



# Chronic exposure to inorganic mercury alters stress responses in male prairie voles (*Microtus ochrogaster*)

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## ABSTRACT

Male, but not female, prairie voles that experience chronic exposure to inorganic mercury display aberrant social behavior - avoiding unfamiliar conspecifics rather than approaching them. The mechanisms that underlie such behavioral changes are unknown, but likely involve the hypothalamus-pituitary-adrenal (HPA) axis. We tested this hypothesis by providing voles of both sexes with mercury chloride in their drinking water for ten weeks and then staging same-sex dyadic encounters after which plasma was assayed for corticosterone as an index of HPA activity. Consistent with sex-specific behavioral responses previously reported, mercury-treated males had lower plasma corticosterone after social encounters than did similarly-treated females or males that consumed normal drinking water. The results suggest that mercury-treated males may be less inclined toward social engagement with conspecifics due to reduced HPA activity.

## 1. Introduction

Exposure to environmental contaminants can alter social behaviors in a variety of species (Gilbert and Grant-Webster, 1995; Delville, 1999; Sloman et al., 2003; Curtis et al., 2010). However, the processes by which such changes are manifested seldom are addressed. The hypothalamus-pituitary-adrenal (HPA) axis likely plays an important role in social interactions. Porges (Porges, 2007) postulated a “social engagement system” involving distinct central processes such as social recognition interacting with the HPA axis to control approach/avoidance behaviors. Environmental contaminants such as heavy metals can affect the activity of the HPA axis as evidenced by reports of changes in basal circulating levels of the stress hormone corticosterone, or in patterns of corticosterone release in response to various stressors (Hontela et al., 1992; Wayland et al., 2002; Franceschini et al., 2017). These observations may derive from the fact that heavy metals accumulate in the adrenal gland (Kozma et al., 1996) and interfere with steroid hormone metabolism (Veltman and Maines, 1986). Accordingly, measures of HPA activity may provide insights into the mechanisms by which heavy metals alter social engagement behaviors.

Because biomagnification/bioaccumulation of environmental contaminants is quite common in aquatic environments, many studies on heavy metals-HPA interactions have focused on fish and seabirds

(Lockhart et al., 1972; Hontela et al., 1992). Studies in terrestrial animals are less common, and tend to focus on birds (Janssens et al., 2003; Franceschini et al., 2009). Studies in mammals provide equivocal results. Mercury reduced the viability of cells isolated from the rat adrenal gland (Ng and Liu, 1990), while a study in bats found no relationship between tissue mercury and stress-induced corticosterone release (Wada et al., 2010). However, the impact of heavy metals on social behaviors have not been examined in these species. Thus, further study of metals-HPA interactions in the context of mammalian social behavior is warranted.

Here we examined the effects of chronic inorganic mercury exposure on circulating corticosterone after dyadic interactions in a small mammal species, the prairie vole. Sexually naïve, non-pair-bonded prairie voles are highly social and seek out close contact with conspecifics (Carter and Getz, 1993). Our previous work showed, however, that vole social behavior changes after chronic exposure to metals. Specifically, male, but not female, prairie voles significantly reduce social contact with a same-sex stranger after mercury- or cadmium-exposure (Curtis et al., 2010). We hypothesize that metals-treated males find such encounters to be more stressful than do water-treated males or females, accounting for the sex-specific behavior. To test this hypothesis, we examined circulating levels of the stress hormone, corticosterone, in mercury- and water-treated voles after dyadic encounters

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with same-sex conspecifics. If our hypothesis is correct, we predicted that 1) stress hormone responses by males and females would differ after mercury-exposure; and 2) mercury-treated males would display elevated circulating corticosterone after interaction with a same-sex conspecific.

## 2. Methods

Subjects were adult male ( $n = 10$ ) and female ( $n = 13$ ) prairie voles from a captive breeding colony. Breeding pairs were housed in shoebox-style cages ( $20 \times 25 \times 45$  cm) containing pine chip bedding and timothy hay. Food (Purina rabbit chow and black-oil sunflower seeds) and water were provided *ad libitum*. Photoperiod was 14:10 L:D with lights on at 06:00. Pups were weaned at ~20 days of age and housed as same-sex pairs in  $17 \times 12 \times 22$  cm cages containing pine chip bedding. Males were housed separately from weaned females and the breeding stock.

Voles (mean age – 169 days) were randomly assigned to one of two treatment groups. The first group ( $n = 7$  females, 4 males) received normal drinking water. The second group ( $n = 6$  females, 6 males) was given a 60 ppm  $\text{HgCl}_2$  solution as their sole drinking water source for 10 weeks as was used in our previous study of mercury effects in prairie voles (Curtis et al., 2010).

