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Oxytocin administration prevents cellular aging caused by social isolation

Jennie R. Stevenson[a,*,1], Elyse K. McMahon[b,1], Winnie Boner[c], Mark F. Haussmann[b]

[a] Department of Psychology, Bucknell University, Lewisburg, PA, USA
[b] Department of Biology, Bucknell University, Lewisburg, PA, USA
[c] Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow, Scotland, United Kingdom

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ABSTRACT

Chronic stressors, such as chronic isolation in social mammals, can elevate glucocorticoids, which can affect cellular mechanisms of aging, including increased levels of oxidative stress and shortened telomere lengths. Recent work in the selectively social prairie vole (*Microtus ochrogaster*) suggests that oxytocin and social support may mitigate some of the negative consequences of social isolation, possibly by reducing glucocorticoid levels. We investigated the influences of isolation, social support, and daily oxytocin injections in female prairie voles. Glucocorticoid levels, oxidative damage, telomere length, and anhedonia, a behavioral index of depression, were measured throughout the study. We found that six weeks of chronic isolation led to increased glucocorticoid levels, oxidative damage, telomere degradation and anhedonia. However, daily oxytocin injections in isolated voles prevented these negative consequences. These findings demonstrate that chronic social isolation in female prairie voles is a potent stressor that results in depression-like behavior and accelerated cellular aging. Importantly, oxytocin can completely prevent the negative consequences of social isolation.

1. Introduction

Poor social support contributes to disease and accelerated aging (Hawkley and Cacioppo, 2010). Specifically, recent work reports that poor social support is linked to reduced telomere length, a key biomarker of cellular aging (Mitchell et al., 2018). Conversely, social support is an essential component of healthy aging, associated with increased immunity, better overall health, and a longer lifespan (Uchino, 2009). Although the underlying mechanisms by which social support promotes healthy aging are unknown, one candidate is the neuropeptide oxytocin. Oxytocin helps mediate social bonding and attachment, and may be released during positive social interactions (reviewed in Carter et al., 2008). One large unanswered question is how social support prevents telomere degradation. While correlational studies have linked oxytocin and the oxytocin receptor to telomere length (Smearman et al., 2016; Yim et al., 2016), no studies have investigated the causal effects of oxytocin on measures of biological aging.

Telomeres, terminal DNA caps important for chromosomal integrity, progressively shorten throughout the life of a cell due to replication restrictions of DNA polymerase (Aubert and Lansdorp, 2008). Telomere shortening may also be caused by another potential contributor to the aging process: oxidative stress by free radicals, which arise as damaging by-products of energy metabolism in the mitochondria (Boonekamp et al., 2017; but see Reichert and Stier, 2017). When telomeres shorten to a critical length, cells enter senescence: an irreversible change in which the cell ceases to divide and undergoes an altered secretory profile that damages neighboring healthy cells. During aging, the number of senescent cells rises, leading to a loss of the regenerative capacity of tissues and an increased risk of disease (Lopez-Otin et al., 2013). While telomere shortening is only one contributing factor to cellular senescence, it has become a biomarker for senescent cells (Bernadotte et al., 2016) and an integrative measure of the physiological state of an organism (Monaghan and Haussmann, 2006; Haussmann and Marchetto, 2010). Supporting this, a number of human studies have shown that individuals with shorter telomeres or faster attrition rates also have reduced life expectancy (reviewed in Wilbourn et al., 2018).

Social support also impacts psychological health, an essential component of healthy aging. Perceived and objective social isolation are both associated with increased depression and mood disorders (Hawkley and Cacioppo, 2010); while increasing meaningful social connections leads to improvement in depression symptoms (Cruwys et al., 2014). Although it is unclear how social support and social connections safeguard against depression, oxytocin treatment protects against isolation-induced depression in an animal model (Grippo et al., 2009), further suggesting a role for oxytocin as a potential mediator of

* Corresponding author at: 215 O'Leary Center, 1 Dent Drive, Bucknell University, Lewisburg, PA, 17837, United States.
E-mail address: j.stevenson@bucknell.edu (J.R. Stevenson).
[1] Shared first authorship.

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the benefits of social support and as a molecular target for treatment. Interestingly, two recent meta analyses demonstrated that either oxidative stress is significantly increased (Black et al., 2015) or that telomere length is significantly decreased in patients with major depressive disorder (Lin et al., 2016), though it remains unclear which factors connect these measures of cellular aging with depression.

One possible link between depression and cellular aging is psychological stress. Stressful periods are energetically expensive to the body, and this energy demand is met in part through the release of glucocorticoid (CORT) hormones which affect energy mobilization and allocation. During times of stress, CORT also works to suppress other physiological systems, like immune function and reproduction, allowing for redirection of nutrients to coping with the stressor. However, during prolonged stress, the continual diversion of energy away from normal self-maintenance of the body can result in disease (McEwen, 1998) and accelerated aging (Epel et al., 2004). In addition, prolonged CORT release contributes to the effects of chronic stress on depression and aging (Manoliu et al., 2018). In social species, isolation is a profound chronic stressor resulting in elevated CORT levels (Cacioppo et al., 2015). Interestingly, oxytocin can reduce activation of the stress response (Windle et al., 1997), and may underlie the protective effects social support has on stressors (Smith and Wang, 2014). It is not known, however, if oxytocin can prevent the effects of chronic isolation stress on cellular aging.

