

## The neuroimmune changes induced by cohabitation with an Ehrlich tumor-bearing cage mate rely on olfactory information

Glaucie J. Alves, Alison Ribeiro, João Palermo-Neto\*

Laboratory of Applied Pharmacology and Toxicology, School of Veterinary Medicine, University of São Paulo, São Paulo, Brazil

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### ABSTRACT

Cohabitation for 14 days with Ehrlich tumor-bearing mice was shown to increase locomotor activity, to decrease hypothalamic noradrenaline (NA) levels, to increase NA turnover and to decrease innate immune responses and decrease the animals' resistance to tumor growth. Cage mates of a B16F10 melanoma-bearer mice were also reported to show neuroimmune changes. Chemosignals released by Ehrlich tumor-bearing mice have been reported to be relevant for the neutrophil activity changes induced by cohabitation. The present experiment was designed to further analyze the effects of odor cues on neuroimmune changes induced by cohabitation with a sick cage mate. Specifically, the relevance of chemosignals released by an Ehrlich tumor-bearing mouse was assessed on the following: behavior (open-field and plus maze); hypothalamic NA levels and turnover; adrenaline (A) and NA plasmatic levels; and host resistance induced by tumor growth. To comply with such objectives, devices specifically constructed to analyze the influence of chemosignals released from tumor-bearing mice were employed. The results show that deprivation of odor cues released by Ehrlich tumor-bearing mice reversed the behavioral, neurochemical and immune changes induced by cohabitation. Mice use scents for intraspecies communication in many social contexts. Tumors produce volatile organic compounds released into the atmosphere through breath, sweat, and urine. Our results strongly suggest that volatile compounds released by Ehrlich tumor-injected mice are perceived by their conspecifics, inducing the neuroimmune changes reported for cohabitation with a sick companion.

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### 1. Introduction

The ability to sense, analyze and respond to olfactory information is a common function of all brains. The early neuroanatomists astutely recognized a link between olfactory processing and emotionality. Paul Brocca emphasized that the limbic lobe has a strong relationship with the olfactory system (Issacson, 1974). Mammals respond readily to a variety of odors associated with predators, both in the field (Stoddart, 1980; Weldon, 1990; Takahashi et al., 2005) and laboratory environments (Kavaliers et al., 2003; Bimová et al., 2009; Arakawa et al., 2008). Olfactory cues convey information including sex, reproductive health status (Ehman and Scott, 2001; Kavaliers et al., 2005), competitive ability and territory ownership (Beynon and Hurst, 2003). Additionally, these cues convey aspects of individual identity, such as genotype (Thom and Hurst, 2004; Thom et al., 2008), familiarity or kinship (Hurst et al., 2001; Sherborne et al., 2007).

Alarm chemosignals are produced in response to acute and chronic stressors in a wide variety of species, including: insects (Free, 1987); fish (Bardach and Todd, 1970); reptiles (Burghardt, 1970); mammals (Arakawa et al., 2008; Zalaquett and Thiessen, 1991) and specifically, humans (McClintock, 1998; Ackerl et al., 2002). Such stress-induced chemosignals provide information about the physiological conditions facing the odor-donors (Hauser et al., 2002, 2005). Female mice display a reduced interest in, and avoidance of, the urine and other odorous secretions of males infected with endoparasites such as protozoans, nematodes, and influenza virus (Kavaliers and Colwel, 1995a,b; Ehman and Scott, 2001; Kavaliers et al., 2003). Odor cues from long-term infected and immunologically challenged animals also induce avoidance responses in healthy conspecifics (Hernandez and Sukhdeo, 1995; Penn and Potts, 1998; Klein, 2000; Yaamazaki et al., 2002).

Cohabitation for 11 days with an Ehrlich tumor-bearing mouse induces an increase in motor activity in an open field and a decrease in resistance to tumor growth in cage mates (Morgulis et al., 2004). Similar cohabitation with a sick cage mate: (a) decreases the levels and increases the turnover of hypothalamic noradrenaline (NA); (b) decreases neutrophil oxidative burst after phorbol myristate acetate (PMA) or *Staphylococcus aureus* induction; and (c) decreases the percentage and intensity of neutrophil phagocytosis (Alves

\* Corresponding author. Address: Farmacologia Aplicada e Toxicologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Rua Prof. Dr. Orlando Marques de Paiva, 87, CEP: 05508-000, São Paulo, S.P., Brazil. Fax: +55 11 30917829.

E-mail address: [jpalermo@usp.br](mailto:jpalermo@usp.br) (J. Palermo-Neto).

et al., 2006). Peritoneal macrophage phagocytosis is also lower in mice that lived with a tumor-bearing cage mate compared to control mice that lived with a healthy cage mate (Alves et al., 2007). Companions of a B16F10 melanoma-bearing mice were also reported to show (a) an enhanced expression of the CD80 co-stimulatory molecule on IAb + CD11C + splenocytes; (b) an altered differentiation of bone marrow cells in the presence of GM-CSF, IL-4 and LPS *in vitro*, resulting in a lower percentage of IAb + CD80 + cells; and (c) a deficit in the establishment of a delayed-type hypersensitivity response to ovalbumin (Tomiyoshi et al., 2009).