The stimulus for HPA activation, exposure to a stranger, was based on the resident-intruder paradigm commonly used to assess aggression in voles (Winslow et al., 1993). The subject was placed alone in a clean cage ( $17 \times 12 \times 22$  cm) for ten minutes, after which an unfamiliar same-sex conspecific was introduced and the animals were allowed to interact for ten minutes. The interactions were video-recorded. The conspecific was fitted with a wire tie loosely placed around the neck to allow the two animals to be distinguished on the video recordings. Stranger-exposure for both groups occurred concurrently; all testing was done between 13:30 and 16:30. The animals were closely monitored during the interaction and the test was terminated early if excessive aggression was observed. In two cases, males displayed sufficient aggression to necessitate early termination of the interaction (8 and 5 min after pairing). In the first case, the water-treated test subject initiated the aggression; in the other case, the “intruder” initiated aggression toward the mercury-treated subject.

Following stranger-exposure, each pair was separated and the subject was rendered unconscious using  $\text{CO}_2$ , and then decapitated. Trunk blood was collected, placed on water ice for 15–20 min, and then centrifuged (15 min at 5000 rpm). The plasma fraction was collected and stored at  $-80^\circ\text{C}$  until assayed for corticosterone.

Corticosterone was assayed using a commercially available ELIZA kit (Enzo, kit # ADI-900-097) following the manufacturer's instructions. All samples and calibration standards were run in duplicate and the averages were used for statistical analysis.

Two-way ANOVA was used to assess sex and treatment effects on circulating corticosterone. A protected Fisher's LSD was used to probe significant main effects. Effects of age and time of plasma collection on corticosterone were assessed by regression analysis. Data are presented as mean  $\pm$  se and  $p$ -values  $< 0.05$  were considered to indicate statistically significant differences. Effect sizes are presented as  $\eta_p^2$  for the two-way ANOVA and  $r^2$  for regressions.

## 3. Results

There was a small but significant sex difference in the ages of the animals ( $F_{1,19} = 6.09$ ,  $p < 0.03$ ,  $\eta_p^2 = 0.24$ ; females –  $246.8 \pm 5.9$  days; males –  $229.0 \pm 4.9$  days). There was no age difference between mercury- and water-treated animals ( $F_{1,19} = 2.00$ ,  $p = 0.17$ ,  $\eta_p^2 = 0.10$ ) and no sex by treatment interaction ( $F_{1,19} = 2.33$ ,  $p = 0.10$ ,  $\eta_p^2 = 0.11$ ).

There was no correlation between sample collection time and plasma corticosterone levels ( $F_{1,21} = 0.45$ ,  $p = 0.51$ ,  $r^2 = 0.02$ ; Fig. 1),

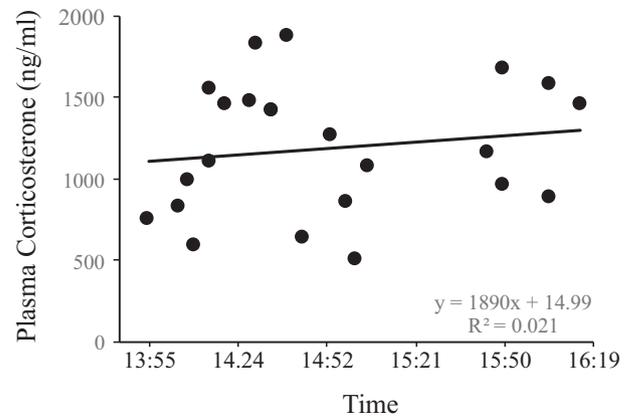


Fig. 1. Circulating corticosterone levels were unrelated to sample collection time. Regression analysis revealed no correlations between the time of day at which plasma samples were collected and circulating corticosterone.

or between age and corticosterone ( $F_{1,21} = 0.28$ ,  $p = 0.60$ ,  $r^2 = 0.01$ ). There were main effects of both sex ( $F_{1,19} = 13.74$ ,  $p < 0.002$ ,  $\eta_p^2 = 0.42$ ) and mercury-treatment ( $F_{1,19} = 5.17$ ,  $p < 0.04$ ,  $\eta_p^2 = 0.21$ ) on circulating corticosterone, but there was no sex by treatment interaction ( $F_{1,19} = 0.01$ ,  $p = 0.91$ ,  $\eta_p^2 < 0.001$ ). Overall, males had lower circulating corticosterone than did females, and animals treated with mercury had lower circulating corticosterone than did control animals (Fig. 2). Pair-wise comparisons revealed that mercury-treated males had significantly lower circulating corticosterone than did mercury-treated females or water-treated control males (both  $p$ -values  $< 0.02$ ). Mercury exposure did not affect circulating corticosterone in females ( $p = 0.08$ ).

