UNIVERSIDADE FEDERAL DO PAMPA

GABRIELA JAQUES SIGARAN

EFEITOS DE UMA SESSÃO DE EXERCÍCIO FÍSICO AGUDO NA MODULAÇÃO DA MEMÓRIA DE RECONHECIMENTO DE RATAS

Uruguaiana, RS

2023

GABRIELA JAQUES SIGARAN

EFEITOS DE UMA SESSÃO DE EXERCÍCIO FÍSICO AGUDO NA MODULAÇÃO DA MEMÓRIA DE RECONHECIMENTO DE RATAS FÊMEAS

Trabalho de Conclusão de Curso apresentado ao Curso de Fisioterapia da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Bacharel em Fisioterapia.

Orientadora: Profa. Dra. Pamela Billig Mello Carpes

Coorientadora: Ma. Karine Ramires Lima

Uruguaiana, RS

2023

Ficha catalográfica elaborada automaticamente com os dados fornecidos
pelo(a) autor(a) através do Módulo de Biblioteca do
Sistema GURI (Gestão Unificada de Recursos Institucionais) .

S574e Sigaran, Gabriela Jaques
Efeitos de uma sessão de exercício físico agudo na modulação da memória de reconhecimento de ratas / Gabriela Jaques Sigaran.
55 p.

Trabalho de Conclusão de Curso(Graduação)-- Universidade Federal do Pampa, FISIOTERAPIA, 2023.
"Orientação: Pamela Billig Mello Carpes".

1. Memória. 2. Exercício físico. 3. Diferença de gênero. 4. Hipocampo. 5. Neurociência. I. Título.

GABRIELA JAQUES SIGARAN

EFEITOS DE UMA SESSÃO DE EXERCÍCIO FÍSICO AGUDO NA MODULAÇÃO DA MEMÓRIA DE RECONHECIMENTO DE RATAS FÊMEAS

Trabalho de Conclusão de Curso apresentado ao Curso de Fisioterapia da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Bacharel em Fisioterapia.

Trabalho de Conclusão de Curso defendido e aprovado em: 06/11/2023

Banca examinadora:

_____

Profa. Dra. Pâmela Billig Mello Carpes

Orientadora

UNIPAMPA

_____

Ft. Ana Carolina de Souza da Rosa

UNIPAMPA

_____

Profa. Dra. Liane da Silva de Vargas

UNIPAMPA

“Acontece com as pessoas. Não sei se vivemos para ser felizes, sei que não vivemos sem aprender, e que quando deixamos de fazê-lo estamos mortos, ainda que continuemos caminhando”

Ivan Izquierdo

AGRADECIMENTOS

Desejo expressar minha sincera apreciação a todos aqueles que tiveram um papel fundamental na minha jornada acadêmica: em primeiro lugar, quero expressar minha gratidão à minha família, que sempre me apoiou de maneira incansável durante toda a minha trajetória acadêmica. Seu apoio e encorajamento foram essenciais, tornando-se possível cada passo desse longo caminho. À minha orientadora Pâmela, sou grata pela inspiração como ser humano e profissional desde as primeiras aulas de Fisiologia Humana I. Sua dedicação incansável e paciência, demonstradas ao longo do tempo, foram inestimáveis, além de me oferecer a oportunidade de me envolver no mundo da ciência. Também gostaria de agradecer ao programa PET (Programa de Educação Tutorial) de Fisioterapia, que desempenhou um papel crucial na minha formação acadêmica desde o primeiro mês de graduação. As oportunidades de aprendizado proporcionadas foram fundamentais para o meu crescimento. Ao grupo de pesquisa que fiz parte, Grupo de Pesquisa em Fisiologia (GPFis), expresso minha gratidão pela experiência enriquecedora que proporcionaram durante minha graduação. O compartilhamento de conhecimento e o apoio ao meu desenvolvimento pessoal e profissional foram inestimáveis, em especial a minha coorientadora Karine. Aos colegas e amigos que compartilharam comigo essa jornada, estendo meu sincero agradecimento. Sua amizade e encorajamento foram fundamentais. Por fim, à UNIPAMPA, agradeço a oportunidade de conhecer pessoas incríveis e adquirir experiências valiosas que contribuíram significativamente para minha formação pessoal e profissional, guardarei na memória todos os momentos vividos no Campus Uruguaiana.

APRESENTAÇÃO

Neste Trabalho de Conclusão de Curso (TCC), nosso objetivo principal é aprofundar a compreensão dos efeitos do exercício físico (EF) agudo na persistência da memória de reconhecimento de objetos (RO) em ratas fêmeas. O TCC está estruturado no formato de artigo científico, no qual é apresentado em três partes essenciais: a introdução, o desenvolvimento e a discussão. Na introdução, apresentamos o contexto e a importância da pesquisa, destacando a necessidade de compreender os efeitos do EF agudo na memória de RO em ratas fêmeas. No desenvolvimento, detalhamos o estudo de experimentação animal, que foi conduzido após aprovação pela Comissão de Ética para Uso de Animais (CEUA), conforme a Resolução 029/2021 (ANEXO I). Exploramos os procedimentos, métodos e resultados obtidos, fornecendo uma análise aprofundada dos efeitos observados. Por fim, na discussão, resumimos as descobertas do estudo e destacamos sua relevância relacionando com achados prévios de outros estudos. Além disso, ao encerrarmos este documento, fornecemos uma lista completa das referências bibliográficas utilizadas tanto nas seções iniciais quanto nas finais. É relevante ressaltar que o artigo deste TCC está em conformidade com os padrões de publicação da revista *Brain Research*, encontrados no ANEXO II, no qual será submetido para publicação em momento futuro.

LISTA DE FIGURAS

SUMÁRIO

ACUTE PHYSICAL EXERCISE ENHANCES MEMORY PERSISTENCE IN FEMALE RATS

Gabriela Jaques Sigaran[a], Karine Ramires Lima[a], Ben-Hur Souto das Neves[a], Marisele dos Santos Soares[a], Murilo Ricardo Sigal Carriço[b], Rafael Roehrs[b], Pâmela Billig Mello-Carpes[a]*

[a]Physiology Research Group, Stress, Memory and Behavior Lab, Federal University of Pampa, Uruguaiana, RS, Brazil
[b]Laboratory of Environmental Chemical and Toxicological Analysis, Federal University of Pampa, Uruguaiana, RS, Brazil

*Corresponding author
pamelacarpes@unipampa.edu.br

Highlights

- Acute physical exercise improves memory persistence in female's rats.
- Acute physical exercise increases noradrenaline levels in the hippocampus of female rats.
- The effects of acute physical exercise on memory are similar in both males and females.

Abstract

Memory is a complex cognitive process with distinct stages, such as acquisition, consolidation, and retrieval. The hippocampus plays a crucial role in memory consolidation and retrieval. Physical exercise (PE) has been shown to enhance memory and cognitive functions, but the available research is mainly developed with males. So, there is limited knowledge about acute PE's effects on females' memory. This study aimed to investigate the impact of acute PE on memory in female rats and explore potential sex differences in PE memory modulation. Forty-two female Wistar rats were subjected to a novel object recognition (NOR) task, with half of them undergoing a single session of 30 minutes of PE after the learning session (memory

acquisition). Behavioral assessments showed that acute PE improved memory persistence in female rats, with increased discrimination of novel objects. Biochemical analysis revealed elevated noradrenaline levels in the hippocampus following acute PE. Notably, the positive effects of acute PE on memory were similar between male and female rats. These findings suggest that acute PE can enhance memory in female rats and underscore the importance of considering sex differences in cognitive research. PE may offer a non-invasive strategy to promote cognitive health in both males and females.

Keywords: Object Recognition Memory. Running. Hippocampus. Gender differences.

1 Introduction

Memory is a complex process that unfolds in three fundamental stages (Squire, 2004; Izquierdo, 2011). The first stage is acquisition, which corresponds to the phase when information is initially obtained – it marks the first encounter with the new information. Next is consolidation, the storage stage, during which complex biochemical processes necessary for information to be stably stored in our memory system take place. Lastly, retrieval can occur – the moment when information is recalled and accessed when needed (Squire & Kandel, 2003). It is important to highlight that the hippocampus, a crucial area of the brain, plays a fundamental role in memory consolidation and retrieval (Squire & Kandel, 2003).

After consolidation, some memories can persist longer than others. Interventions applied following memory acquisition, at the beginning of the consolidation phase, have effectively prolonged information retention and improved memory consolidation (Bekinschtein et al., 2010; Katche et al., 2016). In our research group, we have investigated how a single session of physical exercise (PE) performed after acquiring a novel object recognition (NOR) task affects the duration of this memory in male rats. Our results indicate that male rats subjected to 30 minutes of treadmill exercise maintained memory longer than the control group (Vargas et al., 2017; 2020). The primary mechanisms involved in acute PE include the activation of dopaminergic D1/D5 (Vargas et al., 2020; Lima et al., 2021) and beta-adrenergic (Vargas et al., 2017) receptors in the hippocampus, PKA activation (Lima et al., 2021), in addition to a confirmed dependency on *locus coeruleus* activation (Lima et al., 2023).