While social support is essential for humans, it is a complex and nuanced phenomenon. To help study the effects of social support, social isolation as a manipulation in selectively social animal species can serve as a valuable experimental model. For example, several studies in prairie voles, a socially monogamous species, have demonstrated that they are profoundly affected by social isolation, showing increased depression-like behavior, alteration in neuroendocrine function and autonomic regulation of the heart. Interestingly, treatment with exogenous oxytocin can prevent many of these effects of social isolation (Grippo et al., 2009), indicating that oxytocin is protective during social isolation in a highly social species. However, to date, this design has rarely been employed to explore the role of social isolation and support on cellular aging. Importantly, one recent paper in monogamous African Grey parrots reported that social isolation was associated with shorter telomeres (Aydinonat et al., 2014), demonstrating that social isolation can result in accelerated cellular aging. The purpose of this study was to determine if isolation stress in prairie voles increases oxidative stress or shortens telomeres, and if oxytocin can protect against the effects of social isolation on these measures of cellular aging and depression-like behavior. In this study, female prairie voles were housed in either isolated or paired conditions for six weeks. Exogenous oxytocin treatment or vehicle was administered daily throughout the six week period. We hypothesized that social isolation in prairie voles would act as a chronic stressor, resulting in elevated baseline CORT levels, increased oxidative stress, shortened telomeres, and increased depression-like behavior. We also hypothesized that oxytocin treatment throughout the isolation period would prevent the effects of isolation stress on these measures in prairie voles.

2. Methods

2.1. Animals

Adult female prairie voles (*Microtus ochrogaster*) were bred and housed at Bucknell University. Animals were F3 generation descendants of prairie voles caught near Champagne-Urbana, Illinois, USA. Females were chosen because previous studies have shown a greater response to social isolation and oxytocin in female prairie voles (Cushing and Carter, 2000; Grippo et al., 2007a, 2007b). Animals were 7–8 weeks old and weighed between 32 and 35 g at the start of the study. Animals were housed throughout the study in polycarbonate cages (19.05 × 29.21 x 12.7 cm, Ancare, Bellmore, NY, USA) with Harlan Teklad aspen sani-chip bedding (Envigo, Somerset, NJ, USA), and given ad libitum access to water and high fiber rabbit chow (Purina Mills, Inc., Gray Summit, MO, USA). All prairie voles were pair-housed with a same-sex sibling until assignment to either paired or isolated housing conditions at the start of the study. Colony rooms were maintained on a 14:10 light cycle (lights on at 0600) at approximately 70 °F. All experiments and procedures were approved by the Bucknell University IACUC and conducted in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research.

2.2. Drugs

Oxytocin acetate salt (Bachem, Torrence, CA, USA) was dissolved in 0.9% saline and injected daily during the experimental housing period (i.p.; 0.05 mg/kg; 0.5 mg/mL). This dose was chosen because it successfully protected against some of the consequences of social isolation in prairie voles in previous studies (Grippo et al., 2009, 2012). Vehicle injections were 0.9% saline (i.p.; 10 mL/kg).

2.3. General procedure

Animals were randomly assigned to one of the following six conditions (n = 10 for each group): isolated or paired with no injections (I, P, respectively), isolated or paired with daily vehicle injections (Iv, Pv, respectively), isolated or paired with daily oxytocin injections (Io, Po, respectively). All voles were treated identically (daily handling, cage changes, food and water changes) except that the Iv, Pv, Io, and Po groups were injected daily. Isolated animals were separated from their sibling into clean individual cages, while paired animals were moved to a clean cage with their sibling at the start of the 42 d experimental housing period. During this period, animals received either vehicle or oxytocin injections daily between 14:00 and 16:00. Baseline blood samples and sucrose preference (for anhedonia testing) were taken prior to the start of the experimental housing conditions. Then blood samples were taken and sucrose preference was measured again after three weeks and six weeks in these housing conditions. Sucrose preference tests were conducted between 13:00 and 15:00 and blood samples were collected between 13:00 and 14:00 one to two d following sucrose preference tests (Fig. 1).

2.4. Blood collection

Blood samples were collected in order to measure corticosterone, the primary glucocorticoid in voles, total antioxidant capacity (TAC), reactive oxytocin metabolites (ROMs), and telomere length. Approximately 100–200 uL of blood was collected by puncturing the submandibular vein with a lancet (5.5 m Goldenrod Animal Lancet, Medipoint, Mineola, NY, USA). Blood collection occurred between 1300 and 1400. Samples were collected within three min of first touching the animal's cage. All blood samples were collected in EDTA coated tubes, then were spun at 800 g for 5 min at 4 °C and plasma was removed and stored at -80 °C. Later, plasma was thawed and used to measure glucocorticoids, TAC and ROMs. Packed red blood cells were mixed with a 500ul cryoprotectant solution of 90% Newborn Calf Serum and 10% Dimethyl sulfoxide and stored at -80 °C. Unfortunately, cryoprotectant buffer was not added to baseline blood samples before they were placed in the freezer, and subsequent DNA integrity analysis determined that the DNA in these samples experienced degradation and was not appropriate for telomere analysis. Therefore, we were only able to measure telomeres at the 21 and 42 d time points.

