Title

Social isolation and oxytocin antagonism increase emotion-related behaviors and heart rate in female prairie voles

Authors

Watanasriyakul, W Tang
Scotti, Melissa-Ann L
Carter, C Sue
et al.

Publication Date

2022-05-01

DOI

10.1016/j.autneu.2022.102967

Peer reviewed

HHS Public Access

Author manuscript

Auton Neurosci. Author manuscript; available in PMC 2023 May 01.

Published in final edited form as:

Auton Neurosci. 2022 May ; 239: 102967. doi:10.1016/j.autneu.2022.102967.

Social isolation and oxytocin antagonism increase emotion-related behaviors and heart rate in female prairie voles

W. Tang Watanasriyakul[1], **Melissa-Ann L. Scotti**[1,2], **C. Sue Carter**[3], **Neal McNeal**[1], **William Colburn**[1], **Joshua Wardwell**[1], **Angela J. Grippo**[1]

[1]Department of Psychology, Northern Illinois University, DeKalb, IL, 60115

[2]Department of Psychiatry, University of Illinois at Chicago, Chicago, IL, 60612

[3]The Kinsey Institute, Indiana University, Bloomington, IN, 47405

Abstract

Social isolation influences depression- and anxiety-related disorders and altered cardiac function. Oxytocin may mediate these conditions through interactions with social behavior, emotion, and cardiovascular function, via central and/or peripheral mechanisms. The present study investigated the influence of oxytocin antagonism using L-368,899, a selective oxytocin receptor antagonist that crosses the blood-brain barrier, on depression- and anxiety-related behaviors and heart rate in prairie voles. This rodent species has translational value for investigating interactions of social stress, behavior, cardiac responses, and oxytocin function. Adult female prairie voles were socially isolated or co-housed with a sibling for 4 weeks. A subset of animals in each housing condition was subjected to 4 sessions of acute L-368,899 (20 mg/kg, ip) or saline administration followed by a depression- or anxiety-related behavioral assessment. A subset of co-housed animals was evaluated for cardiac function following acute administration of L-368,899 (20 mg/kg, ip) and during behavioral assessments. Social isolation (vs. co-housing) increased depression- and anxiety-related behaviors. In isolated animals, L-368,899 (vs. vehicle) did not influence anxiety-related behaviors but exacerbated depression-related behaviors. In co-housed animals, L-368,899 exacerbated depression-related behaviors and increased heart rate at baseline and during behavioral tests. Social isolation produces emotion-related behaviors in prairie voles; central and/or peripheral oxytocin antagonism exacerbates these behavioral signs. Oxytocin antagonism induces depression-relevant behaviors and increases basal and stressor-reactive heart rate in co-housed prairie voles, similar to the consequences of social isolation demonstrated in this model. These results provide translational value for humans who experience behavioral and cardiac consequences of loneliness or social stress.

Author for Correspondence: Angela J. Grippo, PhD, Department of Psychology, Northern Illinois University, 1425 W. Lincoln Highway, DeKalb, IL, 60115, angelagrippo@niu.edu, Phone: 815-753-0372, Fax: 815-753-8088.

Conflict of Interest:
None

7.Conflicts of Interest
The authors declared no conflicts of interest.

Keywords

anxiety; cardiac; depression; L-368,899; oxytocin antagonist; prairie vole; social isolation

1. Introduction

Individuals worldwide experience negative consequences of loneliness and social isolation (Cacioppo et al., 2015a; Cacioppo et al., 2018a, 2018b). Objective social isolation and subjective feelings of loneliness are strong predictors of affective disorders including depression and anxiety, physiological disruptions such as cardiovascular diseases, and premature mortality (Beutel et al. 2017; Cacioppo et al., 2018a, 2018b; Gardener et al., 2018; Lim et al., 2016; Petitte et al., 2015; Sgoifo et al., 2014; Steptoe et al., 2013). The global COVID-19 pandemic has brought recent attention to the importance of adaptive social interactions for promoting psychological and physiological health (Mattos Dos Santos 2020; Özdin & Bayrak Özdin, 2020; Saltzman et al., 2020; Savage et al., 2021). Although loneliness may appear to be a function of personality traits or poor social skills, these assumptions are unwarranted. Loneliness influences people of every demographic, social, and culture group (Cacioppo et al., 2015, 2018a, 2018b; Jeste et al., 2020).

Multiple biopsychological systems may influence the interactions of loneliness, psychological well-being, and physical health. The functionality of the nonapeptide oxytocin (OT) may be one system that plays a role in the associations of sociality, stress, physiology, and emotional health including depression and anxiety (Carter et al. 2020). OT is primarily synthesized in the magnocellular neurons of the hypothalamic paraventricular and supraoptic nuclei, and OT receptors are present in both the central and peripheral nervous systems (Jankowski et al., 2004; Jurek & Neumann 2018). OT acting both centrally and peripherally has several effects on stress reactivity, physiological processes, and emotions related to depression and anxiety. For example, OT promotes pair-bonding, buffers hypothalamic-pituitary-adrenal (HPA) responses to stress, and reduces depressive- and anxiety-like behaviors in rodents (Ellis et al. 2021; Slattery & Neumann 2010; Smith & Wang 2014; Williams et al. 1994; Yan et al. 2014). Additionally, OT plays an important role in the regulation of cardiovascular functions through complex interactions among neural control of sympathetic and parasympathetic processes, hormone release, and peripheral actions (Grippo et al. 2009; Gutkowska et al. 2014; Yee et al. 2016). Binding of OT to receptors in the peripheral nervous system attenuates heart rate (HR) and blood pressure in rodents, and central OT signaling regulates cardiovascular functions through neural control of baroreceptor and chemoreceptor reflexes, which are crucial for determining blood pressure, HR, and blood oxygenation (Japundžić-Žigon 2013). Taken together, these previous observations indicate that both central and peripheral OT processes influence emotions including depressive- and anxiety-related states, stress reactivity, and cardiac and autonomic functions.

Given the versatility of OT in regulating neurobiological and behavioral processes, central and peripheral OT communication may play a role in stress-induced cardiovascular dysfunction, loneliness, and affective symptoms (Quintana et al. 2013; Quintana and

Guastella 2020). Animal models are a useful tool for investigating these aforementioned interactions. Among rodent models, the prairie vole (*Microtus ochrogaster*) is considered to have high translational value for understanding social stress-induced behavioral and physiological consequences. Prairie voles have a similar social structure to humans, including exhibiting social monogamy, biparental care of offspring, and living in extended family groups (Carter et al. 1995; Young et al. 2011). These rodents display behavioral, neuroendocrine, and cardiovascular disturbances similar to humans following long-term social isolation and disruption of established social bonds (Bosch et al. 2009; Grippo et al. 2007a, c; Sun et al. 2014). The OT system has been previously investigated in prairie voles due to its involvement in pair-bonding and parental care behaviors (Cho et al. 1999; Keebaugh et al. 2015; Perkeybile et al. 2015; Young et al. 2011). Recent work has also investigated the complexity of central and peripheral OT communication, sociality, cardiovascular function, and affective behaviors related to depression and anxiety in the prairie vole model. For example, prolonged social isolation from a same-sex sibling or a pair-bonded partner increases depressive- and anxiety-like behaviors, activates central OT neurons, and impairs cardiovascular functions (Donovan et al. 2020; Grippo et al. 2007b, c). Further, peripheral OT administration protects against isolation-induced cardiac abnormalities and behavioral dysfunction in prairie voles, which may be due to central or peripheral communication, or both (Grippo et al. 2009, 2012; Stevenson et al. 2019). Moreover, OT mechanisms underlie several aspects of social and affective behaviors, social buffering of stress responses, and social memory in prairie voles (Burkett et al. 2016; Hirota et al. 2020; Pohl et al. 2019; Smith & Wang 2014).

In both humans and animal models, socially isolated individuals are more likely to develop cardiovascular disease and affective disorders including depression (Grippo et al. 2007c; Kiecolt-Glaser et al. 1998; Steptoe et al. 2004). However, whether the OT system mediates these health consequences warrants further investigation. The purpose of the current study was to examine in female prairie voles the potential role of OT, acting either centrally or peripherally, to increase isolation-induced affective and cardiac disruptions. Female prairie voles were selected specifically for the current study given several lines of evidence suggesting that females may deserve additional attention. Data from both humans and animal models indicate that males and females respond to social stress differently through specific coping behaviors and biomarkers (Altemus et al. 2014; Grippo et al. 2007b; Senst et al. 2016; Shufelt et al. 2018). Additionally, the female population may be under-represented in neuroscience and biomedical research (Beery and Zucker 2011; Will et al. 2017).

In the present study, OT signaling was disrupted using the highly selective OT receptor antagonist, L-368,899. After a peripheral injection, this compound has been demonstrated to cross the blood-brain barrier and antagonize central OT receptors, induce neural activation, and influence social behaviors, and therefore may influence central and/or peripheral OT communication (Boccia et al. 2007; Williams et al. 1994). We predicted that a.) social isolation from a same-sex sibling would increase depressive- and anxiety-like behaviors in female prairie voles in operational behavioral tasks, relative to co-housing with a sibling; b.) peripheral L-368,899 injections would increase depressive- and anxiety-like behaviors in both co-housed and isolated prairie voles, and that social isolation would increase the expression of these affective behaviors relative to co-housing; and c.) peripheral

L-368,899 injections would impair cardiac function in co-housed animals relative to vehicle administration, with consequences similar to those of social isolation.

2. Materials Methods

2.1 Animals

A total of 60 adult, sexually naïve and reproductively intact female prairie voles, ages 60–90 days old, were included in the current study. All animals were bred in-house at Northern Illinois University and were descendants of wild prairie voles captured near Champaign, IL. Animals were weaned on post-natal day 21 and co-housed in same-sex sibling pairs in a standard cage (12 × 18 × 28 cm) until experimentation; only one animal from each sibling pair was included in the study. Animals were housed in a room with controlled temperature of 20–23°C and a relative humidity of 40–50%, under a standard 14:10 light/dark cycle (lights on at 0630). All animals were allowed ad libitum access to water and food (Purina rabbit chow, Purina, St. Louis, MO). Handling, cage changing, and measuring of body weight were standardized for all animals. All procedures were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Northern Illinois University Institutional Animal Care and Use Committee.

As noted above, female prairie voles were studied here given the historical underrepresentation of the female population in neuroscience and biomedical research (Beery and Zucker 2011; Will et al. 2017), and evidence of sex differences in behavioral and biological responses to social stress (Altemus et al. 2014; Grippo et al. 2007b; Senst et al. 2016; Shufelt et al. 2018). Female prairie voles as a model offer advantages over other laboratory rodents because they do not exhibit an estrous cycle or ovulate spontaneously; chemosensory cues and tactile stimulation by an unrelated male are required to induce ovulation in this species (Carter & Getz 1993), allowing for studies of intact animals without the need to control for potential estrous cycle confounds.

2.2 General experimental timeline

The general timeline of the experiment is described in this section, with specific experimental procedures described in the following sections. Figure 1 details the groups, manipulations, sample sizes, and experimental timeline. Sixty adult female prairie voles were randomly assigned to one of two housing conditions for a 4-week period: co-housed continuously with a female sibling (control; n = 42) or isolated from the sibling (n = 18).

