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The influence of social environment on endocrine, cardiovascular and tissue responses in the rabbit

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Abstract

Previous work from our lab demonstrated that social environment influences the progression of atherosclerosis in genetically hyperlipidemic rabbits. The purpose of the current study was to examine behavioral and physiological responses associated with these distinct chronic social conditions. Normolipidemic rabbits were exposed to one of 3 social environments for 4 hours/day over 20 weeks: 1) an Unstable Group in which animals were paired weekly with a different unfamiliar rabbit, 2) a Stable Group in which rabbits were paired with the same littermate for the entire study, and 3) an Individually Caged Group in which animals were socially isolated. It was found that the Unstable Group, characterized by increased agonistic behavior and relatively less affiliative behavior, exhibited physiological responses indicative of chronic stress (increased urinary norepinephrine, plasma cortisol, splenic weight, and decreased visceral fat and body weight compared to the other groups). These animals also had increased acute plasma oxytocin responses relative to the other groups 10 minutes into the social pairing. In contrast, the Stable Group exhibited more affiliative behavior and less stressful physiological and tissue responses. The Individually Caged Group had elevated urinary norepinephrine relative to the Stable Group, and they exhibited higher heart rates at the end of the study compared to the other groups, suggesting that this social environment is also associated with chronic sympathetic arousal. It was concluded that distinct social contexts lead to different patterns of behavioral and physiological responses, and these responses are relevant to the pathophysiology of atherosclerosis and cardiovascular disease.

Keywords

Social Environment; Behavior; Chronic Stress; Catecholamines; Oxytocin; Rabbit

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Introduction

Social relationships have been shown to play an important role in atherosclerosis and coronary artery disease (CAD). For example, social isolation and social support have been found to predict mortality from cardiovascular disease (Berkman and Orth-Gomer, 1996; Syme, 1996), such that a lack of positive social interaction (as well as the expression of social aggression, anger, and hostility) is associated with negative health outcomes (House et al., 1988; Knox and Uvnäs-Moberg, 1998; Smith et al., 2004). Conversely, social support, provided by the presence of a bonding partner, has been shown to reduce the endocrine response to a stressful situation (Sachser et al., 1998). This “social buffering” has a positive influence on both short- and long-term stress (Hennessy et al., 2009).

Despite this accumulating evidence, the pathophysiological mechanisms by which social-emotional behavior influences the course of atherosclerosis and CAD are not clear. In order to identify mechanistic pathways linking behavior to the progression of atherosclerosis, investigators have utilized animal models of disease. The most systematic examination of this type has been a series of studies conducted in the cynomolgous monkey by Kaplan, Manuck and colleagues. In their first study (Kaplan et al., 1982), male cynomolgous macaques fed a moderately atherogenic diet were housed in one of two social environments: an Unstable environment, in which group membership was reorganized regularly requiring re-establishment of social relationships, and a Stable environment, in which group membership was maintained throughout the study. It was found that dominant males in the Unstable group developed significantly more coronary atherosclerosis than subordinate animals in the same group and all animals in the Stable group.

In an attempt to identify the physiological mechanism underlying the social-behavioral effects on atherosclerosis, another study (Kaplan et al., 1987) was conducted in which one-half of the monkeys were chronically treated with the β -adrenergic antagonist, propranolol. It was found that β -adrenergic blockade did not significantly affect behavior or serum lipids, but did attenuate the coronary atherosclerosis in dominant male monkeys in the Unstable social group. It was concluded that social stressors promote coronary atherogenesis, in part, due to chronic sympathetic activation. A separate study addressed (Manuck et al., 1983) whether individual differences in sympathetic reactivity to a standardized laboratory stressor was predictive of future coronary disease risk. It was reported that increased heart rate responsivity to the threat of capture was associated with increased atherosclerosis. This suggests that β -adrenergic mediated endothelial injury may be an important mechanism in behaviorally-related atherosclerosis, however, it is also possible that the SNS may influence the progression of disease through other mechanisms, e.g., changes in lipoprotein binding, altering endothelial permeability to lipoproteins (Kaplan and Manuck, 1994), or stimulating release of cytokines from macrophages and lymphocytes (Ross, 1995).

In our laboratory, we examined whether social environment can influence the progression of atherosclerosis in the Watanabe Heritable Hyperlipidemic rabbit (WHHL) (McCabe et al., 2002). These animals spontaneously develop severe hyperlipidemia and atherosclerosis due to a genetic defect in cholesterol clearance (Havel et al., 1989; Buja et al., 1983). In our study (McCabe et al., 2002), WHHL rabbits were assigned to one of three social/behavioral groups: an Unstable Group, in which unfamiliar rabbits were paired daily, with the pairing switched each week, a Stable Group, in which littermates were paired daily for the entire study, and an Individually Caged Group. The Stable Group exhibited more affiliative social behavior and less agonistic behavior than the Unstable Group, and approximately 50% less aortic atherosclerosis than each of the other two groups. Although the Unstable and Individually Caged Groups had comparable aortic lesion areas, the severity of the disease progressed faster in the Unstable Group, as indexed by a larger area of calcification and

increased fibrous cap thickness in complex lesions. Lesions in the Individually Caged Group were less severe and consisted primarily of foam cells. The Unstable Group showed increased agonistic behavior and signs of chronic adrenocortical and gonadal activation, whereas the Individually Caged Group was relatively sedentary, had low glucocorticoid levels, and was hyperinsulinemic compared to the other groups. The study demonstrated that social environment can slow, as well as accelerate, the progression of atherosclerosis. It also emphasized the importance of behavioral factors in atherogenesis, even in a model of disease with strong genetic determinants.