Studying acute PE is crucial to understanding the immediate and transient effects that physical activity can have on different physiological and cognitive processes (Lima et al., 2021). While chronic PE refers to regular and prolonged practice over time, acute PE represents a single session of intense and immediate activity (Smith et al., 2013). Both, acute and chronic PE are important to brain and cognition, it increases levels of brain-derived neurotrophic factor (BDNF), enhances neurogenesis, and promotes synaptic plasticity (Oliff et al., 1998; Lee and Son, 2009; Vaynman et al., 2006; Venezia et al., 2019) – essential factors contributing to memory improvement.

According to our findings, other studies have also demonstrated that acute PE positively regulates brain processes and enhances memory (Bouchet et al., 2017; Diederich et al., 2017). However, despite extensive research on the effects of PE on memory, especially its significant role in enhancing persistence, there remains limited knowledge about its effects on females. In this sense, it is crucial to investigate possible sexual dimorphism, as sex differences in the central nervous system can significantly impact various biological activities and behaviors, such as stress responses and cognitive performance (Bangasser, 2014; Yagi & Galea, 2019).

This study aims to investigate and comprehend the impact of acute PE on memory in female rats. Here, we examine how a single session of PE, conducted after NOR memory acquisition, influences the duration and retention of information. Moreover, our research aims to explore whether sex differences in the central nervous system play a significant role in the effects of acute PE on memory. Through our analyses, we intend to explore how acute PE can affect the memory retention capacity in female rats. By doing so, we aim to contribute to a more comprehensive understanding of the mechanisms involved in this process.

2 Materials and methods

Forty-two female Wistar rats (3 months old, 170–230 g) were obtained from the University of Santa Maria Central Vivarium (RS, Brazil) and used for this study. The animals were housed in individual cages (n = 4 per cage) and maintained under controlled laboratory conditions, including a 12-hour light/dark cycle (with the light cycle starting at 7 AM), a temperature of 23 ± 2 °C, and relative air humidity of 50 ± 10%. Water and food were provided without restriction. All experimental procedures followed the “Principles of Care of Laboratory Animals” (NIH Publication No. 80-23, 1996).

2.1 Behavioral experiment

We conducted a behavior experiment to investigate the effects of acute PE on recognition memory in female rats. The animals were randomly assigned to one of both experimental groups: (i) control (n = 12) or (ii) acute PE (n = 13). The control group did not exercise and were solely subjected to the NOR task. The acute PE group rats underwent treadmill habituation and a maximal work rate test before initiating the NOR task protocol.

All animals were habituated (20 minutes per day for 4 days), trained (5 minutes on one day), and tested (24 hours and 7 days after training) in the NOR task. After training in the NOR, rats in the acute PE group were subjected to a single 30-minute PE session in a treadmill built for rodents, performed at an intensity level of 60-70% of their previously measured maximal work rate.

For the NOR test sessions, conducted 24 hours and 7 days after training, different sets of objects were used, each containing a novel and a familiar object. Following each NOR test, the animals underwent behavioral control tests: the open field (OF) to assess locomotion and exploration and the elevated plus maze (EPM) to evaluate the animal's anxious behavior.

2.2 Biochemical experiment

In a biochemical analysis, we assessed the influence of PE on noradrenaline and dopamine levels in the hippocampus of female rats. The animals were randomly separated into three experimental groups as follows: (i) naive (n = 5), (ii) control (n = 5), and (iii) acute PE (n = 9).

The naive group consisted of wild animals that underwent no specific protocol. As mentioned earlier, the acute PE group underwent treadmill habituation and a maximal work rate test 5 days before commencing the NOR protocol. All animals, except those in the naive group, were habituated for 20 minutes per day over 4 days and then trained for 5 minutes on one day in the NOR task.

Following the NOR training, rats in the acute PE group underwent a single PE session for 30 minutes, according to the intensity of 60-70% of their previously measured maximal work rate. The rats were euthanized thirty minutes after the exercise session or in equivalent time for other groups, and the hippocampus was dissected for subsequent measurement of dopamine and noradrenaline levels using high-performance liquid chromatography (HPLC).

2.3 Acute physical exercise (PE)

Acute PE was conducted using an ergometric treadmill designed for rodents (Insight Ltda São Paulo, Brazil). The animals were habituated to the treadmill for four days to minimize the potential effects of novelty and/or stress. During the first two days, the animals were introduced to the treadmill at a speed ranging from 2 to 5 m/min for 10 minutes. On the third and fourth day of habituation, the treadmill speed was increased to 8 m/min.

Following the habituation phase, the animals underwent a maximal work rate. For this test, the animals were placed on the treadmill at an initial speed of 1 m/min, with a subsequent increase of 5 m/min every 3 minutes. The point of exhaustion, indicated by the maximum speed achieved by each animal, was recorded.

The acute PE was conducted following the NOR training at an intensity level of 60-70% of the previously measured maximal work rate, and it lasted for 30 minutes (Vargas et al., 2017; Vargas et al., 2020; Lima et al., 2021).

2.4 Novel object recognition (NOR)

We employed the NOR task to assess recognition memory, a well-established method (adapted from Ennaceur & Delacour, 1988). This task capitalizes on the natural tendency of animals to explore novel objects more than familiar ones within a familiar context. Various objects were used in the experiments, represented by the following letters: A - magic cube, B - plastic cup, C - circular can, and D - Lego pieces. The animals did not exhibit any preference for a specific object in a pilot study.

The experimental setup consisted of a wooden box with a transparent glass front measuring 60cm x 60cm, in which the animals underwent a 20-minute daily habituation period for four consecutive days. Following the habituation period, two distinct new objects (A and B) were introduced in the box, allowing the animals to explore them freely for a 5-minute training session. Memory assessment took place 24 hours after the training session; we introduced a familiar and a novel object (A and C). To gauge the persistence of memory, an additional test session was conducted 7 days after the training session (objects A and D). During these sessions, the animals were again presented with the opportunity for free exploration of the objects. The objects were consistently placed in the same positions during all the test sessions. Consolidation or persistence is observed by greater exploration of the new object to the familiar one. In previous studies, in control male rats, this protocol allows memory consolidation, but not persistence, of memory (Lima et al., 2019; Neves et al., 2018).

To gauge the duration of exploration, a video camera positioned above the testing area captured the animals' activities within the unoccupied experimental space. Subsequently, an impartial experimenter, unaware of the study's details, employed a manual stopwatch to measure the time allocated to exploration. Exploration was characterized by the animals' engagement in activities such as touching or sniffing objects using their front paws and noses. Behaviors like sitting on the objects or turning around them were not considered exploratory actions. To prevent any potential olfactory biases, thorough cleaning was done on the objects and the apparatus using 70% alcohol.

2.5 Open field (OF)

To ensure that the exploratory and locomotor activity of each animal remained consistent and did not exert any influence during subsequent tests, we conducted a behavioral control protocol for the OF after the memory test sessions. To evaluate their exploratory and locomotor activity, the animals were placed in an OF apparatus, which consisted of a white-painted wooden box measuring 60 cm x 60 cm and divided into 12 equal quadrants by black painted lines. The behavior of the rats was recorded by a video camera positioned over the apparatus in an empty experimentation room. We recorded measurements of animal rearing and crossings during a 5-minute observation period, according to Hall & Ballachey (1932).

2.6 Elevated plus maze (EPM)

To assess anxious behavior, the animals were placed on the EPM platform, and we recorded the number of entries and time spent in both open and closed arms during a 5-minute session. A video camera above the apparatus captured the rats' behavior in an empty experimental room. Anxious behavior was indicated if the animals spent more time in the closed arms than in the open arms, according to Pellow et al. (1985).

2.7 Quantification of noradrenaline and dopamine

Dopamine and noradrenaline levels were quantified using HPLC. For this, the bilateral hippocampi were rapidly dissected from the brain on an ice-cold surface. The homogenate, containing acetonitrile and 0.5 HCl, underwent centrifugation at 10,000 RPM for 10 minutes at a controlled temperature (4ºC). The resulting supernatant was filtered and then transferred to an amber vial, kept at 4ºC for 2 hours until analysis by HPLC-DAD.

Quantitation analysis was carried out using an A-10 AltusTM solvent and sample module and an A-10 AltusTM PDA detector, both PerkinElmer. The system

had a 5 µm Inertsil ODS-3 column (4.6 × 250 mm) and a guard cartridge. The compounds were separated using the mobile phases of water acidified with phosphoric acid to pH 2.56 and methanol, with an injection volume of 20 µL.

Compounds were identified by comparing their retention time and DAD spectrum with reference standards from the analytical curve. The analytical curve was constructed with noradrenaline (198 nm) and dopamine (198 nm) and comprised 7 points at concentrations of 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 mg/L for each compound.

