al., 2001). Their findings show a stepwise increase in the severity of depression and an increase in cardiovascular disease.

Along these lines, the link between depression and cardiovascular disease mortality is especially significant following myocardial infarction. Depression has an adverse effect on the course and outcome of coronary heart disease (Carney et al., 1988), and notably increases mortality in the years following a diagnosis (Barefoot et al., 1996). Frasure-Smith et al. (1993) assessed mortality in patients six months following hospital admission for a myocardial infarction. They found major depressive disorder predicts a

significant morbidity increase in patients with heart disease, compared to demographically matched non-depressed patients (Frasure-Smith et al., 1993). Conversely, cardiovascular disease is linked with an increased incidence of depression. Demonstrating this, a study by Freedland et al. (2003) assessed a sample of patients experiencing heart failure and determined the prevalence of Diagnostic and Statistical Manual of Mental Disorders (American Psychological Association, 2000) categorized depression. Results indicated that as the severity of heart failure increased, so did the prevalence of depression. These results show a stepwise increase in the severity of cardiovascular disease and an increase in the prevalence of depression (Freedland, 2003).

Another important aspect of major depression is that when left untreated, it will persist in patients with cardiovascular disease (Hance et al., 1996). Further, minor depression is almost as likely to progress to major depression as it is to remit over the course of 12 months (Hance et al., 1996). A prospective cohort study performed by Hance et al. (1996) followed a group of patients undergoing cardiovascular diagnostic assessment for 12 months. These investigators identified patients undergoing coronary heart disease treatment as either: (a) non-depressed, (b) experiencing a minor depressive episode, or (c) diagnosed with a major depressive episode. Of the patients that completed participation in the study, half of those diagnosed with major depression either remained depressed or relapsed within 12 months. Strikingly, 58 percent of the patients diagnosed with minor depression remitted; however, the other 42 percent subsequently developed major depression.

Taken together, the above described studies demonstrate the bi-directional relationship between mood and physiological function. For instance, individuals with cardiovascular disease are likely to become depressed (Freedland, 2003) and individuals with depression have an increased risk of developing cardiovascular disease (Hance et al., 1996). Further, both depression and cardiovascular disease significantly contribute to increased mortality (Freedland, 2003; Hance et al., 1996). Each of these conditions alone has been associated with increased mortality; however, when combined, these two conditions are nearly five times as likely to result in death (Nabi et al., 2010). When considering the significant increase in mortality due to a co-morbid diagnosis of depression and cardiovascular disease, it appears that both disorders may function through a similar neurobiological pathway.

Similar neurobiological and behavioral mechanisms may underlie the association between depression and cardiovascular disease

Social stress can lead to disease states such as depression and cardiovascular disease, which are both linked with negative changes in neurotransmitter regulation, autonomic nervous system function, and adverse heart rate variability alterations (Kitzlerova & Anders, 2007). A specific example of this in rodents shows chronic social stress in rats is associated with reduced serotonin neurotransmitter binding to inhibitory

sertonin$_{1A}$ receptors in the hippocampus of stress-responsive subordinates (i.e., low ranked rats in the dominance hierarchy) (McKittrick, Blanchard, Blanchard, McEwen, & Sakai, 1995). Further, the negative consequences of social stress can be physiologically characterized by biomarkers of cardiac dysfunction (e.g., increased heart rate) and autonomic nervous system impairments that are frequently associated with cardiovascular pathology (Carney et al., 2004; Carney et al., 2005b; Penninx et al., 2001; Porges, 2003). In line with this, social stress negatively influences the regulation of neuronal structures responsible for cardiovascular control (McDougall, Widdop, & Lawrence, 2005).

Social stress is linked with a dysfunction between the interaction of the sympathetic and parasympathetic nervous systems of clinically depressed patients, which is observable in their heart rate and heart rate variability. Exemplifying this, when compared to demographically matched controls, depressed patients display an increased heart rate and decreased heart rate variability, which reflects a reduced capacity to vary the intervals between successive heartbeats and is indicative of autonomic nervous system dysregulation (Carney et al., 2005b; Carney et al., 1995). Decreased heart rate variability is linked to an increased risk of sudden death in patients with cardiovascular impairments such as myocardial infarction, heart failure, and hypertension (Carney et al., 1999; Fox et al., 2007). Indeed, one study has demonstrated roughly 27 percent of the effect of depression on exacerbated mortality rates observed in cardiovascular disease patients is mediated through low heart rate variability (Carney et al., 2005b). This finding supports the concept that depression is a risk factor for cardiovascular disease. Furthermore, it also indicates depression and decreased heart rate variability might have

similar mechanisms of function. The time table (one year) of the study performed by Carney et al. (2005) may suggest decreased heart rate variability plays a causal role in the mechanism responsible for the decline of healthy cardiac function (Carney et al., 2005b). In the context of the current study, a decrease in heart rate variability would support this theory. The decline in heart rate variability is hypothesized to be indicative of dysfunctional autonomic nervous system output and ineffective cardiovascular control, leading to and facilitating disease states. These same negative biomarkers of increased heart rate and reduced heart rate variability have been reported in humans with affective disorders (McDougall et al., 2005). These disturbances are common in cardiovascular disease and can predict mortality following myocardial infarction as well as heart failure (Carney et al., 1999; Fox et al., 2007).

Disruption of cardiac function alone is unlikely to cause both depression and cardiovascular disease. However, cardiac dysfunction combined with disrupted central nervous system function (e.g., deregulated hormone regulation and likely a number of other neurochemical systems) could act as a feed-forward mechanism to perpetuate peripheral nervous system dysfunction (Sapolsky, 2000). In line with this theory, the serotonin neurotransmitter system, which expresses multiple receptor types in all known brain regions, can also display dysregulation after chronic social stress (Beaulieu, Di Paolo, & Barden, 1986; Berger, Gray, & Roth, 2009; Freund, Gulyas, Acsady, Gorcs, & Toth, 1990; Holmes, Di Renzo, Beckford, Gillham, & Jones, 1982; Jacobs & Fornal, 1991; Weidenfeld, Newman, Itzik, Gur, & Feldman, 2002). Taken together, these functional disturbances highlight the association between depression and cardiovascular

disease. The complicated interactions between neurotransmitter and hormonal systems require precise --often targeted to one system-- investigation to glean a clear understanding of how disease states adversely influence health. Importantly, the mechanisms of negative social environment mediated changes in health are not fully understood. However, one possible explanation is that responses to negative social experiences adversely affect the neurobiological mechanisms which facilitate physiological and behavioral function (Cryan, Valentino, & Lucki, 2005b; Siever & Davis, 1985). This hypothesis stems from observed similarities in biomarkers for both disease states.

The serotonergic system

One potential neurobiological system that may influence both mood and cardiovascular function is the serotonergic system. Serotonin is involved in the modulation of virtually all human behavioral processes. The following section will introduce this activity with a general description of serotonin, the transporter, and its associated receptor families. That background information will be useful for introducing the role of serotonin in mood, behavior, and cardiovascular function. In the last several decades, the study of serotonin, its transporter, and receptors has yielded insights into many peripheral and central nervous system processes (Jacobs & Azmitia, 1992; Jacobs & Fornal, 1991).

There has been much media attention regarding the function of serotonin in the brain; however, it is not well advertised that the vast majority of total body serotonin is found outside the central nervous system and that only about one in a million neurons in the brain produce it (Gershon & Tack, 2007). Indeed, serotonin was first characterized as a vasoconstrictor substance contained in platelets (as reviewed in Mohammad-Zadeh, Moses, & Gwaltney-Brant, 2008). Subsequent research has identified other areas and functions of serotonin, expanding its role to include both peripheral and central nervous system activity (as reviewed in Mohammad-Zadeh et al., 2008).

Serotonin is synthesized in the raphe nuclei of the central nervous system (Iverson, Iverson, & Saper, 2000). The raphe nuclei can be divided into two groups based on the general target regions of their projections. The caudal raphe projections originate from the raphe magnus, raphe obscurus, raphe pallidus nuclei and parts of the adjacent lateral reticular formation, and innervate the brainstem and spinal cord nuclei (Iverson et al., 2000). Conversely, the rostral raphe nuclei project from the caudal linear, dorsal raphe, and median raphe nuclei to the majority of forebrain regions, and account for approximately 85 percent of serotonergic neurons within the brain (Hornung, 2003; Molliver, 1987). Of this nuclei group, the median and dorsal raphe regions constitute the majority of cells in this nuclei cluster. The dorsal raphe nucleus has been implicated in a variety of physiological and behavioral process, especially during stress (Adell, Celada, Abellan, & Artigas, 2002; Hornung, 2003; Mason, Gao, & Genzen, 2007; Nalivaiko & Sgoifo, 2009; Tanaka, Nagashima, McAllen, & Kanosue, 2002). The projections from

this region are divided into two pathways based on their projected targets in the central nervous system.

In the brainstem, the dorsal tract travels parallel to the medial longitudinal fasciculus, while the medial projection follows a ventral tract running parallel to the paramedian division of the medial raphe nuclei, and enters the ventral tegmental area at the dorsal edge of the interpeduncular nucleus (Hornung, 2003). It should be noted that in non-human primates, the dorsal tract is larger with more myelinated fibers, potentially due to increased size and complexity of the cerebral cortex (Hornung, 2003). The two pathways then join and overlap in projecting to an enormous number of targets within the brain. The importance of this overlap may be related the different morphological characteristics of the two tracts.

While both the dorsal and medial tracts overlap in their innervation of cortical regions, the dorsal tract distribution is such that it does not synapse with one specific synaptic target, but many (Lambe, Krimer, & Goldman-Rakic, 2000; Smiley & Goldman-Rakic, 1996). Conversely, medial tract projections are point-specific with their synapses, and are more abundant in the hippocampus (DeFelipe & Fariñas, 1992; Lambe et al., 2000) and in monkey prefrontal cortex interneurons (Smiley & Goldman-Rakic, 1996). The different morphology of these projections may be responsible for facilitating diverse functions of the dorsal raphe nuclei during basal and stress periods. For example, a swim stressor (i.e., forced swim test) results in increased serotonin levels in the striatum (a region associated with movement and cognition coordination) and decreased levels in the lateral septum (a region associated with stress responses) (Waselus, Galvez,

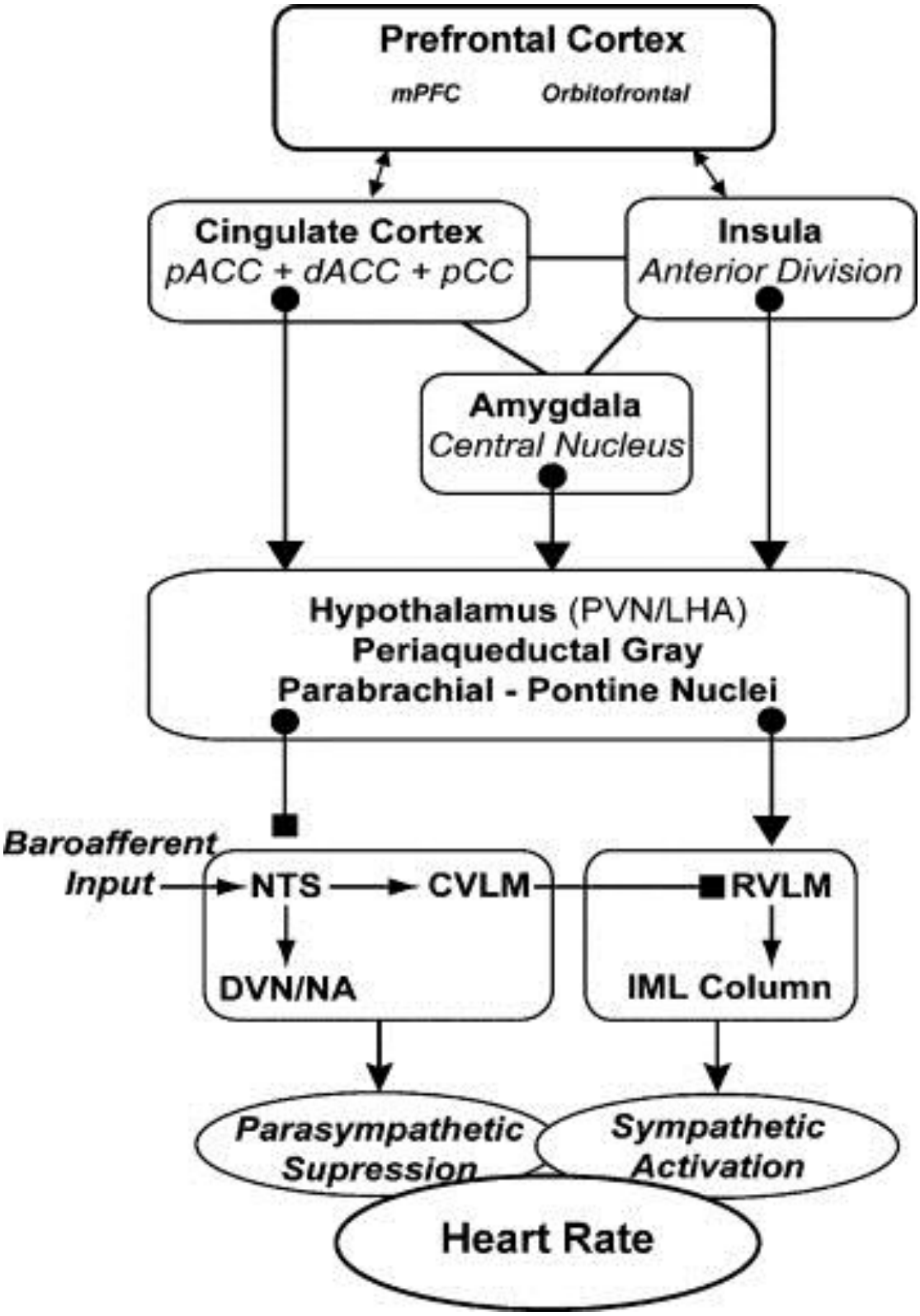
Valentino, & Van Bockstaele, 2006). Retrograde labeling of serotonin projections to these regions yielded greater localization of neurons the rostral region of the dorsal raphe to the striatum and more neurons in the caudal regions of the dorsal raphe to the lateral septum (Waselus et al., 2006). This anatomical organization suggests that the raphe nuclei may facilitate health and behavioral function based on differential serotonin nuclei projections to forebrain structures. While the central nervous system serotonin neurotransmitter system is relatively small numerically, serotonin neurons in the brainstem send ascending projections to cortical, limbic, and hindbrain regions (Jacobs & Azmitia, 1992).

All known brain regions express multiple serotonin receptors, exemplifying the large footprint of this neurotransmitter system (Berger et al., 2009). Moreover, these receptors can induce opposing physiological effects on target neurons. For example, layer five pyramidal cells of the rat medial prefrontal cortex (involved in regulating attention and cognitive performance) express both serotonin$_{1A}$ and serotonin$_{2A}$ receptors, which exert opposing effects on pyramidal neuron firing (Araneda & Andrade, 1991). These two receptors differentially affect cell firing through opposing changes in membrane excitability and may provide a flexible mechanism by which serotonin can regulate how pyramidal neurons in the prefrontal cortex encode incoming excitatory stimuli onto firing activity (Araneda & Andrade, 1991). There are several studies of serotonin receptor mediated regulation of function that exemplify how serotonin exerts its effect on a wide range of systems and behaviors (e.g., cardiovascular, mood, and behavior) (Carver, Johnson, & Joormann, 2008; Chameau & van Hooft, 2006; Lanzenberger et al., 2007;

Magalhaes et al., 2010; Maier & Watkins, 2005; Meltzer et al., 2004; Molliver, 1987; Osei-Owusu, James, Crane, & Scrogin, 2005; Ruhe, Mason, & Schene, 2007). Further, serotonin does not overtly regulate physiological processes but alters these processes through modulating state dependent changes in neuronal activation (Jacobs & Fornal, 1991). As such, serotonin has received the reputation as a neuromodulator, exerting changes (homeostatic or behavioral) through adjustments in neuron firing activities. This is an important aspect when considering possible serotonergic mechanisms that underlie how behavior, cardiovascular function, and mood interact.

An organism's interaction with its environment is theorized to be mediated through a neural circuit controlling cardiovascular function, mood, and behavior (Heim & Nemeroff, 2002; Thayer & Brosschot, 2005). Thayer and Brosschot (2005) theorize this circuit is composed of a reciprocal cortico-subcortical neural circuit (Figure 01) that serves to regulate this interaction. This circuit includes the autonomic nervous system, as well as emotional and cognitive features relevant to both social engagement and modulating responses to the environment. The cortical component of this circuit is composed of the prefrontal cortex while the subcortical component is composed of classic limbic system structures and brainstem nuclei responsible for autonomic nervous system activity. This cortico-subcortical circuit may structurally, as well as functionally, link psychological processes with health outcomes (Thayer & Brosschot, 2005). Importantly, every neuronal structure within this circuit possesses one or more forms of serotonin receptors, theoretically allowing the neurotransmitter to influence the activity

Figure 01. Box plot of prefrontal, subcortical, and brainstem connections, taken from Thayer & Brosschot, 2005. Abbreviations used only in this figure: dorsal anterior cingulate cortex (pACC), dorsal motor nucleus of the vagus (DVN), intermediolateral nucleus column (IML Column), lateral hypothalamic area (LHA), medial prefrontal cortex (mPFC), nucleus ambiguus (NA), nucleus of the solitary tract (NTS), paraventricular nucleus of the hypothalamus (PVN), posterior cingulate cortex (PCC), pregenual anterior cingulate cortex (pACC), and rostral ventrolateral medulla (RVLM).


Prefrontal Cortex
mPFC
Orbitofrontal
Cingulate Cortex
pACC + dACC + pCC
Insula
Anterior Division
Amygdala
Central Nucleus
Hypothalamus (PVN/LHA)
Periaqueductal Gray
Parabrachial - Pontine Nuclei
Baroafferent
Input
NTS
CVLM
RVLM
DVN/NA
IML Column
Parasympathetic
Supression
Sympathetic
Activation
Heart Rate

of every limbic structure associated with cardiovascular function, social behavior, and mood. Serotonin plays a role in regulating the activities of the subcortical nuclei (e.g., amygdala, hippocampus, and hypothalamus) that are involved in behavioral and physiological function (Beaulieu et al., 1986; Freund et al., 1990; Holmes et al., 1982; Jacobs & Fornal, 1991; Weidenfeld et al., 2002). Thus, the serotonin system plays a role in modulating the neurobiological system responsible for how a person behaves within and interacts with its environment. This modulation is achieved through an interplay of the serotonin neurotransmitter, transporter, and receptor families.

Serotonin neurotransmitter, transporter, and receptor families

To describe its role in behavioral and physiological function the following section will introduce the activity of serotonin beginning with its structural conformation. Serotonin is structurally similar to other monoamines such as epinephrine, norepinephrine, and dopamine, and is produced through two steps (Jacobs & Azmitia, 1992). First, the amino acid tryptophan is converted to 5-hydroxytryptophan through the addition of a hydroxyl group by tryptophan hydroxylase. Second, L-aromatic amino acid decarboxylase converts the intermediate substance into 5-hydroxy-tryptamine (serotonin) by removing the carboxyl group (Moroni, 1999).

Serotonin in the central nervous system originates from the raphe nucleus, which produces ascending projections to the forebrain and descending projections to the

medulla and spinal cord (Jacobs & Azmitia, 1992). The activity of these central nervous system serotonin neurons is believed to be controlled though excitatory glutamatergic, tonic noradrenergic, and inhibitory gamma-aminobutyric acid functions (for more in-depth review, see (Adell et al., 2002; Cryan et al., 2005b)). Outside of the central nervous system serotonin synthesis is mostly limited to the intestines and platelets (Mohammad-Zadeh et al., 2008). Serotonin made by the enterochromaffin cells lining the epithelia of the respiratory and digestive tracts is used in secretory and peristaltic processes, and is also readily taken up by platelets that serve as a major peripheral storage site for the neurotransmitter (Jacobs & Azmitia, 1992). As mentioned above, the majority (i.e., 90-95 percent) of the body's total serotonin volume is used in peripheral function (Jacobs & Fornal, 1991). Additionally, 99 percent of total body serotonin is located intracellularly, suggesting very tight regulation of the neurotransmitter (Tyce, 1990).

One control for the amount of serotonin available for activity is monoamine oxidase, which comes in two forms: monoamine oxidase-A located in terminal buttons, and monoamine oxidase-B which has also been localized in human platelets (Arai et al., 2002; McIsaac & Page, 1959). Monoamine oxidase-A is the primary metabolic pathway for the inactivation of serotonin (and several other monoamines) (Sandler, Reveley, & Glover, 1981). Vesicular storage of serotonin protects against the degradation of serotonin into 5-hydroxyindoleacetic acid, the metabolite of serotonin, because monoamine oxidase is localized in the cytosol of the cell and unable to access the monoamines that are bound within a vesicle (Mohammad-Zadeh et al., 2008). This system was an early target for pharmacotherapy, as monoamine oxidase inhibitors were

able to improve mood (Krishnan, 2009). However, those drugs also resulted in a number of adverse side effects and were subsequently replaced with more effective drugs that produced fewer adverse side effects, while still modulating the availability of serotonin for neurotransmission.

Once serotonin is released into the synaptic cleft, it interacts with the various receptor types and the serotonin transporter. The abundance of serotonin in the synaptic cleft is the major determinant of the strength and duration of serotonin activity (Mohammad-Zadeh et al., 2008). This availability of serotonin in the synapse depends on binding of the serotonin receptors (e.g., autoreceptor activation; discussed in the following paragraph) and serotonin transporter activity. Both the serotonin autoreceptor and transporter are located on the presynaptic terminal (Berger et al., 2009). The serotonin transporter is part of a class of monoamine transporters that are selective for a specific substrate; however, there is a small transport affinity for the other monoamines (Torres, Gainetdinov, & Caron, 2003). These transporters function by using the concentration gradient provided by the sodium/potassium pump, and in the case of serotonin, use co-transport of sodium and chloride ions (Torres et al., 2003). Specifically, the probable serotonin transporter mechanism is a symport that first requires the output of potassium, then the subsequent binding of chloride, serotonin, and sodium.

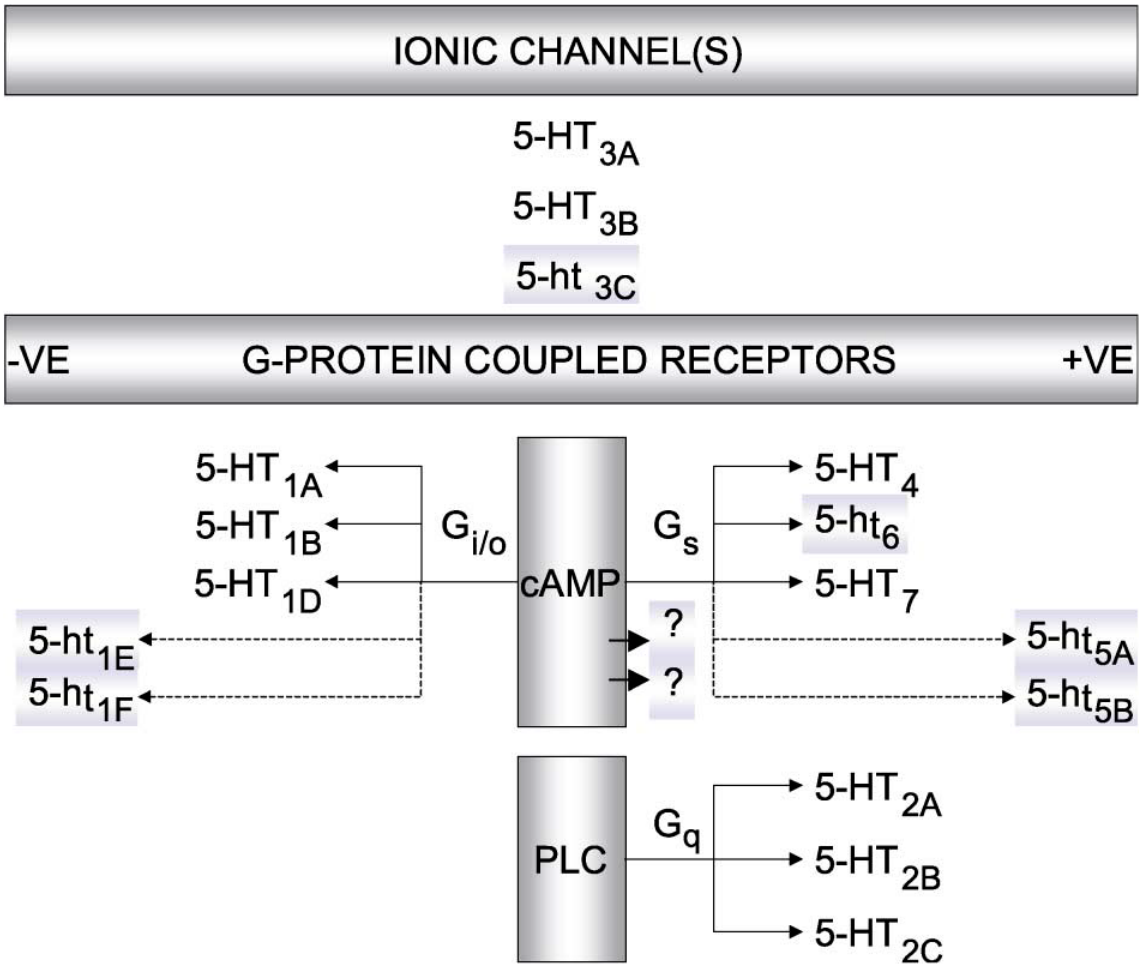
There is no direct evidence suggesting that a specific order is required for solute attachment, but once all are bound, the transporter undergoes a conformational change and the chloride, serotonin, and sodium are released into the cell (Rudnick, 2006). Following dissociation, the transporter reconfigures, waiting for the molecules to attach

and begin the process again (Rudnick, 2006). From there, serotonin is either inactivated by a monoamine oxidase or packaged by vesicular monoamine transporter into a vesicle for rerelease (Bruns, Riedel, Klingauf, & Jahn, 2000; Sandler et al., 1981). Thus, the serotonin transporter is argued to be essential for the fine tuning of serotonergic neurotransmission by directing the magnitude and duration of postsynaptic responses, carrier-facilitated serotonin transport into, and release from, the presynaptic neuron (Heils et al., 1996). This essential role in moderating the availability of serotonin in the synapse has prompted investigations into the activity of the transporter.

