Physiology

Voluntary locomotor activity mitigates oxidative damage associated with isolation stress in the prairie vole (*Microtus ochrogaster*)

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Organismal performance directly depends on an individual’s ability to cope with a wide array of physiological challenges. For social animals, social isolation is a stressor that has been shown to increase oxidative stress. Another physiological challenge, routine locomotor activity, has been found to decrease oxidative stress levels. Because we currently do not have a good understanding of how diverse physiological systems like stress and locomotion interact to affect oxidative balance, we studied this interaction in the prairie vole (*Microtus ochrogaster*). Voles were either pair housed or isolated and within the isolation group, voles either had access to a moving wheel or a stationary wheel. We found that chronic periodic isolation caused increased levels of oxidative stress. However, within the vole group that was able to run voluntarily, longer durations of locomotor activity were associated with less oxidative stress. Our work suggests that individuals who demonstrate increased locomotor activity may be better able to cope with the social stressor of isolation.

1. Introduction

Sociality in animals provides several benefits including greater protection from predators, enhanced success in locating and maintaining access to resources and the creation of mating opportunities [1]. For animals characterized by structured group living however, social isolation is a potent stressor, as individual survival is dependent on the group [1], and separation results in a glucocorticoid stress response [2]. Increased levels of glucocorticoids have recently been linked with increased levels of oxidative stress [3,4]. Oxidative stress results when the production of damaging reactive oxygen species (ROS) exceeds the ability of antioxidant defence and repair mechanisms to neutralize these unstable molecules [5]. While the precise mechanism of glucocorticoid-induced increases in oxidative stress is unclear, both short- and long-term exposure to elevated glucocorticoids can lead to higher levels of oxidative damage and decreased levels of antioxidants [6,7]. Recent work on social stress has shown that chronic periodic isolation results in increased oxidative damage and decreased antioxidant defence in rat brain [8]. In addition to isolation stress, other physiological states such as increased locomotor activity can influence oxidative stress levels, thereby impacting organismal performance [5]. During locomotion, metabolic activity increases, leading to greater rates of aerobic respiration, thus increasing the production of ROS [9]. Interestingly, if the increase
in metabolic activity is routine, it is often accompanied by an upregulation of the antioxidant system [9,10].

Currently, we do not understand how diverse physiological systems like stress and locomotion may interact to affect an individual’s oxidative balance [5]. Both social stress and locomotor activity represent ubiquitous physiological challenges. While both these challenges affect oxidative stress, they do so in contrasting ways; social stress can increase ROS and oxidative damage [8], while routine locomotor activity increases antioxidant defence [9]. Therefore, we test the hypothesis that while chronic isolation stress may increase oxidative stress, routine voluntary exercise could mitigate that effect in the socially monogamous prairie vole (Microtus ochrogaster). Prairie voles represent an excellent model system to study these relationships because they live in structured groups [11] and respond to chronic periodic social isolation with increased glucocorticoids [2]. In addition, metabolic activity can be easily manipulated in prairie voles through voluntary wheel running [12].

2. Material and methods

(a) Animals

Twenty-one male prairie vole (Microtus ochrogaster) sibling pairs aged between five and eight weeks and descended from a wild stock caught near Champaign, Illinois, were used. Vole cages contained Aspen Sani chip bedding (Harlan Teklad, Indianapolis, IN, USA), and voles were provided ad libitum food (high fibre rabbit pellet, Bio-Serv Corporation, Frenchtown, NJ, USA) and water. A 14:1:10 D cycle and temperature of 20–22°C were maintained for the duration of the experiment.

(b) Experimental design

We designed a factorial experiment with two levels of locomotor activity (sedentary (S) and voluntary wheel running (W)) and two types of social environment (isolation (I) and pair housed (P)), since isolation in prairie voles results in social stress and increased glucocorticoid levels [2]. Seven of the 21 sibling pairs (n = 14 individuals) were pair housed, not handled for the duration of the experiment and served as an unmanipulated control. These individuals had the same type of cage as the other treatment groups, including two non-mobile running wheels, food and environmental conditions.

