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UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS CÂMPUS JABOTICABAL

PARTICIPAÇÃO DA MICRÓGLIA DO LOCUS COERULEUS NO CICLO SONOVIGÍLIA E NA RESPOSTA RESPIRATÓRIA E COMPORTAMENTAL A ESTÍMULOS PANICOGÊNICOS

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Bióloga

2026

UNIVERSIDADE ESTADUAL PAULISTA - UNESP CÂMPUS DE JABOTICABAL

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Jaboticabal, 10 de fevereiro de 2026.

DADOS CURRICULARES DO AUTOR

Beatriz Felix Gonçalves de Oliveira, nascida em 08 de junho de 1999 na cidade de São Paulo – SP, filha de José Ricardo Gonçalves de Oliveira e Leonice Aparecida Felix. Graduada Bacharel em Ciências Biológicas pela Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal (FCAV-Jaboticabal). Foi bolsista FAPESP durante a iniciação científica em pesquisa sobre o envolvimento das células microgliais do locus coeruleus nas respostas ventilatórias e comportamentais à estímulos hipercápnicos e panicogênicos, no Laboratório de Fisiologia, Departamento de Morfologia e Fisiologia Animal (UNESP-FCAV), sob orientação da Profª Drª Luciane Helena Gargaglioni Batalhão. Atualmente, realiza mestrado pelo Programa de Pós-graduação em Ciência Animal da Universidade Estadual Paulista “Júlio de Mesquita Filho” (FCAV/UNESP), Câmpus Jaboticabal, com bolsa FAPESP, atuando no Laboratório de Fisiologia do Departamento de Morfologia e Fisiologia Animal, sob orientação da Profª Drª Luciane Helena Gargaglioni Batalhão.

Quando me dei conta, minhas asas estavam prontas e tudo que precisava fazer, era voar. Crescer dói, mas é transformador.
- Leonice Aparecida Felix

Dedico este trabalho ao meu filho, Vicente, que chegou no meio desta jornada e me lembra constantemente de que até as noites mais longas dão lugar à luz.

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Por fim, agradeço à FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo pela bolsa de mestrado concedida (Processo nº 2023/07978-8), e ao CNPq. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Sumário

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

C E R T I F I C A D O

Certificamos que o projeto de pesquisa intitulado **"Participação da micróglia do locus coeruleus no ciclo sono-vigília e na resposta respiratória e comportamental a estímulos panicogênicos"**, protocolo n.º 7674/23, sob a responsabilidade da Profa. Dra. LUCIANE HELENA GARGAGLIONI BATALHÃO, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 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 DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 20 de setembro 2023.

Vigência do Projeto	01/10/2023 a 06/06/2025
Espécie / Linhagem	Camundongo C57BL/6 (Nome científico: *Mus musculus*)
Nº de animais	64
Peso / Idade	20 – 25 g (8 a 12 semanas)
Sexo	Fêmeas
Origem	Biotério do Campus da USP de Ribeirão Preto

Jaboticabal, 20 de setembro de 2023.

Profa. Dra. Paola Castro Moraes
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PARTICIPAÇÃO DA MICRÓGLIA DO LOCUS COERULEUS NO CICLO SONO-VIGÍLIA E NA RESPOSTA RESPIRATÓRIA E COMPORTAMENTAL A ESTÍMULOS PANICOGÊNICOS