The gene for human serotonin transporter (i.e., SLC6A4) was mapped to chromosome 17q11 in the early 1990s (Ramamoorthy et al., 1993). Further investigations into the mechanisms of control for the transporter have yielded insights into its function within the serotonin system (Heils et al., 1996). Specifically, researchers have found differences in the promoter region responsible for regulatory transcription of the gene. Upstream from the transporter gene, a repetitive sequence containing guanine-cytosine rich 20-23 base pair-long elements has shown polymorphisms, in the promoter region (i.e., 5-HTTLPR) (Nakamura, Ueno, Sano, & Tanabe, 2000). These polymorphisms include 14 allelic variants but are generally grouped into long or short allele designations (Nakamura et al., 2000). Importantly, these differences in repeat length can alter promoter activity, and thus alter the expression of the receptor. Numerous studies have observed a link between stress responsivity and susceptibility to negative mood states in individuals with the short allele polymorphisms (Caspi et al., 2003; Furmark et al., 2004; Way & Taylor, 2011).

While pre-synaptic mechanisms are responsible for the amount of serotonin release, a variety of receptor types mediate a diverse effect on postsynaptic cells. There are seven general serotonin receptor groups (i.e., serotonin$_1$ - serotonin$_7$) based on their mechanism of action, and each class has subtypes based on isoform heterogeneity (Barnes & Sharp, 1999). In general, the serotonin receptors are G-protein-coupled receptors that use an intracellular second messenger system to influence cellular excitability. The notable exception to this is the serotonin$_3$ receptor family, that functions as a ligand-gated ion channel (i.e., sodium or potassium) (Hoyer, Hannon, & Martin, 2002). Figure 02 illustrates a graphical representation of serotonin receptors and their general mechanisms of function. Briefly, the serotonin$_1$ receptor family inhibits adenylyl cyclase, resulting in a decrease in cyclic-adenosine monophosphate availability and thus inhibits cell activity (Mohammad-Zadeh et al., 2008). Conversely, serotonin$_{4,5,6,7}$ receptor families stimulate adenylyl cyclase, inducing an increase in cyclic-adenosine monophosphate availability, and thus result in post-synaptic cell excitation (Barnes & Sharp, 1999). Serotonin$_2$ receptor activation up regulates inositol triphosphate and diacylglycerol pathways, resulting in increased intracellular calcium activity, and are thus excitatory as well. Finally, the serotonin$_3$ receptor family are cation channels that induce plasma membrane depolarization (Hoyer et al., 2002). Combined, these receptors can offer a variety of mechanisms for the excitatory or inhibitory modulation of neuron activity. Alteration of neural circuit transmission by serotonergic input thus enables the modulation of a wide range of neuronal systems and behaviors (Jacobs & Azmitia, 1992; Jacobs & Fornal, 1991; Lambe et al., 2000).

Figure 02. Graphical representation of serotonin receptors and mechanisms of function from Hoyer et al. (2002), modified with explanation of G-coupled protein activity. Abbreviations used only in this figure: cyclic-adenosine monophosphate (cAMP), diacylglycerol (DAG), membrane potential (VE) serotonin receptor (5-HT), and phospholipase C (PLC).



G_i/G_o	=	Decreases cellular levels of cAMP	→ Inhibitory
G_q/G_{11}	=	Increase cellular levels of IP_3 and DAG	→ Excitatory
Ionic	=	Ligand-gated Na^+ and K^+ channel	→ Excitatory
G_s	=	Increasing cellular levels of cAMP	→ Excitatory

In summary, the serotonergic system is involved in the regulation of most behavioral and physiological processes. In short, serotonin typically influences the activity of a system through relatively small modifications in cell activity that can influence neuronal circuit function, which ultimately influences behavior (Lesch, 2007). In addition to serotonin receptor interactions, its transporter has also been shown to be a key cellular element regulating the concentration of transmitters in the extracellular fluid, influencing the activity of the system (Berger et al., 2009; Daws & Gould, 2011; Lau, Horschitz, Berger, Bartsch, & Schloss, 2008). In the last several decades, the study of serotonin, its transporter, and the various receptor types has led to improvements in the understanding of how this system influences neurobiological mechanisms underlying psychological and physiological function. The insight yielded from developing an understanding of the molecular interaction of serotonin is directly relevant to elucidating the role of serotonin in physiological and behavioral processes.

The serotonin system influences both cardiovascular and psychological processes and is negatively altered by social stress

It has been well established that the serotonergic system is involved in the regulation of most behavioral and physiological processes (Jacobs & Fornal, 1991). For instance, serotonergic projections that originate from the raphe nuclei modulate physiological function of the cortical, limbic, and hindbrain regions. As such, a route for

modulating cardiovascular function, mood, and social behavior as a concerted system is created (Lesch, 2007; Thayer & Brosschot, 2005). Further, the serotonergic system is uniquely placed to influence the function of brain areas that are involved in emotional and physiological activity (e.g., cardiac control and behavioral responses). Both within the central nervous system and throughout the periphery, serotonin plays a number of roles in cardiovascular function, including the modulation of blood pressure homeostasis, platelet function, and vascular resistance (Jacobs & Fornal, 1991).

A wide variety of empirical research has demonstrated that within the central nervous system, the two principal serotonin receptors involved in the modulation of cardiac and vascular control are serotonin$_{1A}$ and serotonin$_{2A}$ receptors (Nalivaiko & Sgoifo, 2009). In general, activation of central serotonin$_{1A}$ receptors causes a decrease in cardiovascular activity, such as decreases in blood pressure and heart rate (Raymond, Mukhin, Gettys, & Garnovskaya, 1999). Conversely, the activation of serotonin$_{2A}$ receptors produces an increase in cardiac function. This was demonstrated by increased blood pressure, heart rate and cardiac output (Côté, Fligny, Fromes, Mallet, & Vodjdani, 2004). Corroborating these physiological responses, cellular electrophysiological studies demonstrate that serotonin$_{1A}$ and serotonin$_{2A}$ receptors induce opposing changes (i.e., increases and decreases, respectively) in cell membrane potential (Barnes & Sharp, 1999; Nalivaiko & Sgoifo, 2009), which alters the firing of the resident neurons. These mechanisms influence limbic structures involved with cardiovascular function, such as the HPA axis.

Serotonin$_{1A}$ and serotonin$_{2A}$ receptors have also been localized in the rat paraventricular nucleus of the hypothalamus, a nucleus involved in the regulation of the HPA axis and other hormonal systems (Zhang et al., 2004). Additionally, Zhang et al. (2004) also found that on neurons that coexpressed the two receptor types (i.e., oxytocin and corticotropin-releasing factor), serotonin$_{2A}$ receptor activation results in the heterologous desensitization. This demonstrates that activation of the serotonin$_{2A}$ receptor results in a decrease in signaling of the serotonin$_{1A}$ receptor without a change in serotonin$_{1A}$ receptor number or activity, removing its inhibitory influence. Thus, serotonin$_{2A}$ receptor activation will induce an increase in endocrine activity (Damjanoska et al., 2003; Van de Kar et al., 2001). Indeed, Van de Kar et al. (2001) observed that serotonin$_{2A}$ activation on corticotropin-releasing factor and oxytocin neurons in the hypothalamic paraventricular nucleus stimulates the secretion of corticotropin-releasing hormone and oxytocin in the paraventricular nucleus of the hypothalamus. This finding demonstrates that serotonin$_{2A}$ receptor stimulation induces the activation of hormone cascades associated with stress and cardiovascular disease (Damjanoska et al., 2003). The related consequence of this cascade is increased HPA axis activity, which has been linked with deleterious changes in neuronal function, behavior, and overall health (Williams et al., 2001).

The serotonin system has also been more directly linked with influencing heart rate variability and cardiac morphology. Recent research has found that individuals who are homozygous for the serotonin transporter short allele genotype have reduced heart rate variability at baseline (versus homozygous long allele genotype) and attenuated

autonomic reactivity when exposed to a pharmacological panic challenge (Agorastos et al., 2014). The authors assessed heart rate variability at baseline and after the administration of cholecystokinin tetrapeptide (fragment, Tryptophan-Methionine-Aspartic acid-Phenylalanine), a drug used to study panic attacks. Briefly, the drug is believed to induce this effect through activation of the HPA axis (Strohle et al., 2003), inducing a significant glutamate increase in the anterior cingulate cortex (a brain region associated with assigning the salience of information) (Zwanzger et al., 2013), and possibly through affecting the activity of cholecystokinin system receptors in the central nervous system (Bradwejn, 1993). Agorastos et al. (2014) found that cholecystokinin tetrapeptide administration enhanced sympathetic tone in both short and long allele carrier groups, resulting in lower heart rate variability. However, the attenuated reactivity to cholecystokinin tetrapeptide in the short allele group (compared to the long allele group) indicates enhanced sympathetic and/or diminished parasympathetic nervous system activity under basal and stressed conditions. Thus, a homozygous short allele genotype represents a genetic vulnerability to impaired sympathoadrenal pathway regulation (Agorastos et al., 2014).

The serotonergic system is also involved in peripheral cardiovascular function, namely vascular activity. In the periphery, serotonin causes either vasoconstriction or vasodilation depending on the receptor type and its expression in endothelium (i.e., relaxation) or surrounding smooth muscle (i.e., contraction) (Amstein, Fetkovska, Lüscher, Kiowski, & Bühler, 1988). Indeed, serotonin was first identified as a "serum vasoconstrictor" (Rapport, Green, & Page, 1948). Similarly, serotonin is also linked with

platelet activity. Platelets have significant vesicular serotonin stores but lack the enzymes to synthesize serotonin; instead, they take up serotonin from the plasma through a transporter (Jacobs & Fornal, 1991). Serotonin can then be secreted by the platelet during times of activation (e.g., wound healing). Platelet activation promotes aggregation (i.e., forming a clot) and vasoconstriction of blood vessels, thus facilitating homeostasis (Jacobs & Azmitia, 1992).

Selective serotonin reuptake inhibitors can increase bleeding time by inhibiting the uptake and storage of serotonin in platelets. This finding has been linked to a reduced incidence of recurrent myocardial infarction in patients suffering from depression following a myocardial infarction (Maurer-Spurej, 2005). As such, it is hypothesized that selective serotonin reuptake inhibitors help to inhibit the formation of platelet clots, and thus can function as an anti-thrombotic and/or clot reducer (Maurer-Spurej, 2005). Moreover, selective serotonin reuptake inhibitor (i.e., sertraline hydrochloride)-induced attenuation of platelet aggregation is dose dependent (Serebruany, Gurbel, & O'Connor, 2001). Together central and peripheral serotonin activity have the potential to influence the regulation of cardiovascular function. More specifically, serotonin receptors have been found in structures responsible for reflexive control of cardiovascular function.

Cardiovascular responses to serotonin may involve the simultaneous activation of more than one receptor subtype. For example, serotonin$_3$ receptor activation in the nucleus of the solitary tract increases mean arterial pressure and inhibits the down regulation of chronotropic regulation (i.e., parasympathetic input to the heart) (Nosjean, Bernard, & Laguzzi, 1995). However, the exact mechanisms of this action have not been

fully elucidated, and serotonin$_3$ receptors have also been localized in the hippocampus and amygdala (Miquel et al., 2002). This diverse localization of serotonin$_3$ receptors could indicate a larger control network utilizing those receptor types to influence responses to the organism's environment. Alternatively, the serotonin$_3$ receptors could be responsible for facilitating effects of other serotonin receptors or interneuron activities (Bétry et al., 2011). For example, while serotonin$_3$ receptor antagonists do not have an effect on the hippocampal serotonin release, they can increase serotonin levels induced by the selective serotonin reuptake inhibitor paroxetine (Bétry et al., 2011). Findings such as these underscore the complicated interaction between serotonin and the numerous areas of the brain that serve to influence peripheral organ systems. For example, it is currently unknown whether selective serotonin reuptake inhibitors influence the activity of serotonin$_3$ receptor function in the nucleus of the solitary tract. Future investigations in this field of study may offer another method for the pharmaceutical treatment of patients experiencing cardiovascular disease.

Because of the vast influence of the serotonergic system, reduction in the effectiveness of serotonin receptors and transporters may lead to dysregulation of nuclei that control biological systems (Nalivaiko, 2006; Nosjean, Franc, & Laguzzi, 1995). This systemic dysregulation is therefore linked with negative behavioral and health states (Carvalho & Pariante, 2008; Forgas & East, 2008; Ruhe et al., 2007). Similar to the findings noted above, chronic social stress can deregulate the serotonin system and can lead to a variety of maladies that adversely affect health (e.g., depression and cardiovascular disease) (Carvalho & Pariante, 2008; Ruhe et al., 2007; Sapolsky, 1996;

Tedeschini et al., 2011; Young & Leyton, 2002). Fortunately, pharmacological manipulation of this system has facilitated improvements in human health. In the next section, the impact of psychopharmacological intervention as it relates to beneficial health outcomes will be discussed.

Serotonin drug treatment improves health biomarkers

Increasing evidence showing the importance of the interactions between social environments and health outcomes has resulted in a dramatic increase in collaborative projects to understand these phenomena (Cacioppo et al., 2007). As noted previously, stress negatively influences a wide range of biological systems; therefore, identifying a single, specific physiological mechanism responsible for these effects has proven difficult. One hypothesis is that the serotonin system, which is involved in modulating the aforementioned health processes, may serve as a critical link between the social environment and the neurobiological pathways that affect health (Way & Taylor, 2010b). Based on this hypothesis, serotonin system acting drugs (e.g., antidepressants) have historically been used in the treatment of affective disorders (Cipriani et al., 2010; Culang-Reinlieb, Sneed, Keilp, & Roose, 2012; Flament, Lane, Zhu, & Ying, 1999; Kornstein et al., 2000).

Antidepressant drugs that influence serotonin, such as sertraline hydrochloride, have been effective in ameliorating symptoms of significantly depressed, melancholic,

and anxious patients (Flament et al., 1999; Papakostas & Fava, 2008). Further, accumulating evidence suggests that in addition to improving mental health, selective serotonin reuptake inhibitor treatment may also improve quality of life after a myocardial infarction (Glassman et al., 2002; Roose et al., 1998). Several case-controlled studies have observed better health outcomes of patients with cardiovascular disease who took serotonin reuptake inhibitors, specifically sertraline hydrochloride (Glassman et al., 2002; Shapiro et al., 1999) Further, pharmacotherapy treatment (including sertraline hydrochloride) has been associated with improved cardiac outcomes and reduced mortality, even when cognitive behavioral therapy alone failed to improve health outcomes (Berkman et al., 2003). Parallel to these findings in humans, sertraline hydrochloride has also been found to be effective in reducing isolation-induced depressive behaviors and cognitive deficits in rats (Ramanathan, Kumar, & Suresh, 2003). These beneficial treatment outcomes are believed to be the result of restored function in the serotonin system through the pharmacodynamics of the drugs. While the importance of pharmacotherapy is undeniable, the intricacies of exactly how beneficial outcomes are attained are not yet fully understood.

Possible mechanisms mediating the efficacy of drug treatment

The exact mechanisms of serotonin acting drug treatment efficacy have yet to be fully elucidated. The beneficial changes observed from selective serotonin reuptake

inhibitor treatment may be the result of neurotransmitter availability changes that modify receptor activity, specifically the serotonin$_{1A}$ receptor (Nalivaiko, 2006; Way & Taylor, 2010b). Investigations using three-dimensional positron emission tomography scans have found that serotonin$_{1A}$ receptor binding potency is reduced in a number of cortical (e.g., cingulate gyrus), subcortical (e.g., hippocampus and amygdala), and brainstem (e.g., dorsal raphe) regions in depressed individuals (Lanzenberger et al., 2007; Meltzer et al., 2004; Neumeister et al., 2004). It is currently unknown whether this binding deficiency is localized within these brain structures or if the deficit affects the brain and/or body globally (Nalivaiko, 2006). Another important question is how do serotonin$_{1A}$ receptors become dysfunctional? One potential answer for this is the activity of the serotonin transporter.

Similar to findings with the serotonin$_{1A}$ receptor, dysfunction within serotonin transporter activity has been firmly linked with susceptibility to negative mood states and stress responsivity (Caspi et al., 2003; Daws & Gould, 2011; Drevets et al., 2007; Furmark et al., 2004; Way & Taylor, 2011; Williams et al., 2001). Investigations into the mechanisms of control for this transporter have yielded insights into its function within the serotonin system and its involvement in the etiology of social stress. Central to this relationship is the finding that individuals who possess the transporter promoter genotype of one or two short alleles (versus long alleles) are at an increased risk for depression (Caspi et al., 2003).

Within the central nervous system, a short allele genotype is linked with dysfunctional serotonergic neurotransmission and is associated with negative health and

wellness (e.g., cardiovascular disease, depression, metabolic syndrome) (Smith et al., 2004; Way & Taylor, 2010a; Whale, Quested, Laver, Harrison, & Cowen, 2000). Functionally, the short allele polymorphism reduces the transcriptional efficiency of the serotonin transporter gene promoter, thus resulting in a decreased expression of the transporter and movement of the transmitter within the synapse (Lesch et al., 1996). Exactly how this gene expression has the potential to result in dysfunctional neurotransmission (and subsequent negative health outcomes), is still an open question. One prevailing theory is that prolonged stress exposure induces the desensitization of serotonin$_{1A}$ receptors. One hypothesis for the efficacy of selective serotonin reuptake inhibitors in antidepressant drug treatment is that they induce changes in synaptic transmission through mechanisms that are secondary to the transporter inhibition.

When a serotonin reuptake inhibitor is administered, the blockade of the target transporter occurs within several minutes; however, symptom relief is not observed until weeks later (Frazer & Benmansour, 2002; Harmer, Goodwin, & Cowen, 2009). One explanation for this time delay could be changes in serotonin transporter intracellular trafficking. For example, chronic administration of a selective serotonin reuptake inhibitor (i.e., citalopram) has been shown to induce internalization of serotonin transporters from the cell surface as well as redistribution of transporters from neurite extensions into the body of the cell (Lau et al., 2008). Additionally, drug administration did not alter serotonin transporter messenger-ribonucleic acid expression. Therefore, treatment could be influencing the serotonergic system via mechanisms other than directly interfering with the expression of the transporter molecule. Thus one mechanism

of function for antidepressant medication efficacy is believed to lie in receptor activity changes (Reid & Stewart, 2001). As such, serotonin$_{1A}$ receptors are hypothesized to be strongly involved in the mechanisms of antidepressant drugs.

Serotonin$_1$ receptors are located on neurons of the midbrain raphe nuclei and on neurons that are postsynaptic to serotonin nerve terminals, typically in the cortico-limbic areas (Celada, Puig, Amargos-Bosch, Adell, & Artigas, 2004). An important mechanism of control of serotonin neurons is self-inhibition through serotonin$_{1A}$ autoreceptors. Activation of these receptors induces a decrease in cellular activity levels and thus neurotransmission (Adell et al., 2002). This local inhibition is also accompanied by a decrease of serotonin release from the nuclei responsible for serotonin output (raphe nuclei) via axonal collaterals (sometimes referred to as "crosstalk") among serotonin neurons. Thus, in addition to terminal neurotransmitter release inhibition, the axonal collaterals decrease neurotransmission by attenuating neuronal firing and produce a negative feedback regulation of transmitter release (Celada et al., 2004). However, the attenuation of cell firing and terminal serotonin release diminishes after long-term treatment with a serotonin reuptake inhibitor (Haddjeri, Blier, & de Montigny, 1998).

Chronic serotonin reuptake inhibitor administration results in the eventual recovery of serotonin cell firing in the dorsal raphe nucleus and an increase in extracellular serotonin, comparatively greater than from acute administration (Bel & Artigas, 1993; Celada et al., 2004). Thus, an acute administration of a serotonin reuptake inhibitor reduces cell firing immediately; however, when administered chronically it results in an increase in serotonergic neurotransmission. The intracellular mechanisms for

this change could be the up regulation of cyclic-adenosine monophosphate second messenger and cyclic-adenosine monophosphate second messenger response element binding protein, leading to the expression of brain-derived neurotrophic factor (Duman, 1998). In turn, this enhancement of brain-derived neurotrophic factor could reverse stress induced changes in neuronal transmission (Duman, 1998). Together, these findings indicate that antidepressant drug treatment improves synaptic transmission mechanisms that are associated with improvements in depressive symptomatology (Carvalho, Garner, Dew, Fazakerley, & Pariante, 2010; Culang-Reinlieb et al., 2012; Tedeschini et al., 2011).

Due to its role in modulating virtually all important behavioral and health processes, the serotonin system has received considerable interest as a target for drug intervention, not only for depression but for many other psychological and physiological conditions. Indeed, prescription drugs have been used to treat a variety of conditions, including co-morbid cardiovascular disease and depression. Accumulating evidence suggests that selective serotonin reuptake inhibitor treatment may decrease myocardial infarction risk and improve health outcomes (Berkman, et al., 2003; Glassman et al., 2002; Roose et al., 1998; Shapiro et al., 1999). Sertraline hydrochloride has also shown increased efficacy, when compared to other drug treatments, in ameliorating symptoms of severely depressed, melancholic, and anxious patients (Flament et al., 1999), and rodent models (Ramanathan et al., 2003). This evidence, when considered together, indicates that the serotonin system plays an important role in mediating the interactions of the social environment, emotion, and cardiovascular function.

In summary, investigating the mechanisms through which pharmacological treatment influences the serotonin system while an animal is experiencing social stress, will provide necessary insight into the effects of sertraline hydrochloride on behavior, emotion, and cardiovascular function. The present study, coupled with continued investigation of the behavioral and biological consequences of negative social experiences using valid and reliable animal models, will encourage novel treatment strategies and improve the quality of life for individuals experiencing social stress and associated emotional or cardiovascular health consequences. This is an important endeavor as both depression and cardiovascular disease impart a costly toll on society. The total number of people in the United States who report major depression symptoms at any given time is two-to-three percent of men and five-to-nine percent of women (Association, 2000). The number of people affected by one or more forms of cardiovascular disease was estimated to be as high as 81,100,000 in 2006 (Go et al., 2013). Further, in 2006 cardiovascular disease was the leading cause of mortality in the United States, claiming the lives of 631,636 people (approximately 26 percent of all reported deaths) (U.S. Department of Health and Human Services, 2007). Based on these reports, there is a clear and immediate need to improve the treatments of these individuals and to better understand how the social environment contributes to these conditions.

CHAPTER 2

CURRENT EXPERIMENT

Disruption of social relationships can negatively influence both emotional and cardiovascular health (Cacioppo et al., 2010; Gore, 1978; Shankar et al., 2011). For example, individuals who are less socially engaged or who feel lonely experience an increased risk of depression, as well as mortality from general and cardiovascular causes (Cacioppo et al., 2010; Ramsay et al., 2008). Similarly, animal models have shown that social isolation and other forms of social stress lead to depressive behaviors, dysfunction of the heart, and precursors to cardiovascular disease (Grippo et al., 2010; Shively, Musselman, & Willard, 2009). Social stress is theorized to mediate these negative health effects by deleteriously altering neurobiological mechanisms regulating social behavior, mood, and cardiac function.

Social stress is linked with cardiovascular disease and depression, which also share similar pathologies, such as altered behavior, increased heart rate, decreased heart rate variability, and autonomic nervous system imbalance (Bunker et al., 2003; Carney & Freedland, 2003; Carney et al., 2005b; Kitzlerova & Anders, 2007; Orth-Gomer, Rosengren, & Wilhelmsen, 1993; Shear & Shair, 2005). These negative biomarker

changes are associated with dysfunctional neurotransmitter (e.g., serotonin) and hormonal (e.g., cortisol/corticosterone) systems, which display improvements in function after pharmacotherapy treatment (Bel & Artigas, 1993; Celada et al., 2004; Frazer & Benmansour, 2002; Harmer et al., 2009; Lau et al., 2008). Due to the beneficial therapeutic effects of serotonin system acting drugs in depression and cardiovascular disease, the next step is to apply this treatment to social stress research.

Given the important interactions of social stress, depression, and cardiovascular disease, the present experiments used prairie voles as a translational rodent model to assess the mechanisms through which sertraline hydrochloride may improve behavioral and cardiac responses to negative social experiences. Previous investigations with this animal model demonstrate that it is an excellent translational model for the study of the consequences of social stress. This is the first study to investigate the effects of sertraline hydrochloride in prairie voles, and therefore provides novel insights regarding the effects of this drug on behavior and cardiac regulation.

Specific aim 1: To determine whether the negative cardiac effects of social bond disruption can be remediated with pharmacological intervention.

Prediction 1a) Does sertraline hydrochloride administration prevent the isolation induced changes in basal measures of cardiac function in isolated males, relative to the vehicle only group?

Prediction 1b) Does sertraline hydrochloride administration prevent the isolation induced changes in cardiac responses during the behavioral

assessment of depression-relevant behavior (i.e., tail-suspension and forced swim test), relative to the vehicle only group?

Prediction 1c) Does sertraline hydrochloride shorten the latency for cardiac measures (i.e., heart rate and heart rate variability) to return to basal levels following the tail-suspension test?

Specific aim 2: To determine whether the negative behavioral effects of social bond disruption can be remediated with pharmacological intervention.

Prediction 2a) Does sertraline hydrochloride administration prevent the anticipated isolation induced changes in activity during the behavioral assessment of depression-relevant behavior (i.e., tail-suspension and forced swim test), relative to the vehicle only group?

Based on previous research, this experiment is appropriately designed to investigate the specific aims listed above. The recovery and baseline periods follow previously used time tables for implanting transmitters in this animal species (Grippo et al., 2011; Grippo et al., 2007c,d; Grippo et al., 2012b; McNeal et al., 2014). Prior studies have also observed that five days of male-female prairie vole pairing is sufficient time for the animals to form a social bond (Bosch et al., 2009; McNeal et al., 2014; Williams et al., 1992). Indeed, the initial portion of the current experiment is extending the methodology employed in McNeal et al. (2014). That study found isolation of a male prairie vole from its female partner for five days induced an increase in heart rate and

depression-relevant behavior. Further, this five day isolation period before drug administration also offers a time course that mirrors events in human disorders: waiting until there is a problem before seeking treatment. Pharmacotherapy is a novel addition to this experimental paradigm; however, prior research indicates that 14 days of drug treatment was sufficient to improve measures of health similar to those used in the present study. For example, sertraline hydrochloride improved depressive behaviors and improved measure of neuronal health (i.e., decreased cell apoptosis) in a rat model of myocardial infarction (i.e., temporary ligation of a primary coronary artery) (Wann et al., 2009). Together, these components indicate that the current study will extend methodology used in prior studies to offer novel findings.

This experiment enables a better understanding of how a negative social environment adversely influences the behavioral and cardiac function in an animal model. Importantly, this experiment investigated the deleterious changes in cardiac regulation following partner loss and possible remediation through serotonin reuptake inhibitor treatment. Increasing our understanding of how pharmacotherapy may improve the neurobiological mechanisms underlying behavioral and cardiac consequences of partner loss will improve the cardiovascular and emotional health, and life quality, of individuals experiencing social stress.