Although the previously described studies demonstrated that social interactions influence the progression of atherosclerosis, the underlying physiological mechanisms are not clear. It has been suggested that chronic aggressive/aversive social interactions may accelerate disease via the sympathetic nervous system (Kaplan and Manuck, 1994), however, this has not been established in the rabbit model. Similarly, it has been suggested that stable social interactions and affiliative behavior may slow the progression of disease through increased plasma oxytocin (Knox and Uvnäs-Moberg, 1998). In our laboratory, we have demonstrated that oxytocin is anti-inflammatory and anti-oxidant in vascular cells and macrophages (Szeto et al., 2008), and chronic oxytocin infusion in hyperlipidemic mice attenuates aortic atherosclerosis (Nation et al., 2010). Therefore, the current study examined: 1) whether rabbits in an Unstable social environment exhibit physiological responses consistent with chronic stress (e.g., sympathetic activation, elevated glucocorticoids, differences in body weight/visceral fat, increased inflammatory markers), and 2) whether rabbits in a Stable social environment exhibit less stressful responses than animals in unstable or isolated social conditions, and have both acute and chronic increases in plasma oxytocin relative to the other social groups.

Methods

Subjects

Thirty eight male New Zealand White (NZW) rabbits (3 months old, approximately 2 kg) were purchased through Covance Research Products, Inc. (Denver, PA) and were acclimated to experimental procedures for one week prior to the study. For the current behavioral study, NZW rabbits were chosen over WHHL rabbits because disease outcome was not assessed in this study. We have previously reported comparable stress responses between these two strains of rabbits (Szeto et al., 2004). The rabbits were housed in individual cages (6 sq. ft.) on a 12-hour light/dark schedule (lights on at 7 AM). Standard rabbit chow (Purina, 2.5% fat, 0% cholesterol) and water were provided *ad libitum* and the rabbits were weighed weekly. All procedures were approved by the University of Miami Laboratory Animal Care and Use Committee.

Social Manipulation and Behavioral Scoring

The current study used an established behavioral paradigm, as previously described (McCabe et al., 2002). Briefly, animals were assigned to one of three social environments. Rabbits in the Unstable Group ($n = 14$) were paired with an unfamiliar rabbit from a different litter for 4 hours each day. The pairings for this group were switched weekly, forcing the rabbits to continually re-establish dominance/subordination relationships. Rabbits in this group were never paired with a littermate. The Unstable rabbits also alternated between home and away cages, ensuring that no rabbit obtained a systematic home/intruder advantage. Rabbits in the Stable Group ($n = 10$) were paired with the same rabbit for 4 hours each day. In order to enhance the affiliative social interactions in the Stable Group, littermates were paired together throughout the study. Therefore, the Stable Group consisted of pairs of rabbits that were familiar with one another in addition to the constant social

pairings. Animals in this group also alternated between home and away cages. Animals in the Individually Caged Group ($n=14$) were always housed alone and remained in isolation in their home cages during the social pairing of the other two groups. Finally, all animals in the Unstable and Stable groups returned to their individual cages after social pairings.

Following baseline hormonal and cardiovascular measures, the behavioral pairings were begun (i.e., week 1 of the study) and continued for 20 weeks (endpoint of study). Behavior was scored three times per week for each animal during the first 10 minutes of pairing. Behaviors were classified into the following major categories: agonistic behavior, affiliative behavior, other nonagonistic behavior, and inactivity. Agonistic behaviors included biting, combat, chasing, escaping, escape mounting, mounting, enuresis, approaching, aggressive grooming, freezing, attempting to mount, and thumping. Affiliative behaviors included mutual passive rest, nuzzling, and sniffing or grooming. Other nonagonistic behavior included circling, self-grooming, leaping, and cage exploration. Inactivity consisted of passive rest and sitting quietly in the cage. Rabbits in the wild live in warrens with potentially hundreds of family members. Although they are social creatures, rabbits are territorial and aggressive while establishing dominance-subordinance hierarchies within the group (especially males) (Lockley, 1961; Myktowycz & Hesterman, 1975).

The behavioral measures were converted into percent time units based on frequency and type of behavior displayed to account for time spent performing each behavior. Animals in the Individually Caged condition were excluded from analyses of agonistic and affiliative behavior because they were by definition not paired with a conspecific; therefore, they could not engage in these behaviors. It should also be noted that because the total percent time that each animal spends in the various behaviors must equal 100%, the individually caged animals exceeded the proportions of nonagonistic behaviors and inactivity observed in the other two groups. The total percent time spent in each behavior was calculated for each week, behaviors were then combined into the four main categories (i.e., agonistic, affiliative, other nonagonistic, or inactivity). The initial pairing data were the average of the three behavior scores derived during the first week of pairing, and similarly, the midpoint and endpoint data represented the mean of the three scores derived during those weeks.

Blood and Urine collection

For resting tonic measurements of cortisol, oxytocin and C-reactive protein (CRP) blood was collected from the marginal ear vein the week before behavioral pairing was begun (baseline), midpoint, and endpoint (after the 20 weeks of behavioral pairing). Rabbits secrete glucocorticoids in a circadian rhythm that is approximately 12 hours out of phase with humans (Szeto et al., 2004), and thus for cortisol measurement blood was collected between 1 and 3 PM. Blood was collected for measurement of CRP following an overnight fast to avoid the potential effects of a post-prandial state on inflammatory markers (Blackburn et al., 2006). Oxytocin was measured in the same fasted sample to evaluate chronic changes in hormone level.

In order to assess acute changes in plasma oxytocin as a function of behavioral pairing, blood was drawn just prior to, 10 minutes into, and 2 hours into the 4 hour social pairing of animals. These acute changes in oxytocin were assessed every two weeks of the study, beginning with the first week of behavioral pairings (week 1).

