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Short-term social memory deficits in adult female mice exposed to tannery effluent and possible mechanism of action

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HIGHLIGHTS

- Direct contact with tannery effluent cause short-term social memory deficits.
- Tannery effluent affects the central nervous system of female Swiss mice.
- Exposure to tannery effluent may be causing oxidative stress.

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ABSTRACT

The accumulated organic residues in tannery-plant courtyards are an eating attraction to small rodents; however, the contact of these animals with these residues may change their social behavior. Thus, the aim of the present study is to investigate whether the exposure to tannery effluent (TE) can damage the social recognition memory of female Swiss mice, as well as to assess whether vitamin C supplementation could provide information about how TE constituents can damage these animals' memory. We have observed that resident females exposed to TE (without vitamin supplementation) did not explore the anogenital region, their body or chased intruding females for shorter time or with lower frequency during the retest session of the social recognition test, fact that indicates social recognition memory deficit in these animals. Such finding is reinforced by the confirmation that there was no change in the animals' olfactory function during the buried food test, or locomotor changes in females exposed to the pollutant. Since no behavioral change was observed in the females exposed to TE and treated with vitamin C (before or after the exposure), it is possible saying that these social cognitive impairments seem to be directly related to the imbalance between the cellular production of reactive oxygen species and the counteracting antioxidant mechanisms (oxidative stress) in female mice exposed to the pollutant (without vitamin supplementation). Therefore, the present study evidences that the direct contact with tannery effluent, even for a short period-of-time, may cause short-term social memory deficits in adult female Swiss mice.

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1. Introduction

According to Durai and Rajasimman (2011), bovine skin processing is one of the oldest human activities worldwide. Leather tannery was performed in ancient times to fulfil local demands, for instance, to produce leather shoes and music instruments. The demand for leather products kept on growing due to population

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growth, so new commercial tannery plants were launched because of it. Nowadays, the bovine leather production can be highly profitable and plays an important role in many countries such as India, China, Turkey, Brazil, Ethiopia, Pakistan and Bangladesh (Lofrano et al., 2013).

However, the tannery activity is considered one of the most environmentally polluting, since large amounts of residues (solid and liquid) is generated by it and, in many cases, these residues are discharged in the environment without any previous treatment or after inefficient treatment. Therefore, such discharge becomes of high environmental contamination risk because, even after treatment, it may contain high loads of organic and inorganic components used in leather tannery processing (Mwinyihija, 2010).

Recently, several studies have been showing the harming impacts of the exposure to tannery effluent on mammals' health (Souza et al., 2016a). These studies have found different effects on mice models, but, based on the observation of predictive anxiety behaviors, (Siqueira et al., 2011; Silva et al., 2016; Guimarães et al., 2016a, 2017), anxiolytic behaviors (Almeida et al., 2016), depression predictors (Souza et al., 2016b), antidepressants (Mendes et al., 2016), memory deficit (Rabelo et al., 2016; Silva et al., 2016; Guimarães et al., 2017), as well as on the observation of neurobehavioral effects detrimental to the offspring of parents exposed to the xenobiotic (Guimarães et al., 2016b,c), they all converged to the potential risk of the exposure to tannery effluent. Accordingly, there are strong evidences that these pollutants harm the functioning of the exposed animals' central nervous system.

A particularity in these studies lies on the experimental use of rodents to collect data that can be extrapolated to humans. Previous studies have established experimental designs to simulate the intake of water contaminated with tannery effluent (at different concentrations) or the dermal exposure to the pollutant in order to further relate these studies to the occupational health of tannery-plant workers. It is noteworthy that these studies help broadening the knowledge about the possible impacts of tannery effluent on human health. However, little attention has been given to the analyses of the impact these contaminants have on aspects related to the biology of animals living in areas close to tannery plants, although the ecotoxicological risks associated with tannery effluent are undeniable (Shakir et al., 2012).