CHAPTER 3

METHODS

General methods

Male prairie vole subjects were implanted with wireless radiotelemetry recording devices. This technology enabled the collection of cardiac data (via electrocardiograph signal) throughout the pairing, isolation, drug injection, and behavioral assessment periods. As such, heart rate and heart rate variability were assessed to indirectly determine how chronic social stress and subsequent pharmacotherapy treatment affects the output of neuronal structures involved in the regulation of cardiac function. Chronic social stress was administered by first pairing all male prairie vole subjects with a female partner for five days, then isolating the males for the remainder of the experiment. After a five day isolation period subjects were then divided into two groups, control and drug conditions, for another 14 days. The pharmacotherapy group was used to determine whether the antidepressant sertraline hydrochloride could prevent the increase in cardiac dysregulation --observed in rodents and humans-- and negative behavior changes during a laboratory assessment of depression-relevant behavior in rodents (i.e., tail-suspension and forced swim tests). Further, pharmacotherapy treatment began five days after isolation, potentially offering an experimental paradigm similar to a timeline that humans

experience (e.g., not seeking treatment until depressive symptoms or other negative consequences manifest).

The current study investigated behavioral and physiological improvements in a rodent model of social stress. In order to limit undue stress on research animals, isolated prairie voles were the focus of this experiment for three reasons. First, the study attempted to glean insight into mechanisms for improvements between socially stressed animals and similarly housed animals that are administered drug treatment. Thus, it was reasonable to focus solely on isolated animals. Second, selective serotonin reuptake inhibitors are not convincingly linked with improving mood in healthy control subjects (Farah & Wolpe, 2004). Third, previous studies from our laboratory have demonstrated that several forms of treatment have beneficially reduced the negative behavioral and physiological consequences of isolation in prairie voles, including both administration of oxytocin (Grippo et al., 2012b; Grippo et al., 2009) and environmental enrichment (Grippo et al., 2014). However, in all of these studies, the treatments had no effect (neither positive nor negative) on paired control animals. Together, these three reasons indicated it would have been unnecessarily wasteful to include paired animals in the present study, solely for the purpose of comparing variables not directly relevant to the hypothesis of the study. Details of these specific methods used in the current study are described below, while the experimental timeline is depicted in Table 1.

Table 1. Schedule of procedures. All procedures were conducted during the light period, three to six hours following light onset.

Procedure	Schedule
Telemetric transmitter implantation	Days 1–2
Recovery period	Days 1–12 (depending on date of transmitter implantation)
-Subjects in custom made divided cages	Days 1–6
-Subjects in standard cages (with siblings)	Days 6–12
3-day Baseline period -Continuous physiological measurements	Days 12–15
5-day Pairing period -Continuous physiological measurements	Days 15–20
20-day Isolation period -Continuous physiological measurements	Days 20–40
15-day Drug administration period -Sertraline/vehicle administration -Continuous physiological measurements	Days 26–42
Tail-suspension test -Continuous physiological measurements	Day 41
Forced swim test -Continuous physiological measurements	Day 42
Euthanization -Euthanize under anesthesia -Collect samples for future studies -Remove transmitters	Day 42

Subjects

Subjects were a total of 23 male prairie voles, each paired with a female partner. All animals used in this protocol were bred in-house by experienced investigators at Northern Illinois University. Per standard operating procedure in prairie vole laboratory research, the subjects remained with breeder parents until they were developmentally capable of living independently from the breeders, at 21 days of age. At 21 days of age all animals were weaned into same-sex sibling pairs. This housing is used as the "normal" social environment for prairie voles in the laboratory setting (Grippo et al., 2011; Grippo et al., 2007a,b,c,d; Grippo et al., 2012a,b; Grippo et al., 2010; Grippo et al., 2009; McNeal et al., 2014). The paired housing can closely mimic their social environment in nature without being too cumbersome (e.g., unnecessarily stressing the breeder parents every time a study manipulation is performed) or adding potential experimental confounds (e.g., families of varying sizes and sexes). As such, animals were housed with the family unit until 21 days of age, and then in same-sex sibling pairs for approximately 60-90 days, to control for prior social and endocrine/sexual experiences before the study began (Grippo et al., 2007a,b,c,d; Grippo et al., 2009; Grippo et al., 2010; Grippo et al., 2011; Grippo et al., 2012a,b).

Each male prairie vole was housed with a male sibling during the recovery period after transmitter implantation, and during the baseline period. Following this period, the male sibling was removed and replaced with an unrelated female during the male-female

pairing period (see *Pairing Period* description below). This housing is used as a "normal" social environment for prairie vole male-female pairs in the laboratory setting, mimicking their pairing behaviors observed in the wild (Bosch et al., 2009; McNeal et al., 2014; Williams et al., 1992). Five days after male-female pairing, the female prairie vole partner was removed and the male subject was housed individually (see *Isolation Period* description below). This was the stress period of the experiment, in which the social bond between the male and female was disrupted, and the male was in a state of isolation (Bosch et al., 2009; McNeal et al., 2014). Additionally, five days after the initiation of the isolation period, half of the experimental animals (n = 11) received daily sertraline hydrochloride (16 mg/kg) injections while the other half (n = 12) received only the vehicle. Following the drug administration period, all animals underwent the same experimental procedures as described in the detailed methods section.

Power analysis indicated that group sizes of approximately 12 was an appropriate use of animals in this experiment. For this analysis, effect sizes (via Cohen's d) were calculated from previously published experiments using similar methodology and/or dependent measures. A desired statistical power of 0.8 was set to reduce the likelihood of type II error; however, a power size of 0.6 would also be appropriate for new investigations such as this. Finally, a probability level of p = 0.05 was used to ensure the results were not due to chance. Specifically, Cohen's ds were calculated from: (a) Grippo et al. (2009) for heart rate changes in response to drug treatment = 5.88, for an n size of 2; (b) Bosch et al. (2009) for the tail-suspension test = 5.06, for an n size of 2; (c) McNeal et al. (2014) for the heart rate during the forced swim test = 6.96, for an n size of 2; (d)

Grippo et al. (2012a) for heart rate variability change = 2.94 , for an *n* size of 3; (e) Grippo et al. (2007d) for heart rate to basal level following a stressor = 3.15, for an *n* size of 3. Along with previous studies conducted by our laboratory that have employed multiple dependent measures in the same animal, the results of these power analyses indicate the *n* sizes used were appropriate for this experiment. This sample size ensured there was adequate power for all dependent measures used in the current study, and accounting for minimal sample size attenuation due to surgical difficulties, issues with drug injections, and technical difficulties during behavioral tasks.

Handling and cage changes were matched between the sertraline hydrochloride and vehicle treated groups throughout the experiment to ensure uniform environmental conditions. For the entirety of the experiment, animals were allowed *ad libitum* access to food and tap water, maintained at a room temperature of 20-21 degree Celsius (^{o}C), and under a standard 14:10 light/dark cycle (lights on at 0630). All animal body weights are reported excluding the weight of the transmitter devices. All experimental protocols were approved by the Northern Illinois University Institutional Animal Care and Use Committee, conformed to United States Department of Agriculture regulations, and followed National Institute of Health guidelines as stated in the *Guide for the Care and Use of Laboratory Animals.*

Transmitter implantation

Animals were anesthetized with a mixture of isoflurane (Baxter, IL USA) and oxygen throughout surgery. Subjects were placed into an anesthesia chamber at two percent isoflurane until rendered unconscious and then transferred to a nose cone, both of which were mounted above a heated surgical pad (37 ^{o}C). When the subject was no longer responsive to tail pinching, the surgical area was shaved and scrubbed with betadine. The isoflurane mixture was adjusted throughout the procedure (typically lowered) to avoid respiratory distress in the subject. The procedures for implantation of the transmitters were similar to methods described previously for prairie voles (Grippo et al., 2007b). A rostral-to-caudal skin incision was made on the ventral surface of the animal, along the midline. The subject's skin was then separated from the underlying muscle, and another rostral-to-caudal incision made through the muscle. Through this incision, internal organs were retracted to prevent damage or obstruction and a radiotelemetry transmitter (Data Sciences International, St. Paul, MN; model TA10ETA-F10) was placed in the abdominal cavity. Leads from the transmitter were directed though a small puncture in the abdominal muscle wall, then the transmitter body and incision into the muscle wall was sutured closed. A trochar (with a plastic sleeve) was used to tunnel under the skin, and then the leads were directed rostrally to lie atop the muscle on the chest of the animal. Positive and negative leads were then sutured to the muscle on the left and right (respectively) of the heart. Finally, skin incisions were

sutured closed using non-absorbable silk suture. During this procedure respiration and activity level were visually assessed to ensure proper animal safety.

Following radiotelemetry transmitter implantation, the subject's vital signs were closely monitored until the animal regained consciousness. At that point, the subjects were housed with their respective siblings for five days in custom-designed cages with a divider to permit adequate healing of suture wounds. This configuration enabled sibling reintroduction with minimal physical contact between the animals. After this initial recovery period, subjects were then returned to a standard cage with the same-sex sibling to recover for an additional five to seven days. During the 10-11 day post-surgical recovery period, subjects were assessed for the following characteristics of proper recovery: (a) adequate urination/defecation, (b) adequate activity level (approximately 2 counts per minute or higher, according to the radiotelemetry data acquisition system), (c) adequate body temperature (approximately 37 ^{o}C after metabolism of the anesthetic), and (d) stabilization of heart rate (approximately 350-400 beats per minute or higher after metabolism of the anesthetic), to ensure appropriate recovery from surgical procedures.

Radiotelemetry recordings and quantification of cardiac variables

Electrocardiographic signals were recorded continuously with a telemetry receiver (Data Sciences International, St. Paul, MN). The analog signal from the receiver was digitized with 12-bit precision at a sampling rate of 5 kilohertz. This system allowed for

multiple physiological parameters --including electrocardiogram, locomotor activity, and body temperature-- to be monitored in conscious, freely moving animals. Unless otherwise noted, electrocardiogram data were collected for one minute at hourly intervals throughout the experiment. Cardiac measures were derived from these one-minute samples through a multistep verification process.

Telemetry data were evaluated using vendor software (Data Sciences International, St. Paul, MN) to determine the activity level of male prairie vole subjects. This measure was derived from small changes in signal strength as the animal moved around the cage. The change in signal strength was represented in an arbitrary unit of counts per minute and can be used as a general indicator of physical activity. Prairie vole activity measures can range from 1 to over 50 counts per minute. Previous experiments using prairie voles have used low counts per minute (e.g., less than five counts per minute) as indicative of low activity or resting periods (Grippo et al., 2007a,c,d; Grippo et al., 2010; Grippo et al., 2012a,b). As such, basal measures of cardiac function were analyzed from time points during which the male prairie voles displayed low activity counts (i.e., five counts per minute or lower), from multiple segments of data. Electrocardiogram wave peak detections were calculated by proprietary Data Sciences International software to provide an index of heart rate, and was expressed in beats per minute. Heart rate variability was calculated from the standard deviation of all electrocardiogram wave peak intervals from data segments, and was expressed in milliseconds (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The electrocardiogram wave peak-to-

wave peak intervals (i.e., heart rate variability) and heart rate were then analyzed for between group differences.

Baseline measurement period

Following recovery from radiotelemetry transmitter implantation, cardiac signals were recorded with a radiotelemetry receiver (described above). Physiological parameters were recorded for one minute at hourly intervals during an undisturbed baseline period (approximately three days) similar to previously published investigations (Grippo et al., 2007c,d; Grippo et al., 2009; Grippo et al., 2010; Grippo et al., 2011; Grippo et al., 2012b). Activity level is high in prairie voles and occurs in approximately two-to-three-hour ultradian rhythms throughout the light and dark periods (Grippo et al., 2007b). Therefore, resting cardiac parameters were derived from cardiac data sampled during a period of minimal activity (five counts per minute or lower) and were presented as an average of multiple one-minute segments of cardiac data. These data collection procedures were repeated for all additional phases of the experiment, unless otherwise noted.

Pairing period

After baseline measurements, all male animals were separated from their respective siblings and paired with unrelated females of approximately the same age and body weight, for five days. These steps were taken to ensure the most compatibility possible between animals. During this period, male-female prairie vole pairs were allowed to behave naturally within the confines of the laboratory cage. Previous investigations have shown that five days is sufficient time for male and female prairie voles to form a pair bond (Bosch et al., 2009; McNeal et al., 2014; Williams et al., 1992). Cardiac and activity data were collected via radiotelemetry at hourly intervals throughout this period.

Isolation period

Following the pairing period, female prairie voles were removed from all the male partners and those animals were housed in isolation. Subjects remained in this experimental condition until the conclusion of the experiment. Cardiac and physical activity data were collected via radiotelemetry at hourly intervals throughout this period.

Drug administration period

Five days into the isolation period, all male animals were randomly divided into two groups: vehicle or sertraline hydrochloride. This time course is based on McNeal et al. (2014) which found that five days of isolation from a female prairie vole partner was sufficient to induced depression-relevant changes in behavior and cardiac function in males. Subjects remained in these experimental conditions for the remaining 15 days of the isolation period. A solution of two percent sucrose was also made available to animals during this period. This was done in order to minimize body weight loss observed during the administration of sertraline hydrochloride. Cardiac and activity data were collected via radiotelemetry at hourly intervals throughout this period.

Male prairie voles in the sertraline group received sertraline hydrochloride (16 mg/kg; generously donated by Pfizer Inc., New York, NY) administered via daily intraperitoneal injections for 15 days. The drug dose and time course were selected to be consistent with therapeutically-effective dosages in humans and animal models (Glassman et al., 2002; Sanders-Bush, Breeding, Knoth, & Tsutsumi, 1989; Sitges, Aldana, Gomez, & Nekrassov, 2012; Yildirim, Erol, & Ulupinar, 2012). The vehicle group was administered only the vehicle (distilled water) that was used to house the drug.

Sertraline hydrochloride was dissolved in distilled water each day at a concentration of four milligrams per milliliter, and sonicated for at least five minutes. Animals received these daily injections of either sertraline hydrochloride or the vehicle

(distilled water) intraperitoneally. This typically resulted in a total fluid injection of 0.16 milliliters for a 40 gram animal, though the injection volume was modified for appropriate animal weight (i.e., dosage was increased for heavier animals and lowered for lighter ones). This solubility information was provided by Pfizer who produced and supplied the drug for use in this study.

Behavioral assessments

Tail-suspension test

The tail-suspension test was used to evaluate depression-relevant behaviors in each animal (Bosch et al., 2009; Steru, Chermat, Thierry, & Simon, 1985). The animal was suspended by its tail using adhesive tape to a metal bar (5 millimeters in diameter) and hung in the middle of a clear plastic box (32 x 28 x 29 centimeters), approximately 25 centimeters above the apparatus floor. The apparatus was stationed on a laboratory bench during testing, with a video camera suspended directly above the animal on a tripod. Between trials, it was cleaned with a ten percent bleach solution. Each five-minute trial was digitally video recorded and then imported into coding and analysis software (Noldus Observer XT8.0, Noldus Information Technology, 6709 PA, Wageningen, Netherlands). Trials were scored for the duration of immobility exhibited by each animal (i.e., no movement besides those required for respiration) and the duration of active behaviors (i.e., active movements characterized by either contortions of the body and/or

flailing of the limbs) (Cryan, Markou, & Lucki, 2002). For example, if the animal was hanging without movement it was scored as immobile. Conversely, if the animal actively attempted to escape the apparatus by flailing its limbs or contorting its body in an attempt to release itself, it was scored as active behavior.

Forced Swim Test

Social and biological factors that influence a rodent's behavioral state produce changes in forced swim test performance (i.e., behaviors in the apparatus) (Cryan et al., 2002; Cryan et al., 2005b; Grippo et al., 2014). Subjects were scored for active (i.e., swimming, struggling, or climbing) versus passive (i.e., floating or immobility) behavioral responses while in the swim tank apparatus. The passive behavioral responses (i.e., floating) are considered to be an operational index of behavioral despair (Bielajew et al., 2003).

The forced swim tank apparatus used in this experiment was a clear Plexiglas cylinder (20 x 45 centimeters) filled with 18 centimeters of 25 ± 1 ^{o}C tap water. Subjects could neither touch the bottom of the tank nor climb out. The apparatus was stationed on a laboratory bench during testing, with a video camera stationed parallel to the apparatus on a tripod. Between animal trials, the tanks were cleaned with a 10 percent bleach solution. Behaviors were video recorded and then imported into behavior coding and analysis software (Noldus Observer XT8.0, Noldus Information Technology, 6709 PA,

Wageningen, Netherlands). Behaviors were scored according to the following criteria: (a) struggling, defined as movements during which the forelimbs broke the water's surface; (b) climbing, defined as movements during which the forelimbs broke the water's surface and were in direct contact with the wall of the apparatus; (c) swimming, defined as movements of the fore and hind limbs resulting in purposeful motion without breaking the water surface; and (d) floating, defined as idle floating or treading water- the behavior during which the animal used limb movement to maintain its equilibrium without any directed movement of the trunk.

Behavior scoring

For behavioral assessments, electrocardiographic, activity, and body temperature data were collected continuously throughout the five-minute test periods. All video files during the behavioral test periods were categorized manually by trained observers, who were blind to the subject's group membership. Further, these trained observers were trained to produce a quality inter-rater reliability score (i.e., a Cohen's *k* greater than 0.70). Physical activity measures were derived from the changes of the transmitter's signal strength as it moved around the testing apparatus (within in the animal). This measure of physical activity was compared with the manually scored active behaviors. Thus, the dependent measures of counts per minute were assessed to determine if it

corresponds with the manually scored indices of depression-relevant behavior during behavioral assessments.

Euthanization

Similar to previous experiments (McNeal et al., 2014), 10 minutes following the end of the 5-minute forced swim test, all animals were anesthetized with a mixture of ketamine (67 mg/kg, subcutaneous injection; NLS Animal Health, Owings Mills, MD) and xylazine (13.33mg/kg, subcutaneous injection; NLS Animal Health). Blood was sampled within two minutes of the anesthetic injection, from the periorbital sinus via a heparinized capillary tube, and was collected during a one and a half minute time period. Samples were immediately placed on ice, and then centrifuged at 4 ^{o}C, at 3500 rotations per minute, for 15 minutes to obtain plasma. Plasma aliquots were stored at negative 80 ^{o}C for an assay of circulating adrenocorticotropic hormone and corticosterone to be performed in future studies (that are outside the scope of the present experiment). After blood was collected, brain tissue was also removed for future assessments that are outside the scope of the present experiment.

Brains were dissected and preserved using passive perfusion in four percent paraformaldehyde with five percent acrolein, according to procedures published previously by our laboratory (Grippo et al., 2007a). Tissue was postfixed in 4 percent paraformaldehyde, sunk in 25 percent sucrose, and sliced into 40 micrometer sections

using a cryostat. In other studies that are beyond the scope of this dissertation project, these procedures will enable the analysis of potential changes in brain structures as a function of social isolation and treatment with sertraline hydrochloride. Following tissue collection, the transmitters were removed and sterilized according to the vendor instructions.

Statistical procedures

A value of $p < 0.05$ was used for the testing of unidirectional changes (i.e., one-tailed test) in dependent measures. For all cardiac measures, electrocardiographic data were synchronized with experimental manipulations and behavioral tests, and evaluated using multiple (usually three) segments of continuous data. These three segments of one minute data were then averaged together and used for statistical analysis. The measures of cardiac function were acquired via the implanted radiotelemetry transmitters and presented as time measurements between the electrical activity associated with heart muscle contraction (i.e., the electrocardiograph R wave), by the computer software. Heart rate was calculated as the number of heart beats during one minute. Heart rate variability was generated by calculating the standard deviation of all the inter-beat intervals, during the same one minute time period. These measurements were used to generate heart rate and heart rate variability scores, presented as beats per minute and millisecond, respectively.

Cardiac function during both behavioral assessments (i.e., tail-suspension and forced swim tests) and latency for returning to pre-stressor cardiac function levels following the tail-suspension test were analyzed in one minute segments. The five minutes of the tail-suspension and forced swim tests have cardiac measurements corresponding to each full minute of the assessment. Additionally, recovery to basal cardiac function levels was measured in one hour segments that consisted of one minute of electrocardiographic data. Any periods of electrocardiographic data involving animal movement artifact (i.e., signal distortion not due to experimental manipulation) were excluded from the analyses. Behavioral activity in the tail-suspension and forced swim tests were split into active and depression-relevant (e.g., immobility) behaviors. Data were analyzed with mixed-design analyses of variance and Student's *t*tests for all follow-up between group comparisons. All manually scored behavior tests were evaluated by at least two experimentally blind researchers, that were trained to produce a quality inter-rater reliability score (i.e., a Cohen's *k* greater than 0.70) (Viera & Garrett, 2005).

Several types of statistical procedures were used for the analyses described within the result section. A mixed-design analysis of variance was used to test for mean differences between the two groups while accounting for the subjects' previous measures. For this method, variance associated with pre-existing measures (i.e., previous heart rate, heart rate variability, and physical activity) is removed from the dependent variable being assessed. Thus, any remaining differences in the dependent variables (i.e., heart rate, heart rate variability, and physical activity) between the two groups is therefore not due to variations in pre-existing measures, but may instead be attributed to the changes

occurring as a result of the independent variables (i.e., group, experimental period, or time during behavior tests). Further, to control for familywise error (i.e., multiple comparisons within a variable) a Šidák correction was applied. Similar to a Bonferroni correction, the Šidák is also a safeguard against multiple tests of statistical significance on the same data erroneously yielding significant results. However, the Šidák correction assumes independence of the test statistics, resulting in a modest gain in power (Abdi, 2007). Main effects of group, time point, and any interaction were further analyzed with the use of *t*tests for follow-up comparisons.

In general, data involving repeated measures were analyzed with two-factor mixed-design analyses of variance, with group (control/drug) as the independent variable, and the following as repeated dependent variables: (a) heart rate on the last day of the pairing period, isolation period, days 5, 10, and 14 of the drug administration period, and behavioral assessments; (b) heart rate variability on the last day of the pairing period, isolation period, days 5, 10, and 14 of the drug administration period, and behavioral assessments; (c) physical activity on the last day of the pairing period, isolation period, days 5, 10, and 14 of the drug administration period, and behavioral assessments. This analysis was used to compare within-group and between-subject changes in cardiac function and physical activity measures across the experimental time periods.

Student's *t*tests with group (control/drug) as the independent variable or time as the repeated measures variable were used for (a) behaviors and cardiac variables (heart rate, heart rate variability) during the tail-suspension test and the forced swim test; (b) manually scored tail-suspension and forced swim test behaviors (as described above); and

(c) physical activity during the tail-suspension and forced swim tests. Two types of *t*test were used to analyze differences in dependent variable scores (e.g., heart rate) between groups or within groups. The first was an independent-groups *t*test, used to compare unrelated groups in only one measure. The second was a paired-samples *t*test, used to compare dependent variables (e.g., heart rate) for the same subject at different time points.

In order to quantitatively evaluate the relative strength of the statistical findings effect sizes are also reported. Effect sizes were calculated for statistically significant findings. To assess effect sizes, a Cohen's *d* was used with 0.2 (small), 0.5 (medium), and 0.8 (large) results considered as qualitative measures of the effect (Green, Salkind, & Jones, 1996; Rice & Harris, 2005). To assess the effect size of a mixed-design analysis of variance, a Partial Eta squared was used with 0.02 (small), 0.13 (medium), and 0.26 (large) results considered as qualitative measures of the effect (Green et al., 1996). These values were chosen because when assessing analyses of variance main effects, Cohen's *d* can report smaller effects than are actually observed (Green et al., 1996).

Finally, correlations between manually scored behavioral responses and physical activity were assessed using a Pearson *r* correlation coefficient to determine the strength of the relationship between those two variables. This procedure was used to determine the strength of a linear relationship between variables, and thus whether physical activity measured by the radiotelemetry transmitters was comparable to coding observable behaviors for measuring behavioral activity in the forced swim test and tail-suspension test.

CHAPTER 4

RESULTS

General summary of results

The results of the current experiment indicate that socially isolated male prairie voles display disrupted cardiac function related to depression, but that sertraline hydrochloride administration was only partially effective in treating it. To begin, all isolated male prairie voles displayed increased heart rate, and sertraline hydrochloride administration lowered it, though not to a statistically significant degree. When reviewing the data for the drug administration period, the sertraline hydrochloride group was observed to display a higher heart rate with little difference in heart rate variability and physical activity, for at least the first five hours after the injection. However, this acute increase in heart rate following the drug injection (versus the vehicle group) was lowered on the 14th day of the drug administration period. For all male prairie voles (regardless of drug treatment) there were typically no statistically significant changes in heart rate variability during the baseline, pairing, isolation, and later portion of the drug injection periods of the experiment. The notable exception to this was that all male prairie voles isolated from their female partners displayed a lowering of heart rate variability on day five of the drug injection period. This decrease in heart rate variability was significantly

lower in all isolated male prairie voles (regardless of drug treatment), relative to their own heart rate variability in the pairing period.

During the tail-suspension test, the sertraline hydrochloride group displayed a slightly lower heart rate and higher heart rate variability across the trial versus the vehicle group; however, this group difference was not statistically significant. Both groups displayed a decrease in heart rate variability across time in the tail-suspension test. There was no difference in manually scored behavioral responses or physical activity during the tail-suspension test. Following the tail-suspension test, the sertraline hydrochloride group took longer to recover to baseline cardiac values following the behavioral stressor, but subsequently displayed a slightly higher heart rate variability. During the forced swim test both groups displayed similar heart rates, heart rate variability, and manually scored behavioral responses to the stressor. However, the sertraline hydrochloride group displayed a slightly higher heart rate and lower heart rate variability, but this group difference was not statistically significant. Further, the sertraline hydrochloride group displayed significantly less physical activity during the first three minutes of the forced swim test. The following sections will present these specific findings in greater detail.

Basal cardiac function and measures of physical activity during experimental periods

Summary

Male prairie voles in the sertraline hydrochloride group did not display significantly different cardiac function from the vehicle group throughout the different

experimental periods. However, after male prairie voles were isolated from their female partners during the isolation and drug administration periods, the sertraline hydrochloride group displayed a slightly, but not significantly, lower heart rate and higher heart rate variability. During the drug administration period, the sertraline hydrochloride group displayed higher heart rates in the five hours after the injection on days 5 and 10, but not 14. This finding occurred with little difference in heart rate variability and physical activity between the groups. When male prairie voles were isolated from a female partner, basal heart rate variability was largely unaffected across the baseline, pairing, isolation, and later portions of the drug injection periods of the experiment. However, all male prairie voles (regardless of drug treatment) displayed a significantly lower heart rate variability on day five of the drug injection period, relative to their own values during the pairing period.