Urinary catecholamines and cardiovascular measurements were also measured during baseline, midpoint, and endpoint. For catecholamine assessment, rabbits were placed in a metabolic cage for 24 hours and urine was collected. Heart rate and systolic, diastolic, mean arterial blood pressures were measured using an automated tail-cuff system (Model 29SSP,

IITC, Inc., Woodland Hills, CA). These measures were taken while the animals were loosely restrained and provided only mean blood pressure and heart rate values.

Biochemical Assays

Our lab has recently addressed important issues in the measurement of plasma oxytocin (Szeto et al., 2011). First, it is essential to extract plasma samples to eliminate compounds that artificially increase the apparent plasma oxytocin levels. Second, there appears to be several molecules present in plasma extracts that together account for oxytocin-like immunoreactivity. We have preliminary data to suggest that these molecules represent oxytocin precursors or degradation products, and together reflect circulating oxytocin levels. Thus, for the current study when we refer to oxytocin, we are actually measuring “oxytocin-like immunoreactivity.”

Oxytocin enzyme-linked immunoassay (EIA; Enzo Life Sciences, Farmingdale, NY), C-Reactive Protein EIA (Immunology Consultants Laboratory, Inc., Portland, OR), and radioimmunoassay for epinephrine and norepinephrine (Alpco Diagnostics, Salem, NH) assays were performed following the manufacturers' protocol. Catecholamine values were adjusted by the concentration of urinary creatinine. Plasma cortisol was measured by immunoassay using an automated analyzer (Roche Diagnostics, Indianapolis, IN).

Necropsy

At the endpoint of the study following euthanasia, the epididymal and retroperitoneal fat depots, spleen, liver, adrenal glands, and testes were removed from each animal and weighed. For analysis, tissue weights were expressed as a percent of total body weight.

Statistical Analysis

Values are reported as mean $\pm$ SEM. A repeated measures analysis of variance (ANOVA) with a group by time mixed design was used to evaluate whether change in dependent variables over time differed as a function of group. When a test of the interaction was significant, we used *post-hoc* one way-ANOVA and LSD tests to assess differences between social groups at specific timepoints. If the interaction was not significant, we then examined the main effects; a significant result indicating differences collapsed over time or over group (marginal means reported as *M*).

For the assessment of acute oxytocin responses to social pairing, data were collapsed across the 7 time points after confirming no group by time by response interaction. Thus, the mean values for each animal were calculated for pre-pairing levels as well as their 10-minute and 120-minute responses. Groups were then compared using one-way ANOVA and LSD tests at each time point. We used repeated measures to examine change over time within groups.

There were some missing data points due to insufficient sample volume or values outside the assay limits of detection. For acute changes in oxytocin sampled during social pairing, 2% of the 798 possible values were missing. For oxytocin measured at baseline, midpoint and endpoint, 7% of the 114 possible values were missing. Similarly, about 5% of the 228 of the possible catecholamine values were missing. Given the small sample size, we did not exclude animals that had specific instances of missing data. The approach taken was a variation of hot deck imputation where the missing value was replaced with a value derived from animals within the same group. Within each group, a prediction equation used data from all available time points, excluding outliers, to predict the missing value. Unfortunately, our initial sample sizes were too small to implement modern methods of handling missing data (FIML, multiple imputation). Degrees of freedom were adjusted to

account for missing data. In repeated measures analyses, adjustments were made to degrees of freedom when the sphericity assumption was violated.

Results

Behavior as a function of social environment

For agonistic behavior, there was a significant group by time interaction ($F(2,44) = 4.57, p = 0.01$), such that animals within the Unstable Group spent significantly more time in agonistic behavior at endpoint compared to animals within the Stable Group, $p < .01$ (Table 1). Conversely, for affiliative behavior there was a significant group effect ($F(1,22) = 16.40, p < 0.01$) with animals in the Stable Group ($M = 27.5\%$) exhibiting more overall affiliative social behavior than the Unstable Group ($M = 15.9\%$). As expected, Individually Caged animals ($M = 55.2\%$) spent more time in overall inactivity (group effect, $F(2,35) = 99.23, p < 0.01$), compared to the Stable Group ($M = 21.7\%$) and Unstable Group ($M = 23.9\%$), $p < 0.01$ for both comparisons, and the Unstable and Stable Groups did not differ in overall activity level, $p > 0.05$. For other non-agonistic behavior there was a significant group by time interaction ($F(3.53,61.93) = 6.30, p < 0.01$). At the first week of pairing, midpoint and endpoint, there was a significant difference among groups (F ranged from 27.19 to 48.32, $p < 0.01$ at each time point) such that the Individually Caged Group spent significantly more time in these behaviors than the other two groups at all time points, $p < 0.01$ for all comparisons. In addition, the Stable Group spent significantly more time in these behaviors than the Unstable Group at endpoint, $p < 0.01$. These findings were expected and are consistent with previous studies from our laboratory (McCabe et al., 2002; Paredes et al., 2006; Nation et al., 2008).

Resting endocrine measures as a function of social environment

Catecholamines—For urinary norepinephrine, there was a significant group by time interaction ($F(4,64) = 2.45, p = 0.05$). Subsequent mean comparisons revealed a significant difference among groups at baseline, $F(2,29) = 5.92, p < 0.01$, such that the Unstable Group exhibited higher norepinephrine relative to the Stable Group ($p < 0.01$) and the Individually Caged Group ($p = 0.02$; Figure 1A, Table 2). There was also a significant group effect ($F(2,29) = 4.85, p = 0.01$). Animals in the Stable Group ($M = 11.4$) had lower norepinephrine across the study compared to the Unstable Group ($M = 19.2$), $p < 0.01$, as well as the Individually Caged Group ($M = 17.0$), $p = 0.02$. For urinary epinephrine, there was no significant group by time interaction or group effect ($p > 0.05$; Figure 1B, Table 2).

