UNIVERSIDADE FEDERAL DO PAMPA

RITHIELE GONÇALVES

UMA DOSE ÚNICA DE METILPREDNISOLONA MELHORA A CONSOLIDAÇÃO E A EXTINÇÃO DA MEMÓRIA

TRABALHO DE CONCLUSÃO DE CURSO

URUGUAIANA

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Trabalho de conclusão de curso apresentado ao curso de Fisioterapia da Universidade Federal do Pampa, como requisito para obtenção do título de bacharel em Fisioterapia.

Orientadora: Dra. Pâmela Billig Mello-Carpes
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Área de concentração: Fisioterapia

Trabalho de conclusão de curso defendido e aprovado em: 30/11/2017.
Banca examinadora:

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AGRADECIMENTOS

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APRESENTAÇÃO

O presente trabalho de conclusão de curso é apresentado na forma de um artigo cientifico, conforme normas de TCC do Curso de Fisioterapia da UNIPAMPA. Trata-se de um trabalho experimental, que foi desenvolvido após aprovação da Comissão de Ética para o Uso de Animais da Unipampa (ANEXO I). O artigo está de acordo com as normas da Revista Journal of Neurophysiology (ANEXO II).

A SINGLE DOSE OF METHYLPREDNISOLONE IMPROVES MEMORY CONSOLIDATION AND EXTINCTION

NEW & NOTEWORTHY

A single dose of Methylprednisolone promoted the improvement of aversive memory consolidation, as well as of memory extinction, representing an alternative to use in the medical clinic treatment of disorders such as post-traumatic stress.

ABSTRACT

Aversive memory is essential for survival, but in some situations an exacerbation of this can be potentially dangerous. There are several ways to modulate memory, among them, stress-related hormones and substances analogous to them. Recently, our group has shown that a chronic treatment with a low dose (5mg/kg) of methylprednisolone (MP) is able to promote memory persistence. Here, we evaluate if a single dose of MP is able to improve aversive memory consolidation and promote memory persistence and extinction. For this, two experiments were carried out. In the first one, we demonstrated that 5mg/kg of MP administrated 2h after inhibitory avoidance training improves memory consolidation, promoting memory persistence. In the second experiment, we verified that a single dose of MP promotes memory extinction. Thus, a possible new clinical applicability is suggested for MP, however, more studies are needed to verify the mechanisms involved.

KEYWORDS

Aversive memory, Inhibitory avoidance, Corticoids, Memory persistence

INTRODUCTION

Memory consolidation requires, among other things, the synthesis of new proteins in the hippocampus and changes in the production of different neurotransmitters. These processes are closely related and both can be affected in different ways by the use of certain substances and by the endogenous release of some substances, such as hormones (Carter et al. 2005; Gold 2008; Kandel and Schwartz 1982).

The aversive memory corresponds to our ability to identify dangerous situations when certain trigger stimuli promotes the evocation of memories related to a fear experience. These

memories are fundamental in certain situations, where there is a need to activate circuits that trigger sensory-motor responses related to survival and necessary to avoid harmful circumstances to the individual (Ozawa et al. 2017). In this sense, the formation and persistence of this type of memory represents a fundamental characteristic of the mammalians is essential to the evolution and survival (Do Monte et al. 2016).

On the other hand, in certain situations, a strong fear memory can generate exacerbated responses to non-harmful stimulus (Keller et al. 2015), as in Post-Traumatic Stress Disorder (PTSD) (Bisson et al. 2015). The PTSD is very harmful to the patient as it is more likely to have health problems in general (Karstoft et al. 2015). The extinction of fear memories has been widely used in the clinic as a form of treatment of PTSD. People with PTSD often remember their traumatic experiences over and over again, intensively and out of context, which can be severely disabling (Davis 2011; Furini et al. 2014; Milad and Quirk 2012; Sher 2010). In exposure therapy the extinction is used in a way by which the individuals are exposed to stimuli related to the one that have led them to a traumatic experience until they suppress the inadequate responses upon perceiving the absence of danger, becoming able to lead a normal life.