Heart rate during experimental periods

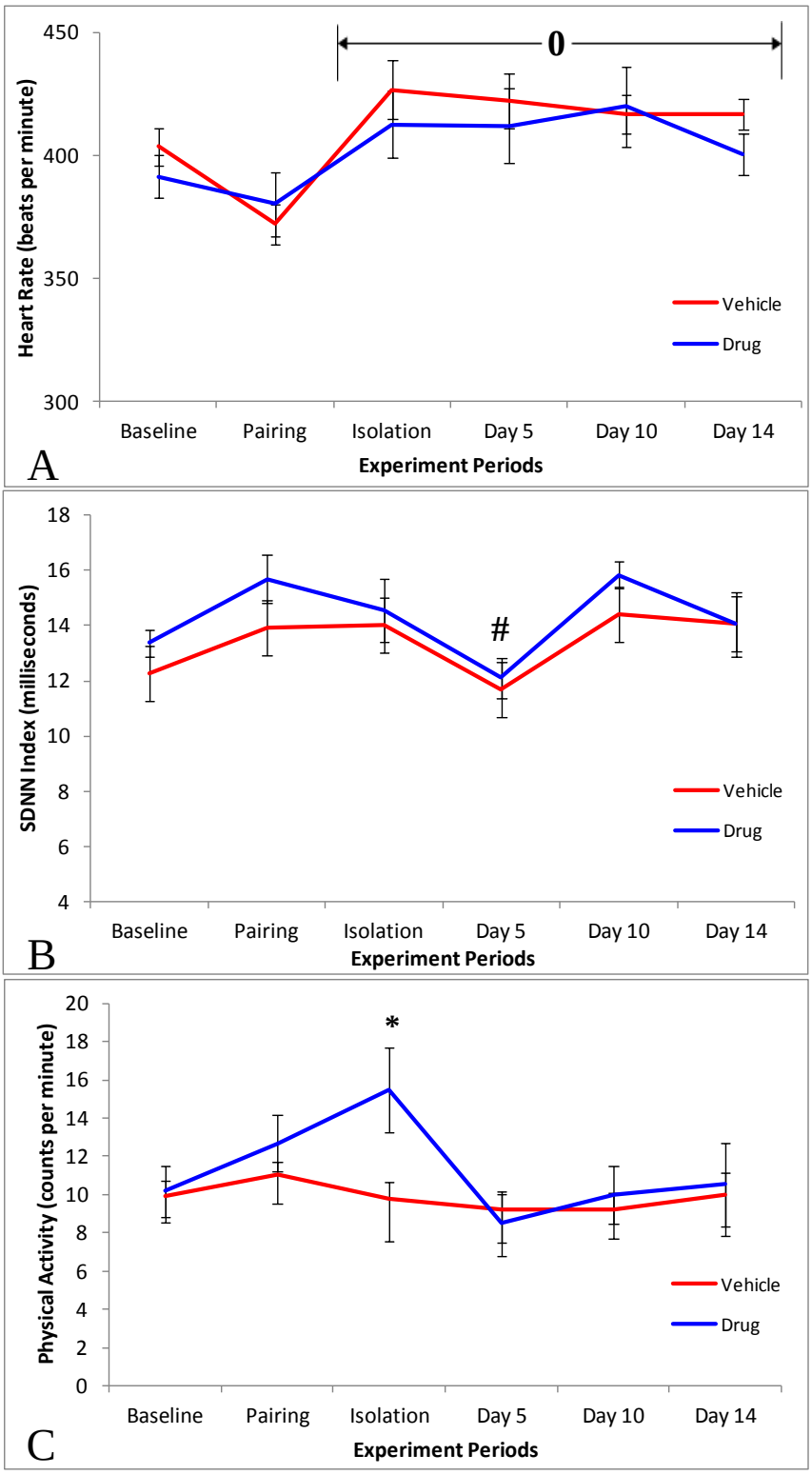
The basal measures of heart rates were assessed on the final day of the baseline, pairing, and isolation periods, and days 5, 10, and 14 of the drug injection period. Both groups displayed similar heart rates throughout the different periods of the experiment. Social isolation significantly increased heart rates in isolated male prairie voles, relative to their own scores during the pairing period. The sertraline hydrochloride group

displayed a slightly, but not significantly, lower heart rate than the vehicle group at the end of the drug injection period.

The mixed-design analysis of variance for heart rate (Figure 03A) yielded a significant main effect for the different periods of the experiment [$F(5,105) = 10.210$, $p = 0.001$, Partial Eta squared = 0.327 (large effect size)], but no main effect for group [$F(1,21) = 0.315$, $p = 0.581$], and no interaction between group and periods of the experiment [$F(5,105) = 0.905$, $p = 0.481$]. These results indicate that while the sertraline hydrochloride group displayed a lower heart rate than the vehicle group, it was not statistically significant. Based on the *a priori* hypothesis that both groups would display an increase in heart rate following removal of the female prairie vole partner (i.e., isolation) and that the sertraline hydrochloride group would be protected from this adverse change, both groups were further assessed with *t*tests.

To determine if isolation from the female prairie vole partners induced increased heart rates for all male prairie voles, the sertraline hydrochloride and vehicle groups were combined in a paired-samples *t*test for the experimental time points during which the males were isolated from their female partners. Specifically, all male prairie vole heart rates on the last day of the pairing period were compared to their own scores on the last day of the isolation period, and days 5, 10, and 14 of the drug injection period. Relative to their own pairing period values, isolated male prairie voles displayed a significant increase in heart rate during the isolation period [$t(1,22) = -7.943$, $p = 0.001$, Cohen's d =

Figure 03. Mean (± SEM) heart rate, heart rate variability, and physical activity of male prairie voles across the baseline, pairing, and isolation periods, and 14 days of either sertraline hydrochloride (16 mg/kg) or vehicle administration. A= Heart rate during the experiment periods, B = Heart rate variability during the experiment periods, and C = Physical activity during the experiment periods. SDNN = standard deviation of the beat-to-beat intervals. **0** = a significant increase in male prairie vole heart rate across the isolation, and drug injection periods, relative to their own values during the pairing period. **#** = a significant decrease in isolated male prairie vole heart rate variability on the fifth day of the drug injection period, relative to their own values during the pairing period. ***** = a significantly higher physical activity for the sertraline hydrochloride group during the isolation period, versus the vehicle group.


450
400
350
300
Heart Rate (beats per minute)
0
Vehicle
Drug
Baseline
Pairing
Isolation
Day 5
Day 10
Day 14
Experiment Periods
A
18
16
14
12
10
8
6
4
SDNN Index (milliseconds)
#
Vehicle
Drug
Baseline
Pairing
Isolation
Day 5
Day 10
Day 14
Experiment Periods
B
20
18
16
14
12
10
8
6
4
2
0
Physical Activity (counts per minute)
*
Vehicle
Drug
Baseline
Pairing
Isolation
Day 5
Day 10
Day 14
Experiment Periods
C

-1.116 (large effect size)], and on day 5 [$t(1,22) = -5.506$, $p = 0.001$, Cohen's $d = -1.032$ (large effect size)], day 10 [$t(1,22) = -6.167$, $p = 0.001$, Cohen's $d = -1.095$ (large effect size)], and day 14 [$t(1,22) = -4.246$, $p = 0.001$, Cohen's $d = -1.064$ (large effect size)] of the drug injection period.

Heart rate variability during experimental periods

The basal measures of heart rate variability were assessed on the final day of the baseline, pairing, and isolation periods, and days 5, 10, and 14 of drug injection period. Both groups displayed similar heart rate variability throughout the different periods of the experiment. Social isolation significantly decreased heart rate variability in isolated male prairie voles --relative to their own values during the pairing period-- 10 days after partner removal (i.e., day 5 of the drug injection period), but not at any other period in the experiment. Further, the sertraline hydrochloride group did not display a significant improvement in heart rate variability due to drug treatment.

The mixed-design analysis of variance for heart rate variability (Figure 03B) yielded a significant main effect for the different periods of the experiment [$F(5,105) = 5.371$, $p = 0.001$, Partial Eta squared = 0.204 (medium effect size)], but no main effect for group [$F(1,21) = 0.738$, $p = 0.400$], and no interaction between group and periods of the experiment [$F(5,105) = 1.191$, $p = 0.319$]. These results indicate that while the sertraline hydrochloride group displayed a higher heart rate variability score than the

vehicle group, it was not statistically significant. Based on the *a priori* hypothesis that both groups would display a decrease in heart rates following the isolation period and that the sertraline hydrochloride group would be protected from this adverse change, both groups were further assessed with *t*tests.

To determine if removal of the female prairie vole partners resulted in decreased heart rate variability for all male prairie voles, the sertraline hydrochloride and vehicle groups were combined in a paired-samples *t*test for the experimental time points during which the males were isolated from their female partners. Specifically, all male prairie vole heart rate variability scores on the last day of the pairing period were compared to their own scores on the last day of the isolation period, and days 5, 10, and 14 of the drug injection period. Isolated male prairie voles displayed a significantly lower heart rate variability on day 5 of the drug injection period (i.e., 10 days after all male prairie voles were isolated) [$t(1,22) = -5.506$, $p = 0.001$, Cohen's $d = 0.840$ (large effect size)], relative to their own values during the pairing period. However, male prairie vole (regardless of drug condition) heart rate variability during the last day of the isolation period [$t(1,22) = 1.589$, $p = 0.126$], and day 10 [$t(1,22) = 0.570$, $p = 0.575$] and day 14 [$t(1,22) = 1.567$, $p = 0.131$] of the drug injection period were not significantly different from the final day of the pairing period. These finding indicates that all isolated male prairie voles displayed a decrease in heart rate variability during day 5 of the drug injection period, followed by a return to pre-isolation values.

Physical activity during experimental periods

The basal measures of physical activity were assessed on the final day of the baseline, pairing, and isolation periods, and days 5, 10, and 14 of drug injection period. Physical activity for each period of the experiment was calculated by averaging the mean hourly physical activity for 24 hours into one score per animal, per day. Both groups displayed a similar average 24 hour physical activity throughout all experimental periods. However, during the isolation period, the sertraline hydrochloride group displayed a greater average 24 hour physical activity level than the vehicle group. This is likely due to one animal in the sertraline hydrochloride group displaying a much larger physical activity measure than all other animals.

The mixed-design analysis of variance for the mean 24 hour physical activity (Figure 03C) yielded a significant main effect for periods of the experiment [$F(5,105) = 5.475$, $p = 0.001$, Partial Eta squared = 0.207 (medium effect size)], and a significant interaction between group and periods of the experiment [$F(5,105) = 3.296$, $p = 0.008$, Partial Eta squared = 0.136 (medium effect size)], but no main effect for group [$F(1,21) = 0.815$, $p = 0.377$]. These results indicate that for the majority of experimental periods both groups displayed similar physical activity; however, during one of the periods the sertraline hydrochloride group displayed more physical activity than the vehicle group.

To test the *a priori* hypothesis that there would be no group difference in physical activity during experimental conditions, independent-groups *t*tests were used to assess

group differences during the periods of the experiment. During the isolation period the sertraline hydrochloride group displayed significantly greater [$t(21) = -2.433$, $p = 0.024$, Cohen's $d = -0.983$ (large effect size)] physical activity than the vehicle group. It should be noted, during that period one animal (study identity 01c2) in the sertraline hydrochloride group displayed a mean 24 hour physical activity count twice as high as the highest animal score in the vehicle group. However, the average 24 hour physical activity of the two groups did not differ during any other period of the experiment ($p > 0.05$).

Acute post-injection heart rate changes

During data analysis, the sertraline hydrochloride group was observed displaying increased heart rates in the hours following the injection (drug or vehicle). In order to explore this unexpected observation, *post hoc* analyses were conducted to quantify the changes of heart rate, heart rate variability, and physical activity during the 5 hours immediately following injection times (either sertraline hydrochloride or vehicle) on the 5th, 10th, and 14th days of the drug injection period. In general, the sertraline hydrochloride group displayed a significantly higher heart rates following the drug or vehicle injections on days 5 and 10 of the drug injection period; however, this change was attenuated on day 14 of the drug injection period. There were no significant group differences in heart rate variability or physical activity. Information on each individual day and a comparison of all three days is detailed below.

Five hours post-injection on day five of the drug injection period

On day five of the drug injection period, the sertraline hydrochloride group displayed a significant increase in heart rate but not heart rate variability, compared to the vehicle group in the five hours after the drug or vehicle injection. Further, both groups displayed a decrease in physical activity in the hours following the drug or vehicle injection. However, the vehicle group tended to display more physical activity, but this difference was not statistically significant.

On day 5 of the drug injection period, the mixed-design analysis of variance for heart rate in the exploratory post-injection time period (5 hours after drug or vehicle injection) (Figure 04A) yielded a significant main effect for group [$F(1, 20) = 7.439$, $p = 0.013$], but not for hours after injection [$F(4,80) = 1.186$, $p = 0.324$], and no interaction between group and hours after injection [$F(4,80) = 0.472$, $p = 0.756$]. This indicates that the sertraline hydrochloride group displayed a significantly higher heart rate across all five hours of the exploratory post-injection time period. The mixed-design analysis of variance for heart rate variability in the exploratory post-injection time period (5 hours after drug or vehicle injection) yielded no main effect for group [$F(1, 21) = 0.834$, $p = 0.371$], hours after injection [$F(4,84) = 0.620$, $p = 0.649$], and no interaction between group and hours after injection [$F(4,84) = 0.497$, $p = 0.738$]. This indicates that heart rate variability did not differ between groups or across the five hours following the drug or

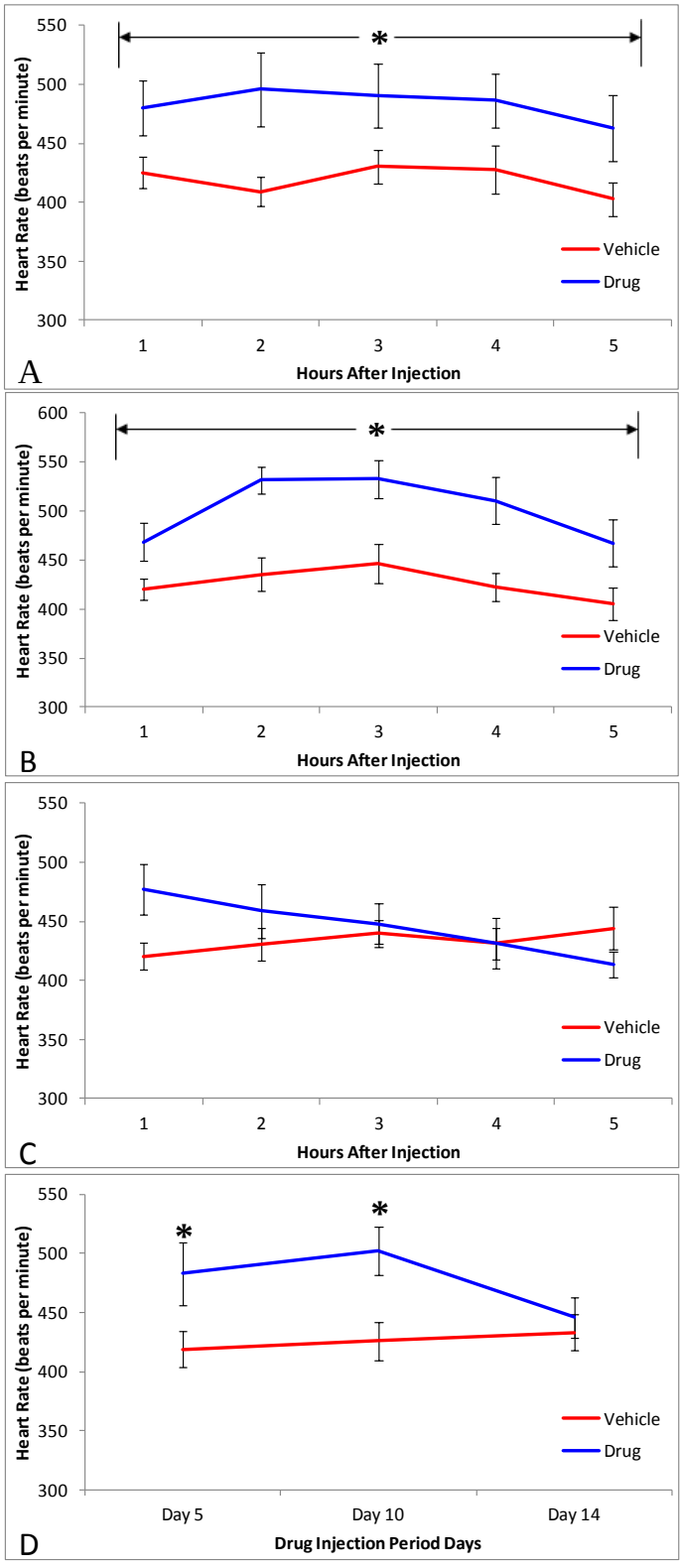
vehicle injection. Finally, the mixed-design analysis of variance for physical activity in the exploratory post-injection time period (5 hours after drug or vehicle injection) yielded a significant main effect for hours after injection [$F(4,80) = 7.640$, $p = 0.001$, Partial Eta = 0.276, (large effect size)], but no main effect for group [$F(1, 20) = 7.439$, $p = 0.061$], and no interaction between group and hours after injection [$F(4,80) = 1.908$, $p = 0.117$]. This indicates that physical activity decreased in the hours following drug injection for all isolated male prairie voles.

5 hours post-injection on day 10 of the drug injection period

On day 10 of the drug injection period, the sertraline hydrochloride group displayed a significantly greater heart rate compared to the vehicle group in the 5 hours following drug or vehicle injection. Additionally, both groups displayed a subsequent decrease in heart rate in the hours following the drug or vehicle injection on day 10 of the drug injection period. There were no between group differences in heart rate variability or physical activity.

On day 10 of the drug injection period, the mixed-design analysis of variance for heart rate in the exploratory post-injection time period (5 hours after drug or vehicle injection) (Figure 04B) yielded a significant main effect for group [$F(1, 21) = 17.88$, $p = 0.001$, Partial Eta squared = 0.992 (large effect size)], and hours after injection [$F(4,84) = 5.112$, $p = 0.001$, Partial Eta squared = 0.196 (medium effect size)], but no interaction

Figure 04. Mean (± SEM) heart rate of isolated male prairie voles in the exploratory post-injection time period 5 hours after drug (sertraline 16 mg/kg) or vehicle injection on days 5, 10, and 14 of the drug injection period. A = Heart rate on day 5 of the drug injection period, B = Heart rate on day 10 of the drug injection period, C = Heart rate on day 14 of the drug injection period, and D = Average heart rate on days 5, 10, and 14 of the drug injection period. ***** = significantly higher heart rate for the sertraline hydrochloride group, compared to the vehicle group.


550
500
450
400
350
300
Heart Rate (beats per minute)
*
Vehicle
Drug
1
2
3
4
5
Hours After Injection
A
600
550
500
450
400
350
300
Heart Rate (beats per minute)
*
Vehicle
Drug
1
2
3
4
5
Hours After Injection
B
550
500
450
400
350
300
Heart Rate (beats per minute)
Vehicle
Drug
1
2
3
4
5
Hours After Injection
C
550
500
450
400
350
300
Heart Rate (beats per minute)
*
*
Vehicle
Drug
Day 5
Day 10
Day 14
Drug Injection Period Days
D

between group and hours after injection [$F(4,84) = 0.937$, $p = 0.447$]. This indicates that the sertraline hydrochloride group displayed a significantly higher heart rate across all five hours of the exploratory postinjection time period and that both groups' heart rates decreased in the hours following the drug injection.

The mixed-design analysis of variance for heart rate variability in the exploratory post-injection time period (5 hours after drug or vehicle injection) yielded no main effect for group [$F(1, 18) = 0.728$, $p = 0.405$], hours after injection [$F(4,72) = 0.212$, $p = 0.931$], and no interaction between group and hours after injection [$F(4,72) = 0.549$, $p = 0.701$]. This indicates that heart rate variability did not differ between groups or across the five hours following the drug or vehicle injection. Finally, the mixed-design analysis of variance for physical activity in the exploratory post-injection time period (5 hours after drug or vehicle injection) yielded no significant main effect of group [$F(1, 21) = 0.153$, $p = 0.699$], hours after injection [$F(4,84) = 2.305$, $p = 0.065$], and no interaction between group and hours after injection [$F(4,80) = 1.277$, $p = 0.286$]. This indicates that there was no difference in physical activity between groups or in the hours following drug or vehicle injection.

5 hours post-injection on day 14 of the drug injection period

On day 14 of the drug injection period there were no group differences in heart rates, heart rate variability, or physical activity in the hours after the drug or vehicle injection.

On day 14 of the drug injection period, the mixed-design analysis of variance for heart rate in the exploratory post-injection time period (5 hours after drug or vehicle injection) (Figure 04C) yielded no main effect for group [$F(1, 8) = 1.526$, $p = 0.252$], hours after injection [$F(4,32) = 0.650$, $p = 0.631$], and no interaction between group and hours after injection [$F(4,32) = 1.473$, $p = 0.233$]. This indicates that the groups did not display a significant change in heart rate in the hours following the drug injection. The mixed-design analysis of variance for heart rate variability in the exploratory post-injection time period (5 hours after drug or vehicle injection) yielded no main effect for group [$F(1, 8) = 0.891$, $p = 0.373$], hours after injection [$F(4,32) = 1.559$, $p = 0.209$], and no interaction between group and hours after injection [$F(4,32) = 0.945$, $p = 0.451$]. This indicates that heart rate variability did not change in the hours following the drug or vehicle injection. Finally, the mixed-design analysis of variance for physical activity in the exploratory post-injection time period (5 hours after drug or vehicle injection) yielded no main effect for group [$F(1, 8) = 0.891$, $p = 0.373$], hours after injection [$F(4,32) = 1.559$, $p = 0.209$], and no interaction between group and hours after injection [$F(4,32) = 0.945$, $p = 0.451$]. This indicates that there was no difference in physical activity between groups or across the hours following drug injection.

Average heart rate for days 5, 10, and 14 of the drug injection period

To compare group differences in heart rate across days 5, 10, and 14 of the drug injection period, the five hourly heart rate samples collected following drug injection were averaged into one score per day, per animal. The sertraline hydrochloride group displayed a significantly higher heart rate following the drug or vehicle injection on days 5 and 10 of the drug injection period; however, that change was attenuated on day 14 of the drug injection period.

The mixed-design analysis of variance for mean daily heart rate for the exploratory post-injection 5 hour time period (5 hours after drug or vehicle injection) (Figure 04D) yielded a significant main effect for group [$F(1, 21) = 12.602$, $p = 0.002$, Partial Eta = 0.375 (large effect)], and a significant interaction between group and hour after injection [$F(2,42) = 3.386$, $p = 0.029$, Partial Eta of 0.154 (medium effect)], but no main effect for day of the drug injection period [$F(2,42) = 1.966$, $p = 0.153$]. These findings indicate the sertraline hydrochloride group initially displayed a higher heart rate in the hours following drug or vehicle injections on days 5 and 10, versus the vehicle group.

Tail-suspension test

The tail-suspension test was performed one hour after sertraline hydrochloride or vehicle injection. This five minute behavioral assessment used video, physical activity, and electrocardiographic data to assess subject responses to the stressor. Following the tail-suspension test, a recovery period of 22 hours was used to assess the time to recover to basal cardiac activity levels following the stressor. During the tail-suspension test and recovery period, heart rate, heart rate variability, and physical activity were analyzed. Additionally, manually scored active behaviors and physical activity measures during the tail-suspension test were compared to determine their relationship. There was no statistically significant group difference in heart rates, heart rate variability, manually scored behavioral responses, or physical activity in the tail-suspension test. However, the sertraline hydrochloride group exhibited a slightly lower heart rate and higher heart rate variability versus the vehicle group during the assessment. During the recovery period there was no difference in physical activity; however, the sertraline hydrochloride group displayed a longer latency to return to basal heart rate levels. Conversely, during the later portion of the recovery period the sertraline hydrochloride group also displayed a slightly higher heart rate variability. Finally, during the tail-suspension test manually scored active behaviors and physical activity measures did not agree with one another.

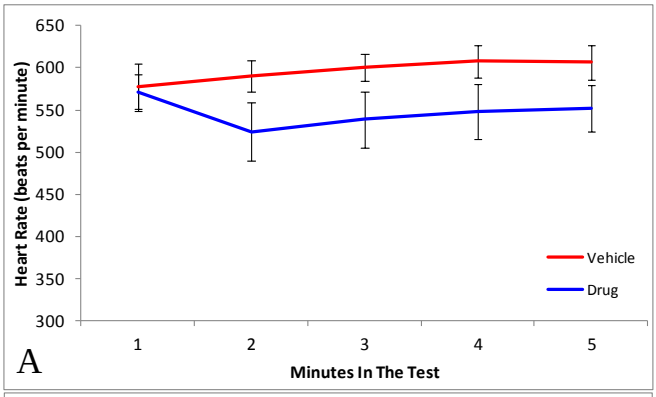
Heart rate during the tail-suspension test

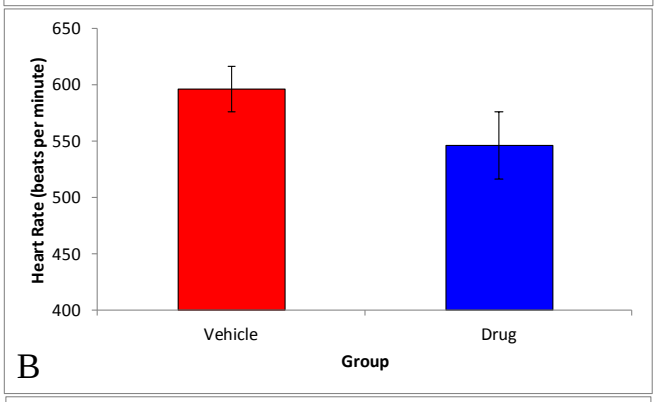
There were no statistically significant group heart rate differences during the tail-suspension test. However, the sertraline hydrochloride group exhibited a slightly lower heart rate than the vehicle group.

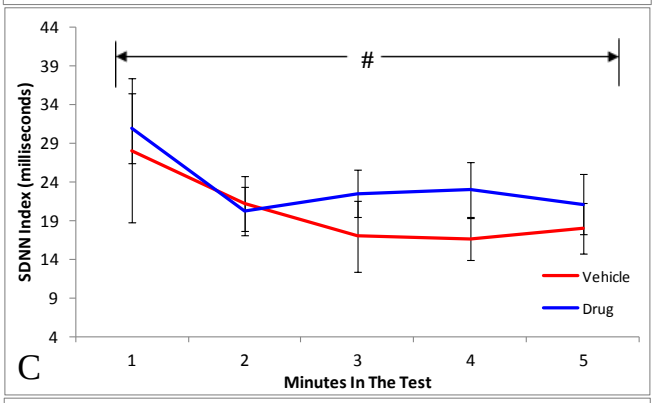
The mixed-design analysis of variance for heart rate (Figure 05A) yielded no main effect for group [$F(1, 20) = 2.458$, $p = 0.133$], minutes in the test [$F(4,80) = 0.771$, $p = 0.547$], and no interaction between group and minutes in the test [$F(4,80) = 1.451$, $p = 0.225$]. These results indicate that there were no within or between group heart rate differences. Based on the *a priori* hypothesis that male prairie voles would display an increased heart rate during the tail-suspension test and that the sertraline hydrochloride group would be protected from this adverse change by exhibiting a lower heart rate, both groups were further assessed with *t*tests.