Following this 4-week housing period, a subset of animals underwent 4 behavioral tests to assess the influence of social isolation and OT disruptions on behavioral coping and adaptation (n = 19 co-housed and n = 18 isolated). All behavioral tests were conducted during the light period, and each behavioral test was separated by 48 hours. The tests were conducted in the following order for the animals in this subset: open field test (OFT), elevated plus maze (EPM), tail-suspension test (TST), and forced swim test (FST).

A subset of co-housed prairie voles (n = 23) was used to examine whether OT disruptions would influence behavioral adaptation and cardiac function. The prairie voles in this subset

were implanted with a radiotelemetry transmitter for the recording of electrocardiogram (ECG), body temperature, and general physical activity prior to the beginning of the experimental design. Following recovery from the surgical procedures and recording of baseline (pre-manipulation) cardiac data, animals remained co-housed with a female sibling for 4 weeks, and underwent the following behavioral tests during the light period, each separated by at least 48 hours, in the following order: home cage assessment, EPM, and FST. Radiotelemetry data were recorded continuously during each assessment in this subgroup, and at specific intervals following the assessment.

One hour prior to the start of each assessment listed above, half of the animals in each housing condition received an injection of the selective OT receptor antagonist L-368,899 (20 mg/kg, ip; injected at 2 ml/kg of saline); generously donated by Merck Sharpe & Dohme Corp., Rahway, NJ), and the other half received saline vehicle injections (2 ml/kg, ip). All animals were returned to their housing condition (co-housed or isolated) after each injection prior to the behavioral assessment, and between each assessment.

According to preliminary validation experiments conducted in prairie voles, the short-term behavioral tests used in this study design can be effectively used to measure emotion-related and coping behaviors without adverse effects. The order of tests does not influence the behavioral or physiological outcomes of subsequent tests, nor are carryover effects observed on behavioral, autonomic, cardiac, or neuroendocrine outcome measures when the tests are separated by at least 24 hours (Grippo et al. 2007c; McNeal et al. 2017).

2.3 Specific experimental procedures

2.3.1 Social manipulations.—

Animals in the co-housed control condition were housed with a same-sex sibling for 4 weeks in a standard cage. Animals in the isolated condition were separated from their same-sex sibling and housed alone in a standard cage without visual, auditory, or olfactory cues from their respective sibling for 4 weeks. All animals had access to food, water, and bedding.

2.3.2 Pharmacological manipulations.—

Animals in each housing condition received injections of either saline vehicle (2 ml/kg, ip; n = 20 co-housed, n = 10 isolated) or L-368,899 (20 mg/kg, ip, injected a volume of 2 ml/kg; n = 22 co-housed, n = 9 isolated), each separated by 48 hours. Each day prior to the injections, L-368,899 was dissolved in saline at a volume of 10 mg/ml via sonication for 10 minutes, to ensure proper reconstitution of the drug; the saline vehicle was also sonicated to ensure the same consistency in temperature of the drug and vehicle. L-368,899 is a potent, nonpeptide, rapidly absorbed OTA that has been shown to selectively bind to OT receptors at a high affinity rate and not the similarly structured vasopressin V1a and V2 receptors (Thompson et al. 1997; Williams et al. 1994). Peripheral administration of L-368,899 crosses the blood-brain barrier and binds to central OT receptors, is present in cerebrospinal fluid and brain regions that contain OT receptors after peripheral administration, mediates cardiac and hypothalamic-pituitary-adrenal functions, and alters social behaviors in rodents and non-human primates (Boccia et al. 2007; Grippo et al. 2019; Harshaw et al. 2021; Hodges et al. 2019; Lee et al. 2015; Madularu et al. 2014; Mooney et al. 2014; Smith et al., 2010; Smith et al. 2013).

2.3.3 Open field test.—The OFT has been used to examine coping and adaptive behaviors related to exploration and anxiety in rodents (Prut & Belzung 2003), and has been adapted for the prairie vole model (Grippo et al. 2014; Osako et al. 2018). One hour after the pharmacological treatment, each animal was removed from its home cage and was placed into the open field apparatus for 5 minutes. The testing apparatus consisted of a white-bottomed, square box (40 × 40 cm), with an internal section marked by a red outline (20 × 20 cm) on the floor of the apparatus; this testing floor was surrounded with transparent Plexiglas sides (40 cm), which were tall enough to prevent the subject from escaping the apparatus. Following the conclusion of the test, the animal was immediately returned to its home cage, in its respective housing condition. The apparatus was sanitized with 10% bleach solution and dried between testing each animal.

A digital video camera was used to record behaviors during the OFT, and behaviors were scored by trained, experimentally blind observers. Behaviors were defined as follows: a.) number of instances entering, and duration spent, in the center and surround sections of the apparatus (all 4 paws must cross into the respective section to be coded as entering the section); b.) duration spent autogrooming (including using paws to groom or scratch the head or body, or licking of the fur); c.) instances of rearing, when the two front paws left the ground (including rearing in the air and rearing at the wall or corner of the apparatus); and d.) duration spent in exploratory motion, defined as forward or backward progress (the following behaviors were excluded from this category: upward progress - which occurs during rearing; and circular motion without a physical change in location - which occurs during autogrooming). Reduced exploration of the center section, altered grooming or rearing, and/or altered exploratory motion in the apparatus, were operationally defined as anxiety-related behaviors (Prut and Belzung 2003).

2.3.4 Elevated plus maze.—The EPM has been used to examine coping and adaptive behaviors related to exploration and anxiety in rodent models (Pellow et al. 1985), including prairie voles (Grippo et al. 2014; Smith et al. 2013). One hour following the pharmacological treatment, animal was removed from its home cage and placed in the center of the EPM in a brightly lit room and allowed to freely explore for 5 minutes. The maze, placed 57 cm off the ground, consisted of two opposing open arms of clear Plexiglas (49.5 × 10 cm), two perpendicular, opposing closed arms of black Plexiglas with an open roof (49.5 × 10 × 30.5 cm), and a center section of clear Plexiglas (10 × 10 cm). Following the conclusion of the test, the animal was immediately returned to its home cage in its respective housing condition. The apparatus was sanitized with 10% bleach solution and dried between testing each animal.

A digital video camera was used to record behaviors during the EPM, and behaviors were scored by trained, experimentally blind observers. Behaviors were defined as follows: a.) duration spent in the open, closed, and center sections of the maze (all 4 paws must cross into the respective section for an animal to be coded as entering the section); and b.) number of crosses into the center section (all 4 paws must cross into the center section; and an animal must necessarily cross the center section before entering another arm of the maze). Reduced exploration of the open arms was used as an operational index of anxiety-related

behavior; and the instances of entering the center section were used to characterize general physical activity (Pellow et al. 1985).

2.3.5 Tail-suspension test.—The TST has been used to examine coping and adaptive behaviors related to a depressive phenotype and responses to pharmacological antidepressant treatments in rodents (Steru et al. 1985), and the test has been adapted for use in the prairie vole model (Bosch et al. 2009; Grippo et al. 2019). One hour following the pharmacological treatment, each animal was removed from its home cage and underwent the TST for 5 minutes. Each animal was suspended by its tail using adhesive tape attached to a metal bar (5 mm in diameter), in the middle of a transparent Plexiglas box, approximately 25 cm above the floor. Care was taken to ensure that the animal's tail was not damaged during the placement or removal of the tape, or during the test, by placing the tape in the middle of the tail. The animal was returned to its home cage immediately following the conclusion of the test, in its respective housing condition. The apparatus was sanitized with 10% bleach solution and dried thoroughly between testing each animal.

A digital video camera was used to record behaviors during the TST, and behaviors were scored by trained, experimentally blind observers. Behaviors were quantified as the duration spent exhibiting the following behaviors: a.) active movements, characterized by contortions or movements of the trunk or flailing of one or more limbs, and b.) immobility, characterized by the lack of movement; this category excluded movements required for respiration and residual swinging of the body as a function of immediately preceding active movements (Steru et al. 1985).

2.3.6 Forced swim test.—The FST has been used to assess coping and adaptive behaviors related to helplessness and depression-like behavior, and is hypothesized to operationally represent a depressive phenotype in some rodent models, including prairie voles (Cryan et al. 2005; Grippo et al. 2012). One hour following the pharmacological treatment, each animal was removed from the home cage and exposed to the FST for 5 minutes. The apparatus consisted of a clear, cylindrical Plexiglas tank (46 cm in height; 20 cm in diameter) that was filled with tap water (25–26°C) to a depth of 18 cm. The animal was gently placed in the tank for 5 minutes; the animal was returned to its home cage immediately following the conclusion of the test, with a 15-minute access to a heat lamp to prevent hypothermia. The swim tank was cleaned thoroughly, sanitized with 10% bleach solution, and filled with clean water between testing each animal.

A digital video camera was used to record behaviors during the FST, and behaviors were scored by trained, experimentally blind observers. Behaviors were quantified as the duration spent exhibiting the following behaviors: a.) swimming, characterized by movements of forelimbs and hindlimbs without breaking the surface of the water; b.) struggling, characterized by forelimbs breaking the surface of the water; c.) climbing, characterized by attempts to climb the walls of the tank; and d.) immobility, characterized by the lack of body movement (floating) or using limbs solely to remain afloat without corresponding trunk movements. Time spent swimming, struggling, and climbing were summed to provide an index of active coping behaviors; immobility was used as an operational index of passive or depressive-related behavior (Grippo et al. 2012).

2.3.7 Telemetric transmitter implantation and data quantification.—All animals in the co-housed subset received an intraperitoneal implantation of a wireless radiofrequency transmitter prior to the beginning of the social housing manipulation (TA10ETA-F10; Data Sciences International, St. Paul, MN) to monitor the electrocardiogram (ECG), locomotor activity, and body temperature, using procedures previously described (Grippo et al. 2007c) and with modifications of procedures described in other rodent models (Gehrmann et al. 2000; Sgoifo et al. 1996). Animals were anesthetized throughout the surgical procedures with a mixture of isoflurane (Henry Schein, Dublin, OH) and oxygen. Briefly, the body of the transmitter was implanted into the intraperitoneal cavity, and negative and positive leads were tunneled subcutaneously and sutured to the muscle on the exterior thorax, to the left and right of the heart. All incisions were closed using sterile suture, and an injection of carprofen (5 mg/kg, ip; Zoetis Inc., Parsippany, NJ) was administered immediately following the procedure to minimize pain and inflammation.

Following immediate recovery from anesthesia, animals were housed for 5 days in custom-designed divided cages (25 × 45 × 60 cm) as previously described (Grippo et al. 2007c); allowing for the surgical wounds to heal while still maintaining limited social interactions with the sibling. Additionally, a heat lamp was made available (covering approximately 1/3 of the cage) for the first 2 nights after the surgical procedures to allow the animal to self-regulate its body temperature, and a solution of 2% sucrose was also made available to the animal (in addition to regular food and water) for the first 5 days following the surgical procedures to ensure adequate fluid intake. Subsequently, all animals were returned to standard cages with their respective siblings to recover for an additional 5–7 days.

Throughout the recovery period, animals were assessed daily for the following characteristics of recovery (with further details of the acquisition of data from the radiotelemetry system below): a.) visible signs of eating and drinking; b.) visible signs of adequate urination and defecation; c.) adequate activity level, measured using data from the radiotelemetry transmitter (approximately 2 counts per minute, with increases in activity level approximately every 2–3 hours); d.) adequate body temperature, measured using data from the radiotelemetry transmitter (approximately 37.5°C); and e.) stabilization of heart rate across the recovery period, measured via the radiotelemetry transmitter.