2.5. Anhedonia

Anhedonia, a decreased sense of pleasure, is a hallmark of depression in humans, and sucrose preference is a validated measure of anhedonia in rodents (Willner et al., 1992). To measure sucrose

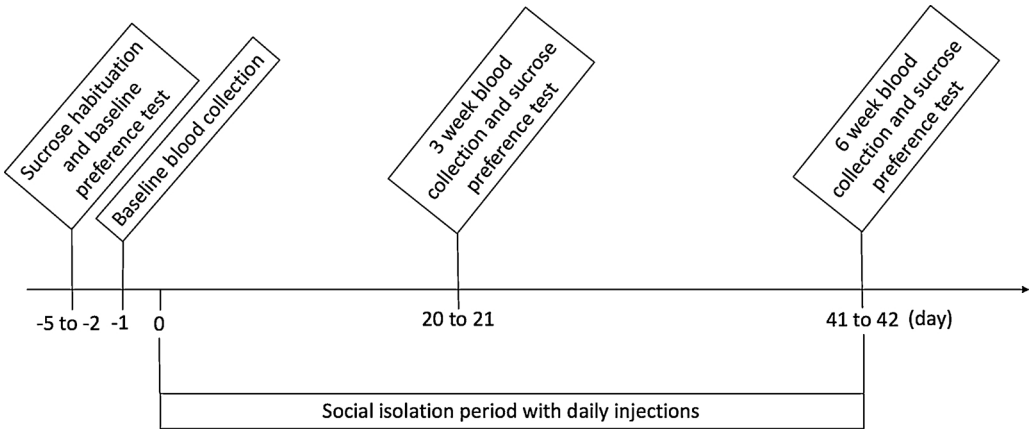

Fig. 1. Study timeline. The duration of the study period was 42 d. Habituation to sucrose and baseline sucrose preference tests occurred in the 5 d preceding the study period, and baseline blood samples occurred 1 d before the study period. During the study period, sucrose preference tests and blood sampling occurred at 21 d and 42 d.

preference, animals were first given unlimited access to a solution of 1% sucrose in tap water in the home cage for 48 h five d before experimental housing conditions began (Fig. 1). The day after the 48 h habituation period, and following a brief 2 h food and water deprivation period, animals were moved to an individual cage for a two h period during which each animal had access to a graduated bottle of 1% sucrose and a graduated bottle of tap water. Consumption of both fluids was measured during the two h testing period. Sucrose preference was calculated using the following equation: ((mL 1% sucrose consumed) / (mL of 1% sucrose consumed plus mL of water consumed)) × 100. The two h sucrose preference test was repeated the following day, and the average of the two preference tests was used to determine baseline sucrose preference. A 2 h sucrose preference test was then administered on d 20 and 41 to determine if changes had occurred as a result of housing or injection conditions. Two tests were used to establish baseline sucrose preference. Because there was no statistical difference between the two baseline tests and because we wanted to limit manipulations once the treatment period had begun, a single test was used to assess preference on 20 and 41 d.

2.6. Corticosterone radioimmunoassay

Plasma CORT levels were determined by radioimmunoassay based on the protocol of Wingfield and Farner (1975), and optimized for prairie voles in our laboratory. Briefly, CORT was extracted from diluted plasma samples with anhydrous diethyl ether, dried under nitrogen gas, resuspended in 90% ethanol, and stored at 6 °C overnight. The samples were then dried again under nitrogen gas after centrifugation and resuspended in phosphate buffered saline with glucose (PBSg). Samples were run in a competitive-binding RIA using CORT-specific antibody Corticosterone-3-Carboxymethyl-oxime:BSAhost: rabbit (MP Biomedicals, Solon, OH, USA) and tritiated corticosterone (2000 cpm, NET 399, New England Nuclear Research Products, Boston, MA, USA). Bound and free CORT were separated using dextran coated charcoal. Samples were then centrifuged and radioactivity was determined via use of a liquid scintillation counter. All samples were run in duplicate which were averaged for analysis and the intra-assay and inter-assay coefficients of variation were 4.9 and 5.4%, respectively.

2.7. ROMs and TAC assays

We assessed reactive oxygen metabolites (ROMs) and total antioxidant capacity (TAC), using the d-ROMs and OXY-adsorbent tests (Diacron International, Grosseto, Italy), respectively, in all blood samples as described previously (Fletcher et al., 2015). The d-ROMs test measures intermediate oxidative damage compounds, mostly represented by hydroperoxides, derived from lipids, proteins and nucleotides. Specifically, we diluted 10 µl of plasma in 200 µl of the provided acidic buffer solution, and then read the plate kinetically (one read per min) for the next 10 min. Absorbance was measured at 490 nm (BioTek ELx800, VT, USA) and we calculated change in ROMs concentrations (in mM of H2O2 equivalents) from these absorbencies by taking the difference between the reading at min 20 and min 0, dividing by 20 min, and multiplying by the constant 9000 (all per the manufacturers specifications). All samples were run in duplicate which were averaged for analysis and the intra-assay and inter-assay coefficients of variation were 5.5 and 4.3%, respectively.