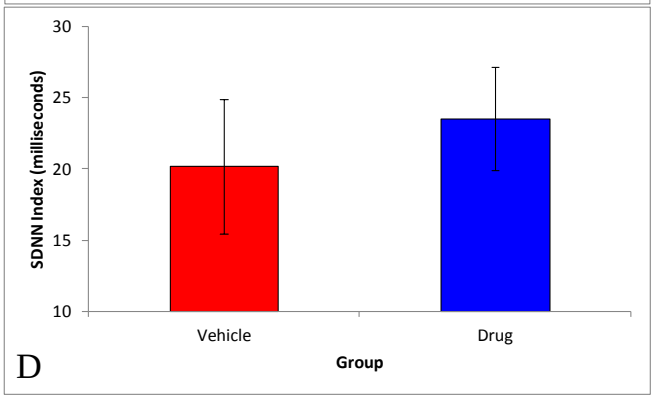
A mean heart rate for all animals was calculated by averaging their heart rate from the entire five minute time span of the tail-suspension test into one score that was tested between groups. An independent-samples *t*test yielded no significant group difference [$t(20) = 1.568$, $p = 0.113$] in the average heart rate for the 5 minute tail-suspension test (Figure 05B). This indicates that sertraline hydrochloride did not significantly decrease heart rate during the tail-suspension test versus the vehicle groups.

Figure 05. Mean (± SEM) heart rate and heart rate variability of male prairie voles across 5 minutes of the tail-suspension test following 5 days of isolation from a female partner and 14 days of either sertraline hydrochloride (16 mg/kg) or vehicle administration. A = Minute-by-minute measure of mean heart rate during the tail-suspension test, B = Average heart rate for the entire five minutes of the tail-suspension test. C = Minute-by-minute measure of mean heart rate variability during the tail-suspension test, D = Average heart rate variability for the entire five minutes of the tail-suspension test. SDNN = standard deviation of the beat-to-beat intervals. # = a significant decrease in SDNN index across the minutes of the tail-suspension test, for all isolated male prairie voles.


650
600
550
500
450
400
350
300
Heart Rate (beats per minute)
Vehicle
Drug
1
2
3
4
5
Minutes In The Test
A


650
600
550
500
450
400
Heart Rate (beats per minute)
Vehicle
Drug
Group
B


44
39
34
29
24
19
14
9
4
SDNN Index (milliseconds)
#
Vehicle
Drug
1
2
3
4
5
Minutes In The Test
C


30
25
20
15
10
SDNN Index (milliseconds)
Vehicle
Drug
Group
D

Heat rate variability during the tail-suspension test

There were no group heart rate variability differences during the tail-suspension test. All isolated male prairie voles displayed a decrease in heart rate variability at the end of the trial, compared to their own value during the first minute of the assessment. However, the sertraline hydrochloride group exhibited a slightly, but not significantly, higher heart rate variability than the vehicle group during the tail-suspension test.

The mixed-design analysis of variance for heart rate variability (Figure 05C) yielded a significant main effect of minutes in the test [$F(4,80) = 3.066$, $p = 0.021$, Partial Eta squared = 0.133 (medium effect size)], but no main effect for group [$F(1,20) = 0.520$, $p = 0.479$], and no interaction between group and minutes in the test [$F(4,80) = 0.338$, $p = 0.852$]. These results indicate that all isolated male prairie voles displayed a decrease in heart rate variability during the tail-suspension test. Based on the *a priori* hypothesis that both groups would display a decrease in heart rate variability during the tail-suspension test and that the sertraline hydrochloride group would be protected from this adverse change by exhibiting a higher heart rate variability, both groups were further assessed with *t*tests.

A mean heart rate variability for all animals was calculated by averaging their heart rate variability from the entire five minute time span of the tail-suspension test into one score per animal that was tested between groups. An independent-samples *t*test yielded no significant group difference [$t(20) = 1.568$, $p = 0.113$] in the mean heart rate

variability during the 5 minute tail-suspension test (Figure 05D). This indicates that sertraline hydrochloride did not significantly increase heart rate variability during the tail-suspension test, versus administration of vehicle.

Manually scored behavior and physical activity during the tail-suspension test

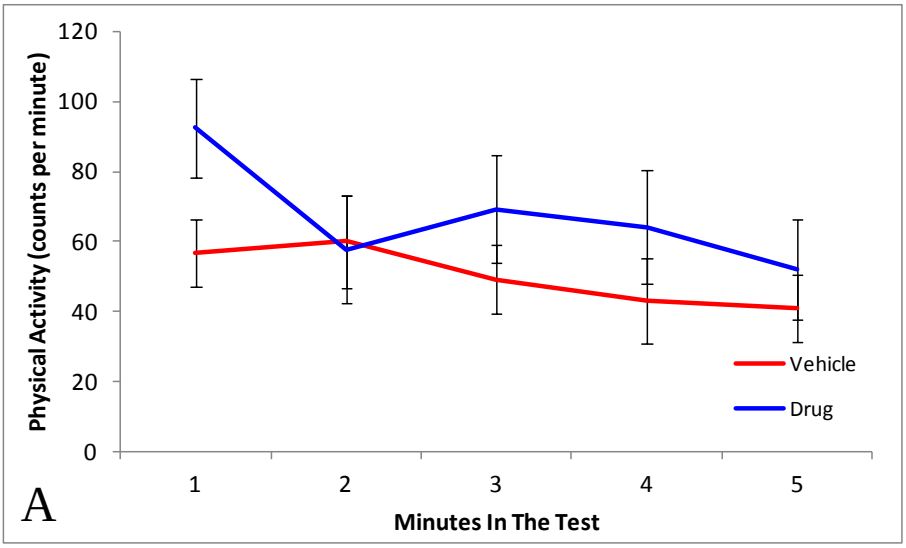
In the video recorded and manually scored behavioral trial, immobility time was compared to active movements of the trunk and/or limbs as an index of behavioral despair, the rodent analogue to human depressive behavior. Both groups displayed similar manually scored behavioral responses (active and immobility measures) and physical activity (generated from the radiotelemetry transmitter) during the tail-suspension test. However, the physical activity measurement did not agree with manually scored active behavior in the tail-suspension test.

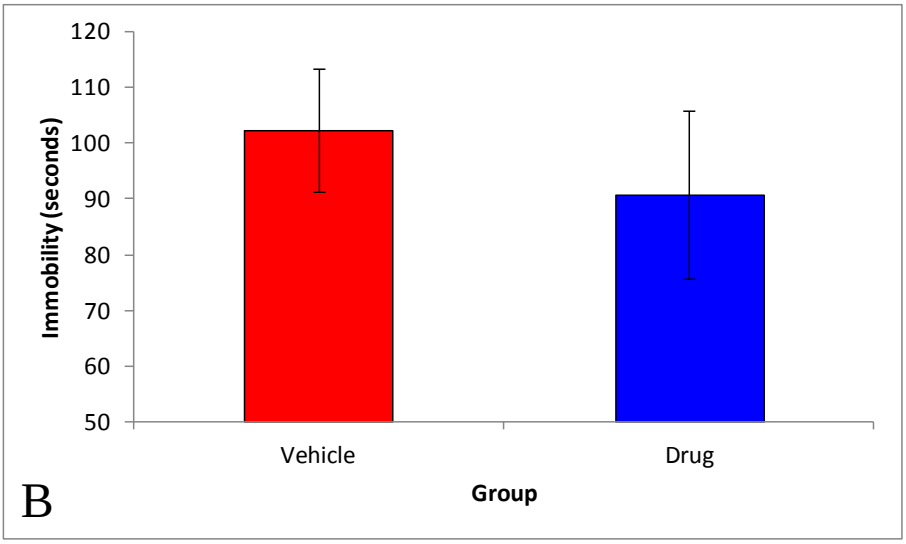
First, when comparing isolated male prairie vole physical activity on a minute-by-minute basis, a mixed-design analysis of variance for physical activity (Figure 06A) yielded no main effect for group [$F(1,19) = 1.952$, $p = 0.178$], minutes in the test [$F(4,76) = 1.695$, $p = 0.160$], and no interaction between group and minutes in the test [$F(4,76) = 0.911$, $p = 0.462$]. These results indicate that there were no within or between group changes in physical activity during the tail-suspension test. Based on the *a priori*

hypothesis that sertraline hydrochloride administration would buffer the adverse behavioral responding in the tail-suspension test, both groups were assessed with *t*tests.

The manually scored immobility behavioral responses were summed for each individual animal during the entire five minute time span of the tail-suspension test into one score that was tested between groups. An independent-groups *t*test (Figure 06B) yielded a non-significant difference between the immobility times of the sertraline hydrochloride and vehicle groups [$t(16) = 0.407$, $p = 0.689$]. This indicates that the immobility levels of isolated male prairie voles did not significantly change during the tail-suspension test. Similarly, the total physical activity score for each animal (i.e., the sum of counts per minute of physical activity across the 5 minute trial; Figure 06C) also yielded no group differences [$t(16) = 0.341$, $p = 0.472$]. The values for all tail-suspension test dependant measures are depicted in Table 02.

Figure 06. Mean (± SEM) immobility behavior and physical activity of male prairie voles across 5 minutes of the tail-suspension test following 5 days of isolation from a female partner and 14 days of either sertraline hydrochloride (16 mg/kg) or vehicle administration. A = Minute-by-minute measure of physical activity during the tail-suspension test, B = Manually scored immobility behavioral response during the tail-suspension test, and C = Average physical activity for the entire five minutes of the tail-suspension test.


120
100
80
60
40
20
0
Physical Activity (counts per minute)
1
2
3
4
5
Minutes In The Test
Vehicle
Drug
A


120
110
100
90
80
70
60
50
Immobility (seconds)
Vehicle
Drug
Group
B

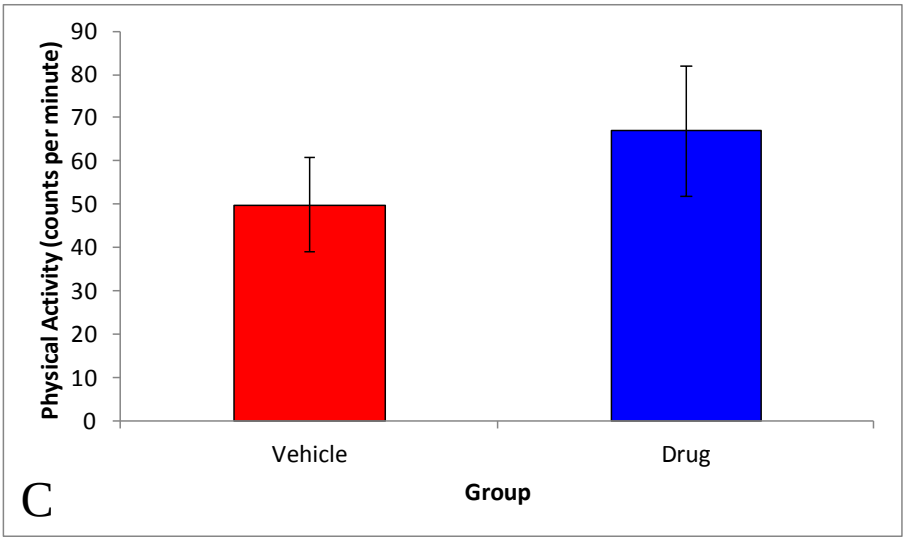

90
80
70
60
50
40
30
20
10
0
Physical Activity (counts per minute)
Vehicle
Drug
Group
C

Table 02. Mean (± SEM) total behavior of male prairie voles across 5 minutes of the tail-suspension test following 5 days of isolation from a female partner and 14 days of either sertraline hydrochloride (16 mg/kg) or vehicle administration.

Behavior	Vehicle Group		Drug Group	
	Mean	SEM	Mean	SEM
Immobility	102.375	18.274	90.800	20.737
Active Behavior	196.00	18.379	207.200	20.728
Total Physical Activity	251.500	51.123	313.000	62.260
Minute 1 Physical Activity	56.719	9.788	92.546	14.227
Minute 2 Physical Activity	60.003	13.125	57.727	15.353
Minute 3 Physical Activity	49.276	10.001	69.273	15.384
Minute 4 Physical Activity	43.182	12.112	64.181	16.199
Minute 5 Physical Activity	40.927	9.571	52.091	14.353

Total physical activity is the summation of the counts per minute score for the entire five minutes of the trial. The physical activity for each minute represents the summation of the counts per minute score for only that minute.

This experiment also assessed the relationship between manually scored active behavior and physical activity measures generated by the transmitters. A statistically significant ($p = 0.005$) Pearson r correlation coefficient of -0.631 (strong negative correlation) between the two measures was observed (Figure 07). In contrast to the initial hypotheses, the measure of physical activity did not agree with manually scored behavior, and rather was inversely correlated.

Tail-suspension test recovery

Data collected during the 22 hours following the tail-suspension test were used to assess recovery of heart rate, heart rate variability, and physical activity after the behavioral test. There was no between group difference in physical activity during this period. However, the sertraline hydrochloride group displayed a longer latency to return heart rate to basal levels following the tail-suspension test. Conversely, the sertraline hydrochloride group displayed a slightly higher heart rate variability in the later portion of the recovery period.

The mixed-design analysis of variance for heart rate in the 22 hours following the tail-suspension test (Figure 08A) yielded a significant main effect for hours after the test [$F(21,357) = 3.106$, $p = 0.001$, Partial Eta squared = 0.154 (medium effect size)], and an interaction between group and hours after the test [$F(21,357) = 1.584$, $p = 0.050$, Partial

Figure 07. Correlation between manually scored behavior and physical activity of male prairie voles across 5 minutes of the tail-suspension test following 5 days of isolation from a female partner and 14 days of either sertraline hydrochloride (16 mg/kg) or vehicle administration.

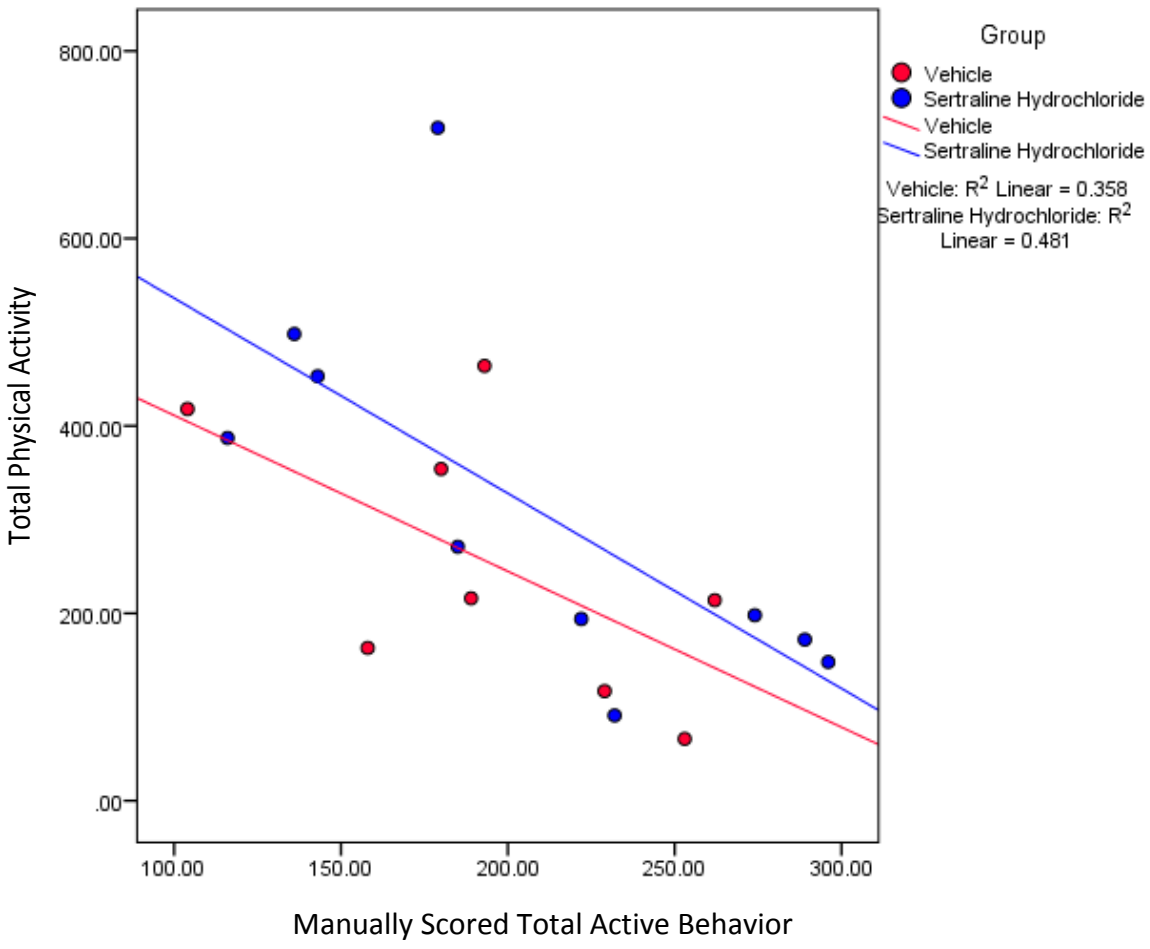
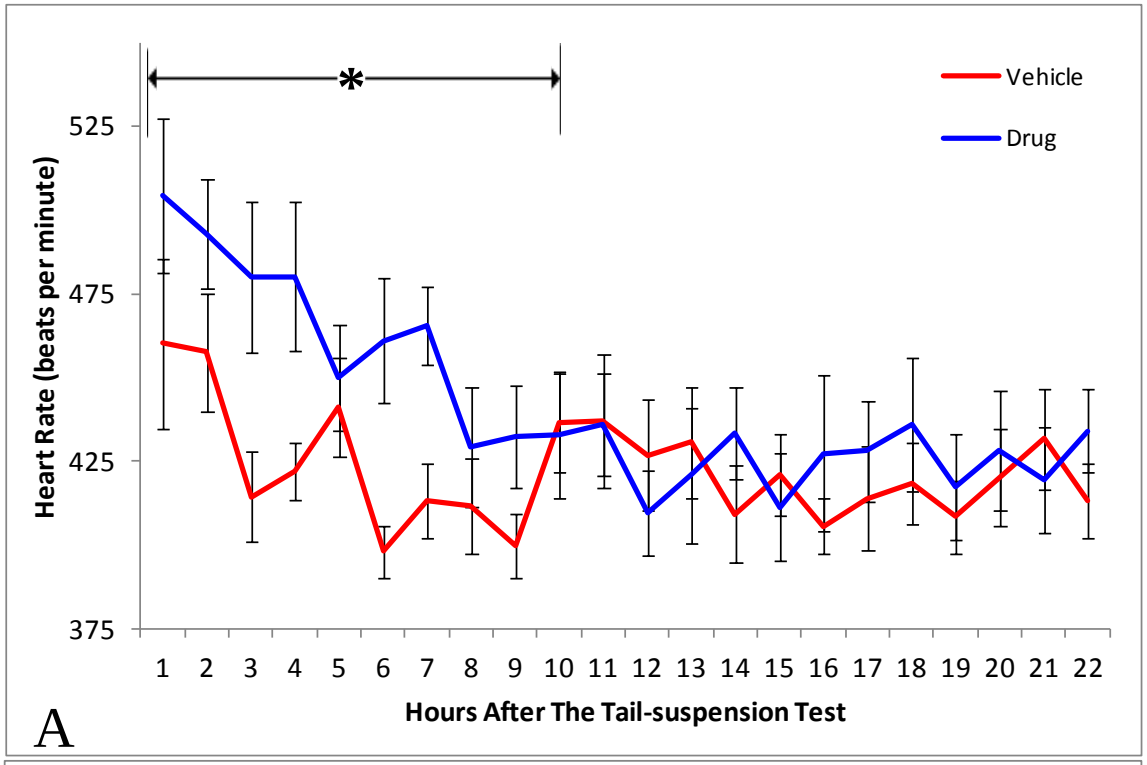
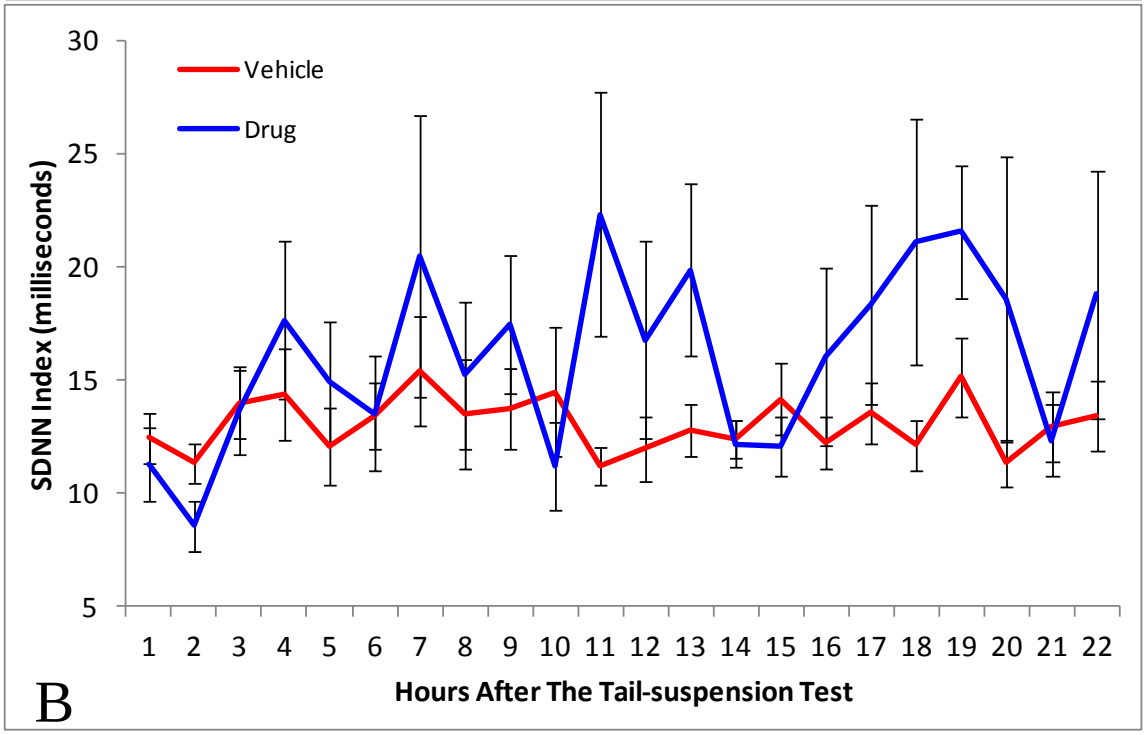

Group
Vehicle
Sertraline Hydrochloride
Vehicle
Sertraline Hydrochloride
Vehicle: R2 Linear = 0.358
Sertraline Hydrochloride: R2 Linear = 0.481
800.00
600.00
400.00
200.00
.00
Total Physical Activity
100.00
150.00
200.00
250.00
300.00
Manually Scored Total Active Behavior

Figure 08. Mean (± SEM) heart rate and heart rate variability of male prairie voles in the 22 hours after the tail-suspension test, following 5 days of isolation from a female partner and 14 days of either sertraline hydrochloride (16 mg/kg) or vehicle administration. A = Heart rate in the tail-suspension test recovery period and B = Heart rate variability in the tail-suspension test recovery period. SDNN = standard deviation of the beat-to-beat intervals. ***** = a significant increase in latency for the sertraline hydrochloride group in returning to baseline heart rate.


Vehicle
Drug
*
525
475
425
375
Heart Rate (beats per minute)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Hours After The Tail-suspension Test
A


Vehicle
Drug
30
25
20
15
10
5
SDNN Index (milliseconds)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Hours After The Tail-suspension Test
B

Eta squared = 0.085 (small effect size)], but no main effect for group [$F(1,17) = 0.965$, $p = 0.340$]. These results indicate that the sertraline hydrochloride group took longer to return to basal heart rate levels after undergoing the tail-suspension test stressor. The mixed-design analysis of variance for heart rate variability (Figure 08B) yielded a significant main effect for hours after the test [$F(21,336) = 2.541$, $p = 0.001$, Partial Eta squared = 0.137 (medium effect size)], but no main effect for group [$F(1,16) = 0.778$, $p = 0.391$], and no interaction between group and hours after the test [$F(21,336) = 1.291$, $p = 0.178$]. These results indicate that the sertraline hydrochloride group displayed a slight increase in heart rate variability as time progressed. Finally, The mixed-design analysis of variance for physical activity yielded no main effect for group [$F(1,17) = 0.118$, $p = 0.741$], hours after the test [$F(21,147) = 0.819$, $p = 0.693$], and no interaction between group and hours after the test [$F(21, 147) = 0.548$, $p = 0.945$]. These results indicate that there were no physical activity differences within or between groups during the recovery period.

Forced swim test

After the tail-suspension test recovery period, a five minute forced swim test was performed. Video, physical activity, and electrocardiographic data were used to assess heart rates, heart rate variability, manually scored behavioral responses and physical activity during the stressor. Additionally, manually scored active behavior and physical

activity were compared during the forced swim test to determine their relationship. There was no statistically significant group difference in heart rate or heart rate variability during the forced swim test. However, the sertraline hydrochloride group exhibited a slightly higher heart rate and lower heart rate variability during the assessment. The groups did not display significantly different mean manually scored behavior or mean physical activity across the entire five minutes of the forced swim test. However, when assessing the minute-by-minute change in physical activity, the sertraline hydrochloride group exhibited less physical activity in the first three minutes of the trial, compared to the vehicle group. Finally, during the forced swim test manually scored behavior and physical activity measures strongly agreed with one another.