Data generated from the telemetric transmitter were recorded with a radiotelemetry receiver using the vendor software (Dataquest ART Acquisition, Data Sciences International). ECG signals were obtained with a sampling rate of 5 kHz and 12-bit precision digitizing; HR was evaluated using the number of beats per minute. General activity level was monitored via the receiver (sampling rate 256 Hz). Motor activity was evaluated using data from the vendor software based on a proprietary data acquisition formula; this index is calculated from the amount of deviation of the transmitter away from a centralized point in the receiver and is reported in counts per minute (therefore this index represents a gross measure of motor activity, where lower counts per minute represent low levels of activity and higher counts per minute represent greater levels of activity). Body temperature was obtained from the vendor software, which reports an index of core body temperature based on the intraperitoneal location of the telemetric transmitter.

Data segments were recorded at hourly intervals during an undisturbed baseline period, which occurred in the home cage of the animals on the morning of each test session (2–5 minutes of data recorded each hour). During each behavioral test, data were recorded continuously during the entire 5-minute testing session. Data segments were recorded for an undisturbed period following each testing session (3–10 minutes of data recorded each hour) while the animals were in the home cage, co-housed with the respective siblings.

2.4 Data analyses

Behavioral data in the present study were coded by at least 2 trained experimenters who were blind to the experimental conditions, using Noldus Observer XT version 8.0 (Noldus Information Technology, Leesburg, VA). Coders were trained to a level of at least 90% agreement, and the mean of all scores was used for each variable.

All behavioral data were analyzed using two-way independent group analyses of variance (ANOVA), with housing condition (co-housed or isolated) and treatment (vehicle or L-369,899) as independent factors. Preliminary comparisons indicated that the behavioral data in the subgroups exposed to the EPM and FST did not differ, therefore these behavioral data were pooled for the purpose of conducting group-level comparisons. Pairwise comparisons were conducted using independent group Student's t-tests, for hypothesis-driven comparisons.

HR data in the co-housed subgroup were analyzed using Dataquest ART Analysis software, version 4.1 (Data Sciences International). Repeated measures in vehicle- and L-368,899-treated animals were analyzed using mixed-design ANOVAs, with treatment (vehicle or L-368,899) as the independent factor and time as the repeated factor. Pairwise comparisons were conducted using either independent group or repeated measures Student's t-tests, as relevant, with a Bonferroni correction applied to all multiple comparisons.

Exploratory correlations were conducted using Pearson's r correlation coefficients to determine the inter-relationships of behavioral and cardiac variables in the co-housed subgroup. The following correlations were computed for vehicle and L-369,899 conditions separately: a.) HR following the injection alone vs. HR during the EPM; b.) HR following the injection alone vs. HR during the FST; c.) HR during the EPM vs. HR during the FST; d.) HR during the EPM vs. duration in the open arms of the EPM; e.) HR during the FST vs. duration of immobility in the FST; and f.) duration in the open arms of the EPM vs. duration of immobility in the FST. Correlations are reported for illustrative purposes; Cohen's (1992) advice was adopted to describe a correlation of approximately 0.1 as weak, approximately 0.3 as moderate, and approximately 0.5 as strong.

Data are reported as means and standard error of the mean (SEM) for all analyses and figures. A probability value of $p < 0.05$ (2-tailed) was considered to be statistically significant. Student's t-statistics that exceeded the Bonferroni-adjusted probability value were reported to be statistically significant for all multiple comparisons.

3. Results

3.1 Open field test

A two-way ANOVA evaluating duration spent exploring the center section of the OFT apparatus as a function of pharmacological treatment and housing condition yielded a main effect of housing condition [$F(1,36) = 7.49$, $p < 0.01$], but no main effect of treatment ($p > 0.05$) and no treatment by housing condition interaction effect ($p > 0.05$; Figure 2a). The duration spent in the center section of the apparatus was significantly lower in the isolated condition, relative to the co-housed condition [$t(34) = 2.8$, $p < 0.008$].

A two-way ANOVA evaluating number of crosses into the center section of the apparatus as a function of treatment and housing condition did not yield any significant main effects or an interaction ($p > 0.05$ for all comparisons; no follow-up tests were conducted; Figure 2b).

A two-way ANOVA evaluating the duration of exploratory motion during the OFT as a function of treatment and housing condition yielded a main effect of housing condition [$F(1,36) = 6.69$, $p < 0.01$], but no main effect of treatment ($p > 0.05$) and no interaction effect ($p > 0.05$; Figure 2c). The duration of exploratory motion was significantly higher in the isolated-vehicle condition relative to the co-housed-vehicle condition [$t(17) = 2.0$, $p < 0.05$). The duration of exploratory motion did not significantly differ between the isolated-L-368,899 and co-housed-L-368,899 conditions ($p > 0.05$).

A two-way ANOVA evaluating the duration of grooming during the OFT as a function of treatment and housing condition yielded a main effect of housing condition [$F(1,36) = 16.5$, $p < 0.0003$], but no main effect of treatment ($p > 0.05$) and no interaction effect ($p > 0.05$; Figure 2d). The duration of grooming was significantly lower in the isolated condition, relative to the co-housed condition [$t(34) = 4.1$, $p < 0.0002$].

A two-way ANOVA evaluating instances of rearing in the apparatus as a function of treatment and housing condition did not yield any significant main effects or an interaction ($p > 0.05$ for all comparisons; no follow-up tests were conducted; data not shown).

3.2 Elevated plus maze

A two-way ANOVA evaluating the duration spent in the open arms of the EPM as a function of pharmacological treatment and housing condition yielded a main effect of housing condition [$F(1,60) = 19.1$, $p < 0.0001$], but no main effect of treatment and no treatment by housing condition interaction ($p > 0.05$ for both comparisons; Figure 3a). The duration spent in the open arms was significantly lower in the isolated condition versus the co-housed condition [$t(60) = 4.25$, $p < 0.0001$].

A two-way ANOVA evaluating the number of center crosses during the EPM as a function of treatment and housing condition did not yield any significant main effects or an interaction effect ($p > 0.05$ for all comparisons; no follow-up tests were conducted; Figure 3b).

A mixed-design ANOVA evaluating HR prior to, during, and following the EPM in a subset of co-housed animals as a function of treatment yielded a main effect of treatment [(F(1.69) = 3,7, $p < 0.05$; a main effect of time [F(2,69) = 98.4, $p < 0.0001$], and an interaction effect [(F(1,69) = 3.6, $p < 0.05$] (Figure 3c]. No baseline differences in HR were observed ($p > 0.05$). Both treatment conditions displayed an increased HR during the EPM relative to the respective baseline HR values [vehicle: t(10) = 9.8, $p < 0.0001$; L-368,899: t(11) = 8.6, $p < 0.0001$], however HR in the L-368,899 condition was significantly higher during the EPM versus the vehicle condition [t(21) = 2.2, $p < 0.02$]. Three hours following the EPM, the HR values of both groups were significantly lower than the respective EPM HR values [vehicle: t(10) = 5.4, $p < 0.0003$; L-368,899: t(11) = 8.4, $p < 0.0001$], but both values at this time point were significantly higher than the respective baseline values [vehicle: t(10) = 3.5, $p < 0.006$; L-368,899: t(11) = 2.4, $p < 0.01$)].

3.3 Tail-suspension test

Analysis of the TST data excluded 3 animals that gained access to the bar during the test (2 animals from the co-housed-saline condition and 1 animal from the co-housed-L-368,899 condition). A two-way ANOVA evaluating the duration of immobility during the TST as a function of pharmacological treatment and housing condition yielded a main effect of housing condition [F(1,39) = 10.4, $p < 0.003$], and a treatment by housing condition interaction [F(1,39) = 4.2, $p < 0.05$], but no main effect of treatment ($p > 0.05$; Figure 4). The duration of immobility was significantly higher in the isolated-vehicle condition versus the co-housed-vehicle condition [t(16) = 3.7, $p < 0.0006$]. The duration of immobility was slightly higher in the co-housed-L-368,899 group relative to the co-housed-vehicle group [t(15) = 2.0, $p = 0.05$], however the duration of immobility in the isolated-L-368,899 group did not significantly differ from the isolated-vehicle group ($p > 0.05$).

3.4 Forced swim test

A two-way ANOVA evaluating the duration of immobility during the FST as a function of pharmacological treatment and housing condition yielded a main effect of housing condition [F(1,60) = 8.2, $p < 0.006$], and a main effect of treatment [F(1,60) = 6.1, $p < 0.02$], but no interaction effect ($p > 0.05$; Figure 5a). The isolated condition displayed greater levels of immobility relative to the co-housed condition [t(59) = 2.8, $p < 0.008$). The L-368,899 condition displayed greater levels of immobility relative to the vehicle condition [t(59) = 2.3, $p < 0.02$].

A mixed-design ANOVA evaluating HR prior to, during, and following the FST in a subset of co-housed animals as a function of treatment yielded a main effect of treatment [F(1,69) = 11.3, $p < 0.0001$], a main effect of time [F(2,69) = 32.7, $p < 0.0001$], and an interaction effect [F(1,69) = 3.2, $p < 0.05$; Figure 5b). No baseline differences in HR were observed ($p > 0.05$). Both treatment conditions displayed an increased HR during the FST relative to the respective baseline HR values [vehicle: t(10) = 3.9, $p < 0.003$; L-368,899: t(11) = 8.8, $p < 0.0001$], however HR in the L-368,899 condition was significantly higher during the FST versus the vehicle condition [t(21) = 3.2, $p < 0.005$]. Three hours following the EPM, the HR values of both groups were significantly lower than the respective HR values during

the FST [vehicle: $t(10) = 2.5$, $p < 0.02$; L-368,899: $t(11) = 6.0$, $p < 0.0001$], and did not significantly differ from the respective baseline HR values ($p > 0.05$ for both comparison).

3.5 Heart rate following L-368,899 or vehicle injections

A mixed-design ANOVA evaluating HR at baseline and following either vehicle or L-368,899 in the co-housed subgroup yielded a main effect of pharmacological treatment [$F(1,46) = 10.2$, $p < 0.003$; a main effect of time [$F(1,46) = 8.2$, $p < 0.006$], and an interaction effect [$F(1,46) = 3.9$, $p < 0.05$; Figure 6]. No baseline differences in HR existed between the vehicle and L-368,899 conditions ($p > 0.05$). Following the injection, the L-368,899 condition displayed a significantly elevated HR relative to the vehicle condition [$t(21) = 3.2$, $p < 0.004$], and relative to this condition's respective baseline HR value [$t(11) = 3.2$, $p < 0.008$]. HR in the vehicle condition following the injection was not significantly different from this condition's respective baseline HR value ($p > 0.05$).

3.6 Exploratory Correlations

Pearson's r correlation coefficients were used to investigate associations of behavioral and cardiac variables in saline- and L-368,899-treated groups separately (Table 1). Briefly, HR following the injection alone was weakly-to-moderately positively correlated with HR during the EPM in the saline group, but not in the L-368,899 group. HR during the EPM was moderately negatively correlated with duration in the open arms of the EPM, and HR during the FST was moderately positively correlated with duration of immobility in the FST, in both groups. HR during the EPM was strongly negatively correlated with HR during the FST, and behavior during the EPM (open arm duration) was strongly negatively correlated with behavior during the FST (immobility duration), in the L,368–899 group but not the saline group.