The remaining 14 pairs of voles served as the experimental group. One vole from each experimental pair was randomly assigned to either the W or S group. Over a 26-day period, once each day for 2 h between 15.00 and 18.00 h, the two voles were separated into opposite sides of the cage by the use of a plastic opaque divider. Each side of the cage included food, water, bedding material and a running wheel. The wheel on the side of the W vole was unlocked and allowed to freely spin, but the wheel on the side of the S vole remained locked and stationary. A wireless odometer was attached to the exercise wheel to record the distance run (kilometres per day). No individual had any previous wheel-running experience, and when the wheel was unlocked, all W voles spent their time either running on the wheel or interacting with the wheel.

This design provided three distinct experimental groups: voles that were sedentary and pair housed (PS, unmanipulated control, n = 14), voluntary wheel running during isolation (IW, n = 14) and sedentary during isolation (IS, n = 14). Because pair-housed voles consistently attempted to run in the wheel simultaneously, a pair-housed, wheel-running experimental group (PW) could not be included.

One day after the last day of exercise, voles were weighed, euthanized with a mixture of ketamine (300 mg kg⁻¹, i.p.) and xylazine (22.5 mg kg⁻¹, i.p.), and trunk blood samples were collected for all oxidative stress assays.

(c) Oxidative stress measures

We assessed reactive oxygen metabolites (ROMs) and total antioxidant capacity (TAC), using the d-ROMs and OXY-adsorbent tests (Diachron International, Grosseto, Italy), respectively, in all blood samples as described previously ([13]; see the electronic supplementary material for more details). All analyses were run in duplicate and the intra-assay coefficients of variation were 5.6% for ROMs and 1.7% for TAC. We used the single cell gel electrophoresis (SCGE) assay ([13]; see the electronic supplementary material for more details) to measure three distinct aspects of DNA damage and repair: in vivo DNA damage levels, vulnerability of DNA to a H₂O₂ challenge, and DNA repair efficiency. The SCGE assay uses fresh blood samples and because only a limited number of samples can be run at one time we only used the IW and IS group.

(d) Statistical analyses

We analysed the effect of our experimental treatments on ROMs, total antioxidant capacity (TAC) and the three characteristics of DNA damage and repair separately by using linear mixed effect models in SPSS 22. In all models, treatment (two or three levels; see above) was included as a fixed factor and body mass and its interaction with treatment as covariates. Cage identity was also included as a random effect to account for the non-independence of voles from the same cage. Post-hoc comparisons were carried out using Fisher’s LSD tests. Previous studies have shown that the positive effects of physical exercise on oxidative status, particularly antioxidant defences, may depend on the amount of exercise and its intensity [9]. Hence, complementary to our analyses, we also used linear models to test whether the exercise distance (kilometres per day; log-transformed) was related with the level of ROMs, TAC and the three measures of DNA damage and repair in the IW voles.

3. Results

There was no effect of treatment on TAC or measures of DNA damage (table 1). However, treatment group had a significant effect on ROMs with IS voles showing the highest levels of ROMs, PS voles having the lowest levels and IW showing an intermediate level of ROMs compared to the other groups (figure 1, Fisher’s LSD, p < 0.05).

Interestingly, when we looked within the IW voles, which all spent time voluntarily wheel running, there were significant effects of the distance run on TAC and ROMs levels and a marginally significant effect on DNA repair efficiency. Thus, individuals who voluntarily ran longer distances had lower levels of ROMs (F₁,₁₃ = 5.03, p = 0.045; figure 2a), higher levels of TAC (F₁,₁₃ = 18.87, p = 0.001; figure 2b) and tended to have an improved DNA repair efficiency (F₁,₁₃ = 4.49, p = 0.056; figure 2c).