Resumo: Os transtornos relacionados ao estresse são fortemente influenciados por circuitos noradrenérgicos e apresentam diferenças sexuais marcantes; no entanto, a contribuição da micróglia do locus coeruleus (LC) para as respostas fisiológicas e comportamentais ao estresse em fêmeas permanece pouco compreendida. Neste estudo, investigamos o papel da micróglia do LC em camundongos fêmeas na regulação das respostas ventilatórias, comportamentais e do ciclo sono–vigília diante de desafios hipercápnicos e de estresse precoce na vida. Utilizando a depleção farmacológica da micróglia com clodronato dissódico, avaliamos as respostas ventilatórias e comportamentais à exposição moderada (7%) e elevada (20%) ao CO_2, bem como a arquitetura do ciclo sono–vigília. Paralelamente, examinamos fêmeas submetidas ao protocolo de adoção cruzada repetida como modelo de estresse precoce na vida, avaliando a responsividade ao CO_2 e a morfologia microglial do LC na idade adulta. A manipulação microglial no LC não alterou as respostas ventilatórias ao CO_2, mas atenuou o comportamento de fuga durante o estímulo panicogênico. Além disso, os animais que receberam microinjeção do fármaco depletor de micróglia, clodronato dissódico (CDS), apresentaram aumento do tempo de sono NREM durante a fase escura. Notavelmente, o estresse precoce na vida não potencializou as respostas ventilatórias do tipo pânico ao CO_2 em fêmeas, sugerindo uma dissociação sexo-específica entre alterações neuroimunes e reatividade quimiossensória. Em conjunto, esses achados indicam que a micróglia do locus coeruleus não constitui um determinante primário das respostas ventilatórias ou comportamentais do tipo pânico evocadas pelo CO_2 em fêmeas, mas pode contribuir para a regulação da arquitetura do sono, particularmente do sono NREM. A ausência de potencialização das respostas ao CO_2 induzida pelo estresse precoce reforça a existência de uma dissociação sexo-específica entre alterações neuroimunes e reatividade quimiossensória, destacando mecanismos regulatórios distintos subjacentes ao estresse, ao sono e ao processamento de ameaças interoceptivas em fêmeas.

Palavras-chave: Locus coeruleus (LC); Micróglia; Sono e vigília; Transtorno do Pânico; Estresse precoce; Adoção Cruzada Repetida

Participation of locus coeruleus microglia in the sleep–wake cycle and in respiratory and behavioral responses to panicogenic stimuli

Abstract: Stress-related disorders are strongly influenced by noradrenergic circuits and neuroinflammation and show marked sex differences, yet the contribution of locus coeruleus (LC) microglia to physiological and behavioral stress responses in females remains poorly understood. Here, we investigated the role of LC microglia in female mice in regulating ventilatory, behavioral, and sleep–wake responses to hypercapnic challenges and early-life stress. Using pharmacological microglial depletion with disodium clodronate, we assessed ventilatory and behavioral responses to moderate (7%) and high (20%) CO_2 exposure, as well as sleep–wake architecture. In parallel, we examined females subjected to repeated cross-fostering as a model of early-life stress, assessing CO_2 responsiveness and LC microglial morphology in adulthood. Microglial manipulation in the LC did not alter ventilatory responses to CO_2, but attenuated escape behavior during the panicogenic stimulus; animals that received microinjections of the microglial-depleting drug clodronate disodium (CDS) exhibited increased time spent in NREM sleep during the dark phase. Notably, early-life stress did not potentiate panic-like ventilatory responses to CO_2 in females, suggesting a sex-specific dissociation between neuroimmune alterations and chemosensory reactivity. Together, these findings indicate that LC microglia are not a primary driver of CO_2-evoked ventilatory or panic-like behaviors in females but contribute to the regulation of sleep architecture, particularly NREM sleep. Moreover, the lack of early-life stress–induced potentiation of CO_2 responses supports a sex-specific dissociation between neuroimmune alterations and chemosensory reactivity, highlighting distinct regulatory mechanisms underlying stress, sleep, and interoceptive threat processing in females.

Keywords: neuroinflammation; glia; Sleep/awake cycle; Panic Disorder; Early life stress; Repeated Cross-Fostering, Hypercapnia

CAPÍTULO 1- CONSIDERAÇÕES GERAIS

1. Introdução

O locus coeruleus (LC) abriga a maioria dos neurônios produtores de noradrenalina (NA) no encéfalo e envia projeções noradrenérgicas generalizadas para todas as principais divisões do sistema nervoso central (SNC) (Schwarz & Luo, 2015; Poe, 2020). Essa região regula funções fundamentais, como o controle respiratório, por meio da quimiossensibilidade ao CO_2, e respostas emocionais e físicas durante episódios de estresse (Gargaglioni et al., 2010; Ripamonte et al., 2024).