Cortisol—There was a significant group by time interaction ($F(4,70) = 3.52, p = 0.01$) in cortisol levels. At midpoint there was a significant difference among groups, $F(2,35) = 13.20, p < 0.01$, such that the Unstable Group exhibited elevated plasma cortisol compared to the two other groups ($p < 0.01$ for both comparisons; Figure 1C, Table 2).

Oxytocin—There was no significant group by time interaction or group effect for plasma oxytocin sampled during resting conditions at baseline, midpoint or endpoint ($p > 0.05$; Figure 1D, Table 2).

Physiological measures as a function of social environment

It should be noted, there was a significant time effect for systolic blood pressure ($F(1,35) = 93.11, p < 0.01$), diastolic blood pressure ($F(1,35) = 36.56, p < 0.01$), mean arterial pressure ($F(1,35) = 60.68, p < 0.01$), and heart rate ($F(1,35) = 53.19, p < 0.01$), such that for each cardiovascular measure collapsed across groups, the values during baseline were elevated relative to values at the end of the study ($p < 0.01$; Table 3).

For diastolic and mean arterial blood pressures, there were significant group by time interactions ($F(2,35) = 5.66, p < 0.01$, and $F(2,35) = 5.10, p = 0.01$, respectively). At baseline, significant group differences were found for diastolic blood pressure ($F(2,35) = 17.95, p < 0.01$), and mean arterial pressure ($F(2,35) = 17.51, p < 0.01$), such that Individually Caged animals displayed lower pressures when compared to the Stable and Unstable Groups ($p < 0.01$ for all comparisons). Finally, there was a significant group by time interaction for heart rate ($F(2,35) = 6.36, p < 0.01$). At the endpoint of the study there was a significant difference among groups ($F(2,35) = 6.25, p < 0.01$), such that Individually Caged animals had higher heart rate when compared to animals in the Stable ($p = 0.02$), and Unstable Groups ($p < 0.01$).

After controlling for the change in body weight from baseline to endpoint, a similar pattern for blood pressures remained. Additionally, group differences in systolic, diastolic, and mean arterial pressures were observed across all time points, where Individually Caged animals displayed lower pressures when compared to the Stable and Unstable Groups ($p < 0.05$ for all comparisons). However, after controlling for behavioral inactivity, we did not find significant differences in any of the cardiovascular measures ($p > 0.05$). Therefore, the group differences in blood pressures were not related to body weight, but were dependent upon behavioral inactivity.

C-reactive protein—There was no significant group by time interaction or group effect for plasma CRP ($p > 0.05$; Figure 1E, Table 2).

Body Weight—There was a significant group by time interaction for body weight ($F(3.61, 63.29) = 8.36, p < 0.01$). At midpoint, there was a significant difference among groups ($F(2,35) = 5.35, p < 0.01$), such that animals housed in the Unstable condition weighed less than both the Stable Group ($p < 0.01$), and Individually Caged animals, $p = 0.01$ (Figure 1F, Table 2). A group difference was also found at endpoint ($F(2,35) = 5.79, p < 0.01$), such that animals housed in the Unstable condition weighed less than those housed in the Stable condition ($p < 0.01$).

Tissues—Tissues collected at necropsy were weighed and adjusted for total body weight prior to analysis. A significant difference among groups was found for epididymal fat ($F(2,35) = 3.58, p = 0.03$) and for retroperitoneal fat ($F(2,35) = 21.28, p < 0.01$). Animals housed in the Stable condition had more epididymal fat compared to the Unstable Group ($p = 0.01$), while the Stable Group and Individually Caged animals had increased retroperitoneal fat compared to the Unstable condition ($p < 0.01$) for both comparisons, Table 4). Additionally, there was a significant group difference in spleen weight ($F(2,35) = 4.78, p = 0.01$), such that animals housed in the Unstable condition had 30% larger spleens compared to the Stable Group ($p = 0.02$), and Individually Caged Group ($p < 0.01$; Table 4). There were no significant group differences in adrenal gland, testicular, or liver weights ($p > 0.05$).

Acute changes in oxytocin as a function of social pairing

In order to assess acute changes in plasma oxytocin, blood was collected just prior to, 10 minutes into, and 2 hours into the 4-hour social pairing of animals. There was a significant mean difference among groups for the 10 minute response ($F(2,35) = 3.25, p = 0.05$) such that the Unstable Group displayed significantly higher oxytocin levels than animals in the Stable Group ($p = 0.04$), and Individually Caged Group ($p = 0.02$; Figure 2). There were no significant group differences in mean pre-pairing levels or mean 2-hour levels ($p > 0.05$). A significant change over time was found within the Unstable ($F(2,26) = 4.41, p = 0.02$), as well as the Stable Groups ($F(2,18) = 6.13, p < 0.01$). There was a significant increase from 0

min to 10 min within the Unstable ($p = 0.01$), and Stable Groups ($p < 0.01$). Additionally, there was a significant increase from 0 min to 120 min within the Unstable ($p = 0.02$), and Stable Groups ($p = 0.03$). There were no significant differences from 10 min to 2 hours in either group ($p > 0.05$), and there was no significant change over time within the Individually Caged Group ($p > 0.05$).