Heart rate during the forced swim test

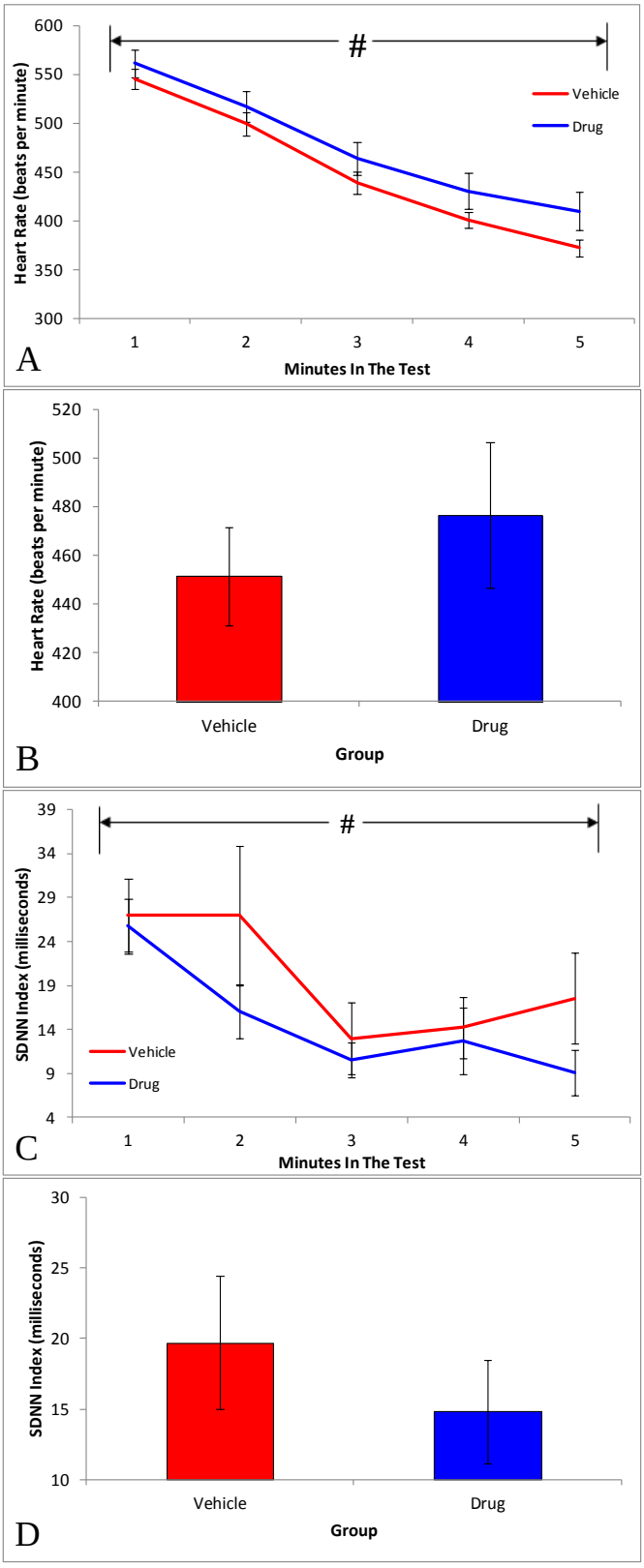
Both the sertraline hydrochloride and vehicle groups displayed a similar heart rate throughout the forced swim test, with both groups displaying a decrease in heart rate across the trial. The decrease in heart rates exhibited by all isolated male prairie voles was expected, as cardiac function is known to decrease in correlation with a body temperature decrease in an environment that is colder than the animal's core body temperature (Budd & Warhaft, 1966; Edelhäuser, Goebel, Scheffer, & Cysarz, 2012). Further, this finding has been previously observed in male prairie voles (McNeal et al.,

2014). Contrary to expectations, the sertraline hydrochloride group displayed a slightly higher heart rate throughout the trial, versus the vehicle group.

The mixed-design analysis of variance for heart rate (Figure 09A) yielded a significant main effect of minutes in the test [$F(4,80) = 226.131$, $p = 0.001$], but no main effect for group [$F(1, 20) = 1.903$, $p = 0.183$], and no interaction between group and minutes in the test [$F(4,80) = 0.989$, $p = 0.418$]. These results indicate that all isolated male prairie voles displayed a decrease in heart rate across the minutes of the forced swim test, but there was no statistically significant between group difference. Based on the *a priori* hypothesis that both groups would display a change in heart rate during the forced swim test but that the responses of the sertraline hydrochloride group would be attenuated, both groups were further assessed with *t*tests.

An average heart rate for all animals was calculated by averaging their heart rate from the entire five minute time span of the forced swim test into one score per animal that was tested between groups. An independent-samples *t*test yielded no significant group difference [$t(20) = -1.380$, $p = 0.183$] in the mean heart rate of the 5 minute forced swim test (Figure 09B). This indicates that sertraline hydrochloride did not significantly decrease heart rate during the forced swim test, versus administration of the vehicle.

Figure 09. Mean (± SEM) heart rate and heart rate variability of male prairie voles during the forced swim test that was performed 22 hours after the tail-suspension test, 5 days of isolation from a female partner, and 15 days of either sertraline hydrochloride (16 mg/kg) or vehicle. A = Minute-by-minute measure of mean heart rate during the forced swim test, B = Average heart rate for the entire five minutes of the forced swim test. C = Minute-by-minute measure of mean heart rate variability during the forced swim test, and D = Average heart rate variability for the entire five minutes of the forced swim test. SDNN = standard deviation of the beat-to-beat intervals. # = a significant decrease in heart rate across the minutes of the forced swim test and a significant decrease in SDNN index across the minutes of the forced swim test, for all isolated male prairie voles.


600
550
500
450
400
350
300
Heart Rate (beats per minute)
#
Vehicle
Drug
1
2
3
4
5
Minutes In The Test
A
520
500
480
460
440
420
400
Heart Rate (beats per minute)
Vehicle
Drug
Group
B
39
34
29
24
19
14
9
4
SDNN Index (milliseconds)
#
Vehicle
Drug
1
2
3
4
5
Minutes In The Test
C
30
25
20
15
10
SDNN Index (milliseconds)
Vehicle
Drug
Group
D

Heart rate variability during the forced swim test

There were no heart rate variability differences between the sertraline hydrochloride and vehicle groups during the forced swim test. All isolated male prairie voles displayed a decrease in heart rate variability at the end of the trial, compared to their own values during the first minute of the assessment. Versus the vehicle group, the sertraline hydrochloride group exhibited a slightly, but not significantly, lower heart rate variability during the assessment.

The mixed-design analysis of variance for heart rate variability (Figure 09C) yielded a significant main effect of minutes in the test [$F(4,80) = 9.120$, $p = 0.001$, Partial Eta squared = 0.313 (large effect size)], but no main effect for group [$F(1,20) = 1.120$, $p = 0.303$], and no interaction between group and minutes in the test [$F(4,80) = 1.138$, $p = 0.345$]. These results indicate that all isolated male prairie voles exhibited a decrease in heart rate variability during the forced swim test, but there were no between group differences. Based on the *a priori* hypothesis that isolated male prairie voles would display a decrease in heart rate variability during the forced swim test and that the sertraline hydrochloride group would be protected from this adverse change by displaying an increased heart rate variability, both groups were further assessed with *t*tests.

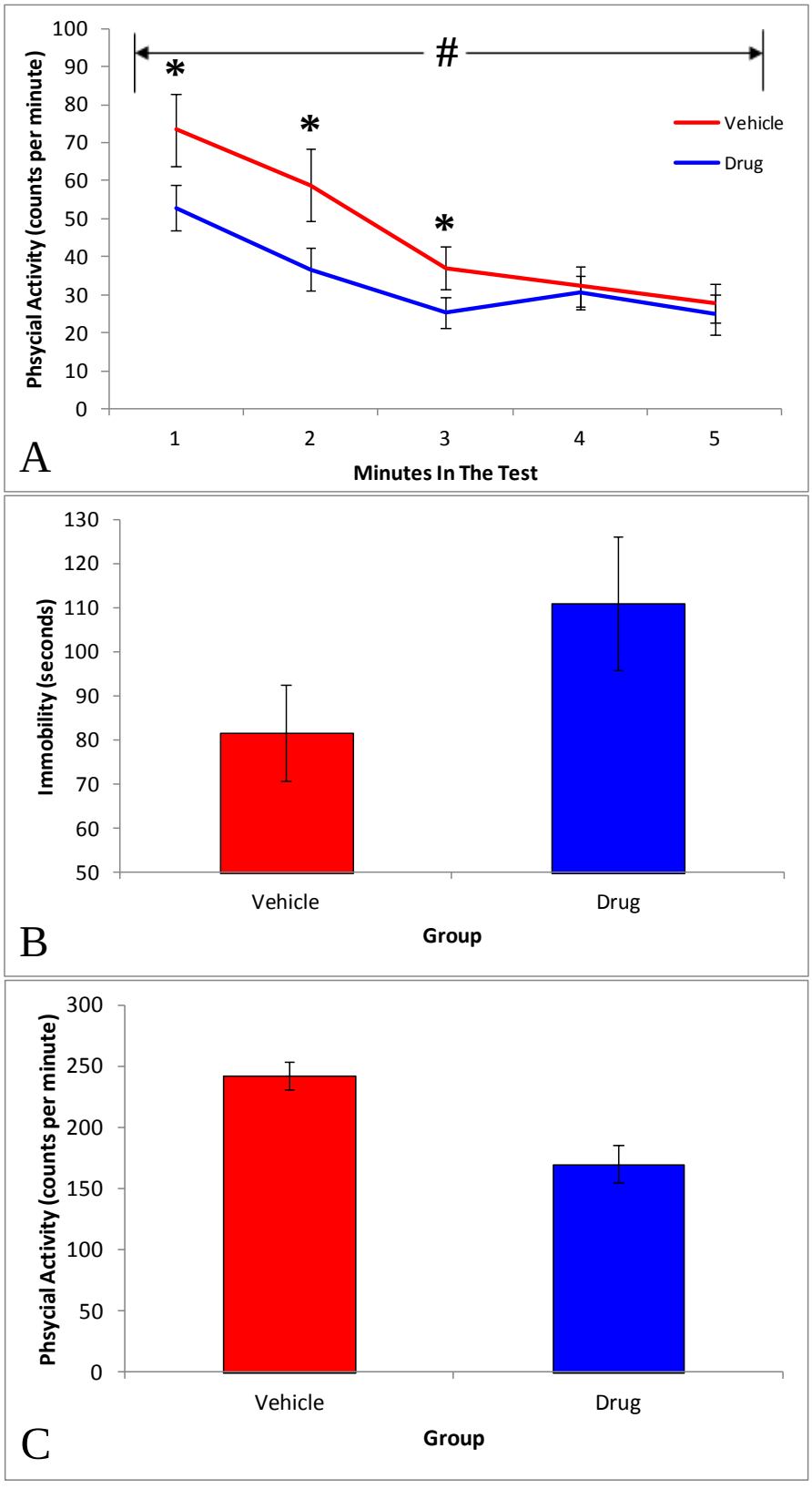
An average heart rate variability for all animals was calculated by averaging their heart rate variability from the entire five minute time span of the forced swim test into one score per animal that was tested between groups. An independent-samples *t*test yielded no significant group difference [$t(20) = 1.058$, $p = 0.303$] in the mean heart rate variability for the 5 minute tail-suspension test (Figure 09D).

Manually scored behavior and physical activity during the forced swim test

In the video recorded and manually scored behavioral trial, immobility (i.e., floating) time was compared to active movements of the subject (i.e., swimming, climbing, and struggling) as an index of behavioral despair, the rodent analogue to human depressive behavior. Both groups displayed similar manually scored behavior and physical activity during the entire five minutes of the forced swim test. However, when assessing the minute-by-minute changes in physical activity, the sertraline hydrochloride group displayed significantly less physical activity in the first three minutes of the trial, compared to the vehicle group. Finally, the physical activity measurement strongly agreed with manually scored active behavior in the forced swim test.

When comparing isolated male prairie vole physical activity on a minute-by-minute basis, a mixed-design analysis of variance for physical activity (Figure 10A) yielded a significant main effect for group [$F(1,19) = 185.919$, $p = 0.001$, Partial Eta

Figure 10. Mean (± SEM) immobility behavior and physical activity of male prairie voles during the forced swim test that was performed 22 hours after the tail-suspension test, 5 days of isolation from a female partner, and 15 days of either sertraline hydrochloride (16 mg/kg) or vehicle injections. A = Minute-by-minute measure of physical activity during the forced swim test, B = Manually scored immobility behavioral response during the forced swim test, and C = Average physical activity for the entire five minutes of the forced swim test. ***** = a significant decrease in sertraline hydrochloride group physical activity, compared to the vehicle group. **#** = a significant decrease in physical activity across the minutes of the forced swim test.


Phsycial Activity (counts per minute)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
Minutes In The Test
#
*
*
*
Vehicle
Drug
A
Immobility (seconds)
130
120
110
100
90
80
70
60
50
Vehicle
Drug
Group
B
Phsycial Activity (counts per minute)
300
250
200
150
100
50
0
Vehicle
Drug
Group
C

squared = 0.907 (large effect size)], minutes in the test [$F(4,76) = 20.871$, $p = 0.001$, Partial Eta squared = 0.523 (large effect size)], and an interaction between group and minutes in the test [$F(4,76) = 3.226$, $p = 0.017$, Partial Eta squared = 0.145 (medium effect size)]. This indicates that the vehicle group displayed more physical activity than the sertraline hydrochloride group at the beginning of the forced swim test and that both groups decreased across the minutes of the assessment. Based on the *a priori* hypothesis that the sertraline hydrochloride group would display more active behavioral responding during the forced swim test, both groups were further assessed with independent-group *t*tests.

First, compared to the vehicle group, the sertraline hydrochloride group displayed decreased physical activity for the first 3 minutes of the forced swim test: minute 1 [$t(20) = 2.138$, $p = 0.045$, Cohen's $d = 1.216$ (large effect size)], minute 2 [$t(20) = 2.734$, $p = 0.013$, Cohen's $d = 1.256$ (large effect size)], minute 3 [$t(20) = 2.377$, $p = 0.028$, Cohen's $d = 1.199$ (large effect size)], but there were no significant physical activity differences between groups in the final 2 minutes: minute 4 [$t(20) = 0.636$, $p = 0.532$] and minute 5 [$t(20) = 0.710$, $p = 0.486$].

Next, the manually scored immobility behavioral responses were summed for all animals during the entire five minute time span of the forced swim test into one score that was compared between groups. An independent-groups *t*test (Figure 10B) yielded no significant difference between the immobility times of the groups [$t(20) = -1.263$, $p = 0.221$]. This indicates that manually scored behavioral responses of the isolated male prairie voles did not significantly change during the forced swim test. Similarly, the total

physical activity (i.e., the sum of counts per minute of physical activity across the 5 minute trial) also yielded no group differences [$t(20) = 0.215$, $p = 0.832$]. These results indicate that there were no statically significant group differences in the sum of active behavioral responses in the forced swim test. Further, because swimming behavior is linked with selective serotonin reuptake inhibitor treatment, it was also independently assessed. There were no significant group differences [$t(20) = -0.877$, $p = 0.391$] observed in the amount of swimming behavior during the forced swim test. All forced swim test behavioral response values are depicted in Table 03.

This experiment also assessed the relationship between manually scored active behavior and physical activity measures generated by the transmitters. A statistically significant ($p = 0.042$) Pearson r correlation coefficient of 0.436 (strong positive correlation) was observed between the two measures (Figure 11). This result indicates that the physical activity count strongly agreed with manually scored behavior in this test.

Body weight during the experiment

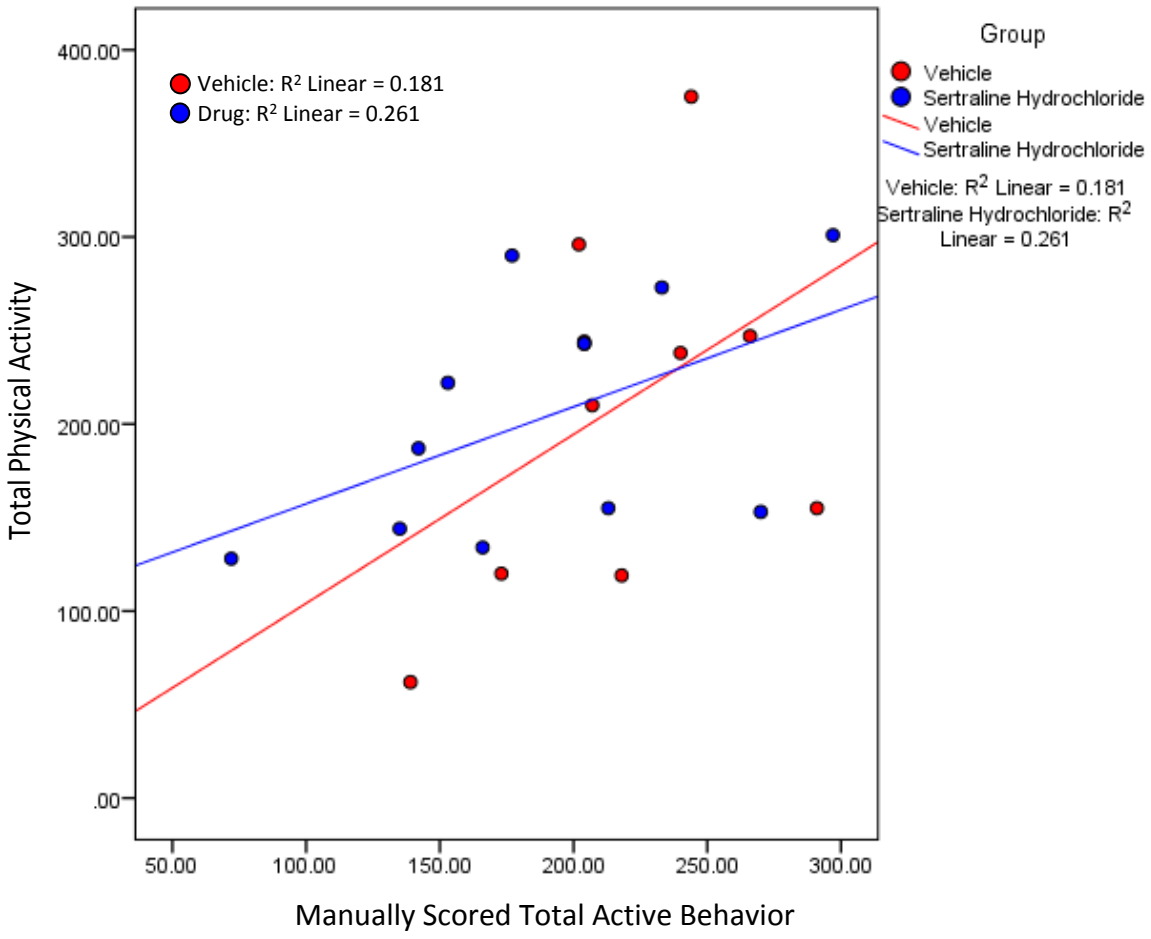
All male prairie voles were weighed at the end of the baseline, pairing, and isolation periods, and on days 5, 10, and 14 days of drug injection period. The sertraline hydrochloride group weighed more during the pairing and isolation periods. However, there were no significant group differences in animal body weight at any other time point in the experiment.

Table 03. Mean (± SEM) total behavior of male prairie voles during the forced swim test that was performed 22 hours after the tail-suspension test, 5 days of isolation from a female partner, and 15 days of either sertraline hydrochloride (16 mg/kg) or vehicle injections.

Behavior	Vehicle Group		Drug Group	
	Mean	SEM	Mean	SEM
Swimming	36.818	6.731	47.273	9.846
Climbing	175.182	13.324	131.636	13.703
Struggling	5.091	1.875	8.546	7.759
Total Active Behavior	217.091	12.728	187.455	19.475
Immobility	81.546	12.675	110.909	19.501
Total Physical Activity	209.909	27.023	202.727	19.762
Minute 1 Physical Activity	73.455	7.484	52.909	6.031
Minute 2 Physical Activity	64.091	8.304	36.636	3.987
Minute 3 Physical Activity	40.091	4.793	25.273	3.987
Minute 4 Physical Activity	34.818	4.793	30.636	3.987
Minute 5 Physical Activity	29.909	4.793	24.818	5.327

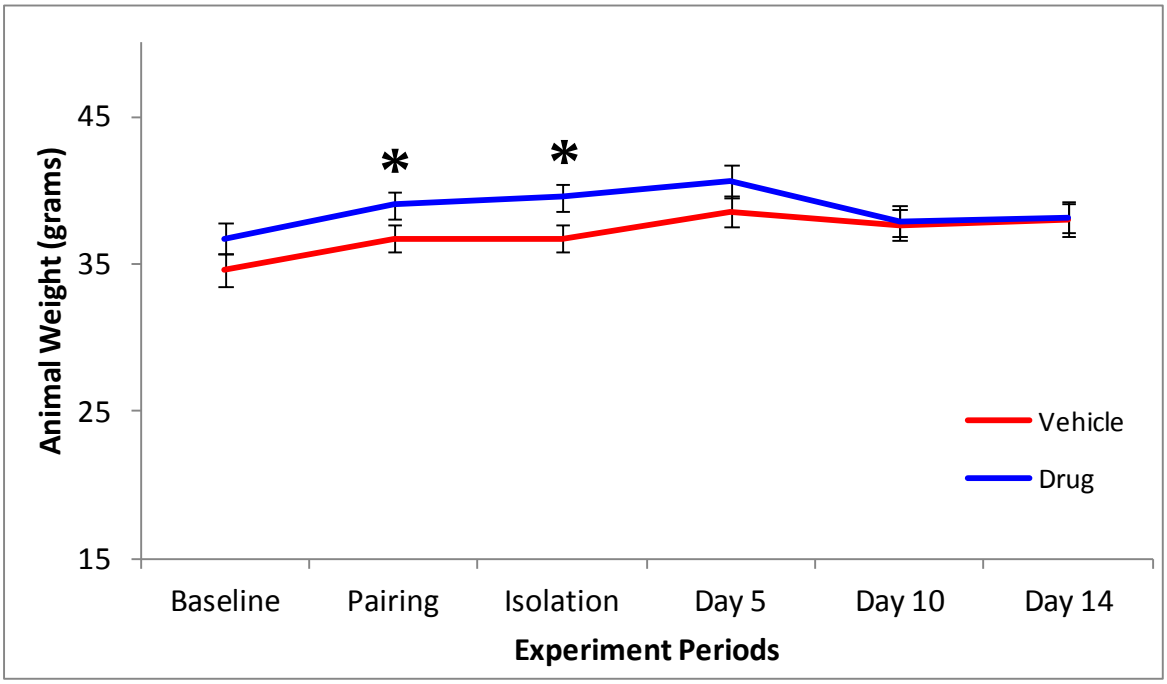
Total active behavior is the summation of swimming, struggling, and climbing manually scored behavioral responses, while total physical activity is the summation of the counts per minute score for the entire five minutes of the trial. The physical activity for each minute represents the summation of the counts per minute score for only that minute.

Figure 11. Correlation between manually scored behavior and physical activity of male prairie voles across 5 minutes of the forced swim test that was performed 22 hours after the tail-suspension test, 5 days of isolation from a female partner, and 15 days of either sertraline hydrochloride (16 mg/kg) or vehicle injections.


Vehicle: R² Linear = 0.181
Drug: R² Linear = 0.261
Total Physical Activity
400.00
300.00
200.00
100.00
.00
50.00
100.00
150.00
200.00
250.00
300.00
Manually Scored Total Active Behavior
Group
Vehicle
Sertraline Hydrochloride
Vehicle
Sertraline Hydrochloride
Vehicle: R² Linear = 0.181
Sertraline Hydrochloride: R² Linear = 0.261

The mixed-design analysis of variance for the subject weight (Figure 12) yielded a significant main effect for periods of the experiment [$F(5,100) = 31.394$, $p = 0.001$, Partial Eta squared = 0.611 (large effect size)], and a significant interaction between group and periods of the experiment [$F(5,100) = 5.768$, $p = 0.001$, Partial Eta squared = 0.224 (medium effect size)], but no main effect for group [$F(1,20) = 0.815$, $p = 0.377$]. This indicates that compared to the vehicle group, the setrtraline hydrochloride group weighed more during the initial periods of the experiment; however, that difference decreased during the drug injection period.

Figure 12. Mean (± SEM) weight (grams) of male prairie voles across the baseline, pairing, isolation periods, and 14 days of either sertraline hydrochloride (16 mg/kg) or vehicle injections. ***** = significantly increased body weight versus the vehicle group.


45
35
25
15
Animal Weight (grams)
*
*
Vehicle
Drug
Baseline
Pairing
Isolation
Day 5
Day 10
Day 14
Experiment Periods

CHAPTER 5

DISCUSSION

The relationship between mood and cardiovascular function can be influenced by an individual's social environment. Social support can influence both physical and psychological health, and the overall well-being of an individual (Kikusui, Winslow, & Mori, 2006). However, many people who experience negative mood states have limited access to social support, and creating those support networks can be a difficult and lengthy process (Berkman et al., 2003). Consequently, attempting to treat negative mood states and disrupted cardiovascular function through pharmacotherapy may be another option to improving patient health. The mechanisms that underlie the interactions among mood, cardiovascular function, and social behavior are not well understood. Thus, studies such as the current experiment that utilize animal models are valuable for investigating the role of the social environment in mediating the link between negative mood states and cardiovascular disease, and will provide better insight into neurobiological mechanisms underlying possible treatment options.

The general timeline for this experiment was to first pair male prairie vole subjects with unrelated female partners for five days. Male prairie vole subjects were then

isolated from their female partners for 5 days, and then randomly assigned to receive sertraline hydrochloride or vehicle only injections for 15 days. Following these periods, two assessments of depression-like behavior were conducted to examine the subjects' cardiac and behavioral responses to acute stress periods. In general, sertraline hydrochloride was limited in its ability to prevent the negative cardiac and behavioral changes associated with stress. In isolated prairie voles, sertraline hydrochloride administration did not significantly lower heart rate or increase heart rate variability during basal or behavioral stressor periods, nor did it significantly influence depression-relevant behavioral responses. Although these findings are contrary to the initial hypothesis, the results of this experiment can help inform our understanding of the mechanisms by which social stress can negatively influence cardiac and behavioral function. The following sections will discuss these results in greater detail.

Basal measures of physiological function during experimental periods

The basal measures (i.e., resting heart rate and heart rate variability) of cardiac function help inform our understanding of the time course for social stress induced changes in cardiac function. Further, they may also offer an explanation of how sertraline hydrochloride treatment may affect the neurobiological mechanisms that control the relationship between social stress and negative cardiac changes, in this animal model. In general, the current findings indicate that isolated male prairie voles display higher heart

rates and lower heart rate variability than their own scores when paired with their female partner. While the observation of improved cardiac function did not reach statistical significance, the group administered sertraline hydrochloride was partially buffered from those negative changes. However, an unexpected observation occurred while reviewing the cardiac data during the drug administration period. Male prairie voles in the sertraline hydrochloride group exhibited significantly higher heart rates (with no group difference in heart rate variability or physical activity) immediately following injections on the 5th and 10th days of the drug injection period. However, this post-injection elevation in heart rate was attenuated on the 14th day of the drug injection period.

In addition to the theorized beneficial changes in the heart rates for isolated male prairie voles administered sertraline hydrochloride, it was also anticipated that drug treatment would improve (i.e., increase) heart rate variability. Social isolation significantly decreased heart rate variability in all male prairie voles 10 days after partner removal; however, the sertraline hydrochloride group did not display a significant improvement in heart rate variability due to drug treatment. The specific findings for basal measures of physiological function are further discussed below.