Os neurônios noradrenérgicos do LC são essenciais para a regulação do estado de vigília/alerta (Berridge et al., 2012), apresentando atividade dependente do estado de despertar e sendo considerados um dos principais núcleos promotores da vigília (Samuels e Szabadi, 2008). Além dos neurônios, as células da glia tornam-se alvos relevantes em estudos que buscam compreender os mecanismos celulares envolvidos na regulação do ciclo sono-vigília. Nesse contexto, ainda não se verificou se a micróglia do LC está envolvida na modulação do ciclo sono-vigília ou se participa da quimiossensibilidade ao CO_2 de forma ciclo-dependente, uma vez que já foi demonstrado que essas células afetam a duração do sono, aumentando o tempo gasto em sono NREM durante a fase escura, e que são importantes para alterações sinápticas durante a vigília (Corsi et al., 2022).

As informações sobre o LC e sua micróglia tornam-se ainda mais relevantes quando consideradas à luz das evidências de que experiências adversas no início da vida modulam de forma duradoura a suscetibilidade a transtornos psiquiátricos, incluindo o transtorno do pânico (Heim & Nemeroff, 2001; Horesh et al., 1997; McEwen, 2000). Nesse contexto, modelos pré-clínicos que combinam separação materna precoce, hipersensibilidade ao CO_2 e alterações em circuitos de estresse, como aqueles baseados no protocolo de adoção cruzada repetida (ACR), demonstram que o estresse precoce está associado a aumento do volume corrente e hiperventilação em resposta a desafios hipercápnicos ao longo da vida (Battaglia et al., 2007; 2008; 2014;

D’Amato et al., 2011; Dumont et al., 2011; Leibold et al., 2016; Winter et al., 2017; Spiacci et al., 2018; Battaglia et al., 2019). Inseridos nesse cenário, o LC (pela sua posição central em circuitos noradrenérgicos de resposta ao estresse e de controle respiratório) e a micróglia local surgem como possíveis alvos para compreender como o estresse precoce interage com a quimiossensibilidade ao CO_2.

4. Discussion

This study demonstrates that LC microglia are not primary drivers of CO_2-evoked ventilatory or panic-like responses in females, but instead exert a modulatory influence on sleep regulation, particularly NREM sleep. Notably, early-life stress failed to potentiate CO_2 responsiveness, revealing a sex-specific dissociation between neuroimmune alterations and chemosensory reactivity. Together, these findings point to distinct regulatory mechanisms underlying stress-related neuroimmune signaling, sleep architecture, and interoceptive threat processing in females.

The absence of robust changes in the ventilatory response to hypercapnia following LC microglial depletion in females may be explained by the distributed organization of central chemoreception (Nattie, 2012). Several studies indicate that ventilation in response to elevated CO_2 arises from the integration of multiple chemosensitive sites located in the brainstem, including the retrotrapezoid nucleus (RTN), raphe nuclei, nucleus of the solitary tract, and the LC itself, such that lesioning or manipulating a single nucleus tends to attenuate, but not abolish, the ventilatory response (Guynet et al., 2015). Indeed, in rats, selective lesion of LC noradrenergic neurons with 6-hydroxydopamine reduces the ventilatory response to hypercapnia (7% CO_2), primarily through a decrease in tidal volume, without completely eliminating the increase in ventilation (Biancardi et al., 2008), demonstrating the existence of compensatory mechanisms in other chemosensitive neuronal populations. In this context, the fact that LC microglial depletion in females in the present study resulted in a modest reduction in V_T and, consequently, V_E during CO_2 exposure (when compared with the control group), without reaching statistical significance, suggests that microglia may contribute subtly to the modulation of this response but do not constitute a determining element of the ventilatory response. Another possibility is that in females, there is a small participation of LC in the CO_2-ventilatory response. Indeed, previous study from our laboratory showed that females did not exhibit LC noradrenergic

activation after CO_2 challenge (20% CO_2) (Ripamonte et al., 2024). In addition, females exhibited attenuated behavioral responsiveness to CO_2 compared with males (Ripamonte et al., 2024). Accordingly, sex-specific patterns of CO_2-evoked LC recruitment may contribute to the divergent behavioral responses observed between males and females. Such differences in LC activation are likely shaped, at least in part, by sex-dependent variation in the distribution of sex steroid hormone receptors within the LC, as well as by differences in circulating sex hormone levels.

