TÚLIO ROBERTO RIBEIRO MAZUCO

O COMPORTAMENTO DOENTIO INDUZIDO POR LPS É INTERROMPIDO PELO ESTRESSE E POR SUA ASSOCIAÇÃO COM SELÊNIO, SENDO ESSA ASSOCIAÇÃO TÓXICA PARA RATOS

Dissertação apresentada ao Programa de Pós-Graduação em Patologia Ambiental e Experimental da Universidade Paulista – UNIP, para obtenção do título de Mestre em Patologia Ambiental e Experimental

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PREFÁCIO

Este volume refere-se à dissertação de mestrado apresentada como requisito para a defesa de mestrado perante a Banca Examinadora no Programa de Pós-Graduação em Patologia Ambiental e Experimental, Universidade Paulista.

Segundo as normas do Programa, este trabalho é apresentado na forma de artigo científico de autoria do aluno e organizado de acordo com as exigências do veículo de publicação científica escolhido.

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RESUMO

O comportamento doentio é definido pelo conjunto de adaptações comportamentais e neuroimunes específicas, temporárias e benéficas que ocorre em resposta a processos infecciosos/inflamatórios. Porém, o comportamento doentio é um estado motivacional que pode ser modulado pelo contexto ambiental. O comportamento doentio foi induzido em ratos com o lipopolissacarídeo (LPS). Para verificar a melhora na expressão do comportamento doentio nós tratamos os ratos com o selênio. Esses ratos foram expostos ao estresse por contenção, e os seguintes paradigmas foram estudados: cura do organismo x preservação da espécie x luta ou fuga. Estudos da vocalização ultrassônica, dos comportamentos em campo aberto, do peso corporal, dos níveis séricos de IL-1 beta e IFN-gama, e de necropsia e histologia foram realizados. O LPS induziu comportamento doentio em ratos, observado pela diminuição da atividade motora e exploratória e pelo aumento dos níveis de mediadores imunes pró-inflamatórios. A suplementação com o selênio não melhorou os sintomas do comportamento doentio. Portanto, a deficiência de selênio não se relacionou com o comportamento doentio. Com relação ao fator estresse, o comportamento dos ratos foi diferencialmente afetado: a exposição ao LPS não afetou o comportamento dos ratos na presença de estresse. Assim, durante eventos estressores, o comportamento doentio foi interrompido para priorizar comportamentos de sobrevivência, como a luta ou fuga. A associação de LPS, selênio e estresse induziu o comportamento doentio mesmo durante eventos estressores e causou a morte de mais da metade dos ratos deste grupo experimental. A necropsia revelou danos severos nas adrenais, fígado, cérebro e especialmente nos pulmões. A síndrome da angústia respiratória do adulto, secundária ao choque circulatório foi a *causa-mortis*. Assim, a associação de LPS, selênio e estresse foi tóxica para ratos.

Palavras-chave: Lipopolissacarídeo; Estresse por contenção; Vocalização ultrassônica; Campo aberto; Interleucina (IL)-1 beta; Interferon (IFN)-gama.

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1. INTRODUÇÃO

O conceito que os patógenos, infecções e processos inflamatórios induzem doença em um modo específico e semelhante em várias espécies foi documentado há quase 30 anos por Benjamin Hart (1988). Os sintomas apresentados geralmente incluem febre, prostração, redução na atividade exploratória, no comportamento social, na ingesta alimentar e no comportamento sexual, bem como anedonia, piora nas funções cognitivas e elevação nos níveis de alguns mediadores inflamatórios, especialmente citocinas pró-inflamatórias como a interleucina (IL)-1 beta (Dantzer and Kelley 2007; Larson and Dunn 2001). Diferentes espécies animais normalmente apresentam esses vários sintomas em comum, mesmo quando afetados por diferentes processos infecciosos, tais como bactérias, vírus e fungos (Hart 1988; Larson and Dunn 2001; Yirmiya et al. 1999). Essas respostas comportamentais e neuroimunes foram batizadas pelo termo "comportamento doentio", por Kent e colegas (1992).

O comportamento doentio é normalmente um estado temporário, caracterizado por adaptações comportamentais e neuroimunes específicas, orquestradas pelo hospedeiro no combate ao microorganismo invasor, visando à cura e reduzindo a exposição do animal doente à predação e contaminação de sua colônia (Hart 1988; Kent et al. 1992). Entretanto, o comportamento doentio é considerado um estado motivacional que pode ser modulado pelo contexto ambiental no qual o animal se encontra (Aubert 1999). Em outras palavras, em situações em que o animal corre risco de morte ou está envolvido em confrontos hierárquicos (por exemplo, com predadores, competidores e climas extremos), o comportamento doentio é momentaneamente interrompido para priorizar outros comportamentos, tais como luta ou fuga e comportamentos maternos (Aubert 1999; Aubert et al. 1997). Assim, embora o comportamento doentio seja necessário e benéfico para um organismo e sua espécie, em situações de estresse é melhor priorizar a chamada resposta de luta ou fuga.