There were no group differences in heart rate during the basal or pairing periods; however, when the female prairie vole partner was removed during the isolation and drug injection periods, both the sertraline hydrochloride and vehicle groups displayed significant increases in heart rate relative to their own heart rate during the pairing period. This finding has been observed previously (McNeal et al., 2014), and was an expected

result. Indeed, this animal model and experimental time course were selected to investigate the physiological and behavioral disruption that follow social isolation in this species. In the current experiment, sertraline hydrochloride did not significantly lower heart rate by the end of the drug administration period, and thus the hypothesis that drug treatment would ameliorate the negative cardiac effects of social stress was not supported.

Drug treatment also had limited effect on improving heart rate variability in isolated male prairie voles. In line with the finding of heart rate changes following social environment disruption, heart rate variability slightly increased for all male prairie voles during the pairing period, but then subsequently significantly decreased on the fifth day of the drug injection period. Unexpectedly, heart rate variability in all male prairie voles then returned to baseline levels for the remainder of the drug injection period (i.e., days 10 and 14). Across the periods of the experiment, there were no significant between group difference in heart rate variability. These results do not support the hypothesis that sertraline hydrochloride would improve this measure of cardiac function and raises questions as to the actual time course of heart rate variability changes in social stress. This is one of two known studies to investigate male prairie vole cardiac function during social isolation from a female partner, so the comparison of this finding with other data is limited.

In addition to the present study, the other investigation of cardiac function in socially isolated male prairie voles (McNeal et al., 2014) did not observe a significant

change in the SDNN index measure of heart rate variability. However, McNeal et al. (2014) found that female prairie vole partner removal induced a decrease in respiratory sinus arrhythmia, which is a measure of heart rate variability specifically mediated by the parasympathetic nervous system, as well as autonomic imbalance characterized by decreased parasympathetic and increased sympathetic input to the heart. Those findings indicate that, in prairie vole males, social isolation from a female partner will initially disrupt at least the vagal regulation of cardiac function. Further, extending that finding with the results of the current experiment may indicate that given a longer time course of isolation, male prairie voles will also display a decrease in heart rate variability controlled by both the parasympathetic and sympathetic nervous system.

Importantly, the negative cardiac changes described in the current study (i.e., increased heart rate and decreased heart rate variability) are well documented in both humans and animals experiencing social stress and adverse health conditions (Carney et al., 2005a; Carney et al., 1995; Ditzen et al., 2007; Fox et al., 2007; Glassman, Bigger, Gaffney, & Van Zyl, 2007; Grippo et al., 2011; Grippo et al., 2007d; Schwartz et al., 1988). The exact time course for stress-relevant changes in heart rate or heart rate variability has not been fully elucidated in either humans or animal models, therefore the current experiment adds valuable data for investigators. One explanation for the time course of this within group change in cardiac function is that after isolation from the female prairie vole partner, the male experienced the stress of isolation (observable by an increase in heart rate and decrease heart rate variability), and then these variables recovered to some extent, possibly allowing the males to search for a new partner.

Supporting this hypothesis, naturalistic studies have observed that male prairie voles will remain in the nest after the female partner has gone missing for approximately 19 days (Getz & Hofmann, 1986). The time course of decreased heart rate variability followed by recovery observed in the current experiment approximately lines-up with the time that it takes for an isolated male prairie vole, in the wild, to wait for a missing partner, and then move on. This may suggest that the isolated male prairie vole is experiencing stress by the loss of a female partner, but then spontaneously recovers (or possibly engages in some type of coping mechanism) to facilitate looking for another mate. This hypothesis would require further testing to determine its applicability to the present findings. Those naturalistic observations may also be skewed by confounding variables such as the presence of young or a behavioral reason for a delay before establishing a new mate and/or nest location. Potential ways to investigate this in a laboratory setting include exposing a previously isolated animal to a new female after different time points and observing the behavioral interactions, such as defensive behavior or determining latency to the formation of a new social bond. These future studies could inform our understanding of the exact time course of behavioral changes associated with male-female bond disruption in male prairie voles.

There are several reasons why the hypothesis of improved basal changes in heart rate and heart rate variability as a function of sertraline hydrochloride administration is not supported in the present study. First, the short-term effects of the drug may have detrimentally influenced cardiac function, therefore hindering the potential long-term benefits. In the current study, an unexpected observation in basal cardiac function came

in the form of increased heart rate immediately following the drug injection in the sertraline hydrochloride group. While analyzing the drug administration period data, it was observed that both groups displayed an increase in heart rate immediately following the daily injection; however, the sertraline hydrochloride group's heart rate increase exceeded that of the vehicle group. In order to explore this unexpected observation, the 5 hours immediately following injection times on the 5th, 10th, and 14th days of the drug injection period were assessed to quantify these cardiac changes. During days 5 and 10 of the drug injection period, the sertraline hydrochloride group displayed a significantly increased heart rate relative to the vehicle group; however, on day 14 the groups displayed a similar heart rate.

While the available information on the acute effects of sertraline hydrochloride on cardiac function is limited, this is an unusual finding. For example, sedated rats given an intravenous dose of sertraline hydrochloride (3 mg/kg) displayed a significant decrease in heart rate and renal sympathetic nerve firing frequency (Tiradentes et al., 2014). This indicates that the acute effects (i.e., single dose) of sertraline hydrochloride in cardiac function are sympatho-inhibitory, at least in anesthetized rodents. In humans, single doses of sertraline have no effect on heart rate, heart variability, and blood pressure in healthy patients (Ahrens, Frankhauser, Lederbogen, & Deuschle, 2007). These short-term effects of sertraline hydrochloride administration in prairie voles will require further detailed investigation to determine the difference between short- and long-term consequences of the drug on cardiac function.

In contrast to short-term consequences of drug treatment, previous studies have demonstrated that longer administration periods of serotonin reuptake inhibitors are associated with beneficial improvements in cardiac function. In healthy humans, 14 days of sertraline hydrochloride (50 mg) treatment was linked with a decrease in heart rate and other measures of basal sympathetic nervous system activity (e.g., skin conductance) (Siepmann, Grossmann, Muck-Weymann, & Kirch, 2003). Further, rats receiving 14 days of sertraline hydrochloride (10 mg/kg) treatment following an experimentally induced myocardial infarction displayed decreased limbic system cell apoptosis and increased active behavioral responses to stress (Wann et al., 2009). However, one known side effect of selective serotonin reuptake inhibitors negatively influencing cardiac function has been observed in the offspring of patients using these drugs (Czaja, Valuck, & Anderson, 2013), or in rodent models of developmental disorders (via juvenile administration) (Haskell et al., 2013; Martin, Liu, & Wang, 2012).

In humans, negative long term effects of developmental exposure to selective serotonin reuptake inhibitors took the form of an increased likelihood of cardiac events in adulthood for the offspring of mothers who took the drug (Czaja et al., 2013). In rodents, mice that were administered serotonin reuptake inhibitors during adolescence displayed smaller left ventricles, higher heart rates, and increased urinary noradrenaline excretion (Haskell et al., 2013). Similarly, juvenile male prairie voles displayed neurochemical system dysregulation (e.g., decreased oxytocin and vasopressin expression in the hypothalamus) (Martin et al., 2012). Those examples involve sertraline hydrochloride exposure times during developmental periods (i.e., in utero for humans and during post-

natal days 1-14 in rodents), whereas the prairie vole subjects in the current experiment were administered the drug as adults. Therefore, these previous studies may not be directly comparable to the present findings.

Another explanation for the lack of statistically significant changes in heart rate and heart rate variability in this experiment is that sertraline hydrochloride is not effective in changing cardiovascular function for this animal model. This explanation is unlikely for several reasons. This is the first experiment to assess treating prairie voles with sertraline hydrochloride in an attempt to ameliorate depression-relevant cardiac and behavioral disturbances, so there are no other studies available for direct comparison. However, the findings of other analogous studies may help with interpretation of the current results. First, another selective serotonin reuptake inhibitor (fluoxetine) has been observed to influence prairie vole pup retrieval latencies, indicating that serotonin system acting drugs do result in behavioral modifications in this animal species (Villalba, Boyle, Caliguri, & De Vries, 1997). Second, it is possible that to be effective in this species of rodent administration of the drug treatment must extend for a longer period of time. The increased heart rate exhibited by the sertraline hydrochloride group during the drug injection period on days 5 and 10 may be an acute effect of drug administration that diminishes over time, as was observed on the last day of the drug administration period. This explanation is in accord with human selective serotonin reuptake inhibitor treatment, namely that many patients can experience a worsening of mood or other negative side effects until prolonged treatment with the drug eventually yields beneficial results.

Therefore, a longer time course of sertraline hydrochloride administration may be necessary to observe long-term benefits on cardiac function.

Assessments of depression-relevant behaviors and cardiac reactivity in isolated male prairie voles

Taking this investigation beyond drug induced changes in basal cardiac function, the current study used two behavioral tests to assess the effect of sertraline hydrochloride on behavioral and cardiac responses to acute stressors. Following the drug administration period, all study animals underwent two tests of depression-relevant behavior in rodents. The tail-suspension test was performed because it is a widely used method for assessing antidepressant-like activity of drugs in rodents (Cryan et al., 2002; Cryan, Mombereau, & Vassout, 2005a). The sensitivity of the tail-suspension test is argued to be the result of the limited motor coordination necessary for in-test responses (i.e., body contortion/movement vs. immobility) and that those activities are general behavioral responses (i.e., active vs. inactive) (Cryan et al., 2005a). This study attempted to extend the tail-suspension test findings of Bosch et al. (2009) by including measures of cardiac activity and assessing behavior after a longer period of pharmacotherapy treatment. Additionally, Bosch et al. (2009) used a corticotropin-releasing hormone receptor antagonist that was applied through intracerebroventricular administration, whereas the current experiment used a more widely accessible drug and conventional route of

administration. Thus, the methodology of the current experiment enabled an investigation of a more readily accessible drug intervention. Further, the current investigation also attempted to extend that previous literature with the inclusion of an additional behavioral assessment of depression.

After the tail-suspension test and the subsequent recovery period, the forced swim test was conducted. The forced swim test is also utilized for measuring susceptibility to depression like-behavior and responses to drugs that modulate neurotransmitter function, especially the serotonergic system (Cryan et al., 2005b). While the tail-suspension test enables increased detection when assessing drug efficacy, the forced swim test is effective for measuring the effect of various drug types on a rodent's specific behavioral response. In short, the behavioral response strategies are scored as a measure of active (i.e., struggling, swimming, and climbing) versus passive (i.e., floating) responses in this test. These categories were developed because each behavior is differentially modified by distinct neurotransmitter systems, for example the catecholamine system for climbing and the serotonin system for swimming (Cryan et al., 2005b).

In general, sertraline hydrochloride did not have a statistically significant effect on isolated male prairie vole depressive behaviors in the tail-suspension or forced swim tests. However, the sertraline hydrochloride group exhibited opposing behavioral stress responses in the two different assessments. In the tail-suspension test, the sertraline hydrochloride group displayed slightly (but not significantly) lower heart rates and more active behavioral responses. This finding is consistent with the hypothesis that drug

treatment would buffer the adverse effects of social isolation and suggests a slight attenuation of depression-relevant behaviors and associated cardiac function. Conversely, during the forced swim test, the sertraline hydrochloride group displayed slightly (but not significantly) higher heart rates and less active behavioral responses. This finding is contrary to the hypothesis, and suggests a slight exaggeration of depression-relevant behaviors and associated cardiac responses. Additionally, following the tail-suspension test, the sertraline hydrochloride group took significantly longer to return to basal heart rate activity versus the vehicle group, a finding which is also contrary to the research hypothesis. However, the sertraline hydrochloride group subsequently displayed slightly higher heart rate variability versus the vehicle group in the later portion of the recovery period.

This is the first study to assess the actions of a selective serotonin reuptake inhibitor on cardiac function in assessments of depression-relevant behavior in the prairie vole species. While there were limited between group differences in the tail-suspension test, the sertraline hydrochloride group did display lower heart rate, indicative of lower cardiac responses to stress; and lower immobility, indicative of less behavioral despair. While not statistically significant, an improvement in cardiac and behavioral function during a the tail-suspension test in isolated male prairie voles administered sertraline hydrochloride does support the research hypothesis of drug treatment improving the responses to a short-term stressor.

Additionally, it is possible that the improvement in cardiac and behavioral function exhibited by the sertraline hydrochloride group would have been more robust given longer access to drug treatment or potentially a slightly higher dose. For example, in rats, 28 days of sertraline hydrochloride (17 mg/kg) treatment resulted in serotonin system signaling changes in rat neocortex; however, these changes were not observed after only 21 days of treatment (Sanders-Bush et al., 1989). Thus, a longer administration period or larger dose may more strongly influence behavior and cardiac function in the tail-suspension test.

Following the tail-suspension test, both groups were monitored at hourly intervals to assess their recovery from the stressor. The recovery period lasted 22 hours following the tail-suspension test. Contrary to the predicted improvement in post-stressor recovery, the sertraline hydrochloride group displayed a longer latency to return to pre-stressor heart rate levels versus the vehicle group. Conversely, while there was no initial difference in heart rate variability during the first 10 hours of the recovery period, in the later portion of the recovery period the sertraline hydrochloride group displayed slightly higher heart rate variability values than the vehicle group. This recovery information may suggest that, while not initially beneficial to the regulation of immediate post stressor cardiac function, sertraline hydrochloride may be helpful in long term recovery from a stressor. Supporting this hypothesis, sertraline hydrochloride has been linked with improvements in the heart rate variability measure of SDNN index --which is controlled by the sympathetic and parasympathetic nervous system-- following a myocardial infarction in humans (McFarlane et al., 2001).

McFarlane et al. (2001) suggests that sertraline hydrochloride administration has the ability to decrease the elevated sympathetic nervous system activity observed in patients with cardiovascular disease and depression, and thus helps to restore autonomic balance. Further, this change could stabilize control of the heart, decreasing its vulnerability to arrhythmias (McFarlane et al., 2001). However, this experiment investigated the drug treatment in patients with cardiovascular issues (i.e., following a myocardial infarction) and used a longer time course (i.e., 22 weeks) of drug administration than the current experiment, so direct result comparisons are not applicable. It is possible that a similar improvement in cardiac function would have been observed in the present experiment if the drug treatment was administered over a longer time course. With regard to the time course of drug administration in the current experiment, it is possible that sertraline hydrochloride may have had sufficient time to improve heart rate variability recovery but not heart rate recovery, and that a longer treatment period would beneficially improve immediate post-stressor heart rate recovery. Further testing will be necessary to test this theory. For example, future projects could assess how an animal administered sertraline hydrochloride is able to recover from multiple stressors over a longer period of time.

The potential benefits of a longer time course of drug administration could also affect the additional behavioral assessment of the current experiment, the forced swim test. Similar to the tail-suspension test, there were no statistically significant between group differences in heart rate, heart rate variability, and manually scored behavioral responses during the forced swim test. However, unlike the tail-suspension test that

indicated the sertraline hydrochloride group tended to display fewer negative effects (e.g., lower heart rate and lower depressive behaviors) of social isolation compared to the vehicle group, in the forced swim test those findings were reversed. In the forced swim test, the sertraline hydrochloride group displayed more immobility and fewer active behavioral responses than the vehicle group, indicative of greater behavioral despair in that group.

It should be noted that during the forced swim test both groups displayed a decrease in heart rate and heart rate variability across the trial; however, this decrease is normal as cardiac function is known to lower in correlation with a body temperature decrease in an environment that is colder than the core body temperature. Specifically, in colder temperatures heart rate decreases (Budd & Warhaft, 1966), while heart rate variability is observed to increase in healthy humans resting in 33 ^{o}C water (4 degrees colder than the average human body temperature of 37 ^{o}C) (Edelhäuser et al., 2012). Those findings are in line with an overall decrease in cardiac activity and is an expected observation for subjects in the current study. However, the sertraline hydrochloride group displayed a slightly higher heart rate and lower heart rate variability than the vehicle group.

These findings are opposite to the hypothesized buffering of negative stress responses in isolated male prairie voles administered sertraline hydrochloride. The research hypothesis predicted that animals that were administered sertraline hydrochloride would display increased active behavioral responding (along with less

immobility), lower heart rate, and higher heart rate variability during the forced swim test, none of which were observed in this assessment. Instead, most of the observed findings are indicative of more behavioral despair and greater stress responses in the sertraline hydrochloride group during the forced swim test. Similar to the tail-suspension test, perhaps if the drug was administered at a larger dose or for a longer period, and changes to serotonergic system signaling were allowed to further develop, the sertraline hydrochloride group would display significant improvements in cardiac and behavioral responses during the forced swim test.

There may be other considerations for the behavior of the sertraline hydrochloride group, one such explanation could be the temperature of the water inside the swim tank apparatus. It is known that the temperature of the water in the swim tank apparatus influences rodent response behaviors. For example, in rats, swimming in 19 ^{o}C cold water, but not in 25 ^{o}C room temperature one day before a second scored trial (24 hours later) resulted in increased immobility and increased activity in serotonergic nuclei responses (assessed via c*fos* expression) (Drugan et al., 2013).

A more likely explanation for the current findings may be that a second day of forced swim assessment is necessary to observe drug therapy improvements in this rodent model. A modified version of the forced swim test has also been used to measure the behavioral dimension of depression-like behavior in rodents. In this 2 day assessment, a 15 minute pretrial and 5 minute scored trial (24 hours later) is performed. Similar to the standard forced swim test (i.e., one day, five minutes in the testing apparatus), struggling,

swimming, climbing, and floating are measured as the behavioral responses. This test is noted for its ability to screen for antidepressant drugs. Exemplifying this, anti-anxiety drugs show no effect on swimming behavior in the scored trial (i.e., the second day). In contrast, antidepressant administration on the second day of the two day forced swim test results in increased active behavioral responses, especially swimming (Cryan et al., 2005b).

In the current experiment, not all measures of behavior during the forced swim test yielded an increase in depression-relevant responses. While not statistically significant, the sertraline hydrochloride group exhibited increased swimming behavior versus the vehicle group. Previous investigations have linked this active behavioral response to increases in serotonin system activity (Cryan et al., 2005b). When study animals (usually rodents) were administered a selective serotonin reuptake inhibitor, swimming behavior specifically was increased, as opposed to any other type of behavioral response (Cryan et al., 2005b). Thus, it is possible that sertraline hydrochloride may be having an effect on the swimming behavior of isolated male prairie voles, but the two day modified forced swim test is necessary to observe this effect. The test-retest design of the behavioral assessment is argued to be important for detecting the actions of selective serotonin reuptake inhibitors (Bielajew et al., 2003; Cryan et al., 2005b). As such, the findings of the current experiment may be the result of only measuring subject responses in the short term, and another day of testing could yield different results.

Manually scored behavior compared with physical activity measures

In addition to manually scored behaviors that are traditionally used in rodent studies involving the tail-suspension test and forced swim test, this experiment also investigated whether the physical activity measures provided by the radiotelemetry transmitter could be used to measure active behavioral responses in these two behavioral tests. This experiment therefore assessed the relationship between manually scored active behavioral responses and physical activity measures generated by the transmitters. A strong negative correlation was found between active behavioral responses and physical activity measures in the tail-suspension test. These data indicate that the measure of physical activity provided by the radiotelemetry transmitter is not consistent with manually coded behaviors for the assessment of rodent responses in this test.

This finding may be the result of two constraints: (1) physical activity (provided via the radiotelemetry transmitter) is not likely a sensitive enough measure to detect active behavioral responses such as subject limb flailing, which has limited movement of the trunk of the body; and (2) when the subjects cease other active behaviors such as contorting, the body often experiences a pendulum motion that could be erroneously detected as physical activity, though the animal has stopped active behavioral responses. In contrast to the automated physical activity measurement generated from the radiotelemetry transmitters, the manual coding of behaviors is advantageous because it allows for rational interpretation to determine the subject's behavioral responses despite

the potential movement confounds that may lead to inaccuracies in physical activity measures.

In contrast to the tail-suspension test comparisons, physical activity measures and manually scored behavioral responses in the forced swim test yielded a strong positive correlation. Therefore, these two measures may be equally useful for interpreting active behavioral responses by a rodent in the forced swim test. This difference in usefulness for measures of physical activity is likely due to the relatively open environment of the forced swim test apparatus enabling actual detection of movement behavior, while subjects in the tail-suspension test are fixed in place and can thus give spurious measures of physical activity. Future experiments may be able to use physical activity as an effective measure of active behavioral responding during the forced swim test. However, further validation of this measure may be required before it is used in place of manually coded behaviors. While the physical activity measure may be useful for assessing the general physical activity of an animal, it does not offer the same distinctions among behavioral responses as manual coding. The manual coding of behaviors in the forced swim test may be beneficial for projects investigating changes in neurotransmitter systems, because of the ability of the test to reflect changes in behavior that correspond with different neurotransmitter systems. For example, only assessing physical activity in this project would have missed the slight increase in swimming behavior in the sertraline hydrochloride group, which is associated with greater serotonergic system activity.

In summary, this experiment assessed the relationship between manually scored active behavioral responses in laboratory assessments of depression-relevant behavior and physical activity measures generated by the radiotelemetry transmitters. Manually scored active behavioral responses and physical activity measures provided by the radiotelemetry transmitter strongly agreed in the forced swim but not tail-suspension tests. This difference is likely due to the relatively open environment of the forced swim test apparatus enabling actual detection of movement behavior, while subjects in the tail-suspension test are fixed in place and can thus give spurious measures of physical activity. Consequently, future experiments may be able to use physical activity as an effective measure of general (but not specific) active behavioral responding during the forced swim test and possibly other behavioral assessments that employ an open area where the subject is free to move around the apparatus, such as a novel object recognition task or resident-intruder paradigms.

Interpretation of current findings

The present experiment has first demonstrated that disruption of the social environment results in increased depression-relevant behaviors, increased basal heart rate, and decreased heart rate variability controlled by both the sympathetic and parasympathetic nervous systems. These findings are in line with previous studies focused on the long-term disruption of social bonds in investigations using both animal

models (Grippo et al., 2011; Grippo et al., 2007d; McNeal et al., 2014), and humans (Bunker et al., 2003; Orth-Gomer et al., 1993; Shear & Shair, 2005). Importantly, these same biomarker changes (i.e., heart rate and heart rate variability) are observed in both depression and cardiovascular disease in humans (Carney et al., 2005a; McDougall et al., 2005; Sapolsky, 1996). Thus, the present results provide further evidence that the prairie vole is a useful animal model for investigating the neurobiological basis of social behavior, mood, cardiovascular function, as well as possible treatment options for social stress.

The current experiment found that sertraline hydrochloride treatment buffered some, but not all, of the social isolation induced dysregulation of cardiac function, as well as behaviors relevant to depression. Specifically, isolated male prairie voles treated with sertraline hydrochloride displayed slightly, but not significantly, lower heart rates and increased heart rate variability at basal levels. During the behavioral assessments, animals receiving sertraline hydrochloride displayed mixed results. In the tail-suspension test, sertraline hydrochloride animals displayed potentially beneficial effects of drug treatment; however, in the single day forced swim test they displayed negative effects. Similarly, during the recovery period after the tail-suspension test, animals administered sertraline hydrochloride --initially-- did not show an improved recovery of heart rate versus the vehicle treated animals. However, in the latter half of that recovery period the sertraline hydrochloride animals displayed higher levels of heart rate variability than the vehicle animals, indicative of improved recovery. Finally, this experiment also compared the findings of manually scored animal behavior to physical activity measure provided by

the radiotelemetry transmitters, again finding mixed results, but providing limited support for using physical activity measures performed in an open testing apparatus. Together, these findings have important implications for future investigations for several reasons.

First, the current study adds to and supports previous literature linking social stress to impaired behavioral activity and physiological function in male prairie voles (Bosch et al., 2004; Grippo et al., 2011; McNeal et al., 2014). One of the strengths of laboratory models is the ability to infer a causal relationship between social distress and adverse behavioral and physiological function. These types of measures would be unethical in humans, impractically expensive, and not possible with the same level of experimental control over the time course and environmental conditions. Thus, the ability to perform these procedures in an animal model that shares many social and biological characteristics with humans is beneficial for improving human health and welfare.

Second, this study suggests that lack of social support may negatively influence the physiological and psychological responses to stress. Further, the results could indicate that antidepressant pharmacotherapy may be only partially effective in treating the adverse effects of social stress. Currently, there is a dearth of available literature on the investigation of cardiac function during behavioral assessments for this animal model of male-female social bond disruption (Bosch et al., 2009; McNeal et al., 2014), thus direct comparisons between studies are not possible. However, this is not the only investigation to find mixed results when administering drug treatments in prairie voles. For example, female prairie voles isolated from a same-sex sibling for two weeks and then given daily

injections of oxytocin for another two weeks (while still isolated) displayed improvements in cardiac function (i.e., heart rate and heart rate variability), but no change in anxiety-related behaviors (Grippo et al., 2012b).

In this previous study from our laboratory, oxytocin administration was able to buffer isolation induced changes of basal cardiac function. After 14 days of oxytocin injections, isolated female prairie voles displayed heart rate and rhythm values similar to pair-housed controls. This experiment also assessed cardiac function during a behavioral measure of anxiety (i.e., elevated plus maze) and social interaction (i.e., resident-intruder test). In these assessments, isolated female prairie voles administered oxytocin displayed cardiac function comparable to pair-housed females. Further, three hours after the behavioral tests, isolated female prairie voles (both the vehicle group and those administered oxytocin) displayed higher heart rate and lower heart rate variability compared to the pair-housed animals. There was no difference in subject behavior (in any group) in the social interaction test, which is in line with previous literature, as prairie voles typically display limited intraspecies aggression. However, during the behavioral measure of anxiety, oxytocin administration did not change the amount of time that isolated female prairie voles spent displaying anxious behavior --observed by the amount of time spent in the open versus closed arms of an elevated plus maze-- when compared with pair-housed controls.

This previous study also demonstrated that oxytocin injection increased oxytocin cell density in the paraventricular nucleus of the hypothalamus in both isolated and

control groups (i.e., animals housed as a sibling pair). More interestingly, corticotropin-releasing hormone cell expression (also in the paraventricular nucleus of the hypothalamus) was significantly increased in the isolated group; however, the paired, paired plus oxytocin, and isolated plus oxytocin all had approximately the same cell density (Grippo et al., 2012b). These findings highlight the role of oxytocin in the neurobiological mechanisms linking an animal's social environment to its cardiac regulation. Additionally, this study indicates pharmacotherapy has the potential to affect cardiac function and behavior differentially, at least in this species. Further, it also indicates that pharmacotherapy may serve to compensate for adverse physiological responses to stress through changes in hormonal regulation.