The total plasma antioxidant capacity (TAC) was measured using the OXY-Adsorbent test (Diacron International, Grosseto, Italy), which measures the effectiveness of the blood antioxidant barrier by quantifying its ability to cope with oxidant action of hypochlorous acid (HClO). We diluted 10 µl plasma in 990 µl of distilled water; we then mixed 5 µl of this diluted plasma with 195 µl of the provided HClO solution and continued by following the manufacturer's instructions. We measured absorbance at 490 nm (BioTek ELx800) and we calculated TAC (in mM of HClO neutralized). All samples were run in duplicate which were averaged for analysis and the intra-assay and inter-assay coefficients of variation were 3.2 and 7.1%, respectively.

2.8. qPCR analysis of relative telomere length

DNA was extracted using the Gentra Purgene Blood kit (Qiagen) and DNA integrity was assessed using DNA integrity gel analysis (Nussey et al., 2014). DNA samples that did not show degradation were used to measure relative telomere lengths by quantitative real-time PCR amplification (qPCR; Cawthon, 2002). We calculated the ratio of the amount of telomere sequence to the amount of a control gene that is non-variable in copy number in voles (GAPDH), in comparison to a control sample, producing a single variable for each sample, the T/S ratio. The amount of telomere sequence present in the sample (T), and the amount of the control single-copy gene (S) are proportional to the number of qPCR amplification cycles needed to reach a threshold fluorescent signal (Ct value) in the exponential phase. The T/S ratio is widely used as a measure of relative telomere length and is suitable for summarizing within-individual changes in telomere length (Nussey et al., 2014); and we subsequently refer to it as relative telomere length.

Forward and reverse primers for the GAPDH gene were 5′-GCGTC CAATACGGCCAAATC-3′ and 5′- GAGCATTTGTTGACCCAGCC-3′, respectively. Telomere primers were Tel1b 5′-CGGTTTGTTTGGGTTTGG GTTTGGGTTTGGGTTTGGGTT-3′ and Tel2b 5′-GGCTTGCCTTACCCTT ACCCTTACCCTTACCCTTACCCT-3′. qPCR analyses for both telomere and GAPDH sequences were performed using 2.5 ng of DNA with both sets of primers and 1x FastStart Essential DNA green master in a final volume of 20 µl. Primer concentrations in the final mix were 500 nM for the telomere assay and 200 nM for the control gene assay. Primer concentrations in the final mix were 500 nM for the telomere assay and

200 nM for the GAPDH assay. Real-time amplification of telomere sequences and GAPDH were performed on separate 96-well plates. The telomere thermal profile was 5 min at 95 °C, followed by 30 cycles of 15 s at 95 °C, 30 s at 58 °C, and 30 s at 72 °C. The GAPDH thermal profile was 5 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C. Both assays were followed by melt curve analysis of (58–95 °C 1 °C/5 s ramp).

Each sample was assayed in triplicate and we used the mean of the three replicates. Longitudinal samples were randomly assigned across plates. Each plate also included a calibrator (or golden sample) run in triplicate, which was used to correct for variation among plates, as well as a serial dilution (telomere and GAPDH—40 to 1.25 ng per well) of the calibrator also run in triplicate. Both a negative control (water) and a melting curve were run for each plate to check for specific amplification of a unique amplicon and for the absence of primer-dimer artifacts. The average intra-plate variation of the Ct values was 1.52 and 0.44% for the telomere and GAPDH assays, respectively (calculated as the mean of the coefficient of variation of the Ct values for the golden sample on each plate) and the average inter-plate variation was 8.32% for the telomere assay and 3.17% for the GAPDH assay (calculated as the coefficient of variation of the Ct values for the golden sample across all plates).

2.9. Statistical analyses

Statistical analyses were performed using JMP software (version 11.1.1, SAS Institute Inc. 2012, Cary, NC, USA). For all analyses, we performed Generalized Linear Mixed Models of Restricted Maximum Likelihood (REML-GLMM). For every model, we checked for homogeneity of variances (Levene's test), and examined residuals to confirm that they approximated a normal distribution (Kolmogorov-Smirnov test). In each model, individual was introduced as a random factor to control for variance among individuals. Sucrose preference, ROMs, TAC, and telomere length were each modeled over the isolation period (three time points). These models included the fixed effects of time, treatment, CORT, and the time*treatment interaction. Additionally, we tested the effects of time, and treatment, and the time*treatment interaction on CORT. Non-significant interactions were sequentially removed from the models and the analyses were repeated until we obtained a model with only significant terms. Post-hoc comparisons were carried out using Tukey HSD tests.

3. Results

3.1. Social isolation effects on corticosterone

There was a significant treatment by time effect for plasma CORT levels ($F = 8.9_{10,109.2}$; $p < 0.0001$; Fig. 2a). Specifically, within I and Iv vole groups there was a significant increase in CORT between 0 and 21 d of social isolation (Tukey HSD, $p < 0.05$), and another increase between 21 and 42 d (Tukey HSD, $p < 0.05$), whereas within all paired groups and the Io group all had stable CORT levels over the experiment (Tukey HSD, $p > 0.05$). In addition, while CORT levels were similar among all treatments at 0 d (Tukey HSD, $p > 0.05$), by 21 d the I and Iv groups had elevated CORT compared to the Pv group, and by 42 d the I and Iv groups had higher plasma CORT levels compared to all paired vole groups and Io voles (Tukey HSD, $p < 0.05$).