O lipopolissacarídeo (LPS), um componente da parede celular de bactérias gram-negativas, é considerado um potente indutor do comportamento doentio (Bluthe et al. 1994). Quando esta endotoxina entra em contato com o organismo animal, mimetiza uma infecção bacteriana por meio da ativação do sistema imune, liberando citocinas pró-inflamatórias, tais como IL-1 beta, IL-6, fator de necrose tumoral (TNF) alfa e interferon (IFN)-gama (Aderem and Ulevitch 2000; Fischer et al. 2015; Miyake 2003). O LPS também ativa o eixo hipotálamo-pituitária-adrenal (HPA), liberando glicocorticoides, tais como a corticosterona (Kirsten et al. 2013).

O selênio é um elemento químico semimetálico da família dos calcogênios, disponível na natureza raramente em sua forma elementar, encontrado em poucos minerais (Boyd 2011). Ele é inserido em nossa cadeia alimentar em grande parte devido às plantas (White 2016). Para os humanos, as principais fontes alimentares são de origem animal, como carne vermelha, e provenientes do mar, como marisco, bem como em grãos, ovos, frango e alho (Yamashita et al. 2013). A dieta recomendável de selênio é de 55 μg/dia para pessoas de 14 anos ou mais, com um limite máximo de tolerância de 400 μg/dia (MacFarquhar et al. 2010).

A deficiência de selênio em nossos organismos é danosa. Por exemplo, os Andes equatorianos apresentam solo vulcânico e ácido, e consequente baixos níveis de selênio. Crianças que vivem nesta região apresentam deficiência nos níveis séricos de selênio (Sempertegui et al. 2003). Esses indivíduos costumam a apresentar hipotireoidismo, doenças cardiovasculares, deficiências no sistema imune, infertilidade masculina, declínio cognitivo e aumento da incidência de vários tipos de câncer (Beck et al. 2003; White 2016). Por outro lado, a exposição elevada ao selênio também é prejudicial. A intoxicação pode causar desde náuseas, dores abdominais, diarreia, enfraquecimento das unhas, perda de cabelo, neuropatia periférica e irritabilidade, e até mesmo hipotensão, edema pulmonar e colapso renal e cardiovascular, levando o indivíduo a óbito (Fairweather-Tait et al. 2011; Spiller and Pfiefer

2007). Portanto, tem sido demonstrado o substancial papel do selênio na nossa saúde e doença (Wrobel et al. 2016). Considerado um micronutriente essencial para nosso organismo, o selênio exerce múltiplos e complexos efeitos na nossa saúde, com propriedades antioxidantes (Han et al. 2017), anti-inflamatórias (El-Ghazaly et al. 2017), antivirais (Abdullaev et al. 1987) e anticâncer (Lipinski 2017).

No presente trabalho, o comportamento doentio foi induzido a partir da exposição aguda dos ratos ao LPS. Este protocolo, incluindo a via de administração, dose e espécie animal já foi anteriormente utilizado e descrito pelo nosso grupo (Kirsten et al. 2015b; Kirsten et al. 2013). Para verificar eventual mudança de expressão do comportamento doentio e melhora da resposta comportamental e imune, tratamos os ratos com selênio.

O selênio foi escolhido para o eventual tratamento do comportamento doentio induzido pelo LPS pelo fato de, em nossos estudos prévios, ter sido revelada a redução dos níveis séricos de selênio induzida pela exposição aguda de LPS. As ratas tratadas com uma única dose de LPS de 100 μg/kg, mesma dose que a do presente estudo, apresentaram redução de cerca de 10% dos níveis séricos de selênio, comparado aos níveis do grupo controle (Kirsten et al. 2015a). Assim, a deficiência de selênio pode exercer papel importante na expressão do comportamento doentio, e sua suplementação pode mudar esta resposta comportamental e imune.