4. Discussion

Several lines of evidence demonstrate that social stressors, such as loneliness and social isolation, influence emotions, behaviors, and physiological functioning (Beutel et al. 2017; Cacioppo et al. 2015b; Lim et al. 2016). OT communication in the central or peripheral nervous system may underlie the associations of social stress, depression- and anxiety-behaviors, and physiological dysfunction (Carter et al. 2020; Gutkowska et al. 2014; Grinevich and Neumann 2021; Smith and Wang 2014). The prairie vole is a valuable translational model for investigating the role of OT in mediating behaviors and cardiac function in the context of social isolation, given the similarities in social structure between this rodent species and humans (Bosch et al. 2009; Carter et al. 1995; Grippo et al. 2007c; Sun et al. 2014; Young et al. 2011). Therefore, the current study investigated the role of OT communication in depression- and anxiety-related behaviors and cardiac reactivity in co-housed and isolated prairie voles using the selective OT receptor antagonist L-368,899. The present data indicate that social isolation induces both depression- and anxiety-related behavioral changes, and depression-related changes are exacerbated in isolated prairie voles following administration of L-368,899. The data further demonstrate that administration of L-368,899 produces depression-relevant behaviors in co-housed prairie voles, as well as promotes increased resting and stressor-responsive HR. It is possible that antagonism of

central or peripheral OT receptors, or both, mediated the behavioral and cardiac disruptions observed here following L-368,899. The present results offer insight into the influence of OT on affective behaviors and cardiac function in co-housed and isolated female prairie voles.

In the current study, prolonged social isolation was associated with anxiety and depressive phenotypes, including altered behaviors in the OFT, EPM, TST, and FST. These findings support previous observations in prairie voles (Bosch et al. 2009; Grippo et al. 2007a, 2007b; Sun et al. 2014) and other rodent models (Ieraci et al. 2016; Kumari et al. 2016; Kwak et al. 2009), indicating that social isolation is associated with several affective behavioral disruptions in validated operational measures. These findings also support data from human samples, which have discussed the affective consequences of loneliness and social isolation (Steptoe et al. 2004, 2013). With both anxiety- and depression-related behaviors being observed in socially isolated animals (with and without OT disruptions), the present findings may provide insight into the comorbidity of anxiety and depressive syndromes in clinical samples (American Psychiatric Association 2013).

Although social isolation increased affective behaviors in the operational measures used here, OT antagonism produced differential effects on anxiety- vs. depression-related behaviors as a function of housing condition. First, focusing on isolated prairie voles, administration of L-368,899 exacerbated depression-relevant behaviors in the FST (but not in the TST), evidenced by an increase in immobility duration during the FST in isolated prairie voles following L-368,899 administration (vs. vehicle-treated isolated animals). These data extend previous results demonstrating that socially isolated prairie voles display depression-relevant behaviors as well as altered OT communication in the brain and circulation (Donovan et al. 2020; Grippo et al. 2007a, 2007b; Sun et al. 2014). However, in contrast to depression-related behaviors, administration of L-368,899 did not influence anxiety-related or exploratory behaviors in the OFT or EPM in the present study. In line with this pattern of results, upregulation of OT via a peripheral injection of exogenous OT protected against depression-relevant behaviors, but did not protect against anxiety-relevant behaviors, in socially isolated prairie voles (Grippo et al. 2009, 2012). Social isolation and disrupted OT communication may have synergistic consequences on depression-related behaviors in the prairie vole model. However, whether central or peripheral OT disruptions (or both) interact with social isolation requires further investigation.

Focusing on affect-related behaviors in co-housed prairie voles, injections of L-368,899 increased depressive-like behaviors in both the FST and TST (vs. vehicle) — to the same behavioral levels as isolated animals. Coupled with the pattern of results discussed above, disruptions of OT may mediate depression-related behaviors via either central or peripheral mechanisms. OT mechanisms have been discussed previously in humans and animal models (Ellis et al. 2021; Neumann and Landgraf 2012). For instance, elevated hypothalamic and dorsolateral prefrontal OT mRNA expression have been observed in depressed patients (Lee et al. 2018; Meynen et al. 2007; Parker et al. 2010), and altered central OT communication has been observed in rodent models associated with depression (Grippo et al. 2007a; Yan et al. 2014). It is also possible that depression-relevant behaviors are a function of peripheral OT disruptions, given that L-368,899 was injected peripherally in the present study design.

In contrast to inducing depression-related behaviors, L-368,899 administration did not influence anxiety-like behaviors in co-housed prairie voles, evidenced by a lack of change in behavior during the OFT and EPM following drug administration. These results — taken together with the lack of anxiety-related behaviors in isolated prairie voles following OT antagonism — suggest that anxiety-related behaviors may only be partially mediated by OT or that the extent or location of OT antagonism plays a role in its anxiogenic effects. The involvement of the OT system in anxiety disorders has yielded inconclusive findings. Briefly, molecular, genetic, and epigenetic evidence indicates that the OT system may underlie social anxiety disorder; however, the role of the OT system in generalized anxiety disorder is not fully elucidated (Gottschalk & Domschke 2017). Endogenous and exogenous OT may mitigate anxiety signs and symptoms — psychological and physical — in humans and animal models (Yoon & Kim 2020). Interestingly however, OT deficiency does not appear to promote anxiety-related behaviors. For instance, OT receptor knockout mice and prairie voles do not exhibit more anxiety-like behaviors than wildtype controls (Horie et al. 2019; Pagani et al. 2015). Therefore, while the binding of OT to OT receptors has anxiolytic effects, the disruption of OT binding has not consistently produced anxiogenic effects. In addition to OT, the arginine vasopressin (AVP) system also plays an important role in mediating behavior and responses to stress (Carter 2017; Jurek and Neumann 2018), and therefore additional research elucidating the specific role of these peptides in psychological and physiological processes associated with social isolation is warranted (such as cardiovascular and kidney functions, social, reproductive, and parental behaviors) (Japundžić-Žigon et al. 2020; Phelps et al. 2017).

In addition to a focus on depression- and anxiety-related behaviors, the present study investigated basal and stressor-reactive cardiac function in co-housed prairie voles following OT receptor antagonism. L-368,899 injections increased resting HR in co-housed prairie voles, relative to vehicle injections. This observation is in line with previous research demonstrating that injections of an OT antagonist at the brainstem/solitary vagal complex elevated HR in male rats (Higa et al. 2002; Higa-Taniguchi et al. 2009). However, although L-368,899 crosses the blood-brain barrier after peripheral administration, the drug also influences peripheral OT receptors; some OT receptors are located on the uterus and the heart (Jankowski et al., 2004; Williams et al. 1994). It is possible that HR changes observed here were the result of peripheral, rather than central, OT antagonism. The present results in co-housed prairie voles contribute to a body of literature on social experiences, general OT communication, and cardiac function in the prairie vole model. For instance, an increase in resting HR has been observed previously following social isolation in female prairie voles (Grippo et al. 2007c); and peripheral administration of exogenous OT prevented an increase in HR in socially isolated prairie voles, which may have been due to central and/or peripheral mechanisms (Grippo et al. 2009).

The current data support findings indicating that OT communication either in the central or peripheral nervous system plays an important role in cardiovascular homeostasis under both basal and stressor-induced conditions. L-368,899 injections (vs. saline) increased HR in co-housed prairie voles during both the FST and EPM, providing support for the hypothesis that disruptions of OT produce cardiovascular dysfunction during high-stress states. OT plays a critical role in several autonomic processes, including moderation of

sympathetic tone (Japundžić-Žigon 2013; Japundžić-Žigon et al. 2020). Elevated OT activity has been previously observed during high sympathetic demands in humans and rodent models (Hashimoto et al. 1989; Pierrehumbert et al. 2010; Taylor et al. 2006). Moreover, chronically stressed or trauma-exposed individuals may exhibit hyper-reactive OT responses to additional stressors (Grippo et al. 2007b; Pierrehumbert et al. 2010), suggesting that, in some cases, the ability of OT to downregulate HPA and/or sympathetic functions may be ineffective.

While the release of OT to moderate sympathetic demands is crucial during fight-or-flight situations, another important role of OT is to promote parasympathetic functions (Quintana et al. 2013; Quintana and Guastella 2020). For example, OT-deficient mice exhibit exaggerated HR responses to a cholinergic blockade challenge compared to wildtype controls (Michelini et al. 2003). In prairie voles, administration of exogenous OT promotes an increase in parasympathetic tone in socially isolated animals and during short-term stressors (Grippo et al. 2009, 2012); and alters neural control of central autonomic outflow, including regions that influence both sympathetic and parasympathetic processes (Yee et al. 2016). In healthy adults, the release of peripheral OT has been associated with faster cardiovascular recovery following a social stress test (Engert et al. 2016) and increased high frequency HR variability (an index of parasympathetic tone) after listening to slow-tempo music (Ooishi et al. 2017).

Further, OT-parasympathetic communications appear to be faulty in chronically stressed, depressed, and anxious individuals (Alvares et al. 2016; Quintana et al. 2013). Disruptions in OT signaling may be one mechanism that underlies the association between affective disorders and cardiovascular disease; this association is well established (Bradley & Rumsfeld, 2015; Halaris, 2013; Hare et al. 2014). OT may moderate affective symptoms and cardiac dysfunction through central or peripheral processes, for instance by interacting with the HPA axis in the hypothalamic paraventricular nucleus, with HPA-related regions such as the amygdala (Herman and Tasker 2016; Jankord and Herman 2008), via brainstem mechanisms (i.e., the nucleus of the solitary tract/dorsal vagal complex; Japundžić-Žigon, 2013; Japundžić-Žigon et al., 2020), or via peripheral consequences related to central OT functions (Heinrichs et al., 2003).

In conclusion, social isolation was associated with both anxiety- and depression-like phenotypes in the current study. The present data support the hypothesis that OT communication may play a greater mediating role in depression-related behaviors, relative to anxiety-like behaviors, especially in the context of social stress. OT also plays an important role in cardiovascular homeostasis at rest and during short-term stressors. The specific role of central vs. peripheral OT communication in mediating behavioral and cardiac consequences associated with social stressors deserves further attention. Findings from the present study highlight the importance of OT in social stress, affective behaviors, and cardiac function, particularly in females. However, additional studies that directly investigate sex differences in behavior and neurobiological processes are also essential. Further investigation of these research questions using valid and reliable animal models will continue to inform our understanding of social stress-induced behavioral and physiological consequences.

Acknowledgements

We are grateful to Merck Sharpe and Dohme Corp. (Rahway, NJ) for the generous donation of L-368,899. Thanks to the following individuals for valuable assistance in data collection and analysis: Christina Bishop, Ashley Dagner, Elliott Ihm, Nalini Jadia, Alison Knecht, Megan LaRocca, Kristen Lehner, Rachel Murphy, Kristin Preihs, Megha Sharma, Rachel Schultz, Stephanie Steel, Amir Toghraee, Andrew Wegner, and Matthew Woodbury.

5. Funding

Dr. Scotti was supported by a National Institutes of Health (NIH) F32 award, HL108475. This research was also supported in part by NIH grants HL112350 and HL147179, as well as the donation of L-368,899 from Merck Sharpe and Dohme Corp. The funders had no role in the study design, data collection and analysis, data interpretation, decision to publish, or preparation of the manuscript.

5. Funding and Grants:

This research was supported by a National Institutes of Health (NIH) F32 award, HL108475 (M-ALS); NIH grants HL112350 and HL147179 (AJG); and the donation of L-368,899 from Merck Sharpe and Dohme Corp. (AJG).