2.8 Comparison of the effects of acute PE on memory in male and female rats

We meticulously compared memory data between females and males, drawing from a previously published experiment (Lima et al., 2021) where an identical experimental protocol was employed. In both studies, we adhered to the same rigorous experimental design involving adult Wistar rats to scrutinize the impact of acute PE on NOR memory. The uniformity extended beyond the experimental design to encompass the environmental settings, objects utilized, experimental conditions, experimenters involved, and the meticulous analysis of data.

2.9 Statistical analysis

We assessed the data for normal distribution using the Shapiro–Wilk test. The NOR results were expressed as the percentage of total time spent exploring each object. We employed a one-sample Student's t-test, assuming a 50% theoretical mean, to evaluate whether animals spent more than 50% of their total exploration time on the novel object, indicating memory retention. Additionally, we conducted between-group comparisons by converting NOR data into a discrimination index (DI = [(t novel - t familiar)/(t novel + t familiar) x 100]; where 't' represents the time spent exploring the object). The data were analyzed using a t-test. A higher DI implies a greater ability to discriminate between objects and spend more time exploring the novel object.

Control analyses, including total object exploration time, OF, and EPM results, underwent a t-test or Mann-Whitney test according to normality and were employed

as control parameters. HPLC results were normalized based on the mean percentage relative to the control, and the data were analyzed in triplicate and compared using Kruskal-Wallis, followed by Dunn’s multiple comparison tests. One-sample Student's t-tests were applied for males, assuming a 50% theoretical mean. We employed a two-way ANOVA with Sidak's multiple comparisons test to compare males and females. Significance was established at $P < 0.05$ for all analyses.

3 Results

3.1 A treadmill exercise session improved memory persistence in females

We evaluated the acute PE effects on NOR memory (**Figure 1A**). After the habituation period, all animals were submitted to a NOR training session, which served as a learning experience. During this 5-minute training session, they explored two novel objects, denoted as A and B. Memory assessments were conducted at two distinct time points: 24 h and 7 days post-training, to assess memory consolidation and persistence, respectively.

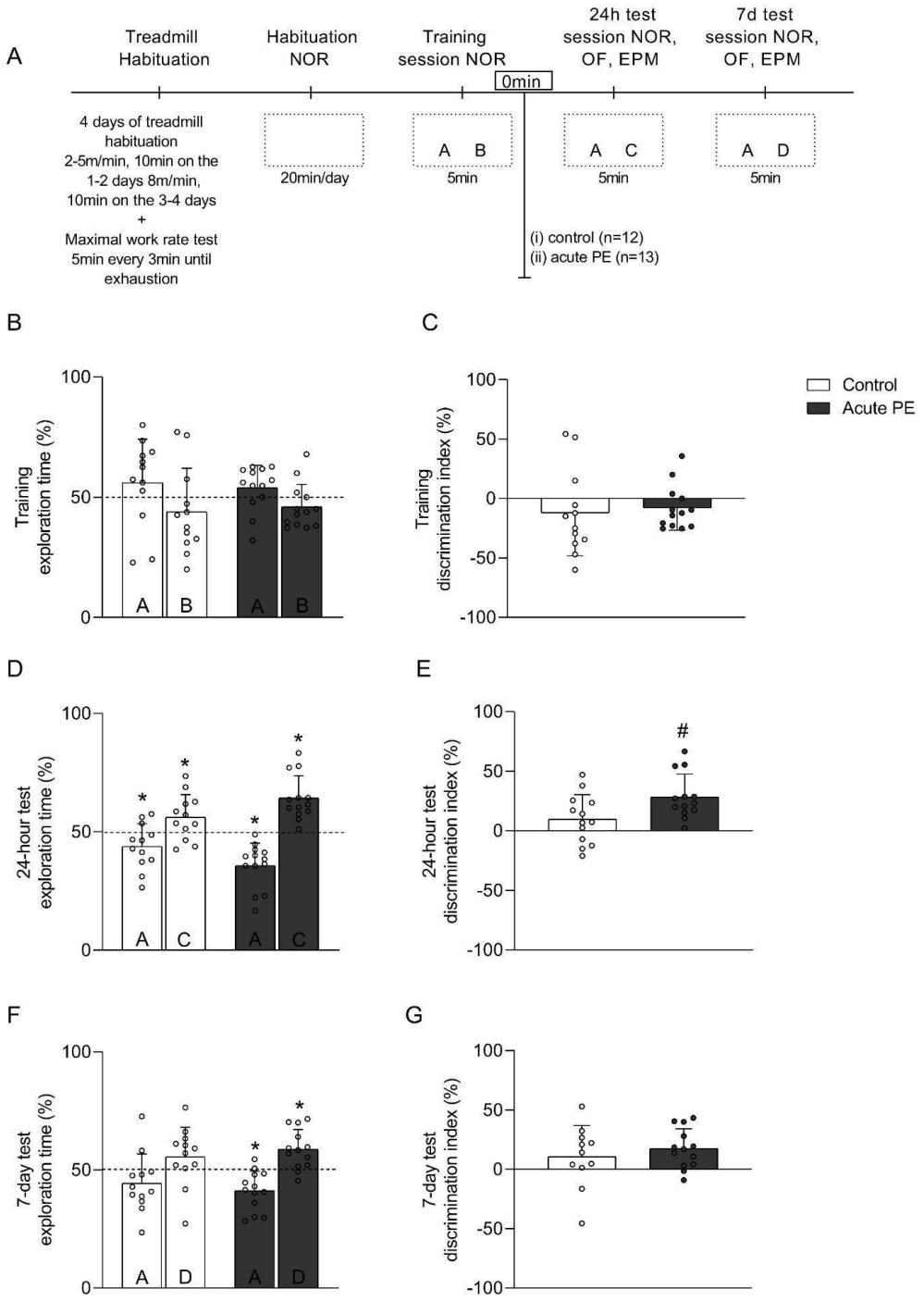

Figure 1 Acute physical exercise (PE) enhances memory persistence in female rats over time. (A) Experimental design. NOR = novel object recognition; OF = open field; EPM = elevated plus maze. (B, D, and F) Memory assessment based on the percentual of total exploration time spent in each object in NOR. (C, E, and G) Group comparisons using the novel object's Discrimination Index (DI). Data are expressed

as mean ± standard deviation (SD). * $P < 0.05$ in One-sample t-test; # $P < 0.05$ in t test (n = 12, to control; n = 13, to acute PE).

During the training session, animals in the control ($P = 0.2703$, $t = 1.161$, $df = 11$; **Figure 1B**) and acute PE ($P = 0.1498$, $t = 1.539$, $df = 12$; **Figure 1B**) groups exhibited similar exploration times for the objects – about 50% of total exploration time. Similarly, when evaluating the DI, no difference was observed between the groups ($P = 0.7183$, $t = 0.3652$, $df = 23$; **Figure 1C**). This outcome was expected since both objects were unfamiliar to them at this experiment stage.

During the 24-hour test, animals from both the control ($P = 0.0490$, $t = 2.212$, $df = 11$; **Figure 1D**) and acute PE ($P = 0.0002$, $t = 5.409$, $df = 12$; **Figure 1D**) explored the novel object for more than 50% of the total exploration time. However, when assessing the DI for this time, animals that underwent acute PE demonstrated significantly greater discrimination toward the new object than the control group ($P = 0.0237$, $t = 2.415$, $df = 23$; **Figure 1E**). These results imply that while both groups exhibited memory consolidation, the acute PE group displayed superior object discrimination.

In the 7-day test, the control group explored about 50% of the total exploration time for each object ($P = 0.1445$, $t = 1.571$, $df = 11$; **Figure 1F**), indicating a potential decline in memory retention. However, in contrast, the acute PE ($P = 0.0026$, $t = 3.79$, $df = 12$; **Figure 1F**) group explored the new object for more than 50% of the total exploration time. Nevertheless, there was no significant difference between the groups in the DI at this time point ($P = 0.4465$, $t = 0.7752$, $df = 23$; **Figure 1G**). These findings suggest that our protocol is sufficient to induce memory consolidation but not persistence in control female rats and that acute PE after learning improves memory retention in this experimental group.

We conducted control tests following the memory assessments to ensure that memory test behavior remained unaffected by exploration, exploratory, or anxiety behavior disparities. Our findings revealed no significant differences between the groups concerning these evaluated parameters ($P > 0.05$); **Table S1 in the supplementary material**).

3.2 A treadmill exercise session increases the levels of noradrenaline in the hippocampus compared to naïve group

In addition to the behavioral experiments, some animals, immediately after acute PE or at an equivalent time to the other groups, were euthanized and had their hippocampus removed. Samples were subjected to analysis using HPLC (**Figure 2A**).