O uso de alguns medicamentos e nutrientes vem sendo receitado sem preocupações com contextos estressores, que, por sua vez, podem interferir inclusive potencializando os efeitos de drogas. Por exemplo, o estresse pré-natal potencializa o comportamento epilético induzido por pilocarpina em ratos jovens (Sadaghiani and Saboory 2010). Neste sentido, a interação das variáveis ambientais, como o estresse, podem exacerbar o efeito tóxico do selênio, ou anular o eventual efeito benéfico deste micronutriente em nossa saúde. Desse modo, o estudo dos efeitos do selênio no comportamento doentio foi avaliado com o contexto

do estresse, ou seja, os animais passando ou não por uma situação de estresse. Portanto, buscou-se estudar os paradigmas: cura do organismo x preservação da espécie x luta ou fuga.

Assim, os ratos receberam o LPS e depois o selênio, e então foram submetidos à sessão de estresse por contenção. As vocalizações ultrassônicas dos ratos, especialmente aquelas envolvidas com contextos aversivos (Takahashi et al. 2010), como parâmetro dos níveis de estresse dos animais foram avaliadas. Foi avaliada também a atividade geral em campo aberto para avaliar os parâmetros exploratórios/motores e de ansiedade (Patti et al. 2005; Prut and Belzung 2003), o peso corporal dos ratos, bem como os níveis séricos de IL-1 beta e IFN-gama. Tomando em conjunto, as avaliações comportamentais, de peso corporal e de mediadores imunes periféricos pró-inflamatórios nos permitem uma análise confiável da expressão do comportamento doentio nos ratos (Bay-Richter et al. 2011; Dantzer and Kelley 2007; Larson and Dunn 2001; Moraes et al. 2017). Por fim, foi conduzida necropsia nos ratos com estudos macroscópicos e histológicos.

To: Psychopharmacology

LPS-induced sickness behavior is switched off by stress and their association with selenium is toxic for rats

Short title: LPS, sickness behavior, selenium, and stress

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Abstract

Sickness behavior is considered part of the specific beneficial adaptive behavioral and neuroimmune changes that occur in response to infectious/inflammatory processes. However, sickness behavior is a motivational state that can be modulated by the environmental context. We induced sickness behavior in rats using lipopolysaccharides (LPS). Rats were treated with selenium in order to verify possible changes of sickness behavior expression. We exposed these rats to a restraint stress challenge and studied the paradigms: cure of the organism x preservation of the species x fight or flight. Studies of ultrasonic vocalizations, open-field behaviors, body weight, IL-1 beta and IFN-gamma serum levels, and necropsy and histology were performed. LPS induced sickness behavior in rats observed by decreased motor/exploratory activity and increased proinflammatory immune mediators' levels. Selenium supplementation did not exert beneficial effects on the sickness behavior symptoms. Therefore, selenium deficiency was not related to sickness behavior expression. When analyzed with stress paradigm, the behavior of rats was differentially affected. LPS exposure did not affect behavior of rats in presence of stress. Thereby, sickness behavior was abrogated during stressor events to prioritize survival behaviors, such as fight or flight. However, the association of LPS, selenium, and stress induced sickness behavior even during stressor events and caused death of more than half rats of this experimental group. Necropsy revealed severe damages in adrenals, liver, brain, and especially in the lungs. Adult respiratory distress syndrome secondary to circulatory shock was the cause-mortis. Thus, the association of LPS, selenium, and stress was toxic for rats.

Keywords: Lipopolysaccharide; Restraint stress; Ultrasonic vocalizations; Open-field behaviors; Interleukin (IL)-1 beta; Interferon (IFN)-gamma.

Introduction

The concept that pathogens, infections and inflammatory processes induce sickness in a specific way in several species was documented about 30 years ago by B. Hart (1988). Animals usually show common symptoms even when affected by different processes, such as bacterial, viral, and fungal infections (Hart 1988; Larson and Dunn 2001; Yirmiya et al. 1999). These behavioral and neuroimmune responses were named 'sickness behavior' by Kent and colleagues (1992). Sickness behavior is generally characterized by fever; prostration; decreases in exploratory activity, social behavior, feeding behavior, and sexual behavior; the induction of anhedonia; poor learning and cognitive functions; as well as increases in proinflammatory mediators levels, such as interleukin (IL)-1 beta (Dantzer and Kelley 2007; Larson and Dunn 2001).

Sickness behavior is normally a temporary state characterized by adaptive behavioral- and neuroimmune-specific changes orchestrated by the host to fight the invading microorganism and heal more quickly, as well as reduce exposure of the sick animal to predation and contamination of their colony (Hart 1988; Kent et al. 1992). However, sickness behavior is considered a motivational state that can be modulated by the environmental context in which the animal is found (Aubert 1999). In other words, in situations where the animal is at risk of death or engaged in a hierarchical confrontation (e.g., with predators, competitors, and climatic extremes), sickness behavior is momentarily interrupted to prioritize other behaviors, such as fight or flight and maternal behavior (Aubert 1999; Aubert et al. 1997). Thus, although sickness behavior is necessary and beneficial to an organism and its species, in stressful situations, it is better to prioritize the so-called fight or flight response.

