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# Cadmium and exposure to stress increase aggressive behavior

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ABSTRACT

Environmental toxicants and stress influence the health and behavior of people from different parts of the world. In the present study, aggressive behavior was evaluated in rats exposed to cadmium (Cd) for four weeks and subjected to immobilization stress (IS) based on the resident/intruder paradigm. Latency to the first bite (LB), total number of attacks (NA), total duration of attack manifestations (DAM), and a composite aggression score (CAS) were used to assess aggressiveness. Cadmium concentrations in the blood and the brain were determined. We observed that the parameters of aggressiveness were not altered by either Cd or IS when administered separately. However, animals exposed to Cd + IS had increased NA, DAM, and CAS. Cadmium was detected in the blood and the brain after treatment and Cd + IS exposure modified Cd distribution in these tissues. These results suggest that exposure to low levels of Cd associated with stress may lead to increased aggressiveness in rats.



## 1. Introduction

Cadmium is a toxic metal that damages many tissues, including the nervous system. In the recent decades, Cd has received increasing attention in studies interested in defining health risks associated with environmental toxicants. In addition to documented industrial problems (Shin et al., 2011), Cd toxicity is of concern to the tobacco-smoking sub-population that includes millions of people worldwide. Cd accumulates in tobacco leaves and smoking a single pack of cigarettes daily is sufficient to double Cd concentration in the body (Satarug and Moore, 2004). Cadmium's biological half-life in mammals can reach decades (Klaassen, 1990). Therefore an intensive research agenda focusing on the potential threat of this xenobiotic contaminant is necessary.

Cd is toxic to several organs. It is primarily nephrotoxic because it causes apoptosis although necrosis is also seen (Prozialeck et al., 2009); induces edema in the lungs (Bus et al., 1978), and testicular pathophysiology in rat (Saïd et al., 2010). Furthermore, this metal is also toxic to the liver (Habeebus et al., 2000a), bones (Habeebus et al., 2000b), and hematopoietic system (Messaoudi et al., 2010). In addition, Cd acts as an endocrine disruptor (Pillai et al., 2003), and is a teratogenic (Simoniello et al., 2011) and carcinogenic agent (Joseph, 2009).

Importantly, Cd penetrates the blood–brain barrier and accumulates in the brain (Méndez-Armenta and Ríos, 2007). Acute Cd toxicity leads to neuronal dysfunction and lethal cerebral edema (Méndez-Armenta et al., 2001). Recent studies demonstrated that Cd administration causes motor hyperactivity (Antonio et al., 2002), increases aggressive behavior (Salvatori et al., 2004), and affects anxiety-like behavior

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(Minetti and Reale, 2006). Pari and Murugavel (2007) investigated the influence of Cd on cholinergic neurotransmission and observed an association between Cd and behavioral impairments in both animal models and humans exposed to Cd.

Although there are few epidemiological studies correlating central disorders with occupational exposure, it is known that Cd exposure can cause peripheral neuropathy and neurobehavioral disturbances such as alterations in attention, psychomotor activity, visuomotor function, speed and memory impairment in exposed workers (Viaene et al., 2000). Cd exposure also increases oxidative stress in the brain and other tissues (Yalin et al., 2006).

On the other hand, although chronic Cd exposure is associated with many adverse health effects in adults, studies involving children are limited (Schoeters et al., 2006). Although prenatal exposure to cadmium is generally low it accumulates in the placenta (Osman et al., 2000) and so the concentration of cadmium in umbilical cord blood increases in the proportion of maternal exposure (Kippler et al., 2010). Salvatori et al. (2004) described that Cd exposure during embryogenesis is toxic to the embryo and cause long-term effects in rats. In addition, when exposure occurs in the early life, behavioral effects of Cd usually persist in adult life (Leret et al., 2003).