3.2. Social isolation effects on anhedonia

There was a significant treatment by time effect for sucrose preference ($F_{10,109.8} = 15.4$; $p < 0.0001$; Fig. 2b). Specifically, within I and Iv vole groups there was a significant decrease in sucrose preference between 0 and 21 d of social isolation (Tukey HSD, $p < 0.05$), and another decrease between 21 and 42 d (Tukey HSD, $p < 0.05$), whereas within all paired groups and the Io groups sucrose preference was stable over the experiment (Tukey HSD, $p > 0.05$). In addition, while sucrose preference was similar among all treatments on d 0 (Tukey HSD, $p > 0.05$), I and Iv voles had a lower preference for sucrose compared to Io and all paired groups at both the 21 d and 42 d sucrose preference test (Tukey HSD, $p < 0.05$).

3.3. Social isolation effects on oxidative stress

There was a significant treatment by time effect for ROMs levels ($F_{10,109.1} = 10.1$; $p < 0.0001$; Fig. 2c), though neither treatment nor time affected levels of TAC (Fig. 2d). Specifically for ROMs, within I and Iv vole groups there was a significant increase in ROMs between 0 and 21 d of social isolation (Tukey HSD, $p < 0.05$), and another increase between 21 and 42 d (Tukey HSD, $p < 0.05$), whereas within all paired groups and the Io groups ROMs levels were stable over the experiment (Tukey HSD, $p > 0.05$). In addition, while ROMs levels were similar among all treatments at 0 and 21 d (Tukey HSD, $p > 0.05$), I and Iv voles had higher ROMs levels compared to all paired groups and the Io voles at 42 d of isolation (Tukey HSD, $p < 0.05$).

3.4. Social isolation effects on relative telomere length

There was a significant treatment by time effect for relative telomere length ($F = 4.3_{5,\ 52.5}$; $p = 0.0006$; Fig. 2e and f). Specifically, the I and Iv voles showed a significant decrease in telomere length during the 21d period of isolation in which telomere length was assessed (Tukey HSD, $p < 0.05$), whereas the P, Pv, Io, and Po voles telomere length did not change over this period (Tukey HSD, $p > 0.05$). At 21 d the Iv group had significantly longer telomeres compared to the P and Po groups (Tukey HSD, $p < 0.05$), but Iv telomere length did not differ from the I, Io, and Pv groups (Tukey HSD, $p > 0.05$). At 42 d Io has significantly longer telomeres compared to I and Iv (Tukey HSD, $p < 0.05$), but Io was not different than the paired groups (Tukey HSD, $p > 0.05$).

3.5. Corticosterone effects on anhedonia, oxidative stress, and relative telomere length

While social isolation (treatment) had clear effects on cellular aging and anhedonia, we also wanted to test the specific effects of CORT on our study endpoints. Taking into account time and treatment, increased levels of CORT were associated with a decreased preference for sucrose ($F_{1,75.24} = 13.1$; $p = 0.0005$), increased ROMs levels ($F_{1,135.2} = 13.5$; $p = 0.0003$), shorter telomeres ($F_{1,102} = 5.4$; $p = 0.02$) and no effect on TAC. In addition, taking into account time and treatment, increased ROMs levels were associated with shorter relative telomere length ($F_{1,88.16} = 5.6$; $p = 0.02$).

4. Discussion

Our study demonstrates that social isolation alters stress physiology and increases the rate of cellular aging in a socially monogamous rodent, the prairie vole. While social support has been consistently linked to better health and aging outcomes, no clear mechanism for those positive effects has been established. Importantly, we report for the first time here, that oxytocin can prevent the negative consequences of social isolation on oxidative stress and telomere length. Our data raise the possibility that oxytocin, a hormone known to be involved in prosocial behavior, may be a possible biological target that mediates the protective effects of social support against stressors. Social isolation is an increasing problem with more disconnected societies in general, and the aging population in particular, and a better understanding of the biological targets of social support and social isolation is essential (Cacioppo et al., 2015).

We found that voles who experienced 42 d of isolation had higher levels of plasma CORT.