Medicines and nutrients have been prescribed without concern for stressful contexts, which may interfere and potentiate the effects of drugs. For example, prenatal stress enhances pilocarpine-induced epilepsy behavior in juvenile rats (Sadaghiani and Saboory 2010).

Lipopolysaccharides (LPS) are an endotoxin that mimics infection by gram-negative bacteria by activating the immune system to release proinflammatory cytokines, such as IL-1 beta, IL-6, tumor necrosis factor (TNF) alpha, and interferon (IFN)-gamma (Aderem and Ulevitch 2000; Fischer et al. 2015; Miyake 2003). LPS also activates the hypothalamic-pituitary-adrenal (HPA) axis (Kirsten et al. 2013). LPS is considered a potent sickness behavior inducer (Bluthe et al. 1994).

Selenium is a semi-metallic element that belongs to the family of chalcogens, rarely found in its elemental form in nature (Boyd 2011). For humans, the main selenium sources are in animal meat, seafood, grains, eggs, and garlic (Yamashita et al. 2013). The recommended dietary allowance is 55 μg/d for persons 14 years or older, with a tolerable upper intake limit of 400 μg/d (MacFarquhar et al. 2010). Selenium deficiency is harmful. For example, selenium deficiency was observed in a population of a volcanic area, which presented acidic soil and consequent low availability of selenium (Beck et al. 2003). This population presented high prevalence of hypothyroidism, cardiovascular diseases, deficiencies in the immune system, male infertility, cognitive impairments, and various types of cancer (White 2016). High levels of selenium are also harmful. It induces nausea, nail thinning, hair loss, irritability, diarrhea, peripheral neuropathy, pulmonary edema, and renal and cardiovascular collapse (Fairweather-Tait et al. 2011; Spiller and Pfiefer 2007). Therefore, selenium is considered an essential micronutrient, exerting multiple effects, including antioxidant (Han et al. 2017), anti-inflammatory (El-Ghazaly et al. 2017), antiviral (Abdullaev et al. 1987), and anticancer properties (Lipinski 2017).

We exposed rats to LPS to induce sickness behavior using the same protocol as previously described by our group (Kirsten et al. 2015b; Kirsten et al. 2013). Rats were treated with selenium in order to verify possible changes of sickness behavior expression. We chose selenium for the sickness behavior treatment based on our previous findings of LPS-exposure inducing decrease in selenium serum levels in rats (Kirsten et al. 2015a). Thus, selenium deficiency may play an important role in the expression of sickness behavior, and its supplementation may improve behavioral and immune responses. We also evaluated the effects of selenium on the sickness behavior of rats including stress context (restraint stress). We studied the paradigms: cure of the organism x preservation of the species x fight or flight.

Ultrasonic vocalizations were evaluated, especially those involved with aversive contexts (Takahashi et al. 2010), as stress levels parameter. The open-field behavior was evaluated as motor, exploratory, and anxiety parameters (Patti et al. 2005; Prut and Belzung 2003). Body weight and IL-1 beta and IFN-gamma serum levels were also evaluated. Taken together, behavioral, body weight, and peripheral proinflammatory mediators analyzes allow us a reliable analysis of sickness behavior expression in rats (Bay-Richter et al. 2011; Dantzer and Kelley 2007; Larson and Dunn 2001; Moraes et al. 2017). Finally, necropsy was performed in rats for macroscopical and histological studies.

Materials and Methods

Ethics statement

The present study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NCR 2011). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Paulista University, Brazil (Permit Number: 024/16). All efforts were

made to minimize suffering, reduce the number of animals used, and utilize alternatives to *in vivo* techniques when available. The experiments were also performed in accordance with good laboratory practice protocols and quality assurance methods.

Animals

A total of 60 Wistar adult male rats (*Rattus norvegicus*) with 80-93 days old from the School of Veterinary Medicine (University of Sao Paulo, Sao Paulo, Brazil) were used. They were housed at Paulista University (Sao Paulo, Brazil) in polypropylene cages (45.5 X 34.5 X 20 cm; maximum of 5 rats per cage) with microisolator system (Tecniplast, Buguggiate, Italy), controlled temperature (23°C ± 2°C) and humidity (55–65%) with artificial lighting (12-hr light/12-hr dark cycle, lights on at 7:00 AM). The animals had free access to irradiated rodent chow (BioBase, Águas Frias, Brazil) and filtered water. Sterilized and residue-free wood shavings were used for animal bedding.