Discussion

The current study examined behavioral and physiological responses to an experimental manipulation of social environment in an animal model. It was found that differing social contexts are associated with distinct patterns of response. In particular, animals in an unstable social environment, characterized by increased agonistic behavior and relatively less affiliative social behavior, exhibited endocrine and tissue responses indicative of a chronic state of stress. These rabbits had elevated urinary norepinephrine and plasma cortisol at times during the 20 weeks of behavioral pairing. In addition, at study endpoint the Unstable Group weighed less than the Stable Group, had less visceral fat, and developed larger spleens than either the Stable or Individually Caged animals. The finding that at the end of the study the Unstable Group had less visceral fat and on average weighed less than the Stable Group is consistent with the hypothesis that these differences might be related to chronic stress rather than to a difference in activity per se, since the Unstable and Stable Groups did not differ in overall activity level. However, it should be kept in mind that the limitations of our general activity measure, which did not distinguish among various types of activities, preclude us from reaching a firm conclusion. Because of its role in immune/inflammatory cell trafficking, increased splenic weight is seen as a sign of a chronic immune/inflammatory response, as well as a result of chronic stress (Azpiroz et al., 1999; Johnson et al., 2006).

In contrast to the Unstable Group, the Stable Group did not exhibit overt physiological arousal indicative of chronic stress. Animals in the Stable Group showed significantly more affiliative social behavior and less agonistic behavior than the Unstable Group, had lower urinary norepinephrine and cortisol, and they gained more weight and had more body fat than the Unstable animals even though the total activity levels of the two groups were similar. Therefore, it appears that a stable, daily 4-hour interaction with a littermate not only leads to affiliative social behavior, but also is associated with significantly less stressful physiological responses.

The Individually Caged Group was socially isolated for the entire study. In rodents and primates, social isolation has been shown to induce chronic stress and depressive-like behavior (Grippe et al., 2009; Shively and Willard, 2011). Although the Individually Caged rabbits in the current study did not appear to be as stressed as the Unstable Group, they did have elevated urinary norepinephrine relative to the Stable Group. In addition, these animals had higher heart rates at the end of the study, which is consistent with a previous finding from our laboratory (McCabe et al., 2002). It is possible that these animals are distressed due to social isolation, however, another plausible explanation is behavioral inactivity. Because they did not have a conspecific with whom to interact, the Individually Caged Group spent a greater percentage of their time behaviorally inactive relative to the other groups. We have hypothesized that this type of sedentary behavior, coupled with increased visceral fat and body weight due to overeating and hyperinsulinemia, could lead to the development of the “metabolic syndrome” and chronic sympathetic nervous system arousal (McCabe et al., 2002; Gonzales et al., 2005).

There is a growing literature that the neuropeptide, oxytocin, works centrally to facilitate a variety of prosocial, affiliative behaviors (Insel and Young, 2001; Carter et al., 2008), and

attenuate peripheral glucocorticoid responses (Neumann, 2002). In addition, it has been suggested that in humans oxytocin is released into the circulation in response to warm physical contact (e.g., touch, massage, warm hug; Uvnäs-Moberg, 1998; Light et al., 2005). In contrast, several animal studies have suggested that oxytocin is released peripherally and centrally in response to stressful stimuli and situations (Callahan et al., 1989; Nishioka et al., 1998; Onaka, 2004; Paredes et al., 2006). In light of its possible role as a stress response, it was suggested that oxytocin may serve as an impetus for the organism to seek out pro-social behavior and/or social support (Taylor, 2006; Taylor et al., 2010).

In the current study, there was no significant group difference in baseline, midpoint, or endpoint plasma oxytocin. Despite this result, the reported mean oxytocin level was higher in the Stable Group than in the other groups at each time point, and was more than twice as high in the Stable Group than in the other groups by endpoint. The standardized difference of .12 units in the endpoint means (Mean difference/SD) is suggestive of a small effect; however, additional research is needed with a larger sample size. Given the relatively small N in the present study, it is possible that the study was underpowered to detect differences in tonic level of oxytocin among groups.

There was a significant group difference in acute oxytocin responses measured during the behavioral pairings, such that the Unstable Group exhibited greater oxytocin responses 10 minutes into the pairing compared to the other two groups. This suggests that in our paradigm, acute plasma oxytocin responses are related to agonistic behavior and chronic stress rather than to affiliative social behavior. Interestingly, both the Unstable and Stable Groups showed significant increases in oxytocin responses at 10 minutes and 120 minutes relative to the pre-pairing levels, whereas the Individually Caged Group did not. This finding may be due to the fact that the Stable Group exhibited a substantial amount of agonistic behavior, although significantly less than the Unstable Group. One caveat to these findings relates to our recent paper (Szeto et al., 2011) that questions the specificity of the antibodies used in commercial oxytocin assay kits. It is likely that these kits are measuring other immunoreactive products, in addition to oxytocin. Notwithstanding, these are the same kits used in most of the other published oxytocin studies.

The patterns of behavioral and physiological responses to social contexts reported in the current study are relevant to social and emotional factors in the progression of disease. In our hyperlipidemic rabbit model of cardiovascular disease, atherosclerosis progressed the fastest in the Unstable Group, as indexed by the severity of the vascular lesions (McCabe et al., 2002). The current findings are consistent with the notion that chronic stress in the Unstable Group is accompanied by sympathetic nervous system and hypothalamic-pituitary-adrenal cortical activation, which may accelerate the pathophysiological mechanisms in the vascular wall underlying atherosclerosis (e.g., inflammation, oxidative stress) in animals predisposed to disease (e.g., WHHLs). In contrast, the lack of stressful physiological responses in the Stable Group may serve to protect these animals by slowing the progression of disease relative to the Unstable Group. Finally, the Individually Caged Group, either through chronic distress related to social isolation or behavioral inactivity leading to increased adiposity and hyperinsulinemia, exhibits some signs of chronic stress and sympathetic arousal. These stress responses, coupled with metabolic factors, may interact to advance the progression of atherosclerosis in this group.