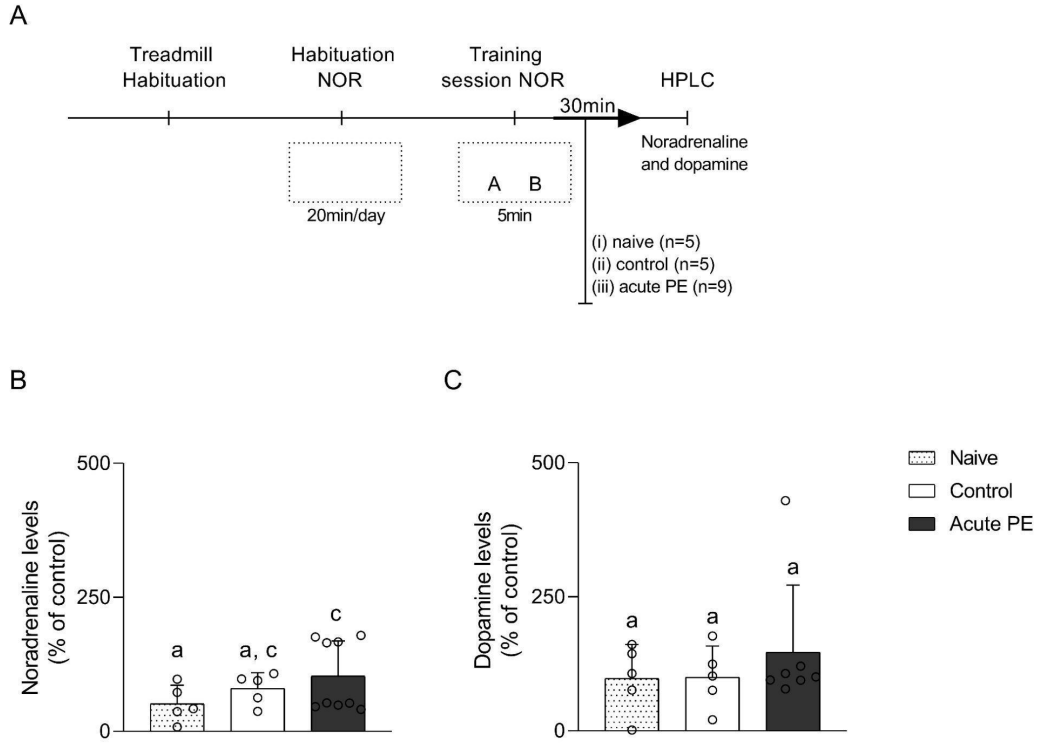

Figure 2 Acute physical exercise (PE) increases the noradrenaline levels in the hippocampus but not increase the dopamine levels. (A) Experimental design. NOR = novel object recognition. (B) Noradrenaline and (C) dopamine neurotransmitter levels in the hippocampus of female rats. The data were compared by calculating the mean percentage relative to the control group and are presented as mean ± standard deviation (SD). Significant differences were indicated by distinct letters ($P < 0.05$) as determined by the Kruskal-Walli's test, followed by Dunn's multiple comparison tests. The analysis was performed in triplicate (n = 5, to naïve; n = 5, to control; n = 9, to acute PE).

Hippocampal noradrenaline levels differed between groups (P = 0.0122, $K_{(3,57)}$ = 8.820; **Figure 2B**). We observed a significant increase in noradrenaline concentration in samples after acute PE compared to the naïve group (P = 0.0113, **Figure 2B**). However, no differences were observed in the comparison's naïve vs control (P = 0.0876; **Figure 2B**) and control vs acute PE (P > 0.9; **Figure 2B**). About the dopamine hippocampal levels, we did not observe any statistically significant differences (P = 9.194, $K_{(3,51)}$ = 0.1681; **Figure 2C**).

3.3 The effect of acute PE did not differ between males and females

In addition to the analyses conducted on females in this study, we compared memory results with data from a previous experiment that used the same NOR memory test protocol and acute PE in male rats, as detailed in a previous article by our research group (Lima et al., 2021).

In both experiments, the animals underwent the NOR task protocol, which included habituation sessions to the apparatus, training sessions, tests conducted 24 hours after training, and tests conducted 7 days later. After training, animals in the acute PE group engaged in a 30-minute treadmill session immediately after training. Conversely, animals in the control groups did not undergo any physical exercise interventions (**Figure 3A**). Overall, it is evident that the behavior and memory performance between males and females are similar.

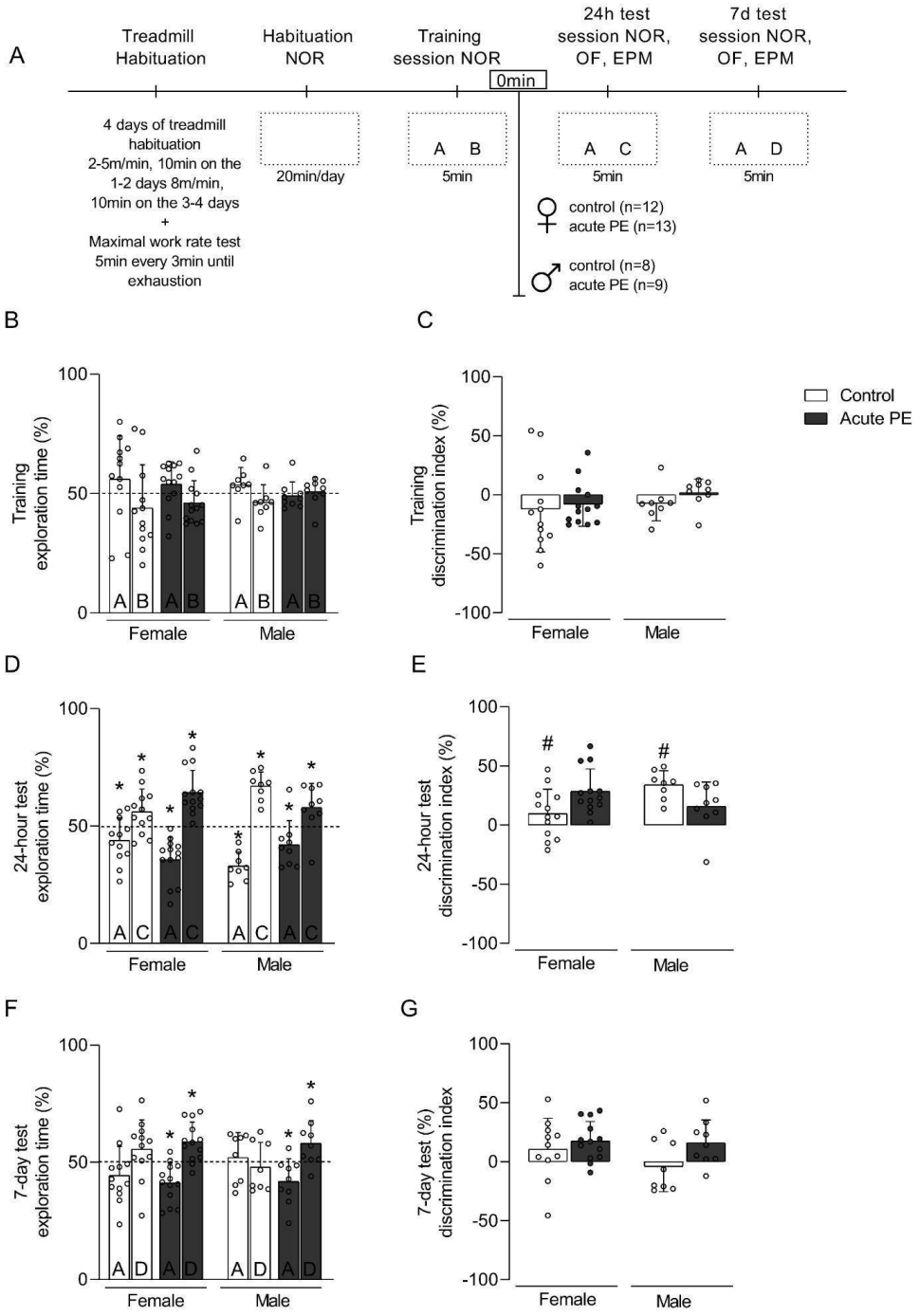

Figure 3 Acute physical exercise (PE) similarly enhances memory persistence in female and male rats. (A) Experimental design. NOR = novel object recognition. (B, D, and F) Memory assessment based on the percentual of the total exploration time spent in each object. (C, E, and G) Group comparisons using the novel object's Discrimination Index (DI). Data from males were collected in previous experiments (Lima et al., 2021). The protocols used were the same. Data are expressed as mean ± standard deviation (SD). * P < 0.05 in One-sample t-test; # P < 0.05 two-way

ANOVA followed by Sidak's multiple comparisons test (females: n = 12, to control; n = 13, to acute PE; males: n = 8, to control; n = 9, to acute PE).