Treatments

Rats were treated with LPS solution, selenium solution, and/or their vehicles. LPS (from *Escherichia coli, ser*otype 0127: B8, Sigma-Aldrich, St. Louis, USA) was dissolved in sterile saline (0.9% NaCl solution, 50 µg/ml) and administered intraperitoneally (i.p.) at a dose of 100 µg/kg, based on our previous studies (Kirsten et al. 2015b; Kirsten et al. 2010). This LPS dose induces sickness behavior for at least 4h, influencing both behavioral and immune parameters, without inducing septic shock (Bay-Richter et al. 2011; Kirsten et al. 2015b; Kirsten et al. 2013; Lenczowski et al. 1997). Sodium selenite (Na2SeO3, cat. no. 214485-100g, Sigma-Aldrich, St. Louis, USA) was dissolved in sterile saline (1.3 mg/ml) and administered i.p. at a dose of 1.3 mg/kg (of Na2SeO3), based on Rasekh and colleagues study (Rasekh et al. 1991). This dose does not induce hyperglycemia, whereas 1.6 and 3.8 mg/kg

does (Rasekh et al. 1991). Moreover, sodium selenite has been used clinically as supplement in patients with selenium deficiency (Duntas and Benvenga 2015; Schrauzer 2001). Sterile saline solution was administered i.p. as vehicle/control groups. Each rat received a 1 mL/kg of each solution, according to schedule treatments.

Groups

The rats were randomly divided into eight groups (Table 1). The first four groups did not receive a stress session and the other four groups received (n = 7 per group). Regardless of exposure to stress, the time interval between the first injection which each rat received and the beginning of the behavioral observations was 4h. The time intervals were chosen based on behavioral and immune expression of sickness behavior (Dantzer et al. 2008; Kirsten et al. 2015b; Kirsten et al. 2010; Lenczowski et al. 1997). All of the experiments were performed between 1:00 P.M. and 6:00 P.M. to minimize the effects of circadian rhythms.

Restraint stress

Restraint stress is considered a simple and painless model of stress that does not cause any lasting impairment (Buynitsky and Mostofsky 2009). It is considered a model of psychological stress due to its similarity to the natural experience of confinement (Dhabhar and McEwen 1996). The restraint stress apparatus consisted of plastic cylindrical restraint tubes (5 cm diameter, 21 cm length) for individual restraints that were fixed on a table with both ends closed and holes for the tail and ventilation. The design of the tubes prevented pain and compression. The rats were subjected to a single restraint session of 2 hours. A 2-hour session is considered sufficient to activate the HPA axis, increasing circulating corticosterone levels (Buynitsky and Mostofsky 2009; Echeverry et al. 2004).

Ultrasonic vocalization

Three hours and fifty-five min after the first injection, which coincides with the last 5 min of restraint stress, rats were observed for ultrasonic vocalizations inside the restraint tube (stress group) or in a round arena (38.5 cm diameter, 29 cm high walls, painted with an acrylic washable cover; no stress groups). The vocalization test room was acoustically isolated. Ultrasonic vocalizations were detected using Ultravox software (XT, version 3.1, Noldus Information Technology, Leesburg, USA) and an ultrasonic microphone (Noldus Information Technology, Leesburg, USA). All ultrasounds up to 125 kHz were detected and evaluated. Rats emit ultrasonic vocalizations in the range of 22-28 kHz during aversive contexts (Antoniadis and McDonald 1999; Takahashi et al. 2010; Thakore et al. 2016). Thus, 22-28 kHz ultrasonic vocalizations were the main interest range evaluated in the study. Several parameters were automatically recorded during the 5-min session, including the number of vocalizations, mean vocalization duration (ms), maximal vocalization duration (ms), and total (ms) vocalizations time.

Open-field behavior

Four hours after the first injection, which coincides with the end of stress session, i.e., immediately after the ultrasonic vocalization test, the rats were removed from the restraint tubes and observed in an open field arena. The study of open-field behaviors evaluates motor, exploratory, and anxiety behaviors (Patti et al. 2005; Prut and Belzung 2003). Moreover, when analyzed together with other behavioral and immune parameters, open-field behaviors study allows a reliable analysis of the expression of sickness behavior in rats (Larson and Dunn 2001; Moraes et al. 2017). The open-field apparatus consisted of a black round acrylic arena (60 cm diameter, 50 cm high walls) subdivided into 12 parts, including central and peripheral zones. The test room was small and with dim lighting, isolated from the

experimenter, and with a video camera mounted above the arena to collect the data, which were analyzed after the end of the experiment. Each rat was individually placed in the center of the apparatus, and the following parameters were evaluated over a period of 5 min: locomotion frequency (number of floor units entered with all four paws), rearing frequency (number of times the rodents stood on their hind legs), total immobility time (s, duration of time spent without active movements), total self-grooming time (s, summing the values of head washing, body grooming, paw/leg licking, and tail/genital grooming), and time (s) spent in peripheral and central zones. The apparatus was cleaned with a 5% alcohol/water solution before placement of the animals to obviate possible biasing effects from odor cues left by previous rats.