## 4. Discussion

Chronic exposure to mercury *via* drinking water significantly decreases social contact by male, but not female, prairie voles (Curtis et al., 2010). We hypothesized that changes in HPA activity might contribute to the decreased social behavior displayed by male voles after metals-exposure. We found significant main effects of both sex and treatment. These data suggest that mercury differentially affects the responses of the HPA axes of prairie vole males and females when they are exposed to strangers. This result is consistent with our previous observation that several weeks of mercury exposure *via* drinking water caused male, but not female, prairie voles to reduce social contact with

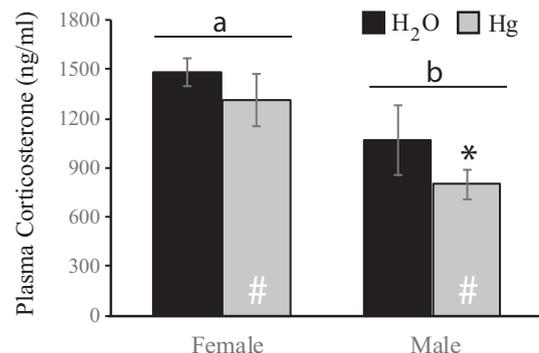


Fig. 2. Chronic mercury-exposure effects on prairie vole corticosterone responses during dyadic encounters with same-sex conspecifics. Overall, females (a) exhibited higher circulating levels of corticosterone than did males (b) after dyadic encounters. Mercury-treated voles exhibited lower circulating corticosterone than did water-treated voles (#). Male voles that consumed mercury displayed lower circulating corticosterone than did similarly-treated females or water-treated males (\*).

a stranger relative to that displayed by voles that drank normal water (Curtis et al., 2010).

Given our hypothesis that metals-treated male prairie voles display reduced social contact due to stress associated with exposure to a stranger, our *a priori* expectation was that mercury-treated males would show higher circulating corticosterone levels relative to untreated controls. We actually found the opposite – mercury-treated males had lower circulating corticosterone than did controls. Similar observations have been made in other taxa. Fish [yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*)] from mercury-contaminated areas displayed reduced stress responses relative to those from less contaminated areas (Hontela et al., 1992). In fact, pike from a contaminated lake had circulating levels of cortisol that were about 1/4 of those fish from an uncontaminated lake (Lockhart et al., 1972). Tree swallows (*Tachycineta bicolor*) displayed a negative relationship between plasma mercury levels and baseline circulating corticosterone, but stress-induced increases in circulating corticosterone were unimpaired (Franceschini et al., 2009).

From a social perspective, an interesting parallel is found between our voles and the social behaviors of rainbow trout (*Oncorhynchus mykiss*). Cadmium-treated trout were less aggressive and significantly more likely to be submissive in dyadic encounters (Sloman et al., 2003). Similarly, trout treated with a glucocorticoid receptor antagonist along with corticosterone were less aggressive than were fish receiving corticosterone only (Schjolden et al., 2009). Thus, one explanation for our observation that mercury-treated male voles display less social contact is that the decrease in corticosterone release makes the animals less aggressive (or more fearful) in approaching unfamiliar conspecifics. Support for this interpretation is the fact that mercury-treatment did not significantly affect female corticosterone levels in the present study, nor did mercury-treated females display reduced social contact in our previous study (Curtis et al., 2010), whereas males were affected in both studies. These results, in conjunction with sex-specific social responses to stress (DeVries et al., 1996), suggest that increased circulating corticosterone may play a central role in male, but not female, prairie vole social interactions.

We found that females had higher circulating corticosterone levels than did males. This observation is not novel (Taymans et al., 1997); however, caution must be used in extending these findings to other species, especially those exposed to environmental contaminants. For example, there were no sex-differences in plasma corticosterone, despite high levels of mercury in blood and tissue, in stressed common loons (*Gavia imer*) although there was a positive correlation between mercury and circulating corticosterone in males (Franceschini et al., 2017). Importantly, the loons displayed no interaction between mercury and the gonadal hormones testosterone and estradiol, suggesting the mercury-effect on corticosterone was not secondary to generalized changes in steroid metabolism.

Two additional factors may have affected the outcome of our study. First, corticosterone levels are known to fluctuate in concert with biological rhythms. Prairie voles exhibit stable corticosterone levels throughout the afternoon (Taymans et al., 1997) when our plasma samples were collected. However, prairie voles also display ultradian behavioral rhythms with a period of about 3 h (Lewis and Curtis, 2016) and blood samples were collected across a similar time-span. It is possible that there is a hormone surge associated with the arousal period of each of these shorter cycles that is not identified by samples collected at longer intervals (Taymans et al., 1997). We found no relationship between circulating corticosterone and sample collection time, suggesting that the timing of sample collection did not drive the treatment effect. We also found that the females were slightly older than the males, however, it is unlikely that an age difference of 17 days in animals that

were 8 months old contributed significantly to the overall outcome.

In summary, male and female prairie voles display sex-specific HPA axis responses during encounters with unfamiliar same-sex conspecifics after chronic exposure to inorganic mercury. As a result, mercury-treated males may be less inclined to approach strangers. This could account for the sex-specific effects of mercury-treatment in behavioral responses during social encounters (Curtis et al., 2010): mercury-treated males may be less likely to initiate social interactions. It should be noted that neither the present nor the previous study examined behavioral or hormonal responses during opposite-sex encounters, suggesting the need for additional studies to assess the effects on metals exposure on other types of social interactions.

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