During the training session, male rats in both groups explored the unknown objects for about 50% of the total exploration time (P = 0.2011, t = 1.411, df = 7, control; P = 0.6513, t = 0.4694, df = 8, acute PE; **Figure 3B**), as females according to data previously mentioned in the previous session. No interaction (P = 0.7396, $F_{(1,38)}$ = 0.1121; **Figure 3C**) or effects of PE intervention (P = 0.3768, $F_{(1,38)}$ = 0.798; **Figure 3C**) or sex (P = 0.3327, $F_{(1,38)}$ = 0.9628; **Figure 3C**) were found.

In the 24 NOR test, as females, male rats in both control and acute PE groups exhibited memory consolidation by exploring the novel object for over 50% of the total exploration time (P < 0.0001, t = 8.018, df = 7, control; P = 0.0481, t = 2.330, df = 8, acute PE; **Figure 3D**). In DI, two-way ANOVA revealed no significant differences for PE intervention (P = 0.9599, $F_{(1,39)}$ = 0.0025; **Figure 3E**) or sex (P = 0.3246, $F_{(1,39)}$ = 0.9955; **Figure 3E**), but interaction was observed (P = 0.0031, $F_{(1,39)}$ = 9.913; **Figure 3E**). Notably, in control groups, the DI of the female was significantly lower than that of the male (P = 0.0312; **Figure 3E**), suggesting that males may exhibit better discrimination of the NOR.

In the 7 days NOR test, we assessed memory persistence. Similar to females, males in the control group showed physiological forgetfulness, exploring novel and familiar objects for a similar time (P = 0.6, t = 0.5491, df = 7; **Figure 3F**). Likewise, male animals subjected to acute PE exhibited memory persistence, exploring the novel object longer (P = 0.0363, t = 2.512, df = 8; **Figure 3F**). In DI, two-way ANOVA revealed no significant interaction between groups (P = 0.3178, $F_{(1,37)}$ = 1.025; **Figure 3G**) and no effect of sex (P = 0.2336, $F_{(1,37)}$ = 1.466; **Figure 3G**). However, a significant effect of intervention was observed (P = 0.0491, $F_{(1,37)}$ = 4.141; **Figure 3G**). No differences were found in post-hoc multiple comparisons.

In summary, this analysis suggests that both males and females exhibit memory consolidation and persistence following acute PE, implying that this intervention may benefit individuals of both sexes.

4 Discussion

In this study, we investigated the effects of acute PE on the memory of female rats, with a particular focus on NOR memory. We utilized the NOR task to assess the rats' memory at two different time points after training: 24 hours and 7 days. The results revealed that the group of rats subjected to acute PE exhibited a significant improvement in memory persistence compared to the control group. This effect was evident through the increased discrimination regarding the new object during the test session conducted 7 days after training. The findings of this study indicate that a single session of PE can potentially enhance memory persistence in female rats.

The improvement in memory persistence can be partially understood through the effects of exercise on the release of norepinephrine, a neurotransmitter that plays a fundamental role in memory consolidation (Sara, 2009). Biochemical analysis revealed a significant increase in norepinephrine levels in the hippocampus of rats after an acute session of PE compared to the naïve group but not the control group. According to a previous study (Titualaer et al., 2021), we hypothesized that learning in the NOR task itself might have contributed to the increased neurotransmitter levels. However, the naïve and control did not differ in their norepinephrine levels, suggesting a more prominent role of norepinephrine in the acute response to PE on memory. Surprisingly, no significant differences in dopamine levels were observed in our study, differing from previous findings in male rats (Vargas et al., 2020). Using more robust techniques, like microdialysis or c-fos in immunohistochemistry, in future studies would be valuable for drawing comprehensive conclusions about the catecholaminergic involvement of acute PE in the female hippocampus.

To compare male and female rats, we analyzed the results obtained in a previous study, which has already been published, using the same methodology used for males. This analysis was then compared with the results observed in females in the present study, a methodology already established in the literature (Fulvio, J.M., Akinnola, I., & Postle, B.R., 2021). An interesting finding from this study is that the positive effects of acute PE on memory seem to be consistent across both males and females. Both groups exhibited enhanced memory persistence after PE, indicating that this approach might be equally effective for both sexes. This finding aligns with other research, emphasizing that gender differences in brain function responses can

be subtle (Bangasser, 2014). However, confirming this in practice is crucial, since many physiological aspects present sexual dimorphism, underscoring the significance of including both males and females in studies investigating the effects of PE on memory. Our study aligns with research that underscores the importance of considering sex and biological variability in neuroscience (Shansky & Woolley, 2016; Choleris et al., 2018).

It is well-established that female hormones influence hippocampal memory; however, NOR memory appears to occur in female rats regardless of their cycle phase (Tuscher et al., 2015), although specific emphasis on memory persistence in this context is lacking. In our study, we chose not to assess the estrous cycle, a potential limitation, but a decision to better align with a more ecological approach. Furthermore, research has demonstrated that even when female rats are used in neuroscience experiments without cycle consideration, their data exhibit no more variability than male rats across behavioral, electrophysiological, neurochemical, and histological measures (Becker; Prendergast; Liang, 2016). An interesting point of view is that the estrous cycle need not be a primary concern (Shansky, 2019). This is explained by the fact that both testosterone and estrogen are potent neuromodulators that influence memory processes (Shansky, 2019). Moreover, given that the variability observed in a cohort of male animals is considered acceptable from a scientific rigor standpoint (as historically acknowledged), a similar level of variability should be deemed acceptable in a cohort of females (Shansky, 2019).

In conclusion, this study illustrates that a single session of acute PE can significantly enhance memory persistence in female rats. This improvement in information retention can be linked with changes in noradrenaline levels in the hippocampus, emphasizing the possible role of this neurotransmitter in memory consolidation. Furthermore, the results indicate that these positive effects of exercise on memory do not differ between males and females. Overall, these findings underscore the potential of PE as a non-invasive strategy to improve information retention and promote cognitive health, with relevance to female populations.

Acknowledgment

This research received partial financial support from the Coordination for the Improvement of Higher Education Personnel (CAPES) and through research grants from the Federal University of Pampa, the National Council for Scientific and Technological Development (CNPq/Brazil) and the Fundação de Amparo à Research of the State of Rio Grande do Sul (FAPERGS/RS/Brazil).

Conflict of interest

The authors declare that they have no conflicts of interest to disclose. We certify that the submission represents original work and is not under review in any other publication.

Author Contribution

G.J.S., K.R.L. and P.B.M.C. conceived and planned the experiments. G.J.S., K.R.L, B.S.N. and M.S.S. conducted the behavioral experiments, while M.R.S.C. and R.R. performed the HPLC analysis. G.J.S, K.R.L., B.S.N and P.B.M.C. analyzed and interpreted the data. G.J.S, K.R.L. and P.B.M.C. wrote and revised the document. All authors, G.J.S, K.R.L., B.S.N., M.S.S., M.R.S.C., R.R. and P.B.M.C., approved the final version of the document.

References

Bangasser DA (2014). Sex differences in stress-related receptors: "Micro" differences with "macro" implications for mood and anxiety disorders. Biology of Sex Differences, 5(1), 2.

Becker JB, Prendergast BJ, Liang JW (2016) Female rats are not more variable than male rats: a meta-analysis of neuroscience studies. Biol Sex Differ, 1–7.

Bekinschtein P, Katche C, Slipczuk L, Gonzalez C, Dorman G, Cammarota M, Izquierdo I, Medina JH (2010) Persistence of long-term memory storage: New insights into its molecular signatures in the hippocampus and related structures. Neurotox Res, 18:377–385.

Bekinschtein P, Cammarota M, Igaz LM., Bevilaqua LR., Izquierdo I, & Medina JHH (2007). Persistence of long-term memory storage requires a late protein synthesis- and BDNF-dependent phase in the hippocampus. Neuron, 53(2), 261-277.

Bouchet CA, Lloyd BA, Loetz EC, Farmer CE, Ostrovskyy M, Haddad N, Foright RM, Greenwood BN (2017) Acute exercise enhances the consolidation of fear extinction memory and reduces conditioned fear relapse in a sex-dependent manner. Learn Mem 24:358–368.

Choleris E, Galea LAM, Sohrabji F, Frick KM (2018) Sex differences in the brain: Implications for behavioral and biomedical research. Neurosci Biobehav Rev 85:126–145 Available at: https://linkinghub.elsevier.com/retrieve/pii/S0149763417300945.

Diederich K, Bastl A, Wersching H, Teuber A, Strecker JK, Schmidt A, Minnerup J, Schäbitz WR (2017) Effects of different exercise strategies and intensities on memory performance and neurogenesis. Front Behav Neurosci 11:1–9.

Ennaceur A, & Delacour J . (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behavioral Brain Research, 31(1), 47-59.

Fulvio, J. M., Akinnola, I., & Postle, B. R. (2021). Gender (im)balance in citation practices in cognitive neuroscience. Journal of Cognitive Neuroscience, 33(1), 3-7.