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References

- Azpiroz A, Fano E, Garmendia L, Arregi A, Cacho R, Beitia G, Brain PF. Effects of chronic mild stress (CMS) and imipramine administration, on spleen mononuclear cell proliferative response, serum corticosterone level and brain norepinephrine content in male mice. *Psychoneuroendocrinology*. 1999; 24:345–361. [PubMed: 10101738]
- Berkman, LF.; Orth-Gomer, K. Prevention of cardiovascular morbidity and mortality: role of social relations. In: Orth-Gomer, K.; Schneiderman, N., editors. *Behavioral Medicine Approaches to Cardiovascular Disease Prevention*. New York: Lawrence Erlbaum Associates, Inc.; 1996. p. 51-67.
- Blackburn P, Després JP, Lamarche B, Tremblay A, Bergeron J, Lemieux I, Couillard C. Postprandial variations of plasma inflammatory markers in abdominally obese men. *Obesity (Silver Spring)*. 2006; 14:1747–1754. [PubMed: 17062804]
- Buja LM, Kita T, Goldstein JL, Watanabe Y, Brown MS. Cellular pathology of progressive atherosclerosis in the WHHL rabbit. An animal model of familial hypercholesterolemia. *Arteriosclerosis*. 1983; 3:87–101. [PubMed: 6824499]
- Callahan MF, Kirby RF, Cunningham JT, Eskridge-Sloop SL, Johnson AK, McCarty R, Gruber KA. Central oxytocin systems may mediate a cardiovascular response to acute stress in rats. *Am J Physiol*. 1989; 256:H1369–H1377. [PubMed: 2719134]
- Carter CS, Grippo AJ, Pournajafi-Nazarloo H, Ruscio MG, Porges SW. Oxytocin, vasopressin and sociality. *Prog Brain Res*. 2008; 170:331–336. [PubMed: 18655893]
- Gonzales JA, Szeto A, Mendez AJ, Zaias J, Paredes J, Caperton CV, Llabre MM, Levine JE, Goldberg RB, Schneiderman N, McCabe PM. Effect of behavioral interventions on insulin sensitivity and atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Psychosom Med*. 2005; 67:172–178. [PubMed: 15784780]
- Grippo AJ, Trahanas DM, Zimmerman RR 2nd, Porges SW, Carter CS. Oxytocin protects against negative behavioral and autonomic consequences of long-term social isolation. *Psychoneuroendocrinology*. 2009; 34:1542–1553. [PubMed: 19553027]
- Havel RJ, Yamada N, Shames DM. Watanabe heritable hyperlipidemic rabbit. Animal model for familial hypercholesterolemia. *Arteriosclerosis*. 1989; 9:133. [PubMed: 2643428]
- Hennessy MB, Kaiser S, Sachser N. Social buffering of the stress response: Diversity, mechanisms, and functions. *Front Neuroendocrinol*. 2009; 30:470–482. [PubMed: 19545584]
- House JS, Landis KR, Umberson D. Social relationships and health. *Science*. 1988; 241:540–545. [PubMed: 3399889]
- Insel TR, Young LJ. The neurobiology of attachment. *Nat Rev Neurosci*. 2001; 2:129–136. [PubMed: 11252992]
- Johnson RR, Prentice TW, Bridegam P, Young CR, Steelman AJ, Welsh TH, Welsh CJ, Meagher MW. Social stress alters the severity and onset of the chronic phase of Theiler's virus infection. *J Neuroimmunol*. 2006; 175:39–51. [PubMed: 16631261]
- Kaplan JR, Manuck SB, Clarkson TB, Lusso FM, Taub DM. Social status, environment, and atherosclerosis in cynomolgus monkeys. *Arteriosclerosis*. 1982; 2:359–368. [PubMed: 6889852]
- Kaplan JR, Manuck SB, Adams MR, Weingand KW, Clarkson TB. Inhibition of coronary atherosclerosis by propranolol in behaviorally predisposed monkeys fed an atherogenic diet. *Circulation*. 1987; 76:1364–1372. [PubMed: 3677359]
- Kaplan JR, Manuck SB. Antiatherogenic effects of beta-adrenergic blocking agents: theoretical, experimental, and epidemiologic considerations. *Am Heart J*. 1994; 128:1316–1328. [PubMed: 7977013]
- Knox SS, Uvnäs-Moberg K. Social isolation and cardiovascular disease: an atherosclerotic pathway? *Psychoneuroendocrinology*. 1998; 23:877–890. [PubMed: 9924742]
- Light KC, Grewen KM, Amico JA. More frequent partner hugs and higher oxytocin levels are linked to lower blood pressure and heart rate in premenopausal women. *Biol Psychol*. 2005; 69:5–21. [PubMed: 15740822]
- Lockley RM. Social structure and stress in the rabbit warren. *J Anim Ecol*. 1961; 30:385–423.
- Manuck SB, Kaplan JR, Clarkson TB. Behaviorally induced heart rate reactivity and atherosclerosis in cynomolgus monkeys. *Psychosom Med*. 1983; 45:95–108. [PubMed: 6683414]