8. References

Altemus M, Sarvaiya N, Epperson CN (2014) Sex differences in anxiety and depression clinical perspectives. Front Neuroendocrinol 35:320–330. 10.1016/j.yfrne.2014.05.004 [PubMed: 24887405]

Alvares GA, Quintana DS, Hickie IB, Guastella AJ (2016) Autonomic nervous system dysfunction in psychiatric disorders and the impact of psychotropic medications: a systematic review and meta-analysis. J Psychiatry Neurosci JPN 41:89–104. 10.1503/jpn.140217 [PubMed: 26447819]

American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders, 5th edn. American Psychiatric Publishing, Washington, D.C.

Beery AK, Zucker I (2011) Sex bias in neuroscience and biomedical research. Neurosci Biobehav Rev 35:565–572. 10.1016/j.neubiorev.2010.07.002 [PubMed: 20620164]

Beutel ME, Klein EM, Brahler E, et al. (2017) Loneliness in the general population: prevalence, determinants and relations to mental health. BioMed Cent Psychiatry 17:97. 10.1186/s12888-017-1262-x

Boccia ML, Goursaud AP, Bachevalier J, Anderson KD, Pedersen CA(2007) Peripherally administered non-peptide oxytocin antagonist, L368,899, accumulates in limbic brain areas: a new pharmacological tool for the study of social motivation in non-human primates. Horm Behav 52:344–351. 10.1016/j.yhbeh.2007.05.009 [PubMed: 17583705]

Bosch OJ, Nair HP, Ahern TH, Neumann ID, Young LJ (2009) The CRF system mediates increased passive stress-coping behavior following the loss of a bonded partner in a monogamous rodent. Neuropsychopharmacology 34:1406–1415. 10.1038/npp.2008.154 [PubMed: 18923404]

Bradley SM, Rumsfeld JS (2015) Depression and cardiovascular disease. Trends Cardiovasc Med 25:614–622. 10.1016/j.tcm.2015.02.002 [PubMed: 25850976]

Burkett JP, Andari E, Johnson ZV, Curry DC, de Waal FBM, Young LJ(2016) Oxytocin-dependent consolation behavior in rodents. Science 351:375–378. 10.1126/science.aac4785 [PubMed: 26798013]

Cacioppo JT, Cacioppo S (2018a) Loneliness in the modern age: An evolutionary theory of loneliness (ETL). Adv Exper Social Psychol 58:127–197. 10.1016/bs.aesp.2018.03.003

Cacioppo JT, Cacioppo S (2018b) The growing problem of loneliness. Lancet 391: 426. 10.1016/S0140-6736(18)30142-9.

Cacioppo JT, Cacioppo S, Cole SW, Capitanio JP, Goossens L, Boomsma DI (2015a) Loneliness across phylogeny and a call for comparative studies and animal models. Perspectives Psychol Sci: 10, 202–212. 10.1177/1745691614564876.

Cacioppo JT, Cacioppo S, Capitanio JP, Cole SW (2015b) The neuroendocrinology of social isolation. Annu Rev Psychol 66:733–767. 10.1146/annurev-psych-010814-015240 [PubMed: 25148851]

Carter CS (2017) The oxytocin-vasopressin pathway in the context of love and fear. Front Endocrinol Lausanne 8:356. 10.3389/fendo.2017.00356 [PubMed: 29312146]

Carter CS, DeVries AC, Getz LL (1995) Physiological substrates of mammalian monogamy: the prairie vole model. Neurosci Biobehav Rev 19:303–314 [PubMed: 7630584]

Carter CS, Getz LL (1993) Monogamy and the prairie vole. Sci Am 268:100–106. 10.1038/scientificamerican0693-100 [PubMed: 8516669]

Carter CS, Kenkel WM, MacLean EL, et al. (2020) Is oxytocin “nature’s medicine”? Pharmacol Rev 72:829–861. 10.1124/pr.120.019398 [PubMed: 32912963]

Cho MM, DeVries AC, Williams JR, Carter CS (1999) The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (Microtus ochrogaster). Behav Neurosci 113:1071–1079. 10.1037//0735-7044.113.5.1071. [PubMed: 10571489]

Cohen J (1992) A power primer. Psychol Bull 112(1):155–159. 10.1037//0033-2909.112.1.155 [PubMed: 19565683]

Cryan JF, Valentino RJ, Lucki I (2005) Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev 29:547–569. 10.1016/j.neubiorev.2005.03.008 [PubMed: 15893822]

Donovan M, Mackey CS, Platt GN, Rounds J., Brown AN, Trickey DJ, Liu Y, Jones KM, Wang Z(2020) Social isolation alters behavior, the gut-immune-brain axis, and neurochemical circuits in male and female prairie voles. Neurobiol Stress 13:100278. 10.1016/j.ynstr.2020.100278 [PubMed: 33344730]

Ellis BJ, Horn AJ, Carter CS, van Ijzendoorn MH, Bakermans-Kranenburg MJ (2021) Developmental programming of oxytocin through variation in early-life stress: four meta-analyses and a theoretical reinterpretation. Clin Psychol Rev 86:101985. 10.1016/j.cpr.2021.101985 [PubMed: 33770582]

Engert V, Koester AM, Riepenhausen A, Singer T (2016) Boosting recovery rather than buffering reactivity: higher stress-induced oxytocin secretion is associated with increased cortisol reactivity and faster vagal recovery after acute psychosocial stress. Psychoneuroendocrinology 74:111–120. 10.1016/j.psyneuen.2016.08.029 [PubMed: 27608360]

Gardener C, Geldenhuys G, Gott M. (2018). Interventions to reduce social isolation and loneliness among older people: an integrative review. Health Soc Care Community 26(2):147–157. 10.1111/hsc.12367 [PubMed: 27413007]

Gehrmann J, Hammer PE, Maguire CT, Wakimoto H, Trieddman JK, Berul CI (2000) Phenotypic screening for heart rate variability in the mouse. Am J Physiol Heart Circ Physiol 279:H733–740. 10.1152/ajpheart.2000.279.2.H733 [PubMed: 10924073]

Gottschalk MG, Domschke K (2017) Oxytocin and anxiety disorders. In: Hurlemann R, Grinevich V (eds) Behavioral Pharmacology of Neuropeptides: Oxytocin. Springer International Publishing, Cham, pp 467–498

Grinevich V, Neumann ID (2021) Brain oxytocin: how puzzle stones from animal studies translate into psychiatry. Mol Psychiatry 26:265–279. 10.1038/s41380-020-0802-9 [PubMed: 32514104]

Grippo AJ, Cushing BS, Carter CS (2007a) Depression-like behavior and stressor-induced neuroendocrine activation in female prairie voles exposed to chronic social isolation. Psychosom Med 69:149–157. 10.1097/PSY.0b013e31802f054b [PubMed: 17289829]

Grippo AJ, Gerena D, Huang J, Kumar N, Shah M, Ughreja R, Carter CS (2007b) Social isolation induces behavioral and neuroendocrine disturbances relevant to depression in female and male prairie voles. Psychoneuroendocrinology 32:966–980. 10.1016/j.psyneuen.2007.07.004 [PubMed: 17825994]

Grippo AJ, Ihm E, Wardwell J, McNeal N, Scotti MAL, Moenk DA, Chandler DL, LaRocca MA, Preihs K (2014) The effects of environmental enrichment on depressive and anxiety-relevant behaviors in socially isolated prairie voles. Psychosom Med 76:277–284. 10.1097/PSY.0000000000000052 [PubMed: 24804886]

Grippo AJ, Lamb DG, Carter CS, Porges SW (2007c) Social isolation disrupts autonomic regulation of the heart and influences negative affective behaviors. Biol Psychiatry 62:1162–1170 10.1016/j.biopsych.2007.04.011 [PubMed: 17658486]

Grippo AJ, McNeal N, Watanasriyakul WT, Cacioppo S, Scotti MAL, Dagner A (2019) Behavioral and cardiovascular consequences of disrupted oxytocin communication in cohabitating pairs of male and female prairie voles. Soc Neurosci 14:649–662. 10.1080/17470919.2019.1572031 [PubMed: 30658044]

Grippo AJ, Pournajafi-Nazarloo H, Sanzenbacher L, Trahanas DM, McNeal N, Clarke DA, Porges SW, Carter CS (2012) Peripheral oxytocin administration buffers autonomic but not behavioral responses to environmental stressors in isolated prairie voles. Stress 15:149–161. 10.3109/10253890.2011.605486 [PubMed: 21854168]

Grippo AJ, Trahanas DM, Zimmerman RR, Porges SW, Carter CS (2009) Oxytocin protects against negative behavioral and autonomic consequences of long-term social isolation. Psychoneuroendocrinology 34:1542–1553. 10.1016/j.psyneuen.2009.05.017 [PubMed: 19553027]

Gutkowska J, Jankowski M, Antunes-Rodrigues J (2014) The role of oxytocin in cardiovascular regulation. Braz J Med Biol Res 47:206–214 [PubMed: 24676493]

Halaris A (2013) Inflammation, heart disease, and depression. Curr Psychiatry Rep 15:400. 10.1007/s11920-013-0400-5 [PubMed: 23975043]

Hare DL, Toukhsati SR, Johansson P, Jaarsma T (2014) Depression and cardiovascular disease: a clinical review. Eur Heart J 35:1365–1372. 10.1093/eurheartj/eht462 [PubMed: 24282187]

Harshaw C, Lanzkowsky J, Tran AD, Bradley AR, Jaime M (2021) Oxytocin and “social hyperthermia”: interaction with β3-adrenergic receptor-mediated thermogenesis and significance for the expression of social behavior in male and female mice. Horm Behav 131:104981. 10.1016/j.yhbeh.2021.104981 [PubMed: 33878523]

Hashimoto K, Murakami K, Takao T, Shinya M, Masanori S, Zensuke O (1989) Effect of acute ether or restraint stress on plasma corticotropin-releasing hormone, vasopressin and oxytocin levels in the rat. Acta Med Okayama 43:161–167. 10.18926/AMO/30888 [PubMed: 2548372]

Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U(2003) Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol Psychiatry 54:1389–1398. https//: [PubMed: 14675803]

Herman JP, Tasker JG (2016) Paraventricular hypothalamic mechanisms of chronic stress adaptation. Front Endocrinol Lausanne 7:137. 10.3389/fendo.2016.00137 [PubMed: 27843437]

Higa KT, Mori E, Viana FF, Morris M, Michelini LC (2002) Baroreflex control of heart rate by oxytocin in the solitary-vagal complex. Am J Physiol - Regul Integr Comp Physiol 282:R537–45. 10.1152/ajpregu.00806.2000

Higa-Taniguchi KT, Felix JVC, Michelini LC (2009) Brainstem oxytocinergic modulation of heart rate control in rats: effects of hypertension and exercise training. Exp Physiol 94:1103–1113. 10.1113/expphysiol.2009.049262 [PubMed: 19638362]

Hirota Y, Arai A, Young LJ, Yuri K, Mitsu S (2020) Oxytocin receptor antagonist reverses the blunting effect of pair bonding on fear learning in monogamous prairie voles. Horm Behav 120:104685. 10.1016/j.yhbeh.2020.104685 [PubMed: 31935400]

Hodges TE, Eltahir AM, Patel S, Bredewold R, Veenema AH, McCormick CM (2019) Effects of oxytocin receptor antagonism on social function and corticosterone release after adolescent social instability in male rats. Horm Behav 116:104579. 10.1016/j.yhbeh.2019.104579 [PubMed: 31449812]

Horie K, Inoue K, Suzuki S, Adachi S, Yada S, Hirayama T, Hidema S, Young LJ (2019) Oxytocin receptor knockout prairie voles generated by CRISPR/Cas9 editing show reduced preference for social novelty and exaggerated repetitive behaviors. Horm Behav 111:60–69. 10.1016/j.yhbeh.2018.10.011 [PubMed: 30713102]

Ieraci A, Mallei A, Popoli M (2016) Social isolation stress induces anxious-depressive-like behavior and alterations of neuroplasticity-related genes in adult male mice. Neural Plast 2016:1–13. 10.1155/2016/6212983

Jankord R, Herman JP (2008) Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. Ann N Y Acad Sci 1148:64–73. 10.1196/annals.1410.012 [PubMed: 19120092]

Jankowski M, Danalache B, Wang D, Bhat P, Hajjar F, Marcinkiewicz M, Paquin J, McCann SM, Gutkowska J (2004) Oxytocin in cardiac ontogeny. Proc. Natl. Acad. Sci. 31:13074–13079. 10.1073/pnas.0405324101.