Hall CS, & Ballachey EL (1932). A study of the rat's behavior in a field. A contribution to method in comparative psychology. University of California Publications in Psychology, 6(9), 1-12.

Izquierdo I (2011). Memória. Artmed Editora.

Katche C, Dorman G, Gonzalez C, Kramar CP, Slipczuk L, Rossato JI., ... & Medina JH (2016). On the convergence of the brain networks that underlie episodic memory in the rat. PLoS One, 11(2), e0148637.

Lima KR, da Rosa AC de S, Picua SS, Silva SS, Soares NM, Mello-Carpes PB (2021) One single physical exercise session improves memory persistence by hippocampal activation of D1 dopamine receptors and PKA signaling in rats. Brain Res 1762:1–8.

Lima KR, Neves B-HS das, Carrazoni GS, Rosa AC de S da, Carriço MRS, Roehrs R, Mello-Carpes PB (2023) Acute physical exercise improves recognition memory via locus coeruleus activation but not via ventral tegmental area activation. Physiol Behav 272:114370.

Lima LB, Rodrigues AC, França SM, Marcon JL, Roesler R, & Schroder N. (2019). Effects of acute stress on the consolidation of object recognition memory in male and female rats. Behavioural Brain Research, 356, 89-94.

Lima LB, Rodrigues AC, França SM, Marcon JL, Roesler R, & Schroder N. (2021). Acute physical exercise immediately after object recognition training enhances memory persistence in male rats. Brain Research, 1765, 147494.

Neves B, Muller SD, De Oliveira DL, & Marques CA (2018). Strength training sessions improve the performance of rats in an exhausting task after cerebral ischemia. Fisioterapia em Movimento, 31, e003120.

Oliff HS, Berchtold NC, Isackson P, Cotman CW, & Lynch G. (1998). Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. Molecular Brain Research, 61(1-2), 147-153.

Pellow S, Chopin P, Eichenbaum H, & Briley M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neuroscience Methods, 14(3), 149-167.

Sara SJ (2009). The locus coeruleus and noradrenergic modulation of cognition. Nature Reviews Neuroscience, 10(3), 211-223.

Shansky RM (2019) Animal studies in both sexes. Science, 364:825–826.

Shansky RM, Woolley CS (2016) Considering sex as a biological variable will be valuable for neuroscience research. J Neurosci 36:11817–11822.

Squire LR (2004). Memory systems of the brain: a brief history and current perspective. Neurobiology of Learning and Memory, 82(3), 171-177.

Squire LR, & Kandel ER (2003). Memory: From Mind to Molecules. Macmillan.

Titulaer J, Björkholm C, Feltmann K, Malmlöf T, Mishra D, Bengtsson Gonzales C, Schilström B, Konradsson-Geuken Å (2021) The Importance of Ventral Hippocampal Dopamine and Norepinephrine in Recognition Memory. Front Behav Neurosci 15:1–6.

Smith, J. C., Nielson, K. A., Antuono, P., Lyons, J. A., Hanson, R. J., Butts, A. M., & Verber, M. D. (2013). Semantic memory functional MRI and cognitive function after exercise intervention in mild cognitive impairment. Journal of Alzheimer's Disease, 37(1), 197-215.

Tuscher JJ, Fortress AM, Kim J, Frick KM (2015) Regulation of object recognition and object placement by ovarian sex steroid hormones. Behav Brain Res 285:140–157.

Vargas RS, Lima LB, & Schroder N (2017). Acute physical exercise improves memory persistence in male rats: Independent from neurogenesis. Hippocampus, 27(9), 1087-1097.

Vargas RS, Lima LB, & Schroder N (2020). Acute physical exercise enhances the consolidation of object recognition memory. Physiology & Behavior, 223, 113021.

Venezia AC, Hyer MM, Glasper ER, Roth SM, Quinlan EM (2019) Acute forced exercise increases Bdnf IV mRNA and reduces exploratory behavior in C57BL/6J mice. Genes, Brain Behav 19:1–14.

Yagi S, Galea LAM (2019) Sex differences in hippocampal cognition and neurogenesis. Neuropsychopharmacology.

Supplementary material

Table S1 No significant differences in behavioral control measures were observed across the various experimental groups. This included the total exploration time during the novel object recognition (NOR) task (measured in seconds), as well as locomotor activity (number of crossings) and exploratory behavior (number of rearings). Additionally, anxiety-related behavior, including entries into and time spent in the open arms (in seconds), exhibited similar patterns across all groups. The data were presented as mean values ± standard deviation (SD), and statistical analysis used a t-test or Mann-Whitney test according to their normality. No statistically significant differences were observed (P > 0.05).

	Control	Acute PE	P value
Total object exploration time (s)			
Training	67.69 ± 27.96	74.14 ± 30.40	0.56
24 h test	73.75 ± 32.71	66.69 ± 43.25	0.65
7-day test	67.54 ± 18.82	64.62 ± 24.12	0.38
Crossings (n)			
24 h test	51.00 ± 28.64	61.60 ± 25.59	0.35
7-day test	44.25 ± 20.01	62.89 ± 23.59	0.05
Rearings (n)			
24 h test	10.00 ± 3.68	13.73 ± 6.53	0.08
7-day test	6.37 ± 4.28	10.36 ± 6.15	0.67
Entries in open arms (n)			
24 h test	13.06 ± 23.10	6.30 ± 1.49	0.44
7-day test	8.23 ± 1.85	8.54 ± 1.97	0.68
Time in open arms (s)			
24 h test	142.7 ± 33.99	150.8 ± 23.00	0.51
7-day test	128.6 ± 28.07	123.9 ± 23.03	0.66

ANEXOS

ANEXO I - Certificado de aprovação de protocolo para utilização de animais de laboratório

CERTIDÃO

CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

(ACRÉSCIMO DE ANIMAIS E PRAZO)

Número de protocolo da CEUA: 029/2021

Título: Mecanismos neuroquímicos envolvidos na modulação da persistência da memória induzida pelo exercício físico agudo

Data da aprovação: 05/10/2022

Período de vigência do projeto: 01/12/2024

Pesquisadores(a): Pâmela Billig Mello Carpes

Campus: Uruguaiana

Telefone: (55) 9 9661-2454

E-mail: pamelacarpes@unipampa.edu.br

Finalidade	**( ) Ensino (X) Pesquisa**
Espécie / Linhagem / Raça	Ratos Wistar
Nº de animais	160 Machos e 35 Fêmeas
Peso / Idade	300 a 350g/90 dias
Sexo	Machos e Fêmeas
Origem	Biotério da Universidade Federal de Pelotas/RS

Assinado eletronicamente por **ALESSANDRA SAYURI KIKUCHI TAMAJUSUKU NEIS**, **Coordenador(a)**, em 13/10/2022, às 16:04, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.

A autenticidade deste documento pode ser conferida no site https://sei.unipampa.edu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **0943385** e o código CRC **7DF41D35**.

Referência: Processo nº 23100.014088/2021-57 SEI nº 0943385

ANEXO II - Diretrizes da *Brain Research*

INTRODUCTION

Brain Research is dedicated to publishing the highest quality and greatest impact articles within the ever-evolving field of Neuroscience. We recognize how technology has changed the way scientific breakthroughs are communicated and Brain Research is committed to serving as a dynamic journal meeting the needs of the neuroscience research community. We now offer multiple formats for scientists to share their work with the community. These include Short Reports, Technology Developments, and Commentaries, in addition to standard research articles and reviews that the journal has published in its storied 50-year history.

Brain Research is a broad-format journal accepting manuscripts from across the international neuroscience community that range in scope from issues in fundamental neurobiology to translational and clinical neuroscience, there are contemporary themes within neuroscience that our Editorial Board has identified that are of particular innovation and interest because of their innovation and importance, highlighted below. The journal is further committed to studies that investigate sex-differences as a powerful cross-cutting variable within these high-interest areas. Consistent with our mission, however, Brain Research remains enthusiastic to publish any neuroscience-oriented work that advances the field in a meaningful way.

- **Neurodegenerative Diseases and Dementia -** molecular insights into the mechanism of disease and preclinical models
- **Psychiatric diseases, autism spectrum disorders -** preclinical models and insights into mechanisms underlying neuropsychiatric disorders
- **Neuromodulation -** optogenetics, DREADDs, deep brain stimulation, TMS, tDCS are all creating new levels of control and intervention in brain function in both the preclinical and clinical settings
- **fMRI, ERP and other "windows into the brain" -** quantitative approaches to cognitive neuroscience and real-time measures are rapidly advancing our understanding of the brain
- **Stem cell biology/neurodevelopment -** Emerging developments in stem cell biology are changing the way we view, and study, neurodevelopment, and represent an exciting interface of technology and biology

Article Types

Research Articles: Standard submission, are not limited in size. However, we do strongly recommend to authors be as succinct as possible in the best interest of the readers and the dissemination of the work. Graphical abstract required.

Reviews: Pleaes submit reviews directly.