- McCabe PM, Gonzales JA, Zaias J, Szeto A, Kumar M, Herron AJ, Schneiderman N. Social environment influences the progression of atherosclerosis in the watanabe heritable hyperlipidemic rabbit. *Circulation*. 2002; 105:354–359. [PubMed: 11804992]
- Mykityowycz R, Hesterman ER. An experimental study of aggression in captive European rabbits, *Oryctolagus Cuniculus* (L.). *Behavior*. 1975; 52:104–123.
- Nation DA, Gonzales JA, Mendez AJ, Zaias J, Szeto A, Brooks LG, Paredes J, D'Angola A, Schneiderman N, McCabe PM. The effect of social environment on markers of vascular oxidative stress and inflammation in the Watanabe heritable hyperlipidemic rabbit. *Psychosom Med*. 2008; 70:269–275. [PubMed: 18256340]
- Nation DA, Szeto A, Mendez AJ, Brooks LG, Zaias J, Herderick EE, Gonzales J, Noller CM, Schneiderman N, McCabe PM. Oxytocin attenuates atherosclerosis and adipose tissue inflammation in socially isolated ApoE^{-/-} mice. *Psychosom Med*. 2010; 72:376–382. [PubMed: 20368478]
- Neumann ID. Involvement of the brain oxytocin system in stress coping: interactions with the hypothalamo-pituitary-adrenal axis. *Prog Brain Res*. 2002; 139:147–162. [PubMed: 12436933]
- Nishioka T, Anselmo-Franci JA, Li P, Callahan MF, Morris M. Stress increases oxytocin release within the hypothalamic paraventricular nucleus. *Brain Res*. 1998; 781:56–60. [PubMed: 9507065]
- Onaka T. Neural pathways controlling central and peripheral oxytocin release during stress. *J Neuroendocrinol*. 2004; 16:308–312. [PubMed: 15089967]
- Paredes J, Szeto A, Levine JE, Zaias J, Gonzales JA, Mendez AJ, Llabre MM, Schneiderman N, McCabe PM. Social experience influences hypothalamic oxytocin in the WHHL rabbit. *Psychoneuroendocrinology*. 2006; 31:1062–1075. [PubMed: 16963189]
- Ross R. Cell biology of atherosclerosis. *Annu Rev Physiol*. 1995; 57:791–804. [PubMed: 7778883]
- Sachser N, Dürschlag M, Hirzel D. Social relationships and the management of stress. *Psychoneuroendocrinology*. 1998; 23:891–904. [PubMed: 9924743]
- Shively CA, Willard SL. Behavioral and neurobiological characteristics of social stress versus depression in nonhuman primates. *Exp Neurol*. 2012; 233:87–94. [PubMed: 21983263]
- Smith TW, Glazer K, Ruiz JM, Gallo LC. Hostility, anger, aggressiveness, and coronary heart disease: an interpersonal perspective on personality, emotion, and health. *J Pers*. 2004; 72:1217–1270. [PubMed: 15509282]
- Syme SL. Rethinking disease: Where do we go from here? *Ann Epidemiol*. 1996; 6:463–468. [PubMed: 8915479]
- Szeto A, Gonzales JA, Spitzer SB, Levine JE, Zaias J, Saab PG, Schneiderman N, McCabe PM. Circulating levels of glucocorticoid hormones in WHHL and NZW rabbits: circadian cycle and response to repeated social encounter. *Psychoneuroendocrinology*. 2004; 29:861–866. [PubMed: 15177701]
- Szeto A, McCabe PM, Nation DA, Tabak BA, Rossetti MA, McCullough ME, Schneiderman N, Mendez AJ. Evaluation of enzyme immunoassay and radioimmunoassay methods for the measurement of plasma oxytocin. *Psychosom Med*. 2011; 73:393–400. [PubMed: 21636661]
- Szeto A, Nation DA, Mendez AJ, Dominguez-Bendala J, Brooks LG, Schneiderman N, McCabe PM. Oxytocin attenuates NADPH-dependent superoxide activity and IL-6 secretion in macrophages and vascular cells. *Am J Physiol Endocrinol Metab*. 2008; 295:E1495–E1501. [PubMed: 18940936]
- Taylor SE. Tend and befriend: Biobehavioral bases of affiliation under stress. *Curr Dir Psychol Sci*. 2006; 15:273–277.
- Taylor SE, Saphire-Bernstein S, Seeman TE. Are plasma oxytocin in women and plasma vasopressin in men biomarkers of distressed pair-bond relationships? *Psychol Sci*. 2010; 21:3–7. [PubMed: 20424014]
- Uvnäs-Moberg K. Oxytocin may mediate the benefits of positive social interaction and emotions. *Psychoneuroendocrinology*. 1998; 8:819–835. [PubMed: 9924739]

Highlights

- Distinct social environments led to unique behavioral response patterns in rabbits
- Unstable social environment led to endocrine/physiological stress responses
- Stress responses included increased norepinephrine, cortisol, and splenic weight
- Unstable Group also exhibited acute increases in plasma oxytocin to social pairing
- These patterns are relevant to the pathophysiology of cardiovascular disease

Figure 1.

Urinary catecholamines, plasma cortisol, plasma oxytocin, C-reactive protein, and body weight at baseline, midpoint, and endpoint as a function of social environment. Panel A, urinary norepinephrine (NE). Note the elevated NE level in the Unstable Group at baseline, and the significantly lower NE in the Stable Group collapsed over time. Panel B, urinary epinephrine. Panel C, plasma cortisol. Note the elevated cortisol level in the Unstable Group at midpoint. Panels D & E, plasma oxytocin and C-reactive protein, respectively. Panel F, body weight. Note the lower body weight in the Unstable Group relative to the other groups.

Figure 2.

Acute changes in plasma oxytocin as a function of behavioral pairing. Blood was drawn just prior to, 10 minutes into, and 2 hours into the 4-hour social pairing of animals. The results are collapsed across the 7 time points of data collection. Note the increased oxytocin levels in the Unstable Group at 10 minutes. In addition, both the Unstable and Stable Groups exhibited significant oxytocin elevations from baseline at 10 minutes and 120 minutes.

Table 1Percent Time in Behavior by Group (Mean $\pm$ SEM).