The modern lifestyle of the general population constantly exposes individuals to many sources of stress, in example economic factors, that can contribute to an increase in anxiety and aggressiveness (Veenema and Neumann, 2007). Few studies suggest that Cd increases aggressive behavior (Leret et al., 2003) nevertheless these data are still inconclusive and the mechanisms remain unidentified. Since humans are exposed to both environmental chemicals and daily situations that induce high level of stress and altered behavior, it is important to study the concomitant effects of Cd exposure and stress. Therefore, the present study was designed to investigate the aggressive behavior of rats exposed to Cd and subjected to immobilization stress. We determined Cd levels in the blood and the brain to correlate changes in behavior to tissue accumulation of this metal.

## 2. Materials and methods

### 2.1. Animals

Sixty-four 80-day-old male Wistar rats were obtained from the colony housed at the Sao Paulo State University and kept under a constant 12 h light/dark cycle and controlled temperature ($23 \pm 2\,^{\circ}C$). Standard pellet chow (BioBase®, Santa Catarina/SC, Brazil) and tap water were available *ad libitum*. The Committee of Ethics in Experimentation Animals (CEEA) of the Institute of Biosciences at Sao Paulo State University approved the experimental protocols. All procedures were performed in accordance with institutional guidelines for animal use and care.

### 2.2. Experimental procedure

After an acclimatization period, rats were randomly assigned to two different groups: intruder ($N = 32$) or resident ($N = 32$). Intruder animals were housed four per cage (polypropylene cages) and received no treatment. Residents were housed one per cage and received one of the following treatments: filtered drinking water as control (Ct group, $N = 8$); cadmium acetate, Shynth (Cd group, 50 ppm of Cd in filtered drinking water, $N = 8$); immobilization stress (IS group, animals were immobilized in acrylic cages, 21 cm × 7.5 cm × 6.5 cm for 30 min three times a day with 3 h interval during the treatment period, $N = 8$); and Cd solution plus immobilization stress (Cd + IS group, $N = 8$). Treatments were performed for four weeks and during the treatment period animals were weighed and consumption of food and water (24 h period) was estimated once a week, on the same day of the week. The IS protocol used was based on the protocol previously described by Vyas et al. (2002).

### 2.3. Aggressive behavior assessment

This study used a model of aggression based on the resident-intruder paradigm (Long et al., 1996). This method allows the observation of social interaction and offensive behavior of the resident animal as well as the intruder's defensive elements by reflecting intraspecific aggression (Koolhaas and Bohus, 1991). The resident/intruder paradigm is a good predictive test for validating human aggression (Oliver and Young, 2002) and has the advantage of detecting even mild aggression involving minimal injury to the animals.

To perform the test, resident animals were housed individually throughout the treatment period and evaluations. Intruder animals were housed grouped in cages, received no treatment and were used only once a day for the experiment and never returned to the same resident animal. Residents were housed one animal per cage and treated for 4 weeks. During this period their cages were not washed or exchanged, and during the last week the bedding in their cages was not changed. To assess aggression, an intruder animal was placed in the cage of a resident animal and their interaction for 900 s (15 min) was recorded in tape. Recordings were later evaluated for the following observed parameters: latency to the first bite (LB, seconds), total number of attacks (NA), total duration of attack manifestations (DAM, seconds, including explosions of bite, lateral threats, maintenance of his paws over the other animal, and more intense grooming). A composite aggression score (CAS) was constructed: $NA + 0.2 \times LB + 0.2 \times DAM$ (Long et al., 1996). The home territory defended by residents was the cage each animal since the first day of the experiment.

Twenty-four hours after assessment of aggressive behavior, animals were euthanized by decapitation and Cd levels in the blood and whole brain were determined by graphite furnace atomic absorption spectrophotometry (Spectra AA-220Z, Varian) after wet digestion with perchloric and nitric acids (Corpas et al., 2002).

### 2.4. Statistics comparisons

Data obtained were analyzed using Instat 3.0 (Graph Pad Software). Data were compared by Student's *t* test and one-way analyses of variance (ANOVA); a Tukey–Kramer *post hoc* test was used for comparisons between means when