Japundžić-Žigon N (2013) Vasopressin and oxytocin in control of the cardiovascular system. Curr Neuropharmacol 11:218–230. 10.2174/1570159X11311020008 [PubMed: 23997756]

Japundžić-Žigon N, Lozić M, Šarenac O, Murphy D (2020) Vasopressin & oxytocin in control of the cardiovascular system: an updated review. Curr Neuropharmacol 18:14–33. 10.2174/1570159X17666190717150501 [PubMed: 31544693]

Jeste DV, Lee EE, Cacioppo S. (2020). Battling the modern behavioral epidemic of loneliness: suggestions for research and interventions. JAMA Psychiatry 77(6):553–554. 10.1001/jamapsychiatry.2020.0027 [PubMed: 32129811]

Jurek B, Neumann ID (2018) The oxytocin receptor: from intracellular signaling to behavior. Physiol Rev 98:1805–1908. 10.1152/physrev.00031.2017 [PubMed: 29897293]

Keebaugh AC, Barrett CE, Laprairie JL, Jenkins JJ, Young LJ (2015) RNAi knockdown of oxytocin receptor in the nucleus accumbens inhibits social attachment and parental care in monogamous female prairie voles. Soc Neurosci 10:561–570. 10.1080/17470919.2015.1040893 [PubMed: 25874849]

Kiecolt-Glaser JK, Glaser R, Cacioppo JT, Malarkey WB (1998) Marital stress: immunologic, neuroendocrine, and autonomic correlates. Ann N Y Acad Sci 840:656–663 [PubMed: 9629292]

Kumari A, Singh P, Baghel MS, Thakur MK (2016) Social isolation mediated anxiety like behavior is associated with enhanced expression and regulation of BDNF in the female mouse brain. Physiol Behav 158:34–42. 10.1016/j.physbeh.2016.02.032 [PubMed: 26921097]

Kwak C, Lee SH, Kaang BK (2009) Social isolation selectively increases anxiety in mice without affecting depression-like behavior. Korean J Physiol Pharmacol 13:357–360. 10.4196/kjpp.2009.13.5.357 [PubMed: 19915697]

Lee MR, Sheskier MB, Farokhnia M, Feng N, Marenco S, Lipska BK, Leggio L (2018) Oxytocin receptor mRNA expression in dorsolateral prefrontal cortex in major psychiatric disorders: a human post-mortem study. Psychoneuroendocrinology 96:143–147. 10.1016/j.psyneuen.2018.05.039 [PubMed: 29940428]

Lee S-Y, Park S-H, Chung C, Kim JJ, Choi S-Y, Han J-S (2015) Oxytocin protects hippocampal memory and plasticity from uncontrollable stress. Sci Rep 5:18540. 10.1038/srep18540 [PubMed: 26688325]

Lim MH, Rodebaugh TL, Zyphur MJ, Gleeson JF (2016) Loneliness over time: the crucial role of social anxiety. J Abnorm Psychol 125:620–30. 10.1037/abn0000162 [PubMed: 27124713]

Madularu D, Athanassiou M, Yee JR, Kenkel WM, Carter CS, Mumby DG (2014) Oxytocin and object preferences in the male prairie vole. Peptides 61:88–92. 10.1016/j.peptides.2014.08.015 [PubMed: 25219944]

Mattos Dos Santos R (2020) Isolation, social stress, low socioeconomic status and its relationship to immune response in Covid-19 pandemic context. Brain Behav Immun - Health 7:100103. 10.1016/j.bbih.2020.100103

McNeal N, Appleton KM, Johnson AK, Scotti MAL, Wardwell J, Murphy R (2017) The protective effects of social bonding on behavioral and pituitary-adrenal axis reactivity to chronic mild stress in prairie voles. Stress 20:175–182. 10.1080/10253890.2017.1295444 [PubMed: 28276805]

Meynen G, Unmehopa UA, Hofman MA, Swaab DF, Hoogendijk WJG (2007) Hypothalamic oxytocin mRNA expression and melancholic depression. Mol Psychiatry 12:118–119. 10.1038/sj.mp.4001911 [PubMed: 17252002]

Michelini LC, Marcelo MC, Amico J, Morris M (2003) Oxytocinergic regulation of cardiovascular function: studies in oxytocin-deficient mice. Am J Physiol-Heart Circ Physiol 284:H2269–H2276. 10.1152/ajpheart.00774.2002 [PubMed: 12531722]

Mooney SJ, Douglas NR, Holmes MM (2014) Peripheral administration of oxytocin increases social affiliation in the naked mole-rat (Heterocephalus glaber). Horm Behav 65:380–385. 10.1016/j.yhbeh.2014.02.003 [PubMed: 24530845]

Neumann ID, Landgraf R (2012) Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. Trends Neurosci 35:649–659. 10.1016/j.tins.2012.08.004 [PubMed: 22974560]

Ooishi Y, Mukai H, Watanabe K, Kawato S, Kashino M (2017) Increase in salivary oxytocin and decrease in salivary cortisol after listening to relaxing slow-tempo and exciting fast-tempo music. PloS One 12:e0189075. 10.1371/journal.pone.0189075 [PubMed: 29211795]

Osako Y, Nobuhara R, Arai YP, Tanaka K, Young LJ, Nishihara M, Mitsui S, Yuri K (2018) Partner loss in monogamous rodents: modulation of pain and emotional behavior in male prairie voles. Psychosom Med 80:62–68. 10.1097/PSY.0000000000000524 [PubMed: 28872575]

Özdin S, Bayrak Özdin Ş (2020) Levels and predictors of anxiety, depression and health anxiety during COVID-19 pandemic in Turkish society: the importance of gender. Int J Soc Psychiatry 66:504–511. 10.1177/0020764020927051 [PubMed: 32380879]

Pagani JH, Williams Avram SK, Cui Z, Song J, Mezey É, Senerth JM, Baumann MH, Young WS (2015) Raphe serotonin neuron-specific oxytocin receptor knockout reduces aggression without affecting anxiety-like behavior in male mice only. Genes Brain Behav 14:167–176. 10.1111/gbb.12202 [PubMed: 25677455]

Parker KJ, Kenna HA, Zeitzer JM, Keller J, Blasey CM, Amico JA, Schatzberg AF (2010) Preliminary evidence that plasma oxytocin levels are elevated in major depression. Psychiatry Res 178:359–362. 10.1016/j.psychres.2009.09.017 [PubMed: 20494448]

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–167 [PubMed: 2864480]

Perkeybile AM, Delaney-Busch N, Hartman S, Grimm KJ, Bales KL (2015) Intergenerational transmission of alloparental behavior and oxytocin and vasopressin receptor distribution in the prairie vole. Front Behav Neurosci 9:191. 10.3389/fnbeh.2015.00191 [PubMed: 26257619]

Petitte T, Mallow J, Barnes E, Petrone A, Barr T, Theeke L (2015) A systematic review of loneliness and common chronic physical conditions in adults. Open Psychol J 8:113–132. 10.2174/1874350101508010113 [PubMed: 26550060]

Phelps SM, Okhovat M, Berrio A (2017) Individual differences in social behavior and cortical vasopressin receptor: genetics, epigenetics, and evolution. Front Neurosci 11:537. 10.3389/fnins.2017.00537 [PubMed: 29085274]

Pierrehumbert B, Torrisi R, Laufer D, Halfon O, Ansermet F, Beck Popovic M (2010) Oxytocin response to an experimental psychosocial challenge in adults exposed to traumatic experiences during childhood or adolescence. Neuroscience 166:168–177. 10.1016/j.neuroscience.2009.12.016 [PubMed: 20018229]

Pohl TT, Young LJ, Bosch OJ (2019) Lost connections: oxytocin and the neural, physiological, and behavioral consequences of disrupted relationships. Int J Psychophysiol 136:54–63. 10.1016/j.ijpsycho.2017.12.011 [PubMed: 29330007]

Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 463:3–33 [PubMed: 12600700]

Quintana DS, Guastella AJ (2020) An allostatic theory of oxytocin. Trends Cogn Sci 24:515–528. 10.1016/j.tics.2020.03.008 [PubMed: 32360118]

Quintana DS, Kemp AH, Alvares GA, Guastella AJ (2013) A role for autonomic cardiac control in the effects of oxytocin on social behavior and psychiatric illness. Front Neurosci 7:48. 10.3389/fnins.2013.00048 [PubMed: 23565075]

Saltzman LY, Hansel TC, Bordnick PS (2020) Loneliness, isolation and social support factors in post-COVID-19 mental health. Psychol Trauma: Theory Res Prac Policy 12(S1): S55–S57. 10.1037/tra0000703

Savage RD, Wu W, Li J, et al. (2021) Loneliness among older adults in the community during COVID-19: a cross-sectional survey in Canada. BMJ Open 11:e044517. 10.1136/bmjopen-2020-044517

Senst L, Baimoukhametova D, Sterley TL, Bains JS (2016) Sexually dimorphic neuronal responses to social isolation. Elife 5:e18726. 10.7554/eLife.18726 [PubMed: 27725087]

Sgoifo A, Carnevali L, Grippo AJ (2014) The socially stressed heart. Insights from studies in rodents. Neurosci Biobehav Rev 39:51–60. 10.1016/j.neubiorev.2013.12.005 [PubMed: 24373860]

Sgoifo A, Stilli D, Medici D, Gallo P, Ami B, Musso E (1996) Electrode positioning for reliable telemetry ECG recordings during social stress in unrestrained rats. Physiol Behav 60:1397–1401. 10.1016/s0031-9384(96)00228-4 [PubMed: 8946481]

Shufelt CL, Pacheco C, Tweet MS, Miller VM (2018) Sex-specific physiology and cardiovascular disease. Adv Exp Med Biol 1065:433–454. 10.1007/978-3-319-77932-4_27 [PubMed: 30051400]

Slattery DA, Neumann ID (2010) Chronic icv oxytocin attenuates the pathological high anxiety state of selectively bred Wistar rats. Neuropharmacology 58:56–61. 10.1016/j.neuropharm.2009.06.038 [PubMed: 19589349]

Smith AL, Freeman SM, Voll RJ, Young LJ, Goodman MM (2013a) Carbon-11 N-methyl alkylation of L-368,899 and in vivo PET imaging investigations for neural oxytocin receptors. Bioorg Med Chem Lett 23:902–906. 10.1016/j.bmcl.2012.10.116 [PubMed: 23270988]