Thus, we can say that extinction is the formation of a new memory, without erasing the original memory (de Carvalho Myskiw et al. 2015), but overlapping it. It is characterized by a progressive decrease in the intensity and/or frequency of the conditioned response caused by repeated evocation in the absence of the unconditioned stimulus (Cammarota et al. 2005). Although the extinction is used in exposure therapy, it is efficiency is limited (Maren and Holmes 2016). In this way, several strategies and different protocols have been studied trying to promote and improve extinction, among them is the use of drugs that modulate extinction, such as: serotonergic and noradrenergic drugs, neuropeptides, endocannabinoids, glucocorticoids, histone deacetylase inhibitors, among others (Fitzgerald et al. 2014).

Glucocorticoids have been reported in the literature as important modulators of mnemonic responses (Brunner et al. 2005; Buss et al. 2004). Among these glucocorticoids is Methylprednisolone (MP), a glucocorticoid commonly used in the therapy of allergies, inflammations and autoimmune disorders (Kajiyama et al. 2010; Longui 2007). The effects of MP on memory have also been studied. Zhang et al. (2011) demonstrated that MP may cause memory impairment by causing deficits in synaptic plasticity in the hippocampus. On the other hand, a single corticosterone injection after training in a fear conditioning task seems to improve memory consolidation (Abrari et al. 2009). Instead there are some interesting studies on MP

effects on memory they present apparent controversial results that could be related to doses and time of treatment.

Recently, our group demonstrated that the chronic treatment (10 days) with a low dose of MP (5 mg/kg), but not with a high dose (30 mg/kg) is able to promote the persistence of aversive memory, tested 14 days after learning (de Vargas et al. 2017). In addition, in this same work, we verified an improvement in long-term potentiation (LTP; (de Vargas et al. 2017), an essential phenomenon related to the formation and persistence of memory (Malenka and Bear 2004). We also observed an increase in the influx of calcium into the cell (de Vargas et al. 2017), a signaling event related to the induction of LTP and memory consolidation (Baker et al. 2013).

Based on our previous results, in which we observed an important influence of MP, chronically administrated in low doses, on the persistence of aversive memory, we wonder about the effects of a single MP dose on aversive memory consolidation and persistence, and, also, about the MP effects on the extinction of aversive memory, what could be a possible alternative to the treatment of disorders such as PTSD. In this work we demonstrated that a single dose of MP is able to improve the aversive memory consolidation and extinction, promoting the persistence of these events.

MATERIALS AND METHODS

Experimental design

This study was composed by two experiments. The first one was carried out in order to verify if a single dose of MP is able to improve the aversive memory consolidation, promoting its persistence, as well as the optimal time for drug administration (Fig 1A). The second experiment was carried out considering the results of the first one, in order to verify the effect of this single dose of MP on the aversive memory extinction (Fig 2A).

Animals

Fifty four adult male Wistar rats (three months old, 250-280g) were purchased from the Central Vivarium of Federal University of Santa Maria (RS/Brazil) and housed four per cage under controlled light and environmental conditions (12h light/12h dark cycle at 23 ± 2°C and 50 ± 10% humidity) with food and water *ad libitum*. The study was performed according to and

approved by the Ethics Committee for Animal Use from Federal University of Pampa (protocol #20/2016).

Drugs

Methylprednisolone sodium succinate (MP, Solu-Medrol®) was purchased from Laboratórios Pfizer (São Paulo, SP, Brazil).