Short Report: Short Reports have the following soft limits. The manuscript should ideally contain no more than 3 Figures/Tables and 4000 words, including the title page, all sections of the manuscript (including the references), and Figure/Table legends. The abstract should be limited to 200 words. High impact, expedited review, one-round only, graphical abstract required.

Neurotech Report: These reports provide researchers with a full-length format in which to report significant methodological advances. The basic components of a Research Article will be the presentation of the method, a comparison of the method with related methods when appropriate, and validation. The impact is based purely on a technical or methodological advance, does not need to be hypothesis-driven science, graphical abstract required.

Point-Counterpoint:A pair of articles addressing a contemporary debate or controversy within a field - two individuals need to take opposing sides of a controversial topic; these articles are then published together. The articles can be broad or very focused in nature (no limit in size), merged graphical abstract required.

Brain Research will also regularly publish **thematic special issues** highlighting important new developments in neuroscience research.

The Neuroscience Peer Review Consortium

Brain Research is a member of the Neuroscience Peer Review Consortium (NPRC). The NPRC has been formed to reduce the time expended and, in particular, the duplication of effort by, and associated burden on reviewers involved in the peer review of original neuroscience research papers. It is an alliance of neuroscience journals that have agreed to accept manuscript reviews from other Consortium journals. By reducing the number of times that a manuscript is reviewed, the Consortium will reduce the load on reviewers and Editors, and speed the publication of research results.

If a manuscript has been rejected by another journal in the Consortium, authors can submit the manuscript to *Brain Research* and indicate that the referees' reports from the first journal be made available to the Editors of *Brain Research*.

It is the authors' decision as to whether or not to indicate that a set of referee's reports should be forwarded from the first journal to *Brain Research*. If an author does not wish for this to happen, the manuscript can be submitted to *Brain Research* without reference to the previous submission. No information will be exchanged between journals except at the request of authors. However, if the original referees' reports suggested that the paper is of high quality, but not suitable for the first journal, then it will often be to an author's advantage to indicate that referees' reports should be made available.

Authors should revise the original submission in accordance with the first journal's set of referee reports, reformat the paper to *Brain Research's* specification and submit the paper to *Brain Research* with a covering letter describing the changes that have been made, and informing the Editors that the authors will ask for the referee's reports to be forwarded from the first Consortium journal. The authors then must contact the first journal, and ask that reviews be forwarded, indicating they have submitted to *Brain Research*, and providing the new manuscript ID number.

The Editors of *Brain Research* will use forwarded referees' reports at their discretion. The Editors may use the reports directly to make a decision, or they may request further reviews if they feel such are necessary.

Visit http://nprc.incf.org for a list of Consortium journals, as well as further information on the scheme.

Contact Details for submission
Submission of manuscripts to *Brain Research* is entirely online at https://www.editorialmanager.com/brainres/default.aspx. Queries about the submission or editorial processes may be directed to https://service.elsevier.com/app/home/supporthub/publishing/

checklist
You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address

Manuscript:
- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

While submitting the revision, please include both marked and un-marked version of the manuscript. Also, please respond to all the queries raised by the reviewers via "Response to reviewers" document.

Further considerations
- Manuscript has been 'spell checked' and 'grammar checked'
- All references mentioned in the Reference List are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)
- Relevant declarations of interest have been made
- Journal policies detailed in this guide have been reviewed
- Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

Before you begin

Ethics in publishing

Please see our information on Ethics in publishing.

Policy and ethics
The work described in your article must have been carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans* https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ ; *EC Directive 86/609/EEC for animal experiments* http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; *Uniform Requirements for manuscripts submitted to Biomedical journals* http://www.icmje.org. This must be stated at an appropriate point in the article.

For other policy issues, authors are referred to the policy guidelines of the Society for Neuroscience (see their website http://www.jneurosci.org/misc/itoa.shtml).

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double anonymized) or the manuscript file (if single anonymized). If there are no interests to declare then please state this: 'Declarations of interest: none'. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Declaration of generative AI in scientific writing

The below guidance only refers to the writing process, and not to the use of AI tools to analyse and draw insights from data as part of the research process.

Where authors use generative artificial intelligence (AI) and AI-assisted technologies in the writing process, authors should only use these technologies to improve readability and language. Applying the technology should be done with human oversight and control, and authors should carefully review and edit the result, as AI can generate authoritative-sounding output that can be incorrect, incomplete or biased. AI and AI-assisted technologies should not be listed as an author or co-author, or be cited as an author. Authorship implies responsibilities and tasks that can only be attributed to and performed by humans, as outlined in Elsevier's AI policy for authors.

Authors should disclose in their manuscript the use of AI and AI-assisted technologies in the writing process by following the instructions below. A statement will appear in the published work. Please note that authors are ultimately responsible and accountable for the contents of the work.

Disclosure instructions

Authors must disclose the use of generative AI and AI-assisted technologies in the writing process by adding a statement at the end of their manuscript in the core manuscript file, before the References list. The statement should be placed in a new section entitled 'Declaration of Generative AI and AI-assisted technologies in the writing process'.

Statement: During the preparation of this work the author(s) used [NAME TOOL / SERVICE] in order to [REASON]. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

This declaration does not apply to the use of basic tools for checking grammar, spelling, references etc. If there is nothing to disclose, there is no need to add a statement.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify compliance, your article may be checked by Crossref Similarity Check and other originality or duplicate checking software.

Preprints

Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Preprint posting on SSRN

In support of Open Science, this journal offers its authors a free preprint posting service. Preprints provide early registration and dissemination of your research, which facilitates early citations and collaboration.

During submission to Editorial Manager, you can choose to release your manuscript publicly as a preprint on the preprint server SSRN once it enters peer-review with the journal. Your choice will have no effect on the editorial process or outcome with the journal. Please note that the corresponding author is expected to seek approval from all co-authors before agreeing to release the manuscript publicly on SSRN.

You will be notified via email when your preprint is posted online and a Digital Object Identifier (DOI) is assigned. Your preprint will remain globally available free to read whether the journal accepts or rejects your manuscript.

For more information about posting to SSRN, please consult the SSRN Terms of Use and FAQs.

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Content should make no assumptions about the beliefs or commitments of any reader; contain nothing which might imply that one individual is superior to another on the grounds of age, gender, race, ethnicity, culture, sexual orientation, disability or health condition; and use inclusive language throughout. Authors should ensure that writing is free from bias, stereotypes, slang, reference to dominant culture and/or cultural assumptions. We advise to seek gender neutrality by using plural nouns ("clinicians, patients/clients") as default/wherever possible to avoid using "he, she," or "he/she." We recommend avoiding the use of descriptors that refer to personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability or health condition unless they are relevant and valid. When coding terminology is used, we recommend to avoid offensive or exclusionary terms such as "master", "slave", "blacklist" and "whitelist". We suggest using alternatives that are more appropriate and (self-) explanatory such as "primary", "secondary", "blocklist" and "allowlist". These guidelines are meant as a point of reference to help identify appropriate language but are by no means exhaustive or definitive.

Reporting sex- and gender-based analyses

Reporting guidance

For research involving or pertaining to humans, animals or eukaryotic cells, investigators should integrate sex and gender-based analyses (SGBA) into their research design according to funder/sponsor requirements and best practices within a field. Authors should address the sex and/or gender dimensions of their research in their article. In cases where they cannot, they should discuss this as a limitation to their research's generalizability. Importantly, authors should explicitly state what definitions of sex and/or gender they are applying to enhance the precision, rigor and reproducibility of their research and to avoid ambiguity or conflation of terms and the constructs to which they refer (see Definitions section below). Authors can refer to the Sex and Gender Equity in Research (SAGER) guidelines and the SAGER guidelines checklist. These offer systematic approaches to the use and editorial review of sex and gender information in study design, data analysis, outcome reporting and research interpretation - however, please note there is no single, universally agreed-upon set of guidelines for defining sex and gender.

Definitions

Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth"), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms "sex" and "gender" can be ambiguous—thus it is important for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER guidelines, the resources on this page offer further insight around sex and gender in research studies.

Author contributions

For transparency, we require corresponding authors to provide co-author contributions to the manuscript using the relevant CRediT roles. The CRediT taxonomy includes 14 different roles describing each contributor's specific contribution to the scholarly output. The roles are: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; and Writing - review & editing. Note that not all roles may apply to every manuscript, and authors may have contributed through multiple roles. More details and an example.

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors **after** the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Article transfer service
This journal uses the Elsevier Article Transfer Service to find the best home for your manuscript. This means that if an editor feels your manuscript is more suitable for an alternative journal, you might be asked to consider transferring the manuscript to such a journal. The recommendation might be provided by a Journal Editor, a dedicated Scientific Managing Editor, a tool assisted recommendation, or a combination. If you agree, your manuscript will be transferred, though you will have the opportunity to make changes to the manuscript before the submission is complete. Please note that your manuscript will be independently reviewed by the new journal. More information.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

For gold open access articles: Upon acceptance of an article, authors will be asked to complete a 'License Agreement' (more information). Permitted third party reuse of gold open access articles is determined by the author's choice of user license.