Body weight

Rat's body weight (g) was calculated immediately after the open-field test. This time interval was greater than 4h from the first injection. Throughout all the experimental protocols intervals the rats had free access to rodent chow and filtered water. Exposure to LPS, selenium, and stress may affect body weight. Moreover, when analyzed together with other behavioral and immune parameters, it is considered a reliable analysis of the expression of sickness behavior in rats (Larson and Dunn 2001; Moraes et al. 2017).

IL-1 beta and IFN gamma

Immediately after the body weight measurement, the rats were decapitated, and trunk blood was collected in conical tubes. Twenty min later, the samples were centrifuged (10 min, 3.500 RPM), and serum was obtained. Serum samples of each animal were aliquoted in different conical tubes for separate analyses of IL-1 beta (R&D Systems, cat. no. SRLB00, Minneapolis, MN, USA) and IFN gamma (R&D Systems, cat. no. SRIF00, Minneapolis, MN,

USA) using enzyme-linked immunosorbent (ELISA) commercial kits in duplicate and according to the manufacturer's instructions. Both IL-1 beta and IFN gamma levels are used as sickness behavior biomarkers (Fischer et al. 2015).

Necropsy

A pathologist conducted necropsy in rats as previously described (de Paula Coelho et al. 2017). Macroscopical and microscopical analyzes were performed in several tissues, such as adrenal, liver, lung, and brain. The histological analysis was performed according to conventional paraffin-embedded inclusion and hematoxylin-eosin (HE) staining method.

Statistical analysis

Homogeneity was verified using the F test or Bartlett test. Normality was verified using the Kolmogorov–Smirnov test. Two-way analysis of variance (ANOVA) followed by Newman-Keuls's multiple comparison test was used to compare parametric data. The results are expressed as the mean ± SEM. Results that presented values of 0 were transformed to 1 for the statistical analysis. In all cases, the results were considered significant at $p < 0.05$ and $\alpha = 0.05$.

Results

LPS induced sickness behavior in rats observed by decreased motor/exploratory activity and increased proinflammatory immune mediators' levels. Selenium supplementation did not exert beneficial effects on the sickness behavior symptoms. Therefore, selenium deficiency was not related to sickness behavior expression. When analyzed with stress paradigm, the behavior of rats was differentially affected. LPS exposure did not affect

behavior of rats in presence of stress. Thereby, sickness behavior was abrogated during stressor events to prioritize survival behaviors, such as fight or flight. However, the association of LPS, selenium, and stress induced sickness behavior even during stressor events and caused death of more than half rats of this experimental group. Necropsy revealed severe damages in adrenals, liver, brain, and especially in the lungs. Adult respiratory distress syndrome secondary to circulatory shock was the cause-mortis. Thus, the association of LPS, selenium, and stress was toxic for rats.

Este volume de Dissertação de Mestrado apresenta somente o resumo dos principais resultados obtidos com este estudo. Para maiores detalhes, consultar a publicação em artigo científico, ou contatar o orientador deste estudo via e-mail: Prof. Dr. Thiago B. Kirsten, thiago.kirsten@docente.unip.br ou thik@outlook.com

Discussion

Este volume de Dissertação de Mestrado apresenta somente as conclusões do trabalho, sem sua discussão. Para maiores detalhes, consultar a publicação em artigo científico, ou contatar o orientador deste estudo via e-mail: Prof. Dr. Thiago B. Kirsten, thiago.kirsten@docente.unip.br ou thik@outlook.com

Conclusion

LPS induced sickness behavior in rats observed by decreased motor/exploratory activity and increased proinflammatory immune mediators' levels. Selenium supplementation did not exert beneficial effects on the sickness behavior symptoms. Therefore, selenium deficiency was not related to sickness behavior expression. When analyzed with stress

paradigm, the behavior of rats was differentially affected by the treatments. LPS exposure did not affect motor/exploratory behavior of rats in presence of stress. Thus, sickness behavior was abrogated during stressor events to prioritize survival behaviors, such as fight or flight. However, the association of LPS, selenium, and stress induced sickness behavior even during stressor events and caused death of more than half rats of this experimental group. Necropsy revealed severe damages in adrenals, liver, brain, and especially in the lungs. Adult respiratory distress syndrome secondary to circulatory shock was the cause-mortis. Thus, the association of LPS, selenium, and stress was toxic for rats.

Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

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Table 1 Experimental groups. Effects of LPS (100 μg/kg), selenium (Na2SeO3; 1.3 mg/kg), and stress (restraint) in adult male rats. The experimental groups, their respective treatments (administered solutions), and exposure to stress are presented, divided by exposure intervals and conducted tests. (SAL) saline; (LPS) lipopolysaccharide; (Se) selenium; (Str) stress (n = 7 per group)

	0h	**1h**	**2h**	**3h55**	**4h**	**4h05**
SAL+SAL	saline	saline	no stress	vocalization	open-field	euthanasia
LPS+SAL	LPS	saline	no stress	vocalization	open-field	euthanasia
LPS+Se	LPS	selenium	no stress	vocalization	open-field	euthanasia
SAL+Se	saline	selenium	no stress	vocalization	open-field	euthanasia
SAL+SAL+Str	saline	saline	restraint stress	vocalization	open-field	euthanasia
LPS+SAL+Str	LPS	saline	restraint stress	vocalization	open-field	euthanasia
LPS+Se+Str	LPS	selenium	restraint stress	vocalization	open-field	euthanasia
SAL+Se+Str	saline	selenium	restraint stress	vocalization	open-field	euthanasia

Table 2 Values of two-way analysis of variance. Effects of LPS (100 μg/kg), selenium (Na2SeO3; 1.3 mg/kg), and stress (restraint) on the statistical values of F, DFn, DFd, and *p* of two-way analysis of variance of 22-28 kHz ultrasonic vocalization, open-field behavior, body weight, and serum IL-1 beta and IFN gamma levels in adult male rats (n = 4-7 per group)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ (two-way ANOVA followed by the Newman-Keuls test, $\alpha = 0.05$).

Table 3 Ultrasonic vocalizations. Effects of LPS (100 µg/kg), selenium (Na2SeO3; 1.3 mg/kg), and stress (restraint) on the 22-28 kHz ultrasonic vocalizations in adult male rats. (SAL) saline; (LPS) lipopolysaccharide; (Se) selenium; (Str) stress. 1h between each injection; 1h after the last injection for the beginning of the stress session. (n = 4 per group). Data are expressed as the mean ± SEM

* $p < 0.05$, ** $p < 0.01$ vs SAL+SAL+Str (two-way ANOVA followed by the Newman-Keuls test).

Fig. 1 Open-field behavior. Effects of LPS (100 μg/kg), selenium (Na2SeO3; 1.3 mg/kg), and stress (restraint) on the open-field behaviors in adult male rats. (SAL) saline; (LPS) lipopolysaccharide; (Se) selenium; (Str) stress. 1h between each injection; 1h after the last injection for the beginning of the stress session (n = 7 per group). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ (two-way ANOVA followed by the Newman-Keuls test, $\alpha = 0.05$). Data are expressed as the mean ± SEM

Fig. 2 IL-1 beta and IFN gamma. Effects of LPS (100 μg/kg), selenium (Na2SeO3; 1.3 mg/kg), and stress (restraint) on IL-1 beta and IFN gamma serum levels in adult male rats. (SAL) saline; (LPS) lipopolysaccharide; (Se) selenium; (Str) stress. 1h between each injection; 1h after the last injection for the beginning of the stress session session (n = 7 per group). * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$ (two-way ANOVA followed by the Newman-Keuls test, $\alpha = 0.05$). Data are expressed as the mean ± SEM

Fig. 3 Tissues photomicrographs. Effects of LPS (100 μg/kg), selenium (Na2SeO3; 1.3 mg/kg), and stress (restraint) on peripheral tissues of adult male rats. 1h between each injection; 1h after the last injection for the beginning of the stress session session. Photomicrographs of (A) Adrenal (400x); (B) liver (100x); (C) lung (400x) analyzed by hematoxylin eosin. C: adrenal cortex; M: adrenal medulla; GZ: glomerular zone; FZ: fascicular zone; RZ: reticular zone; Co: (generalized) congestion; P: peliosis (trabecular disorganization); ED: pulmonary edema; E: pulmonary emphysema; T: thrombus

Fig. 4 Brain photomicrographs. Effects of LPS (100 μg/kg), selenium (Na2SeO3; 1.3 mg/kg), and stress (restraint) on neural tissues of adult male rats. 1h between each injection; 1h after the last injection for the beginning of the stress session session. Photomicrographs of cortex (A: 100x and B: 400x); (C) brainstem (100x) analyzed by hematoxylin eosin. G: glia cells; N: neuron; GM: gray matter; WM: white matter; Co: congestion; I: mononuclear inflammation cells and gliosis (glial proliferation)