	Week 1	Week 7	Week 20
Agonistic behavior			
Unstable	37.8% (7.3)	40.2% (6.1)	55.8% (5.9) ^a
Stable	34.4% (4.2)	29.1% (5.0)	24.8% (4.3)
Affiliative behavior			
Unstable ^c	14.2% (2.4)	12.5% (2.4)	20.8% (3.6)
Stable	22.3% (2.3)	23.5% (3.4)	36.7% (2.9)
Inactivity			
Unstable	30.1% (3.6)	32.3% (2.5)	9.2% (2.9)
Stable	25.9% (1.9)	31.2% (1.6)	7.9% (1.5)
Individually Caged ^d	59.1% (1.4)	59.5% (2.9)	47.0% (3.6)
Other non-agonistic behavior			
Unstable	17.9% (3.2)	15.0% (2.0)	14.2% (2.4) ^a
Stable	17.5% (3.1)	16.1% (2.4)	30.6% (2.1)
Individually Caged	40.9% (1.4) ^{a,b}	40.5% (2.9) ^{a,b}	53.0% (3.6) ^{a,b}

^aSignificantly different from Stable Group ($p < 0.01$).^bSignificantly different from the Unstable Group ($p < 0.01$).^cSignificantly different from Stable Group, collapsed over time ($p < 0.01$).^dSignificantly different from both groups, collapsed over time ($p < 0.01$).

Table 2

Measures Collected at Baseline, Midpoint, and Endpoint by Group (Mean $\pm$ SEM), Unstable (n=14), Stable (n=10) and Individually Caged (n=14)

	Baseline	Midpoint	Endpoint
Norepinephrine (ng/mg creatinine)			
Unstable	29.3 (3.8) ^{a,c}	11.4 (1.4)	17.0 (2.8)
Stable ^{e,f}	14.3 (1.6)	6.3 (1.8)	13.7 (1.6)
Individually Caged	20.4 (1.7)	10.7 (2.3)	20.0 (2.4)
Epinephrine (ng/mg creatinine)			
Unstable	1.2 (0.2)	0.9 (0.2)	1.7 (0.5)
Stable	0.6 (0.1)	0.5 (0.1)	1.3 (0.4)
Individually Caged	1.1 (0.2)	0.5 (0.1)	1.3 (0.2)
Cortisol (μ g/ml)			
Unstable	0.36 (0.05)	0.31 (0.05) ^d	0.22 (0.05)
Stable	0.35 (0.05)	0.05 (0.01)	0.30 (0.08)
Individually Caged	0.32 (0.06)	0.13 (0.03)	0.16 (0.03)
Oxytocin (pg/ml)			
Unstable	12.3 (1.4)	11.4 (1.8)	19.8 (7.9)
Stable	18.3 (6.6)	19.7 (9.6)	41.2 (13.7)
Individually Caged	11.4 (2.0)	5.9 (0.9)	19.4 (6.6)
Weight (kg)			
Unstable	2.50 (0.04)	3.15 (0.07) ^{a,b}	3.94 (0.10) ^a
Stable	2.52 (0.07)	3.44 (0.07)	4.43 (0.12)
Individually Caged	2.53 (0.04)	3.38 (0.06)	4.15 (0.09)
C-reactive protein (mg/L)			
Unstable	7.6 (3.6)	7.4 (2.4)	19.0 (4.5)
Stable	8.0 (3.4)	4.2 (1.8)	17.4 (4.7)
Individually Caged	5.4 (1.5)	7.8 (3.3)	9.8 (2.8)

^aSignificantly different from Stable Group ($p < 0.01$).

^bSignificantly different from Individually Caged Group ($p = 0.01$).

^cSignificantly different from Individually Caged Group ($p = 0.02$).

^dSignificantly different from both groups ($p < 0.01$).

^eSignificantly different from Unstable Group, collapsed over time ($p < 0.01$).

^fSignificantly different from Individually Caged Group, collapsed over time ($p = 0.02$).

Table 3Cardiovascular Measurements by Group (Mean $\pm$ SEM)

	Baseline	Endpoint
Systolic blood pressure ^a		
Unstable	102 (3)	82 (2)
Stable	105 (2)	83 (3)
Individually Caged	90 (2)	78 (2)
Diastolic blood pressure ^a		
Unstable	77 (2)	64 (3)
Stable	80 (3)	61 (2)
Individually Caged	62 (2) ^b	59 (2)
Mean arterial pressure ^a		
Unstable	86 (2)	70 (2)
Stable	88 (3)	68 (2)
Individually Caged	72 (2) ^b	66 (2)
Heart rate ^a		
Unstable	236 (6)	207 (4)
Stable	238 (6)	213 (6)
Individually Caged	238 (6)	231 (6) ^{c, d}

^aSignificant change over time, collapsed across groups ($p < 0.01$).^bSignificantly different than both groups ($p < 0.01$).^cSignificantly different from Stable Group ($p = 0.02$).^dSignificantly different from Unstable Group ($p < 0.01$).

Table 4Tissues Collected at Necropsy by Group (Mean $\pm$ SEM), Adjusted by Endpoint Weight

	Epididymal fat (g)	Retroperitoneal fat (g)	Spleen (g)
Unstable	0.10 % (0.01) ^a	2.13% (0.20) ^d	0.04% (0.00) ^{b, c}
Stable	0.14% (0.02)	3.93% (0.26)	0.03% (0.00)
Individually Caged	0.11% (0.01)	3.91% (0.23)	0.03% (0.00)

^aSignificantly different from Stable Group ($p = 0.01$).^bSignificantly different from Stable Group ($p = 0.02$).^cSignificantly different from Individually Caged Group ($p < 0.01$).^dSignificantly different from both groups ($p < 0.01$).