Smith AS, Ågmo A, Birnie AK, French JA (2010) Manipulation of the oxytocin system alters social behavior and attraction in pair-bonding primates, Callithrix penicillata. Horm Behav 57: 255–262. 10.1016/j.yhbeh.2009.12.004 [PubMed: 20025881]

Smith AS, Lieberwirth C, Wang Z (2013b) Behavioral and physiological responses of female prairie voles (Microtus ochrogaster) to various stressful conditions. Stress 16:531–539. 10.3109/10253890.2013.794449 [PubMed: 23647082]

Smith AS, Wang Z (2014) Hypothalamic oxytocin mediates social buffering of the stress response. Biol Psychiatry 76:281–288. 10.1016/j.biopsych.2013.09.017 [PubMed: 24183103]

Steptoe A, Owen N, Kunz-Ebrecht SR, Brydon L (2004) Loneliness and neuroendocrine, cardiovascular, and inflammatory stress responses in middle-aged men and women. Psychoneuroendocrinology 29:593–611. 10.1016/S0306-4530(03)00086-6 [PubMed: 15041083]

Steptoe A, Shankar A, Demakakos P, Wardle J (2013) Social isolation, loneliness, and all-cause mortality in older men and women. Proc Natl Acad Sci U S A 110:5797–801. 10.1073/pnas.1219686110 [PubMed: 23530191]

Steru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacol Berl 85:367–370

Stevenson JR, McMahon EK, Boner W, Haussmann MF (2019) Oxytocin administration prevents cellular aging caused by social isolation. Psychoneuroendocrinology 103:52–60. 10.1016/j.psyneuen.2019.01.006 [PubMed: 30640038]

Sun P, Smith AS, Lei K, Liu Y, Wang Z (2014) Breaking bonds in male prairie vole: long-term effects on emotional and social behavior, physiology, and neurochemistry. Behav Brain Res 265:22–31. 10.1016/j.bbr.2014.02.016 [PubMed: 24561258]

Taylor SE, Gonzaga GC, Klein LC, Peifeng H, Greendale GA, Seeman TE (2006) Relation of oxytocin to psychological stress responses and hypothalamic-pituitary-adrenocortical axis activity in older women. Psychosom Med 68:238–245. 10.1097/01.psy.0000203242.95990.74 [PubMed: 16554389]

Thompson KL, Vincent SH, Miller RR, Colletti AE, Alvaro RF, Wallace MA, Feeney WP, Chiu SH (1997) Pharmacokinetics and disposition of the oxytocin receptor antagonist L-368,899 in rats and dogs. Drug Metab Dispos 25:1113–1118 [PubMed: 9321512]

Will TR, Proano SB, Thomas AM, et al. (2017) Problems and progress regarding sex bias and omission in neuroscience research. eNeuro 4:1–10. 10.1523/ENEURO.0278-17.2017

Williams JR, Insel TR, Harbaugh CR, Carter CS (1994a) Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (Microtus ochrogaster). J Neuroendocrinol 6:247–250. 10.1111/j.1365-2826.1994.tb00579.x [PubMed: 7920590]

Williams PD, Anderson PS, Ball RG, et al. (1994b) 1-((7,7-Dimethyl-2(S)-(2(S)-amino-4-(methylsulfonyl)butyramido)bicyclo [2.2.1]-heptan-1(S)-yl)methyl)sulfonyl)-4-(2-methylphenyl)piperaz ine (L-368,899): an orally bioavailable, non-peptide oxytocin antagonist with potential utility for managing preterm labor. J Med Chem 37:565–571. 10.1021/jm00031a004 [PubMed: 8126695]

Yan Y, Wang YL, Su Z, Zhang Y, Guo SX, Liu AJ, Wang CH, Sun FJ, Yang J (2014) Effect of oxytocin on the behavioral activity in the behavioral despair depression rat model. Neuropeptides 48:83–89. 10.1016/j.npep.2014.01.001 [PubMed: 24444823]

Yee JR, Kenkel WM, Frijling JL, et al. (2016) Oxytocin promotes functional coupling between paraventricular nucleus and both sympathetic and parasympathetic cardioregulatory nuclei. Horm Behav 80:82–91. 10.1016/j.yhbeh.2016.01.010 [PubMed: 26836772]

Yoon S, Kim Y-K (2020) The role of the oxytocin system in anxiety disorders. In: Kim Y-K (ed) Anxiety Disorders: Rethinking and Understanding Recent Discoveries. Springer Singapore, Singapore, pp 103–120

Young KA, Gobrogge KL, Liu Y, Wang Z (2011) The neurobiology of pair bonding: insights from a socially monogamous rodent. Front Neuroendocrinol 32:53–69. 10.1016/j.yfrne.2010.07.006 [PubMed: 20688099]

Highlights

- Social stress and loneliness have emotional and cardiac consequences
- Oxytocin may influence emotional and cardiac responses to isolation
- Social isolation increased emotion-related behaviors in female rodents
- Blocking oxytocin worsened emotion-related behaviors in isolated rodents
- Blocking oxytocin increased heart rate in co-housed rodents

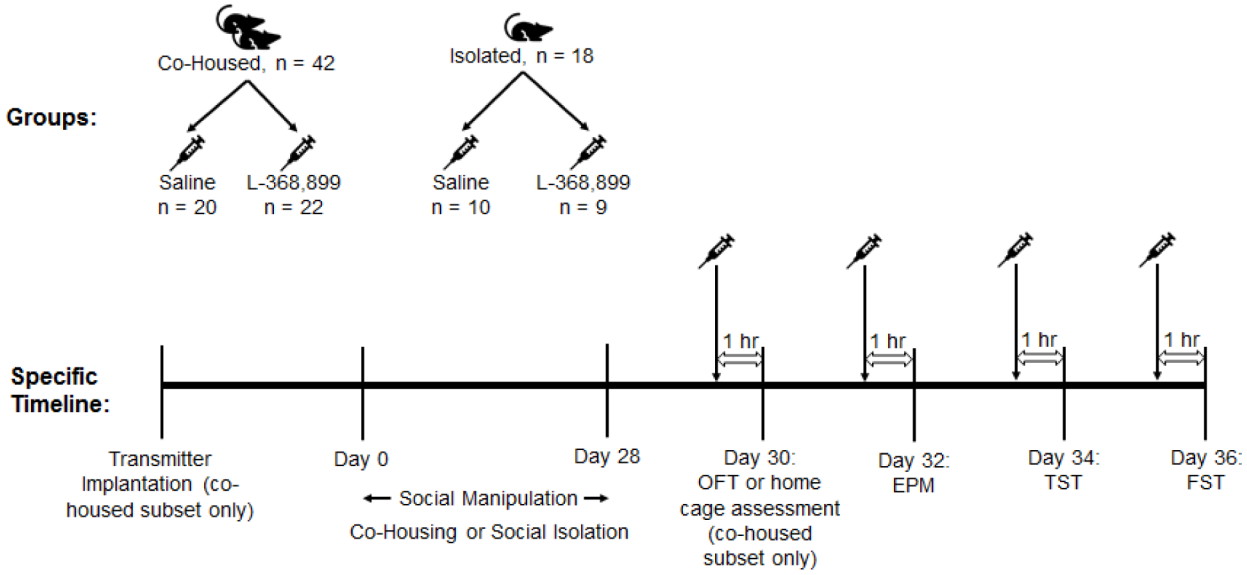

Figure 1.

Housing conditions, pharmacological manipulations, sample sizes, and specific experimental timeline in the present study.

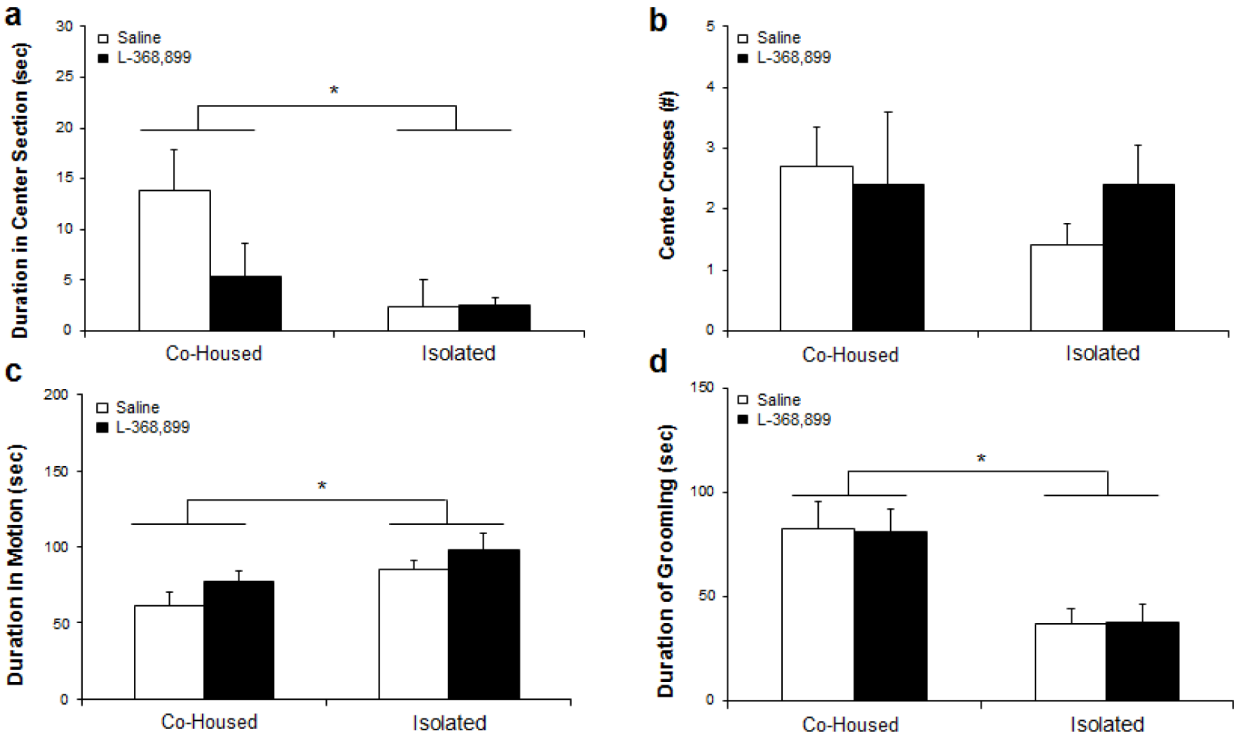

Figure 2.

Behavioral results during the OFT following co-housing or socially isolated conditions, 1 hour after saline or L-368,899 injections (means + SEM). a.) The duration spent in the center section of the open field, which was used as a behavioral index of exploration and low-anxiety state. Socially isolated animals spent significantly less time in the center section of the OFT compared to co-housed control animals. b.) Instances of crosses into the center section of the OFT, which was used as a behavioral index of exploration. Neither social housing condition nor pharmacological manipulation affected this behavior. c.) The duration spent in motion, which was used as a behavioral index of anxiety. Socially isolated animals spent significantly more time in motion compared to co-housed animals; and d.) The duration spent autogrooming, which was used as a behavioral index of an anxiety-related state. Socially isolated animals spent significantly less time autogrooming compared to co-housed animals. *$p < .05$ vs. co-housed condition (main effect of housing condition).