Studies involving microglial manipulation in other regions implicated in respiratory control have predominantly shown modulatory and context-dependent effects that are more pronounced under challenge conditions, such as systemic inflammation (Lorea-Hernández et al., 2015; Marciante et al., 2024). Pharmacological modulation or microglial depletion in brainstem respiratory rhythm–generating regions alters respiratory pattern and plasticity, particularly during hypoxia or inflammatory states, but does not necessarily produce large changes in resting ventilation (Lorea-Hernández et al., 2015; Marciante et al., 2024). Thus, it is plausible that, in healthy females, localized depletion of LC microglia is compensated by other chemosensitive nuclei and by intrinsic neuronal mechanisms within the LC itself, resulting in discrete ventilatory changes in response to CO_2.

Depletion of LC microglia altered panic-like behavior during exposure to a panicogenic hypercapnic challenge (20% CO_2). Although the animals remained responsive to CO_2, the expression of active escape behaviors, specifically running episodes (maximum speed), was reduced, indicating that LC microglia contribute more to the regulation of escape responses than to the initial perception of the threat. These findings suggest that local microglial populations within the LC may play a role in organizing or sustaining defensive strategies. In this context, a recent study by Ripamonte et al. (2024) showed that disruption of noradrenergic signaling in the LC (LC–NA) also attenuates active escape behaviors during CO_2-induced panic-like responses, with animals displaying reduced locomotor escape. These findings indicate that proper LC–NA function is necessary for the expression of panic-related behaviors and suggest that LC microglia may modulate escape responses during panicogenic challenges.

Microglial depletion of the LC in females produced alterations in the sleep–wake cycle consistent with the modulatory role of these cells in sleep homeostasis, particularly via noradrenergic regulation. Animals treated with CDS in the LC exhibited an apparent prolongation of wakefulness during the light phase, with a concomitant reduction during the dark phase, although these effects did not reach statistical significance. At this point, it is important to highlight some limitations of our study, such as (1) the sample size of the groups and, consequently, (2) intra-group variability, which together may have limited the more precise detection of changes in the parameters investigated. This response pattern suggests destabilization of wakefulness, with greater fragmentation and unstable transitions between states, a finding that has been widely reported following systemic or regional microglial depletion (Seifinejad et al., 2025; Liu et al., 2021; Picard et al., 2023). Specifically, microglia-depleted mice exhibit reduced stable nocturnal wakefulness (dark phase) and increased wake→NREM and NREM→wake transitions, resulting in shorter, more unstable wake episodes (Seifinejad et al., 2025; Liu et al., 2021). In the present study, the apparent increase in wakefulness during the light phase may reflect an initial compensatory response or a localized effect within the LC, a key noradrenergic nucleus for wake consolidation, whose microglial modulation affects norepinephrine (NE) signaling (Seifinejad et al., 2025; Gu et al., 2023; Ma et al., 2024).

More robustly, LC microglial depletion significantly increased NREM sleep time during the dark phase and appeared to reduce it during the light phase. This pattern aligns with findings from multiple mouse studies in which microglial depletion (pharmacological or genetic) increases total NREM time and the number of NREM episodes specifically during the active period (dark phase), without major changes in REM sleep (Seifinejad et al., 2025; Liu et al., 2021; Picard et al., 2023). For example, microglial depletion with PLX5622 reduces wakefulness and increases NREM sleep during the dark phase, an effect attributed to wake instability, mediated by alterations in the anterior thalamic reticular nucleus (aTRN) and by ceramide production (Seifinejad et al., 2025; Liu et al., 2021). Similarly, selective microglial depletion in females increases both the duration and number of NREM episodes, with more pronounced effects during the dark phase (Picard et al., 2023). These data suggest that LC microglia,

through suppression of NE signaling or the release of mediators such as adenosine, TNF-α, or ceramide, favor NREM consolidation during periods of higher sleep homeostatic pressure (Gu et al., 2023; Ma et al., 2024; Pinto et al., 2024).