In the context of the current experiment there were no measures of hormonal or neuronal changes, but samples of both were collected for future analysis. In future studies, the effects of sertraline hydrochloride administration on post-stressor hormone production and basal neuronal activity in subcortical structures associated with hormone regulation and the stress response (e.g., hypothalamus) will be assess using those samples. Based on the hypothesis that pharmacotherapy would buffer the adverse cardiac and behavioral effects of social isolation, it is anticipated that isolated animals receiving drug treatment would display similar physiological changes as those shown in Grippo et al. (2012b). Specifically, we would anticipate observing lower circulating level of stress-related hormones (e.g., corticosterone and adrenocorticotropic hormone) and activity in cells responsible for the activation of the HPA axis (e.g., transcriptional activity of cells). This potential finding would indicate that social isolation induces similar neurobiological

changes as depression, and support the theory that social bonds buffer negative social environment changes by limiting the response to stress (Cohen & Janicki-Deverts, 2009; Kaplan et al., 1988; Kikusui et al., 2006).

Third, the finding of limited cardiac function improvements via drug therapy is also not limited to this experiment. For example, in a four week chronic mild stress paradigm (i.e., random mild stressors performed daily), rats that received concurrent injections of fluoxetine (10 mg/kg) displayed improvements in a behavioral measure of depression (Grippo, Beltz, Weiss, & Johnson, 2006). However, drug administration did not completely buffer the adverse changes in cardiovascular function (Grippo et al., 2006). In this experiment, rats experiencing chronic mild stress and administered fluoxetine displayed similar levels of anhedonia as control rats administered the drug. However, fluoxetine treatment only partial prevented chronic mild stress induced cardiovascular dysfunction. Specifically, drug treatment only partially prevented: an increased resting heart rate, exaggerated blood pressure and heart rate response to a behavioral stressor (i.e., air jet), reduced cardiac output and stroke volume, and exaggerated heart rate decrease in response to beta-adrenergic receptor blockade. These findings parallel the general results of the current experiment, suggesting only limited cardiac improvement for the stressed group as a function of antidepressant administration.

In particular, the Grippo et al. (2006) finding of exaggerated heart rate decrease in response to beta-adrenergic receptor blockade is relevant to the findings of the current

investigation. Cardiac function is the result of combined sympathetic and parasympathetic input to the heart; therefore, administering a beta-adrenergic receptor antagonist (i.e., sympathetic nervous system blockade) or cholinergic receptor antagonist (i.e., parasympathetic nervous system blockade) into the peripheral circulation will attenuate the respective autonomic nervous systems limb's input to the heart (Schachinger, Weinbacher, Kiss, Ritz, & Langewitz, 2001; Wilkins et al., 2007). Thus, via these receptor antagonists, it is possible to evaluate the relative input from each autonomic nervous system limb through measuring drug induced changes in heart rate, and inferring the relative influence of either the sympathetic or parasympathetic nervous system. The findings of Grippo et al. (2006) indicated that fluoxetine treatment was unable to ameliorate the stress induced exaggeration of sympathetic input to the heart. Thus, it is possible that selective serotonin reuptake inhibitor treatment has limited cardio-protective effects in rodents, including prairie voles, and possibly humans (Glassman et al., 2007).

In line with results from rodents, a similar lack of improvement in cardiac function (e.g., heart rate and heart contraction activity) has also been observed in non-human primates (Groban, Kitzman, Register, & Shively, 2014). Adult female cynomolgus monkeys were fed a Western-like diet for 3 years, and half were treated with sertraline hydrochloride for 18 months. All animals were assessed for depressive behavior (e.g., behavioral expressions such as slumped posture and not attending to environmental stimuli) in the final 12 months of the experiment, and finally, the study concluded with an echocardiography measure. Monkeys scoring above the mean for

depressive behaviors (19 of the 42 subjects) displayed higher heart rates, smaller left ventricles, and impaired systolic and diastolic (i.e., smaller volumes and end-systolic dimension) function (Groban et al., 2014). The sertraline hydrochloride treatment group displayed improvements in some measures of cardiac function, through significantly decreased heart rates and marginally larger left ventricles. However, sertraline hydrochloride did not improve the impaired systolic and diastolic cardiac function. These findings indicate that long-term sertraline use may have the ability to support heart function in depressed mammals; however, it may not affect all aspects of cardiac function. Along these lines, long-term sertraline hydrochloride treatment in humans has yielded similar results.

Glassman et al. (2007) found that heart rate variability recovery was decreased in depressed patients following a myocardial infarction, and the difference between individuals with and without depression increased following 16 weeks of observation (Glassman et al., 2007). In this study, human patients received assessments of depression (i.e., Hamilton Rating Scale for Depression) and heart rate variability (i.e., frequency domain) at the onset and end of the trial. In this randomized trial, patients were treated with either a placebo or sertraline hydrochloride for 16 weeks following a myocardial infarction. Depressed patients receiving sertraline hydrochloride did not recover to heart rate variability levels comparable to the non-depressed group, but displayed higher scores than patients in the depressed plus placebo group, whose scores actually decreased further over the course of the 16-week paradigm (Glassman et al., 2007). These findings

suggest that sertraline hydrochloride treatment partially buffered the adverse changes in heart rate variability observed in depressed patients following a myocardial infarction.

Additional studies with humans indicate that sertraline hydrochloride use is linked with improvements in mortality risk for patients experiencing negative mood states and cardiovascular disease (Berkman et al., 2003; Glassman et al., 2002; Roose et al., 1998; Shapiro et al., 1999). Some of these investigations have linked sertraline hydrochloride treatment with improvements in ultra low-frequency heart rate variability power (Glassman et al., 2007) while improvements in mood are associated with improvements in low-frequency power (Glassman et al., 2007; Glassman et al., 2002). Importantly, decreased heart rate variability is associated with increased mortality (Carney et al., 2001; Carney et al., 2002; Carney et al., 1999; Jiang et al., 2008; O'Connor et al., 2010; Shapiro et al., 1999). Thus, understanding and improving this significant biomarker of health is important to improving human mortality rates. Together, this previous literature and the findings from the current experiment could offer a mechanistic explanation for achieving beneficial effects of sertraline hydrochloride on patient survival.

Potential mechanisms for pharmacotherapy induced improvement in health

In the present study, the difference in cardiac function can indirectly indicate the relative activity of nuclei responsible for autonomic nervous system regulation. Group

differences in heart rate and especially heart rate variability indicate changes in the output of these nuclei. The decrease in heart rate variability could indicate increased activity in rostroventrolateral medullary nuclei and a concurrent decrease in the activity within the brainstem structures responsible for parasympathetic drive (i.e., dorsal motor nucleus of the vagus and nucleus ambiguus). Importantly, while animals administered sertraline hydrochloride showed no significant improvement in basal or stressor cardiac function, they did display an increase in heart rate variability during the later portion of the post tail-suspension test recovery period. This may suggest sertraline hydrochloride could be linked with increasing parasympathetic nervous system control of post-stressor recovery; however, further investigation will be necessary to determine the veracity of this theory. If founded, this could indicate that while drug intervention yields limited improvement to acute stress related psychological and behavioral activity, it may beneficially improve long term stress recovery.

Chronic treatment with a selective serotonin reuptake inhibitor may thus beneficially change the activity of autonomic nuclei responsible for cardiac control. Specifically, this would indicate pharmacotherapeutic intervention may beneficially suppress the activity of nuclei associated with stress responses (i.e., sympathetic output), and/or enhance the activity of brainstem nuclei responsible for cardiac chronotropic regulation (i.e., parasympathetic activity). This change in nuclei activity through sertraline hydrochloride administration may enable improved long term recovery from acute stressors. Supporting this hypothesis, exercise is purported to be beneficial because it, in part, results in an improvement of recovery after physical activity (Blumenthal et al.,

2012). This is especially salient for heart rate variability, which yields a beneficial increase following exercise (Javorka, Zila, Balharek, & Javorka, 2002). Similarly, initial investigations into drug and exercise combination therapy have shown that it produces benefits in patient outcomes superior to those yielded by each individual therapy type (Blumenthal et al., 2012).

In addition to autonomic regulation, the serotonin system is known to influence nearly all processes in the body, including hormone regulation. While serotonin activity in the raphe nuclei does not directly induce changes in cardiac function, it is linked with homeostatic functions (e.g., hormone regulation) that regulate blood pressure (Mason et al., 2007). For example, Mason et al. (2007) found very few serotonergic cells in the reticular formation that displayed any activity in relation to the cardiac cycle. However, the majority of cells investigated displayed activity that correlated with very slow heart rate variability changes (i.e., very low heart rate variability) and/or respiratory rhythms (Mason et al., 2007). Thus, while not imparting a direct impact on cardiovascular regulation, changes in the serotonin system could indirectly influence its regulation through changes in hormone --especially cortisol-- regulation and long-term blood pressure control through changes in hypothalamic and brainstem nuclei activity, respectively. Supporting this hypothesis, selective serotonin reuptake inhibitor treatment is associated with decreased salivary cortisol levels in depressed women (Dziurkowska, Wesolowski, & Dziurkowski, 2013). Further, prior investigations have linked sertraline hydrochloride treatment with improvements in ultra low-frequency heart rate variability

power, which is associated with circulating hormone regulation (Bigger et al., 1992; Glassman et al., 2007).

The influence of sertraline hydrochloride on autonomic and endocrine regulation may be explained by sertraline hydrochloride's mechanisms of action. One potential mechanism by which sertraline hydrochloride induces beneficial improvements in health could be through changes in central serotonin system neurotransmission (Frazer & Benmansour, 2002; Harmer et al., 2009). Sertraline hydrochloride administration is associated with differential changes in serotonin receptor activity. First, long-term treatment with sertraline hydrochloride results in increased extracellular serotonin expression and less inhibition of intracellular second messenger system activity (Bel & Artigas, 1993; Celada et al., 2004). Thus, chronic selective serotonin reuptake inhibitor treatment is linked with the desensitization of serotonin$_{1A}$ receptors in the raphe nuclei via adaptation to increased serotonin levels at the synapse (Adell et al., 2002; Haddjeri et al., 1998). Second, chronic sertraline hydrochloride treatment in rats results in a desensitization of serotonin$_2$ receptor second messenger system activity in rat neocortex (Sanders-Bush et al., 1989). This could result in a decrease in cortical inhibition of raphe nuclei, thus facilitating serotonergic neurotransmission via decreased inhibitory input from the prefrontal cortex. Together, these changes may combine to enhance serotonergic neurotransmission associated with improvements in mood following selective serotonin reuptake inhibitor treatment.

Desensitization of serotonin$_{1A}$ receptors in the raphe nuclei results in an increase of serotonin because those cells are less inhibited by the activity of those receptors. Similarly, decreasing the inhibitory input from the cortex (i.e., desensitization of serotonin$_2$ receptors) would also disinhibit serotonin neurotransmission. Therefore, drug treatment may facilitate improved behavioral and cardiovascular responses to stressful environments by restoring serotonergic modulation of neuronal activity. Specifically, after drug treatment has been administered long enough, it will result in enhanced serotonergic outflow from the raphe nuclei and thus facilitate active responses to stress and improved cardiovascular function. In the context of the current study, drug treatment may not have been administered for long enough to induce improvement in serotonin neurotransmission, and thus was only partially effective in buffering the isolation induced behavioral and cardiac dysregulation. Potential methods to investigate this possibility are discussed in the future directions section.

Alternative explanations for the present findings

This section will discuss possible alternative explanations for the findings of the current experiment. First, the possibility that sertraline hydrochloride treatment in other investigations was more effective because the subjects of the current study were not as physically unhealthy as the subjects in other investigations is discussed. Next, the possibility that depression and social isolation are distinct constructs that affect health differently will be considered. Following that, the possibility that the negative health

effects of social isolation could be mediated through a different neurobiological mechanisms, rather than directly through the serotonergic system is discussed. The final alternative explanation discussed is that the beneficial health effects of serotonin reuptake inhibitors are primarily mediated through peripheral platelet activity changes.

One possible alternative explanation for the lack of significant findings in this study versus other investigations of sertraline hydrochloride administration in the treatment of cardiovascular disease and negative mood states, could be the relative health states of the subjects. Many of the subjects and participants in previous studies were in comparatively "worse" health states than the animals used here (e.g., experimentally induced myocardial infarction), and therefore therapeutic intervention may yield a greater change than in animals socially stressed for 15 days. As an example, 14 days of sertraline hydrochloride administration following an experimentally induced myocardial infarction in rats resulted in increased displays of active behavioral responses (and less immobility) on the second day of a 2 day forced swim test (Wann et al., 2009). It is therefore possible that the current study did not find statistically significant behavioral and cardiac function improvements because the subjects were not as physically unhealthy as the subjects in other studies.

Another potential explanation for the limited effects of sertraline hydrochloride on improving behavioral and cardiac function in isolated male prairie voles may be that the serotonergic system is not the major mechanism underlying responses to social stress or isolation. For instance, other pharmacotherapy treatments (i.e., peripheral oxytocin injection) in prairie voles have resulted in the amelioration of some of the negative

cardiac and behavioral consequences of social isolation (Grippo & Johnson, 2009; Grippo et al., 2012b). Similarly, intracerebroventricular administration of a corticotropin-releasing hormone receptor antagonist ameliorated the depression-relevant behaviors (i.e., increased immobility in a tail-suspension and forced swim test) in male prairie voles isolated from a female partner (Bosch et al., 2009). It is therefore possible that social isolation could negatively affect health through alternate neurobiological mechanisms, rather than directly through serotonergic system dysregulation. Mechanistically, the responses to social isolation may be mediated via oxytocin and/or corticotropin-releasing hormone regulation of nuclei involved in the stress response, such as changes in hypothalamic mediated HPA axis activity.

Aside from alternative central mechanisms, the lack of significant responses to sertraline hydrochloride in the present experiment may be due to the fact that social isolation and depression are not the same construct. As discussed in the introduction, Cacioppo et al. (2006) demonstrated that depression and loneliness display phenotypically distinct characteristics in humans, so it is possible that the physiological effects of isolation and depressive behaviors are differentially mediated. The findings from the current experiment may support the idea that social isolation and depression are different constructs. Specifically, socially isolated animals displayed cardiovascular dysfunction, and it is clear from previous research that social isolation also results in depressive behaviors (Cacioppo et al., 2010; Grant, Hamer, & Steptoe, 2009; Shankar et al., 2011; Yang, McClintock, Kozloski, & Li, 2013); however, an antidepressant drug was not significantly effective at improving behavioral responses in the present study.

These findings suggest that, although depressive behaviors may be a component of a larger cluster of consequences of social isolation, it is not sufficient to treat the depressive behaviors without considering the additional constellation of isolation-related changes.

Unfortunately, this explanation would not account for the lack of significant improvement in cardiac function observed during the present experiment. While it is possible that social isolation and depression affect health differently, the lack of improvement in cardiac and behavioral measures following drug treatment is at odds with a host of research indicating beneficial results of selective serotonin reuptake inhibitor treatment (Carver et al., 2008; Glassman et al., 2002; Shapiro et al., 1999). The majority of available literature links chronic selective serotonin reuptake inhibitor use with improvements in patient behavioral and cardiovascular health (Carver et al., 2008; Glassman et al., 2002; Miura, Kitagami, & Ozaki, 2007; Miura, Qiao, Kitagami, Ohta, & Ozaki, 2005; Shapiro et al., 1999; Siepmann et al., 2003; Wann et al., 2009; Yildirim et al., 2012). Thus, a more probable explanation is that the drug treatment used in this experimental paradigm will require a longer time course or larger dosage to influence the underlying neurobiological pathway regulating physiological and biological responses to stress.

Finally, serotonin reuptake inhibitors are known to inhibit blood platelet activity (Jacobs & Azmitia, 1992), and it has been speculated that they may function as mild blood thinners (Berkman et al., 2003; Maurer-Spurej, 2005). This mechanism of action would be functionally different from directly modulating the output of central nervous

system structures. In this alternative explanation, it is possible that sertraline hydrochloride decreases the viscosity of the subject's blood to facilitate improved circulation. This reduction in work load could facilitate a lowering of the subject's heart rate. To further explore this hypothesis, our laboratory is currently adapting mouse blood pressure monitoring devices for use in prairie voles. Future studies will be conducted to investigate possible social isolation induced changes in blood pressure and potential vascular effects of serotonin reuptake inhibitors more directly.

Future directions

The current experiment is an important step in understanding how pharmacotherapy may buffer the negative behavioral and cardiac effects of social stress in an animal model. Specifically, it highlights the need for future experiments to consider methodological alterations to the current research paradigm or alternative drug therapy options. To address these considerations, there are several possible options. First, possible methodological improvements for the current experiment are discussed. Second, to determine if the findings of limited drug effectiveness in the current experiment are the result of ineffective sertraline hydrochloride function or of the inhibition of serotonin reuptake in general, additional comparative studies with other serotonin acting drugs are suggested. Third, the possibility of using the current experimental paradigm with different drugs is discussed. Finally, further investigation into fully elucidating the effects

of selective serotonin reuptake inhibitor used, especially as it pertains to the serotonin$_3$ receptor, is presented.

First, future studies using this methodology should extend the time course of drug treatment for an additional 10 to 14 days to mimic more long term drug administration time courses seen in humans. This may result in statistically significant improvement in behavioral and cardiovascular dependent measures. Similarly, rather than a single day of the forced swim test, future investigations should include the two day modified forced swim test. This change may enable the detection of beneficial effects of sertraline hydrochloride administration on depressive behaviors and cardiac regulation.

Second, beyond the potential investigations listed in the discussion above, such as a longer time course or higher drug dose, another future project should focus on other serotonin reuptake inhibitors or pharmacotherapies to determine if the limited changes in cardiac and behavioral function in the current experiment are due specifically to sertraline hydrochloride or the general mechanism of inhibiting serotonin reuptake. For example, a future study could compare sertraline hydrochloride with other serotonin reuptake inhibitors (e.g., fluvoxamine, citalopram, and/or escitalopram) to assess the effectiveness of different drugs that affect the serotonin system and responses to social isolation. This potential study would help determine whether the lack of significant effects of drug treatment in the current experiment are the result of sertraline hydrochloride specifically, or a function of inhibiting serotonin reuptake in general.

Third, in addition to serotonin reuptake inhibitors, other drug classes may be used in the current research paradigm to investigate potentially beneficial effects on the behavioral and cardiovascular responses to social isolation in male prairie voles. For instance, in male prairie voles, intracerebroventricular administration of a corticotropin-releasing hormone receptor antagonist has been shown to buffer the adverse short-term behavioral consequences of isolation from a female partner (Bosch et al., 2009). Future experiments should investigate whether treatment with corticotropin-releasing hormone receptor antagonists also can buffer the adverse cardiac changes linked with partner loss in this species.

Similarly, another drug that has been found to ameliorate some of the negative consequences of social isolation, at least in female prairie voles, is oxytocin. Oxytocin treatment could be used in place of or in combination with sertraline hydrochloride in another study. This study would parallel known findings in prairie voles, thus allowing for greater cross study comparisons. For example, it has already been observed that female prairie voles isolated from a same-sex sibling partner, and receive oxytocin injections, are buffered from some of the adverse cardiac function (i.e., decreased heart rate and heart rate variability) and behavioral (i.e., decreased sucrose intake and immobility in the forced swim test) changes displayed by animals receiving only the vehicle (Grippo et al., 2012b; Grippo et al., 2009). Extending these findings to male animals and/or comparing oxytocin and serotonin system affecting drugs in this paradigm could enable direct assessments of potentially different neurobiological mechanisms of function.

Alternatively, the benefits of other pharmacotherapeutic treatments options could be explored as well. Beyond serotonin, depression has also been linked with norepinephrine and dopamine monoamine system dysfunction (Haenisch & Bonisch, 2011). In line with this, drug treatment that specifically targets the reuptake of those monoamine transporters (i.e., bupropion) while simultaneously displaying limited clinical effects on the serotonin transporter results in an efficacy comparable to antidepressants (Stahl et al., 2004). Thus, administering a drug that targets another monoamine system may be a useful alternative and/or comparison group to sertraline hydrochloride in a future experiment.

Between dopamine and norepinephrine, targeting the dopaminergic system is likely more interesting and clinically relevant. Social bonding is linked with brain reward circuitry, and the dopamine system plays a role in mediating the effects of social isolation (Coria-Avila et al., 2014; Young, Liu, Gobrogge, Wang, & Wang, 2014). For example, early life social isolation impairs pair bonding in adulthood for mandarin voles, potentially through alterations in the corticosterone (i.e., increased expression) and nucleus accumbens dopamine (i.e., altered receptor distributions) signaling pathways (Yu et al., 2013). Thus, potential future investigations using a dopamine reuptake inhibitor in an attempt to treat isolation induced negative behavior and/or cardiovascular health effects could be useful additions to science.

Another available antidepressant option for treatment is a norepinephrine reuptake inhibitor; however, care should be taken when considering the use of this drugs class. A

number of cardiovascular impairments are characterized by increased sympathetic activity and impaired norepinephrine reuptake (Schlaich et al., 2004). As such, in a population of patients with cardiovascular impairments, drug treatment that enhances norepinephrine activity could induce detrimental short-term side effects that outweigh possible long-term improvements.

Another potential pharmacotherapeutic option could be to use a drug to up-regulate intracellular activity associated with improvements in depression (i.e., increased expression of cyclic-adenosine monophosphate). This would be useful because depression is associated with a decrease in cyclic-adenosine monophosphate activity (Duman, 1998; Gao et al., 2014). In this capacity, a phosphodiesterase inhibitor such as sildenafil citrate (e.g., Revatio) or a phosphodiesterase$_{4B}$ specific inhibitor could be used. It is important to note that decreased phosphodiesterase expression is also observed in depression. This is because phosphodiesterase and the ligand it regulates (i.e., cyclic-adenosine monophosphate) co-vary (Takahashi et al., 1999; Zhang, 2009). However, chronic antidepressant treatment increases both cyclic-adenosine monophosphate activity and phosphodiesterase expression. As such, phosphodiesterase inhibitor treatment may be considered as an early intervention treatment on its own. Alternatively, a phosphodiesterase inhibitor could be co-administered with other more traditional pharmacotherapeutic interventions such as selective serotonin reuptake inhibitors to potentially decrease the latency of efficacy in that drug class (Duman, 1998).

Finally, future experiments building upon the current study should also endeavor to further characterize changes in serotonin system activity in the context of social stress. The exact role of serotonin in cardiac function has yet to be fully elucidated. Cardiovascular responses to serotonin may involve the simultaneous activation of more than one receptor subtype. For example, serotonin$_3$ receptor activation in the nucleus of the solitary tract increases mean arterial pressure and inhibits parasympathetic input to the heart (i.e., the limb responsible for a decrease in cardiac chronotropic regulation) (Nosjean et al., 1995). However, the exact mechanisms of these actions have not been fully characterized. Additionally, serotonin$_3$ receptors have also been localized in brain areas involved in the modulation of cardiovascular control nuclei (e.g., hippocampus and amygdala) (Miquel et al., 2002).

As such, future studies should investigate the pathways of communication between the hippocampus, amygdala, and the nucleus of the solitary tract, as well as whether serotonin reuptake inhibitors influence the function of the serotonin$_3$ subtype. Studies such as these underscore the complicated interaction between serotonin and the numerous areas which still need to be understood. For example, it is currently unknown whether selective serotonin reuptake inhibitors influence the activity of serotonin$_3$ receptor function in the nucleus of the solitary tract. Elucidation of this unknown interaction could potentially offer novel drug treatment options such as the specific targeting of a brain region involved in homeostatic balance. Thus, future work in this field of study may offer another route for improved treatment of people experiencing social stress, and who are at risk for or already experiencing cardiovascular disease.

Conclusions

This experiment explored whether the deleterious cardiac and behavioral changes resulting from the disruption of male-female bonds in prairie voles could be treated through the administration of sertraline hydrochloride. The present findings replicate and extend research developed by McNeal et al. (2014) and Bosch et al. (2009) who showed that social isolation from a female prairie vole partner will result in increased depression-relevant cardiac dysfunction and behavior, and that pharmacotherapy can buffer the adverse behavioral changes (respectively).

Contrary to the research hypothesis, after male prairie voles were separated from their female partners, the group administered sertraline hydrochloride did not display a statistically significant reduction in heart rate or increase in heart rate variability. Further, the sertraline hydrochloride displayed increases in heart rate on days 5 and 10, but not 14, of the drug administration period. It is possible this short-term increase in heart rate could be interfering with the long-term benefits of the drug. The behavioral assessments in the present paradigm indicated that sertraline hydrochloride was not able to significantly improve depressive behavioral responses or buffer adverse changes in cardiac function during the behavioral tests. Importantly, while animals administered sertraline hydrochloride displayed a significantly increased latency to return to pre-stressor heart rate levels, they did display a slightly increased heart rate variability in the later portion of the post tail-suspension test recovery period. This could indicate that while drug

intervention yields limited improvement to acute stress related physiological function, it may beneficially improve long term stress recovery ability.

While the research hypothesis was not statistically supported by the findings of this study, the results do help inform our understanding of how social stress negatively influences physical and behavioral health. The current investigation may indicate that the time course (15 days) or dose (16 mg/kg) of sertraline hydrochloride administered in this experiment is not sufficient to ameliorate neurobiological changes associated with partner loss in male prairie voles. Future investigation will therefore be necessary to explore whether a longer time course or a higher drug dose may be required to achieve benefits in the context of social isolation. Additional pharmacotherapeutic interventions, other than serotonin reuptake inhibitors, may beneficially improve the activity of brain regions (e.g., hypothalamus, reticular formation nuclei, and prefrontal cortex regions) associated with stress responses and chronotropic cardiac regulation. Future investigation can therefore extend this experiment by selecting alternative pharmacotherapeutic treatments that may influence other central nervous system mechanisms. For example, a future study could use a drug that enhances the expression of oxytocin or alternatively a phosphodiesterase inhibitor which may have the capacity to up-regulate intracellular activity associated with improvements in depression (e.g., increased expression of second messenger systems and/or brain-derived neurotrophic factor) to ameliorate the adverse effects of social isolation in male prairie voles (Duman, 1998). Finally, future experiments in this animal model should investigate regions of the brain associated with endocrine regulation to

elucidate the effect those treatments have on hormonal function associated with the stress response.

In conclusion, these findings offer a better understanding of potential neurobiological mechanisms underlying the interactions between emotion and cardiovascular function, and how social bond disruption influences the relationship. Understanding how these changes occur in a translational animal model of social environment stress helps to elucidate the underlying mechanisms by which social stressors adversely influence behavior and cardiac function. Further, this experiment improves our understanding of how a negative social environment mediates the adverse changes in human health. This experiment and others like it will help improve clinical understanding and care for individuals who are at risk for cardiovascular disease and/or mood disorders following the loss of a partner.

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