Electronic supplementary material / Online Resource

ESM 1 (Fig.) 22-28 kHz ultrasonic vocalizations. Effects of LPS (100 μg/kg), selenium (Na2SeO3; 1.3 mg/kg), and stress (restraint) on 22-28 kHz ultrasonic vocalizations in adult male rats in adult male rats. Example of ultrasonic vocalizations detected in the frequency range of 22-28 kHz in stressed rats

Electronic supplementary material / Online Resource

ESM 2 (Fig.) Body weight. Effects of LPS (100 μg/kg), selenium (Na2SeO3; 1.3 mg/kg), and stress (restraint) on the body weight of adult male rats. (SAL) saline; (LPS) lipopolysaccharide; (Se) selenium; (Str) stress. 1h between each injection; 1h after the last injection for the beginning of the stress session (n = 7 per group; two-way ANOVA followed by the Newman-Keuls test, $\alpha = 0.05$). Data are expressed as the mean ± SEM

3. CONSIDERAÇÕES FINAIS

- O LPS induziu comportamento doentio em ratos, observado pela diminuição da atividade motora e exploratória e pelo aumento dos níveis de mediadores imunes pró-inflamatórios.
- A suplementação com o selênio não resultou em efeitos benéficos sobre os sintomas do comportamento doentio. Portanto, a deficiência de selênio não apresentou relação com a expressão do comportamento doentio.
- Quando analisado com o paradigma do estresse, o comportamento dos ratos foi diferencialmente afetado pelos tratamentos. A exposição ao LPS não afetou o comportamento motor e exploratório de ratos na presença de estresse. Assim, durante eventos estressores, o comportamento doentio foi interrompido para priorizar comportamentos de sobrevivência, como a luta ou fuga.
- A associação do LPS com o selênio e o estresse induziu o comportamento doentio mesmo durante eventos estressores e causou a morte de mais da metade dos ratos deste grupo experimental. A necropsia revelou danos severos nas adrenais, fígado, cérebro e especialmente nos pulmões. A síndrome da angústia respiratória do adulto, secundária ao choque circulatório foi a *causa-mortis*. Assim, a associação de LPS, selênio e estresse foi tóxica para ratos.

C E R T I F I C A D O

CERTIFICAMOS, que a proposta intitulada "ESTUDO SOBRE OS EFEITOS DO SELÊNIO NO COMPORTAMENTODOENTIO DE RATOS ESTRESSADOS'' e "ESTUDO SOBRE OS EFEITOS DO LPS E DO SELÊNIO NA ATIVIDADE GERAL EM CAMPO ABETO E NOS NÍVEIS DE CORTICOSTERONA DE RATOS ESTRESSADOS", registrada com o nº 024/16, sob-responsabilidade de '' THIAGO BERTI KIRSTEN, TÚLIO ROBERTO RIBEIRO MAZUCO e THALLES FAGUNDES BIONDI '' que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subiflo Vertebrata (exceto humanos), para fins de pesquisa cientifica (ou ensino) encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela Comissão de Ética no Uso Animais (CEUA) da UNIP, em reunião de 18 /05 /2016.

Finalidade	Ensino ()	Pesquisa Cientifica (X)
Vigência de autorização	01/08/2016 A 31/01/2018	
Espécie / linhagem/ raça	RATO H. WISTAR	
Nº de animais	64	
Peso / idade	65-75 DIAS/150-350G	
Sexo	INDEFINIDO	
Origem	BIOTERIO DA FMVZ-SP	

Juliana Guizi

Secretária da Comissão de Ética no Uso de Animais – CEUA

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REVISORES ORTOGRÁFICOS E GRAMATICAIS

DECLARAÇÃO

DECLARO que a *Dissertação* do Programa de *Mestrado* em *Patologia Ambiental e Experimental* da Universidade Paulista – UNIP foi devidamente revisada.

Título: *O COMPORTAMENTO DOENTIO INDUZIDO POR LPS É INTERROMPIDO PELO ESTRESSE E POR SUA ASSOCIAÇÃO COM SELÊNIO, SENDO ESSA ASSOCIAÇÃO TÓXICA PARA RATOS*

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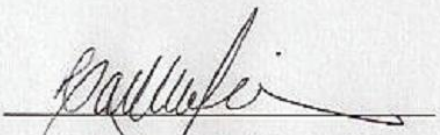
13 de novembro de 2017.



Assinatura do(a) Revisor(a)