4. Discussion

We found that chronic periodic isolation, as a source of social stress, causes increased levels of oxidative stress in the prairie vole. These results agree with previous studies, which demonstrated a link between long-term isolation and
increased levels of oxidative damage in social animals [8]. Isolation in social mammals also results in increased levels of glucocorticoids [1]. Because birds with elevated levels of glucocorticoids during stress show an associated increase in oxidative stress [6,13,14], it is possible that the effect on oxidative stress we report in voles during isolation is also attributable to increased levels of glucocorticoids.

Interestingly in the voles in this study, we found that running further distances appeared to mitigate the negative consequences of isolation through a reduction in oxidative stress. Previous work in field voles reported that short-term voluntary exercise (1–7 days) did not significantly affect oxidative stress levels [15]. However, other studies report that more regular exercise produces moderate levels of ROS that can induce an acclimation response in which antioxidant defences are upregulated, and this is thought to allow the organism to better cope with a subsequent more intense encounter [9]. A non-mutually exclusive alternative is that the ability to perform locomotor activity could be a form of displacement behaviour or a coping mechanism, which lessens the stress of social isolation [16]. Previous studies have found that environmental enrichment prevents and remedies behavioural consequences that result from social isolation and also decreases corticosterone levels in isolated individuals [12]. It is possible that voles that ran more had

### Table 1. Results of generalized linear mixed models (GLMM) on the response to pair-housing (P) or social isolation (I) and sedentary (S) or voluntary wheel running (W). For TAC and ROMs, $n = 42$ voles. For DNA measures, $n = 28$ voles (IW and IS only). Bold values indicate $p < 0.001$.

<table>
<thead>
<tr>
<th>dependent variable</th>
<th>treatment (PS, IS, IW) body mass (g)</th>
<th>treatment × body mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC</td>
<td>$F_{2,39} = 0.970, p = 0.388$</td>
<td>$F_{1,38} = 0.704, p = 0.407$</td>
</tr>
<tr>
<td>ROMs</td>
<td>$F_{2,38} = 11.369, p &lt; 0.001$</td>
<td>$F_{1,37} = 1.903, p = 0.176$</td>
</tr>
<tr>
<td>in vivo DNA</td>
<td>$F_{1,25} = 0.006, p = 0.938$</td>
<td>$F_{1,26} = 0.169, p = 0.685$</td>
</tr>
<tr>
<td>DNA vulnerability to H$_2$O$_2$ challenge</td>
<td>$F_{1,26} = 1.366, p = 0.263$</td>
<td>$F_{1,24,16} = 0.723, p = 0.200$</td>
</tr>
<tr>
<td>DNA repair efficiency</td>
<td>$F_{1,26} = 0.636, p = 0.432$</td>
<td>$F_{1,25} = 0.723, p = 0.403$</td>
</tr>
</tbody>
</table>

**Figure 1.** The effect of pair-housing (P) or social isolation (I) and sedentary (S) or voluntary wheel running (W) on ROMs in male prairie voles (all groups, $n = 14$; mean ± s.d.). *p < 0.05.

**Figure 2.** The relationship between voluntary wheel-running distance and (a) ROMs, (b) TAC and DNA repair efficiency after H$_2$O$_2$ challenge in male prairie voles ($n = 14$). Lines show an adjusted linear regression.
longer interactions with this form of environmental enrichment; though, regardless of distance run, IW voles spent time interacting with the wheel during isolation.

This study is unique in that it attempts to determine whether routine voluntary exercise is able to mitigate the negative effects of isolation stress on oxidative balance. While physical performance has a number of links to fitness, our results indicate that individuals that perform regular locomotor activity in the form of migration, hunting, etc., may better tolerate other physiological challenges through effects on oxidative balance. Specifically, the moderate levels of ROS that are produced through regular exercise appear to upregulate antioxidant defences that may also alleviate oxidative stress induced by other physiological challenges, such as social stress.

References


4. Haussmann MF, Marchetto NM. 2010 Telomeres: oxidative stress induced by other physiological challenges, such as social stress.