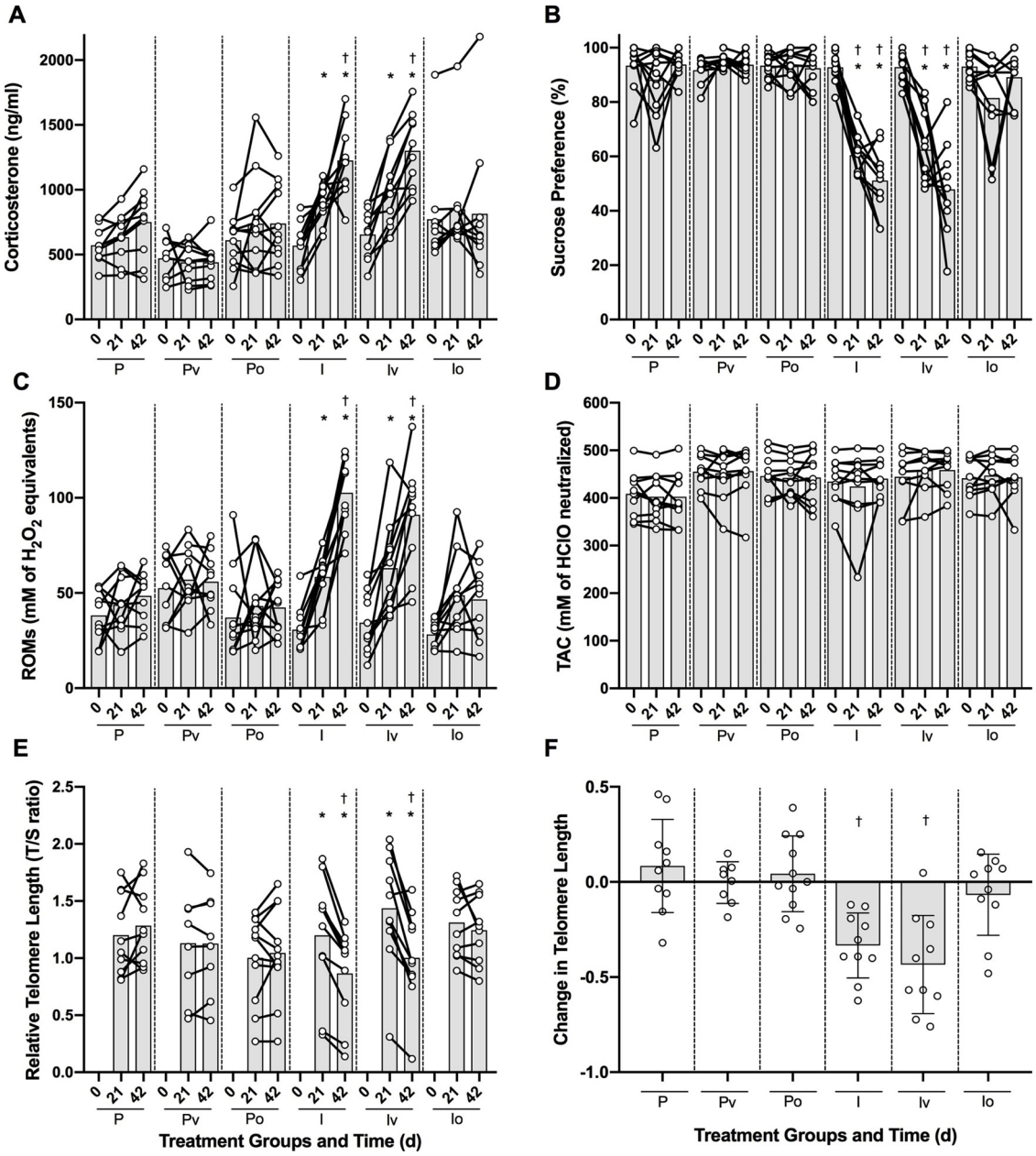

Fig. 2. Social isolation increases corticosterone, depression-like behavior and cellular aging, and oxytocin prevents these consequences. The effects of prairie voles who were sibling paired (P), sibling paired with daily vehicle injections (Pv), sibling paired with daily oxytocin injections (Po), socially isolated (I), socially isolated with daily vehicle injections (Iv), and socially isolated with daily oxytocin injections (Io) on a) corticosterone, b) sucrose preference, a marker of anhedonia and depression like-behavior, c) reactive oxygen metabolites, d) total antioxidant capacity, e) telomere length (T/S ratio), and change in telomere length. Scatter plots are shown, and data points from individuals are joined by lines. Group means are denoted by the gray bars. An * denotes statistically significant difference within groups ($p < 0.05$) and † denotes statistically significantly differences among groups ($p < 0.05$). To see specific differences in corticosterone and telomere length among groups at d 21, see the results.

Although previous findings regarding the effects of social isolation on basal CORT have been mixed, several studies have also found elevated CORT in socially isolated rats and mice (reviewed in Mumtaz et al., 2018). Studies of the effects of social isolation on CORT in prairie voles are limited, but previous investigations found no effects of isolation on basal CORT (Grippo et al., 2007a, 2007b; Pournajafi-Nazarloo et al., 2011; Scotti et al., 2015). This is likely due to methodological differences; specifically, our study employed a 6-week isolation period and assessed CORT levels within individuals at multiple time points, whereas previous prairie vole studies measured CORT at a single terminal time point after a 4 week isolation period. Our longitudinal sampling allows us to detect that isolated animals show elevated CORT between 0 and 21 d, and between 21 and 42 d. If we do not account for individual differences (dropping individual as a random factor, and only analyzing group differences), we only can detect a change in CORT between 0 and 42 d. This suggests that longitudinal sampling offers a more high resolution design to better reflect the progressive effects of isolation on physiological and behavioral measurements..