Experimental protocols

Inhibitory avoidance (IA) learning

IA is a commonly behavioral task used to investigate learning and memory processes in rodents (Gold 1986; McGaugh and Roozendaal 2009). The device used in this study consisted in a 50 x 25 x 50 cm metal box, with a transparent acrylic made front. The floor of the apparatus consisted of an array of parallel electrified bars with a platform box of 5.0 cm height by 7.0 cm wide placed in the left side of the box. During IA training, rats were carefully placed on the elevated platform and they receive a single aversive foot shock (0.5 mA for 2 s) when stepping down from the platform and all four legs contacted with the electrified bars (Rossato et al. 2009). After this event, the animals were immediately placed into their housing-boxes. Even though IA training consists of a single trial, the brain processes underlying task acquisition are complex. Rats must encode different pieces of information in order to acquire a correct association between a particular location within the apparatus and the aversive stimulus of foot shock, which involve the hippocampus, the amygdala and the prefrontal cortex. Retention of the training was tested at 24 h and then every week for the three weeks after training by measuring rats' latency to step down from the platform. Longer retention test latencies (compared to training ones) are interpreted as good memory and/or persistence of memory.

IA extinction learning

To extinguish the aversive memory, which was made in the second experiment, the animals were submitted to non-reinforced test sessions different times after training: 24h, 25h30min and 27h (Cammarota et al. 2005). At each test session, the animals were placed on the training box platform until they came down with all four feet on the railing. No shock was given and the animals could freely explore the box for 30 seconds before being returned to their boxes. To evaluate the extinction of aversive memory, a retention test was performed 24 hours

after the last extinction session. To evaluate the persistence of aversive extinction memory, tests were performed every week for three weeks.

Control behavioral tasks

All animals were subjected to control behavioral experiments to avoid biases of the effect of MP injection on other behavioral parameters that could influence the IA learning or alter the tests results. The control behavior tests were performed in each test day in both experiments (1 and 2). To analyze exploratory and locomotor behavior, each rat was submitted to an Open Field (OF) test, in which crossing and rearing were monitored over 5 min (Bonini et al. 2006). To evaluate anxiety, rats were exposed to an Elevated Plus Maze (EPM). The time spent and the total number of entries into the open and closed arms were recorded over a 5 min session (Pellow et al. 1985). Finally, to ensure that pain sensitivity did not change upon MP administration, we used the Tail Flick (TF) test (Tjolsen et al. 1989), the latency time for tail removal was evaluated.

Statistical analysis

First of all, the Shapiro-Wilk test was used to check the data distribution. After this, to IA statistical analysis, a Wilcoxon test was used for intragroup comparison (training *vs*. test), and Kruskal Wallis followed by Dunn's post-hoc (when there are more than 2 groups), or Mann-Whitney (when there are 2 groups) were used for comparisons between the different groups. The different groups' results on OF, EPM and TF were compared by ANOVA or unpaired t-test. Values of $P < 0.05$ were considered significant.

RESULTS

Experiment 1 - Effects of a single dose of Methylprednisolone (MP; 5mg/kg) in aversive memory consolidation and persistence

In the first experiment we could verify that all the groups that received MP administration improved the memory consolidation and persistence (Fig 1B).