The ventilatory responses of females subjected to the repeated cross-fostering (RCF) protocol showed a significant increase in ventilatory response to hypercapnia, a pattern similar to that observed in the control group. These findings suggest that postnatal stress induced by RCF did not alter ventilatory sensitivity to CO_2 in female mice. These data diverge from those reported by D'Amato et al. (2011) and Luchetti et al. (2021), who observed exacerbated hyperventilation in male rats and C57BL/6J mice subjected to the same environmental stress during exposure to 6% CO_2 from infancy to adulthood. The discrepancy between studies may be attributed to important methodological differences: the present experiment was conducted exclusively in females and used a higher CO_2 concentration (20%), which may influence the ventilatory response. In addition, sex differences in neurophysiological and hormonal responses to stress and hypercapnia are well recognized and may contribute to the observed divergences (Ripamonte et al., 2024). In the present study, females subjected to the RCF did not exhibit a behavioral response to CO_2, in contrast to previous findings. Those earlier studies demonstrated that disturbances in the early postnatal environment, modeled by the cross-fostering protocol, produce sex-dependent effects on the adult phenotype. While male mice develop depression-like characteristics, females exhibit increased sensitivity to rewarding stimuli (Di Segni et al., 2016).

The present findings demonstrate that the RCF protocol applied during early life did not induce long-lasting alterations in the morphology of LC microglial cells in adult females. These results can be interpreted in light of the literature describing baseline sex differences in microglial number, morphology, and developmental maturation trajectories, as well as the existence of critical windows of susceptibility to environmental and inflammatory challenges (Schwarz et al., 2012; Lenz & McCarthy, 2015). Evidence indicates that during early developmental stages, males have more microglia with an amoeboid phenotype than females, whereas females show a relative increase in microglial number only at later stages, such as adolescence and adulthood (Schwarz et al.,

2012; Hanamsagar & Bilbo, 2017). In this context, females have been shown to exhibit greater resilience to the long-term consequences of neonatal immune activation, as early-life immune challenges tend to induce persistent alterations in males, whereas comparable effects are absent or attenuated in females in adulthood (Bilbo et al., 2011; Hodgson & Knott, 2002). These findings suggest that males and females exhibit fundamentally distinct responses to immune activation during early life, with females showing a greater capacity for adaptation or recovery following early neuroimmune perturbations. However, it is important to acknowledge that the interpretation of our findings is limited by the exclusive inclusion of females in the present analyses. Given the evidence for sex differences in microglial dynamics and responses to early-life stressors, the inclusion of males in future studies will be essential for a more comprehensive understanding of the effects of early-life stress on LC microglia, enabling the identification of potential windows of increased male vulnerability and clarifying whether the absence of morphological alterations observed in females reflects a sex-specific pattern of resilience or whether distinct outcomes would emerge in males exposed to the same experimental protocol.

Several preclinical studies have investigated the long-term impact of critical early-life experiences on responses to motivational and aversive stimuli, highlighting the complexity of interactions between early stress and adult behavior (Nelson et al., 2009; Pechtel & Pizzagalli, 2011; Ventura et al., 2013; Di Segni et al., 2016; Novick et al., 2018). Furthermore, both the incidence of and susceptibility to stress-related disorders show marked differences between men and women. Studies indicate that women are at least twice as likely as men to develop psychopathologies involving altered processing of motivational stimuli, such as depression and anxiety (Kessler et al., 2003; Altemus, 2006; Valentino et al., 2012). In contrast to these patterns, the present data indicate that, in the panicogenic response to CO_2, female mice did not exhibit changes relative to the control group, suggesting that ventilatory and behavioral sensitivity associated with early-life stress may be modulated differently by sex and stimulus type. This sex-specific resilience, or decoupling, in CO_2-induced responses aligns with evidence that female rodents often exhibit distinct ventilatory chemosensitivity and active coping strategies (e.g., increased rearing) during interoceptive threats, potentially driven by differential recruitment of noradrenergic systems, such as

the locus coeruleus (Ahlbrand et al., 2024). Future studies examining stimulus-specific interactions between early-life stress, sex hormones, and brainstem circuits will be crucial to elucidate these divergent trajectories and their translational relevance to panic disorder vulnerability in women.

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