Along with elevated CORT, isolated prairie voles also had elevated levels of oxidative damage. In the literature, continuous social isolation in non-selectively social rats can also result in chronically elevated baseline CORT levels and oxidative damage (Zhuravliova et al., 2009). Interestingly, social stress, elevated CORT, and increased oxidative damage may all be linked. Elevated CORT can directly affect the

mitochondria to provide the energy necessary to cope with persistent stressors (reviewed in Picard et al., 2014), however one important downstream consequence is an increase in the production of reactive oxygen species (ROS) which can lead to oxidative damage (Zafir and Banu, 2009; Costantini et al., 2011). In agreement with this, we also found that voles with higher CORT over the study period had higher levels of ROMs, our measure of oxidative damage. Unchecked ROS production results in an oxidative damage cascade, and ROMs are intermediate oxidative damage compounds that are derived from several molecules, including lipids, proteins and nucleotides. Importantly, plasma ROMs appear to reflect oxidative damage in other tissues, including the liver, spleen, heart and kidney (Arguelles et al., 2004), raising the possibility that the increased oxidative damage we measured in the plasma also existed in other tissues in isolated voles. While chronic isolation did result in higher levels of oxidative damage, it did not affect our measure of antioxidant capacity. Because blood samples were collected prior to injections of oxytocin/saline, it is possible that we missed the time window in which oxytocin may have temporarily increased antioxidant capacity, and future investigations should investigate a time course of potential antioxidant effects of oxytocin. The TAC test quantifies the ability of the plasma antioxidant barrier to buffer free radical production from massive oxidation events through non-enzymatic means, and while we did not measure enzymatic antioxidants here, previous work has shown that stress can have diverse effects on antioxidant capacity (Djordjevic et al., 2010).

We found that chronic isolation resulted in reduced white blood cell telomere length over the study period. Psychological stress has been shown to cause telomere shortening in mice (Kotrschal et al., 2007). In humans, a wide variety of stressful conditions are associated with shorter telomeres including lack of social support (Mainous et al., 2011), lower socioeconomic status (Steptoe et al., 2011), major depression (Simon et al., 2006), childhood trauma (Kiecolt-Glaser et al., 2011); and even maternal stress during pregnancy can result in shorter telomere lengths in newborns (Entringer et al., 2011; Marchetto et al., 2016). Given that telomere length is associated with a variety of stressor exposures, telomere length has been suggested as a molecular-level measure of allostatic load, or physiological wear and tear (Tomiyama et al., 2012). Furthermore, work in experimental models points toward CORT exposure as a major contributing factor to the negative effects of stress on telomeres (Haussmann et al., 2012; Haussmann and Heidinger, 2015; Tomiyama et al., 2012). In support of this, we did see a that voles exposed to higher CORT levels also had shorter telomeres.

Unfortunately, we were not able to assess baseline telomere length in this study, and only were able to measure white blood cell telomere length at 21 and 42 d of isolation. The absence of telomere data at baseline (0 d) results in two hurdles to the interpretation of how social isolation and support affect telomeres: (i) we don't know if telomere length differed among groups at 0 d, and (ii) we don't know whether telomere length changed occurred over the first 21d of the study. While knowledge of initial telomere length would be informative, the remaining two longitudinal telomere measurements still illuminates how social isolation and support affect telomeres. We address this by highlighting three results from our telomere analysis: among group differences at 21 d into the study, among group differences at the end of the study (42 d), and within group differences between these times. First comparing groups at 21 d, differences in telomere lengths beg the question of whether telomere elongation may have occurred in some groups during the first half the study. While telomere elongation has been reported in the literature, recent studies have acknowledged that telomere length cannot be measured with perfect precision and reports of telomere elongation may be due to error in the measurement technique (Simons et al., 2014). Because Iv voles have longer telomeres on average in comparison to P and Pv voles, but I voles do not, together with the fact that I and Iv voles were similar in all other measurements makes it unlikely that telomeres elongated due to isolation and more plausible that the differences were already present at the start of the study. Second, comparing groups at the end of the study, Io voles had significantly longer telomeres than the isolated voles without oxytocin (I and Iv), but Io vole telomeres did not differ in length from any of the paired voles (P, Pv, and Po). This argues that by the end of the study period, the isolated voles that were given oxytocin looked no different than the paired groups, while the isolated voles without oxytocin experienced telomere shortening. Finally, the within group data strengthens this conclusion, because over the 21 d we found that there was no change in telomere length in paired groups (P, Pv, and Po), that isolated groups (I and Iv) experienced significant telomere loss, while voles experiencing isolation and daily oxytocin injections had no change in telomere length. Taken together, this argues that isolation shortens telomeres, while oxytocin in combination with isolation negates these effects.

Our measurement of telomeres was taken from circulating leukocytes. Glucocorticoids have well known effects on laboratory rodent and human circulating leukocyte populations, by rapidly increasing the numbers of neutrophils while decreasing the numbers of lymphocytes and other leukocytes (McKay and Cidlowski, 2003). While early work in prairie voles suggested that glucocorticoids may not have the same level of glucocorticoid immunosuppressive effects compared to these other species (Klein et al., 1996, 1997), it is important to acknowledge that some of the telomere differences we saw in this study may be due to changing leukocyte populations. Several studies now suggest that while telomere length may vary depending on cell type, individual differences in telomere lengths are consistent over time (Heidinger et al., 2012; Benetos et al., 2011), and these individual differences are conserved among tissues and cell types (Kimura et al., 2010; Daniali et al., 2013). However, future work should employ leukocyte differentials to identify if leukocyte populations change during isolation stress. Regardless of this, because telomere length marks the replicative age of the cell (Aubert and Lansdorp 2008), may shorten in response to cellular insults such as oxidative stress (Boonekamp et al., 2017), and can signal cellular senescence (Bernadotte et al., 2016), telomere shortening has been suggested to represent both a marker and mechanism of biological aging (Epel, 2009; Haussmann and Marchetto, 2010). Our results, taken together with evidence from the literature, suggests that social isolation in our study resulted in prairie voles aging more rapidly.