Recently, we investigated the effects of tactile, olfactory and visual cues on locomotion and neutrophil activities induced by cohabitation with an Ehrlich tumor-bearing cage-mate (Alves et al., 2010). The olfactory cues released by Ehrlich tumor bearing mice were thought to be responsible for the neuroimmune changes observed. Indeed, mice that were not allowed to perceive odor cues from their sick partners presented no alterations in neutrophil oxidative burst and phagocytosis; this outcome was not observed after visual and physical contact deprivations. The present experiment was specifically designed to extend the analysis of the effects of odor cues on animals host resistance to tumor growth, hypothalamic levels of noradrenaline and plasmatic levels of adrenaline and noradrenaline induced by cohabitation with a sick cage mate. To meet these objectives, devices specifically constructed to analyze the influence of chemosignals released from tumor-bearing mice were employed. Furthermore, we decided to confirm our previous findings on odor cue effects in open field and plus-maze behaviors of mice (Alves et al., 2010) performing novel experiments after the use of this new device; indeed, in the previous work mice were not analyzed in this apparatus for odor cues effects on behavior.

## 2. Materials and methods

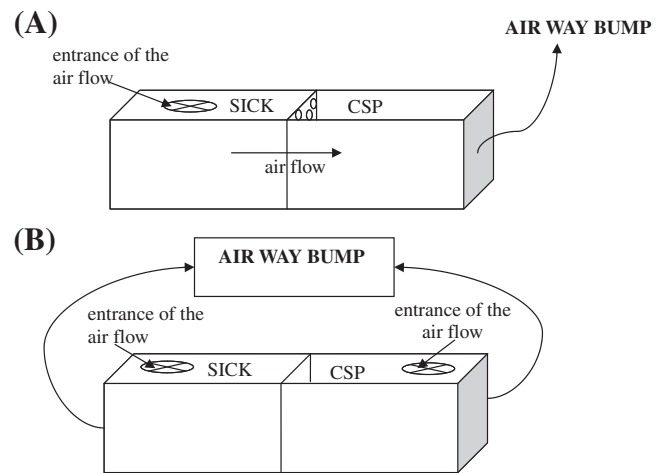
### 2.1. Animals

Naive Swiss female mice (50–60 days old) were used. Female mice were chosen based on our previous studies (Alves et al., 2006, 2007, 2010). The animals were housed under conditions of controlled temperature (22–26 °C), artificial light (12-h light/12-h dark, lights on at 7:00 a.m.), and with free access to rodent chow and water. Mice were transferred to a different (temperature-consistent) room and were acclimated for 10 days before the beginning of the experiments. Animals were housed and treated in accordance with the guidelines of the Bioethical Committee for the Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil; these guidelines are similar to those of the National Institutes of Health (NIH), USA.

### 2.2. Evaluation of odor cue effects

Evaluations of odor cue effects were made as proposed by Alves et al. (2010); briefly, eight wooden rectangular boxes (29 × 18 × 19 cm) painted white and elevated 56 cm from the ground were used. Fig. 1A depicts one box of experimental cage A (box A); as it can be seen, the eight boxes A used were divided along its longitudinal axis into two equal parts by a transparent and perforated acrylic plaque being enclosed by an acrylic top. An airflow (0.03 m/s) entered the top gridded side of the boxes A and passed through the open holes of the acrylic subdivision to the other side of the cage (with the covered top), and afterwards, the air was released through PVC tubes outside of the experimental room.

As can be seen in Fig. 1B the experimental boxes B were divided in the middle by a transparent, but not perforated, acrylic plaque; the eight boxes B used were covered by an acrylic plate with gridded holes (11.5 cm), one for each compartment. The air-



**Fig. 1.** Examples of experimental cages constructed to analyze the influences of odor cues on neuroimmune changes induced by cohabitation with a sick partner. (A) One box of experimental cage A; they were divided along its longitudinal axis into two equal parts by a perforated acrylic plaque, note that CSP1 mice were allowed to receive the odor from their sick partners. (B) One box of experimental cage B; they were also divided in the middle but by an acrylic plate that present no holes, note that CSP2 mice were not allowed to receive the odor cues left by their sick companions.

flow within both sides of boxes B was customized in such a way that the air (0.03 m/s) entered the gridded top holes and then moved outside of the experimental room.

### 2.3. Group formation and experimental design

Four experiments with 24, 24, 16 and 24 pairs of mice, respectively, were conducted in accordance with Good Laboratory Practice (GLP) protocols and quality assurance methods. Mice were initially weighed and paired according to their weights. Seven days later, one animal from each pair of mice was inoculated with  $5 \times 10^6$  Ehrlich tumor cells *i.p.*, as described elsewhere (Alves et al., 2010). The other animal, the subject of this study, was kept undisturbed and referred to as CSP (“companion of sick partner”). Sick mice were analyzed in their home cages for Ehrlich tumor signs and symptoms as proposed elsewhere (Morgulis et al., 2004). Briefly, the following scoring system was employed: 0, predominantly active, no signs of disease; 1, predominantly active with normal feeding and presence of rough hair; 2, active, normal feeding, rough hair, and presence of a small increase in abdominal volume; 3, active, normal feeding, rough hair, and a mild increment in abdominal volume; 4, absence of activity, anorexia, dyspnea, rough hair, and severe increment in abdominal volume. The day on which tumor injections were given was called ED1 (Experimental Day 1). The day on which the majority of the sick mice presented a score of 4 was chosen for behavioral and innate immune studies on CSP animals; *i.e.*, ED14.

The devices described above, which were constructed to analyze the influences of odor cues on neuroimmune changes induced by cohabitation with a sick partner (Alves et al., 2010), were used in all experiments. Thus, within each experiment, the mice were divided randomly and equally into two groups. In the first group, 8 tumor-injected mice were placed on one side of experimental cage A, and their conspecifics (CSP<sub>odor</sub>) were placed on the other side of the same box immediately after tumor injection. These pairs of mice were separated from each other by a perforated and transparent acrylic plaque, which allowed them to receive odor cues from their sick companions. In the second group, 8 pairs of mice were similarly placed in the experimental cage B

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