It is known that tannery-plant courtyards, mostly rudimentary and small scale plants, hold a large amount of organic matter resulting from bovine tannery processing. These matters get mixed to great volumes of water and form the tannery effluent (Farenzena et al., 2005). Thus, the skin, hair, meat and fat tissue scraps from the bovine tannery processing stages constitute a great feeding attraction to animals such as small rodents (i.e.; mice or rats) who end up including these residues in their diet. Furthermore, various insectivorous rodents can be found in the industrial environment (such as the genus *Akodon* sp., *Bibimys* sp., *Blarinomys* sp., *Gyldenstolpia* sp. and/or *Rhagomys* sp.), which are attracted by arthropods that host organic materials of the tanning industry (Hogue, 1993). Accordingly, these animals' exposure to tannery effluents during foraging on tannery plant courtyards may cause behavior changes in them, fact that may put their survival at risk, as well as the social functioning of their population groups (Warren-Hicks et al., 1989). In this case, the exposure of these animals to the organic and inorganic constituents found in the tannery effluent may happen due to the intake of contaminants when they feed on the organic matter or when they ingest tannery effluent during self-cleaning events, or due to the direct (dermal) contact and/or to the inhalation of toxic gases at sub-lethal concentrations.

Assessing these animals' social recognition memory would be one of the ways to infer the impact of tannery effluent on the structuring and populational organization of rodents foraging tannery plants. It has been largely acknowledged that the social recognition memory is fundamental to the survival of animals living in population groups such as rodents (Gheusi et al., 1994; Moura and Xavier, 2010). The recognition memory is important for social hierarchy, mating and offspring recognition, as well as for interspecies recognition, which is vital to the recognition of frequent visitors in the animal's habitat, whether or not the visitor poses threat to the animals or to the colony (Macbeth et al., 2009; Noack et al., 2010). Therefore, the ability of recognizing an individual is important to many animal behaviors, because the absence of this capacity does not enable the animal to distinguish kin from a potential competitor or predator; thus, these animals would not present appropriate responsive behaviors.

Previous studies have already shown that the exposure of rodents to certain pollutants such as heavy metals and pesticides (Holloway and Thor, 1988; Ricceri et al., 2013; Venerosi et al., 2006; Teixeira et al., 2014) may cause social recognition memory deficit. However, there are studies about the possible impacts of complex constitution contaminants such as tannery effluent on rodents' social recognition memory. If one considers that rodents play an important environmental role in ecosystems (Mihalca and Sándor, 2013; Sunyer et al., 2013) since they act as seed dispersers, as pollinators and also as organic matter recyclers, besides making part of the eating chain of other animals, and contributing to the maintenance of other species' populations, it is worth directly or indirectly acknowledging how residues deriving from highly polluting activities may affect these animals' behavior.

Accordingly, the aim of the present study was to investigate whether the exposure to tannery effluent is able to harm the social recognition memory of female Swiss mice. Furthermore, based on the administration of vitamin C - which is a potential antioxidant - before or after the exposure to the pollutant, the current study also aimed at preliminarily assessing the relation between the observed effects and the possible oxidative stress. The initial hypothesis was that the exposure to tannery effluent could cause social memory deficits in female mice and that vitamin C supplementation could provide information about how tannery effluent constituents can damage these animals' memory.

2. Materials and methods

2.1. Animals and experimental design

Sixty (60) nulliparous adult female Swiss mice (2–3 months old) were stored in the bioterium of the Biological Research Laboratory at *Instituto Federal Goiano – Campus Urutaí* (Urutaí, Goiás State, Brazil). The mice were stored in polypropylene boxes (30.3 × 19.3 × 12.6 cm, at most five animals per box). All the animals were kept under 12/12 h light/dark cycle, on a ventilated shelf, and under controlled temperature and humidity conditions (22-25° C and 55–60% humidity).

The animals were counter-balanced according to the covariables "age" and "body mass" so that the mean age and mean body mass of the experimental groups were statistically constant. Subsequently, the mice were distributed in the following experimental groups (n = 12 per group): i) control group, composed of mice that were not exposed to the tannery effluent and did not receive ascorbic acid; ii) control + vit. C group, composed of mice that were not exposed to the tannery effluent, but received vitamin supplement (ascorbic acid - vit. C - 200 mg kg^{-1}, according to Bisong et al. (2016)); iii) effluent group, composed of animals that were dermally exposed to the tannery effluent; iv) effluent + vit. C (before) group, composed of animals that were subjected to the

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