Author rights
As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing
Find out how you can share your research published in Elsevier journals.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement, it is recommended to state this.

Open access

Please visit our Open Access page for more information about open access publishing in this journal.

Elsevier Researcher Academy
Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)
Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the Language Editing service available from Elsevier's Language Services.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail.

Submit your article
Please submit your article via
https://www.editorialmanager.com/brainres/default.aspx

Section, Senior Editor and Reviewers

Authors will be asked during manuscript submission to select a section of the journal, and a senior editor from that section whom they consider most appropriate to edit their manuscript. While every effort will be made to honor authors' selections, the assignment to a handling editor will be made by the Editor-in-Chief. Please submit, with the manuscript , the names, addresses and email addresses of 3 potential reviewers. Note that the handling editor retains the sole right to decide whether or not the suggested reviewers are used.

Suggesting reviewers
Please submit the names and institutional e-mail addresses of several potential reviewers.

You should not suggest reviewers who are colleagues, or who have co-authored or collaborated with you during the last three years. Editors do not invite reviewers who have potential competing interests with the authors. Further, in order to provide a broad and balanced assessment of the work, and ensure scientific rigor, please suggest diverse candidate reviewers who are located in different countries/regions from the author group. Also consider other diversity attributes e.g. gender, race and ethnicity, career stage, etc. Finally, you should not include existing members of the journal's editorial team, of whom the journal are already aware.

Note: the editor decides whether or not to invite your suggested reviewers.

Preparation

Queries

For questions about the editorial process (including the status of manuscripts under review) or for technical support on submissions, please visit our Support Center.

Peer review

This journal operates a single anonymized review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. Editors are not involved in decisions about papers which they have written themselves or have been written by family members or colleagues or which relate to products or services in which the editor has an interest. Any such submission is subject to all of the journal's usual procedures, with peer review handled independently of the relevant editor and their research groups. More information on types of peer review.

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision

Divide your article into clearly defined and numbered sections (e.g. Abstract, 1. Introduction, 2. Results, 3. Discussion, 4. Experimental Procedure, Acknowledgements, References). Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to " the text" . Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide relevant background information. Published studies should be described concisely, and be cited appropriately.

Results

The results should be described clearly and in logical order without extended discussion of their significance. Results should usually be presented descriptively and be supplemented by photographs or diagrams.

Discussion

The results of the research should be discussed in the context of other relevant published work; Extensive citations and discussion of published literature should be avoided. The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion section.

Experimental Procedure

This section should contain all the details necessary to reproduce the experiments. Avoid re-describing methods already published; only relevant modifications should be included in the text.

Essential title page information

- ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
- ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
- ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Highlights

Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

The abstract should state briefly (in no more than 250 words) the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 x 1328 pixels (h x w) or proportionally more. The image should be readable at a size of 5 x 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view Example Graphical Abstracts on our information site.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and", "of"). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, it is recommended to include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Minimal Data Standards

Resources reported in neuroscience articles often lack sufficient detail to enable reproducibility or reuse. To facilitate resource identification in the neuroscience literature , we recommend to include relevant accession numbers and identifiers in your article, which will be converted into links to corresponding data repositories and embedded enrichments once the article is published and appears on SciencDirect. Please consider inclusion of GenBank accession numbers, antibody identifiers, species specific nomenclatures, and software identifiers in the method section of your article. The complete set of recommendations with detailed instructions is available at: https://www.elsevier.com/about/content-innovation/minimal-data-standards

Artwork

Electronic artwork

General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Embed the used fonts if the application provides that option.
- Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Provide captions to illustrations separately.
- Size the illustrations close to the desired dimensions of the published version.
- Submit each illustration as a separate file.
- Ensure that color images are accessible to all, including those with impaired color vision.

A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format.
Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS (or PDF): Vector drawings, embed all used fonts.
TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
TIFF (or JPEG): Combinations bitmapped line/half-tone (color or grayscale), keep to a minimum of 500 dpi.

Please do not:

- Supply files that are optimized for screen use (e.g., GIF, BMP, PICT, WPG); these typically have a low number of pixels and limited set of colors;
- Supply files that are too low in resolution;
- Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF) or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) in addition to color reproduction in print.
Further information on the preparation of electronic artwork.

Figure captions

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, Crossref and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles, publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is highly encouraged.

A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: VanDecar J.C., Russo R.M., James D.E., Ambeh W.B., Franke M. (2003). Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. Journal of Geophysical Research, https://doi.org/10.1029/2001JB000884. Please note the format of such citations should be in the same style as all other references in the paper.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Data references

This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. The [dataset] identifier will not appear in your published article.

Preprint references

Where a preprint has subsequently become available as a peer-reviewed publication, the formal publication should be used as the reference. If there are preprints that are central to your work or that cover crucial developments in the topic, but are not yet formally published, these may be referenced. Preprints should be clearly marked as such, for example by including the word preprint, or the name of the preprint server, as part of the reference. The preprint DOI should also be provided.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software

Most Elsevier journals have their reference template available in many of the most popular reference management software products. These include all products that support Citation Style Language styles, such as Mendeley. Using citation plug-ins from these products, authors only need to select the appropriate journal template when preparing their article, after which citations and bibliographies will be automatically formatted in the journal's style. If no template is yet available for this journal, please follow the format of the sample references and citations as shown in this Guide. If you use reference management software, please ensure that you remove all field codes before submitting the electronic manuscript.
More information on how to remove field codes from different reference management software

.

Reference formatting

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the article number or pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct. If you do wish to format the references yourself they should be arranged according to the following examples:

Reference style

Text: All citations in the text should refer to:

1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.

Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999).... Or, as demonstrated (Jones, 1999; Allan, 2000)... Kramer et al. (2010) have recently shown ...'

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific

article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.
Reference to a journal publication with an article number:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.
Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).
Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.
Reference to software:
Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S., 2020. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo. https://doi.org/10.5281/zenodo.3727209.

Journal abbreviations source
Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide

text for both the electronic and the print version for the portions of the article that refer to this content.

Data visualization

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here to find out about available data visualization options and how to include them with your article.

Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your article to enhance it. Submitted supplementary items are published exactly as they are received (Excel or PowerPoint files will appear as such online). Please submit your material together with the article and supply a concise, descriptive caption for each supplementary file. If you wish to make changes to supplementary material during any stage of the process, please make sure to provide an updated file. Do not annotate any corrections on a previous version. Please switch off the 'Track Changes' option in Microsoft Office files as these will appear in the published version.

Research data

This journal requires and enables you to share data that supports your research publication where appropriate, and enables you to interlink the data with your published articles. Research data refers to the results of observations or experimentation that validate research findings, which may also include software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Below are a number of ways in which you can associate data with your article or make a statement about the availability of your data when submitting your manuscript. When sharing data in one of these ways, you are expected to cite the data in your manuscript and reference list. Please refer to the "References" section for more information about data citation. For more information on depositing, sharing and using research data and other relevant research materials, visit the research data page.

Data linking

If you have made your research data available in a data repository, you can link your article directly to the dataset. Elsevier collaborates with a number of repositories to link articles on ScienceDirect with relevant repositories, giving readers access to

underlying data that gives them a better understanding of the research described.

There are different ways to link your datasets to your article. When available, you can directly link your dataset to your article by providing the relevant information in the submission system. For more information, visit the database linking page.

For supported data repositories a repository banner will automatically appear next to your published article on ScienceDirect.

In addition, you can link to relevant data or entities through identifiers within the text of your manuscript, using the following format: Database: xxxx (e.g., TAIR: AT1G01020; CCDC: 734053; PDB: 1XFN).

Research Elements

This journal enables you to publish research objects related to your original research – such as data, methods, protocols, software and hardware – as an additional paper in a Research Elements journal.

Research Elements is a suite of peer-reviewed, open access journals which make your research objects findable, accessible and reusable. Articles place research objects into context by providing detailed descriptions of objects and their application, and linking to the associated original research articles. Research Elements articles can be prepared by you, or by one of your collaborators.

During submission, you will be alerted to the opportunity to prepare and submit a manuscript to one of the Research Elements journals.

More information can be found on the Research Elements page.

Data statement
To foster transparency, we require you to state the availability of your data in your submission if your data is unavailable to access or unsuitable to post. This may also be a requirement of your funding body or institution. You will have the opportunity to provide a data statement during the submission process. The statement will appear with your published article on ScienceDirect. For more information, visit the Data Statement page..

After acceptance

Online proof correction

To ensure a fast publication process of the article, we kindly ask authors to provide us with their proof corrections within two days. Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor. Web-based proofing provides a faster and less error-prone process by allowing you to

directly type your corrections, eliminating the potential introduction of errors.
If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.
We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

Author inquiries

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.
You can also check the status of your submitted article or find out when your accepted article will be published.