Oxytocin treatment prevented the effects of social isolation on CORT levels, oxidative damage, and telomere degradation. While previous studies have demonstrated that oxytocin can protect against some endocrine and behavioral effects of social isolation (Grippo et al., 2009), our results provide the first evidence that oxytocin can protect against the effects of social isolation on telomeres. These data add to evidence showing that social support and oxytocin can reduce oxidative damage in an animal model of stroke (Karelina et al., 2011), as well as evidence that oxytocin can reduce oxidative stress associated with chronic CORT treatment (Stanić et al., 2016). Taken together, these data suggest an important role for social support and oxytocin in preventing oxidative damage. While specifically testing how oxytocin protects against the effects of social isolation stress is beyond the scope of this study, previous work indicates multiple candidate mechanisms. Oxytocin can inhibit CORT activity, acting in the hypothalamic paraventricular nucleus (PVN) (Windle et al., 1997; Neumann et al., 2000) where it can reduce stress-induced CRF gene expression (Jurek et al., 2015) and may also inhibit CRF neurons in the PVN via actions at $GABA_A$ receptors (Bülbül et al., 2011; Smith et al., 2016). Therefore, it is possible that oxytocin treatment reduced cellular aging effects in our isolated animals by dampening HPA activation. These mechanisms would require that our peripheral injections of oxytocin cross the blood brain barrier or trigger activation of endogenous central oxytocin release, both of which have been suggested or demonstrated. In rats, intraperitoneal injections of oxytocin caused c-Fos activation in PVN oxytocin neurons (Carson et al., 2010). A recent study in non-human

primates, however, showed that labelled oxytocin injected intravenously was detectable in cerebrospinal fluid, suggesting that peripherally injected oxytocin can cross the blood-brain barrier. Interestingly, that study found no increase in endogenous oxytocin in response to oxytocin injections (Lee et al., 2018). It is also possible that oxytocin treatment produced effects via peripheral mechanisms in our study. Peripheral oxytocin administration has been shown to prevent negative consequences of social isolation in prairie voles by enhancing vagal regulation of the heart (Grippo et al., 2009). An additional candidate peripheral mechanism is inflammation. While social isolation, HPA-axis activation, telomere degradation, and anhedonia have been linked to inflammatory factors (Chrousos, 1995; Franceschi et al., 2000; McDade et al., 2006; Cole et al., 2007; Biesmans et al., 2016), oxytocin has anti-inflammatory effects (Karelina et al., 2011). Future research must investigate the role of inflammatory mechanisms in this animal model of the effects of social context on depression and cellular aging. It is likely that multiple mechanisms acting in concert mediate the effects of social isolation we have observed in this study.

While less studied, reactive nitrogen species (RNS) are also important contributors to oxidative damage. Interestingly, oxytocin can scavenge RNS, and may therefore constitute an important part of the antioxidant defense system (Faghihi et al., 2012). Because voles treated with oxytocin had normal levels of oxidative damage, we are unable to determine if this effect was due to oxytocin's effects on CORT, oxidative damage, or possibly both. Future studies should investigate the direct role that oxytocin may play in reducing RNS during psychological stress, and the damage these molecules inflict.

Isolated prairie voles showed significantly increased anhedonia that worsened throughout the six week isolation period, and this, too, was mitigated by treatment with oxytocin. Anhedonia was used here as an index of depression-like behavior. In humans, anhedonia is a central feature of depression. Our data extend findings by Grippo et al. (2009) which demonstrated increased anhedonia during a four week isolation period that was reversed by oxytocin treatment during the last two weeks. Collectively, these findings show that oxytocin can prevent or reverse isolation-induced depression-like behavior. In our study, CORT levels significantly predicted anhedonia, indicating that elevated glucocorticoids may have contributed to increased depression-like behavior. This is consistent with studies from humans and animals showing that elevated CORT precipitates depression (McEwen and Wingfield, 2003). Oxytocin treatment mitigated the effects of isolation stress on depression-like behavior. It is likely that oxytocin decreased anhedonia in isolated animals, in part, by reducing activity of CORT. In humans, social isolation is strongly associated with a decrease in psychological health, as well as accelerated aging (Hawkley and Cacioppo, 2010; Lin et al., 2016). Our findings are in agreement with these human data and also demonstrate that oxytocin is protective against these effects of social isolation.

4.1. Conclusions

Our study identifies a promising experimental animal model that allows us to investigate the complex experience of social isolation with the integrated consequences of physiological stress, cellular aging, and depression. Specifically, we found that social isolation, a relentless stressor in social organisms, results in these individuals showing advanced cellular aging. Future work should also explore whether social isolation in this model not only affects cellular aging, but also organismal aging. Importantly, we were able to demonstrate that oxytocin shows promise in mitigating these negative consequences of social isolation, and thus is likely a key player linking social factors and aging outcomes.

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