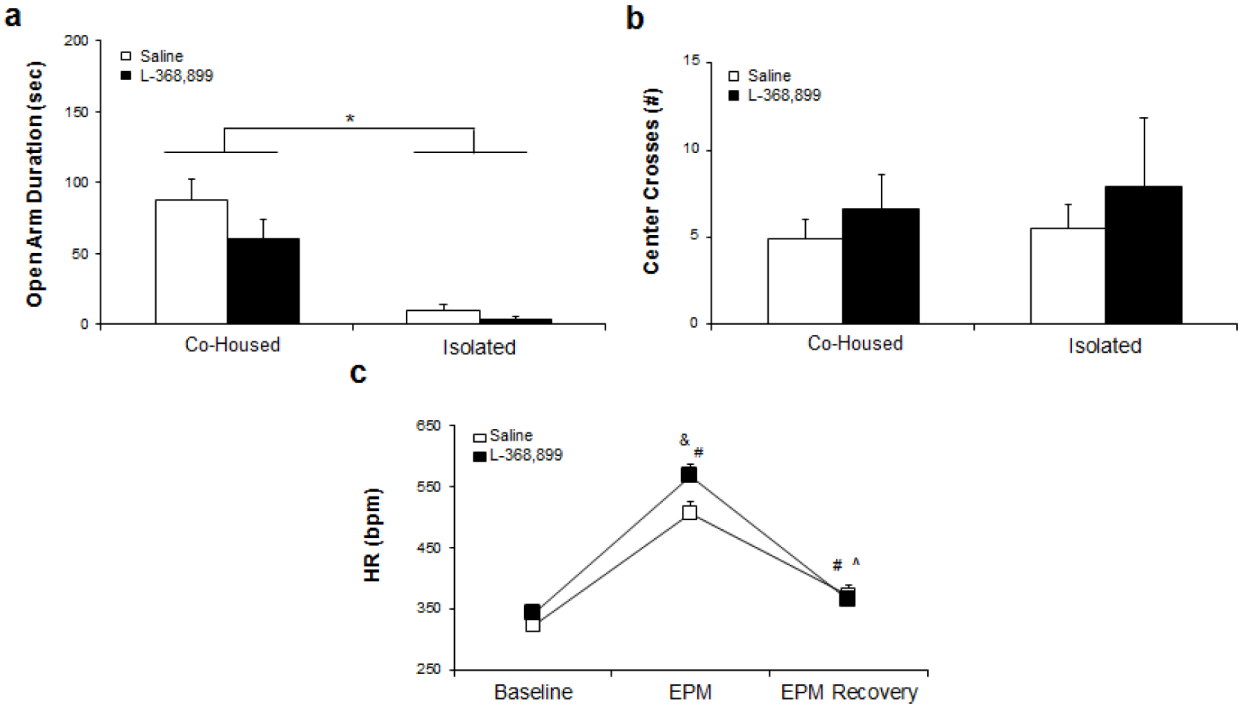

Figure 3.

Behavioral and cardiac results during the EPM following co-housing or socially isolated conditions, 1 hour after saline or L-368,899 injections (means + SEM). a.) The duration spent in the open arms of the EPM, which was used as a behavioral index of exploration and low-anxiety state. Socially isolated animals spent significantly less time in the open arms of the EPM compared to co-housed animals. b.) Instances of crosses into the center section, which was used as a general marker for locomotor activity. Neither social housing condition nor pharmacological manipulation affected this behavior. c.) HR of co-housed subgroup at baseline, during the EPM, and 3 hours following the EPM. All co-housed animals, regardless of pharmacological condition, showed a significant elevation of HR during the EPM, and HR of L-368,899-injected animals was significantly higher during the EPM compared to HR of vehicle-injected animals. HR of all co-housed animals, regardless of pharmacological condition, remained elevated above baseline levels 3 hours following the EPM. *$p < .05$ vs. co-housed condition (main effect of housing condition); $^{\&}p < 0.05$ vs. saline administration; $^{\#}p < 0.05$ vs. respective baseline HR value; $^{\wedge}p < 0.05$ vs. respective EPM HR value.

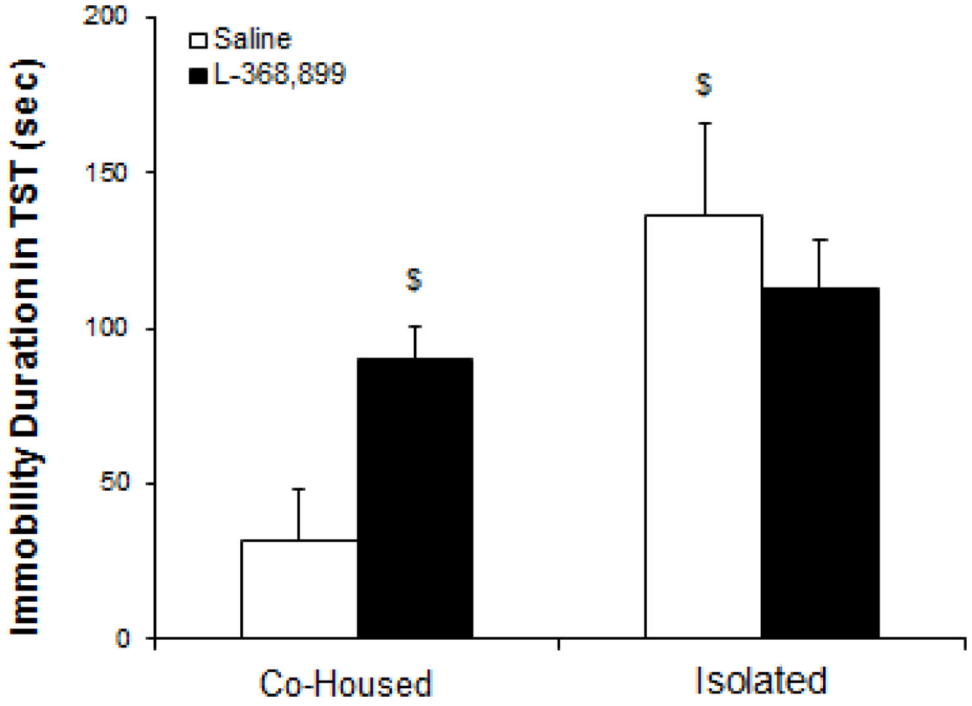

Figure 4.

Behavioral results during the TST following co-housing or socially isolated conditions, 1 hour after saline or L-368,899 injections (means + SEM), showing duration of immobility during the TST, which was used as a behavioral index of helplessness. L-368,899-injected co-housed animals and saline-injected isolated animals displayed significantly more immobility during the TST compared to vehicle-injected co-housed animals. $^{\$}p = 0.05$ vs. saline administration in the co-housed condition.

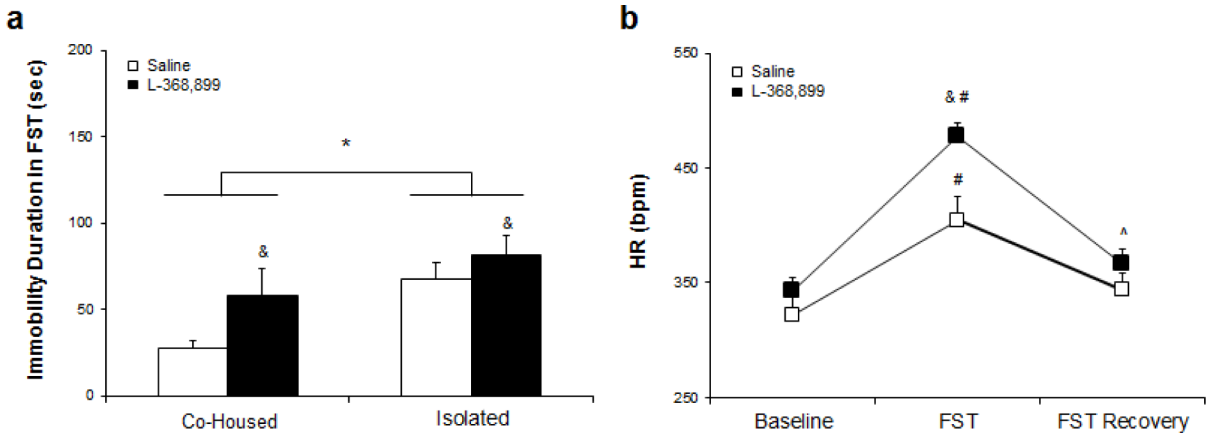

Figure 5.

Behavioral and cardiac responses during the FST following co-housing or socially isolated conditions, 1 hour after saline or L-368,899 injections (means + SEM). a.) Duration of immobility during the FST, which was used as a behavioral index of helplessness. Socially isolated animals displayed significantly more immobility during the FST compared to co-housed animals. L-368,899-injected animals (regardless of social condition) displayed significantly more immobility during the FST compared to vehicle-injected animals. b.) HR of the co-housed subset at baseline, during the FST, and 3 hours following the FST. All animals showed a significant elevation of HR during the FST regardless of pharmacological condition. L-368,899-injected co-housed animals exhibited a significantly higher HR during the FST compared to vehicle-injected co-housed animals. *$p < .05$ vs. co-housed condition (main effect of housing condition); $^{\&}p < 0.05$ vs. saline administration (main effect of pharmacological treatment); $^{\#}p < 0.05$ vs. respective baseline HR value; $^{\wedge}p < 0.05$ vs. respective FST HR value.

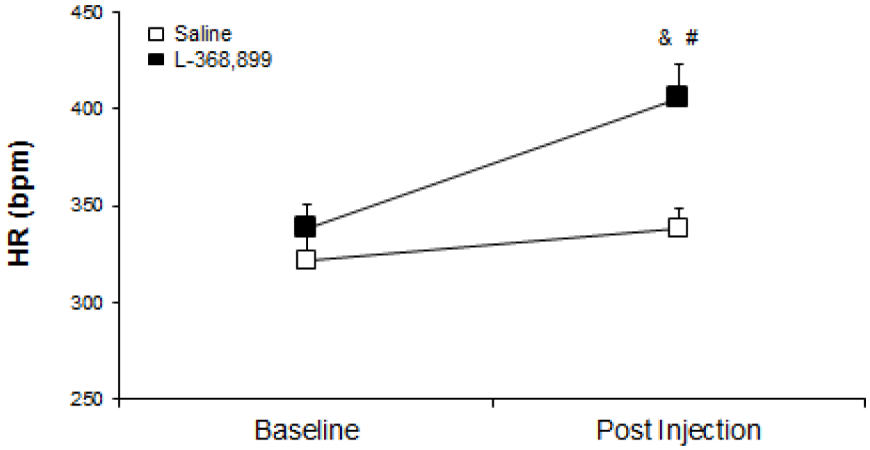

Figure 6.

Resting HR of the co-housed subgroup at baseline and 1 hour following saline or L-368,899 injections (means + SEM). Co-housed L-368,899-injected animals displayed a significant elevation of HR compared to baseline HR and that of co-housed vehicle-injected animals. $^{\&}p < 0.05$ vs. saline administration; $^{\#}p < 0.05$ vs. respective baseline HR value.

Table 1.

Pearson’s r correlation coefficients in co-housed animals administered either saline or L-368,899, comparing behavioral and cardiac variables.

	Saline	L-368,899
HR Following Injection vs. HR During EPM	0.34	0.02
HR Following Injection vs. HR During FST	−0.03	−0.01
HR During EPM vs. Open Arm Duration in EPM	−0.38	−0.26
HR During FST vs. Immobility Duration in FST	0.26	0.36
HR During EPM vs. HR During FST	0.05	−0.47
Duration in Open Arms During EPM vs. Immobility Duration During FST	−0.09	−0.54

Note: probability values for all correlations are greater than 0.05.