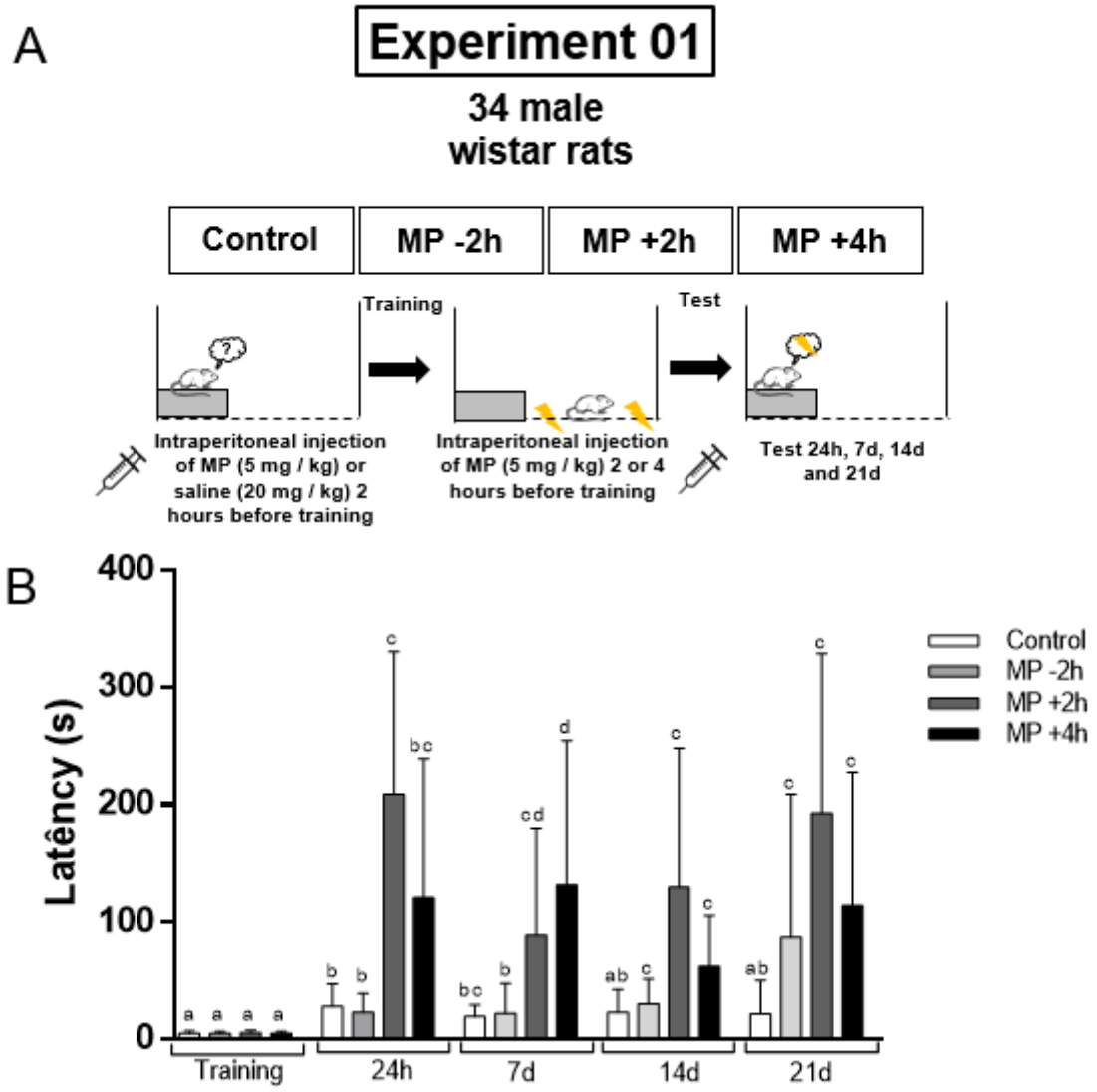

Figure 1. A – Experiment 1. The animals were divided in 4 groups (control group received saline solution and MP groups received Methylprednisolone 5mg/kg, n = 8-9 each), were trained in the task of inhibitory avoidance and tested 24h, 7d, 14d and 21d after training to evaluate memory consolidation and persistence. **B – A single dose of MP enhances the memory consolidation and promotes the memory persistence.** Different letters indicate difference vs training or between groups (Wilcoxon test or Kruskal-Wallis test followed by Dunn post-hoc respectively; $P < 0.05$).

In the 24h test, all groups showed increased step-down latencies ($P = 0.007$ for control; $P = 0.0002$ for MP -2h; $P = 0.003$ for MP +2h; $P = 0.01$ for MP +4h). The comparison of 24h test latencies show differences between groups ($H_{(4)} = 13.58$; $P = 0.003$), and MP +2h group was different from control ($P = 0.05$) and from MP -2h ($P = 0.01$).

In the 7d test, all groups showed increased step-down latencies ($P = 0.007$ for control; $P = 0.01$ for MP -2h; $P = 0.003$ for MP +2h; $P = 0.01$ for MP +4h). The comparison of 7d test

latencies show differences between groups ($H_{(4)}$ = 16.04; P = 0.001), and MP +4h group was different from control (P = 0.05) and from MP -2h (P = 0.01), and MP +2h was different from MP -2h (P = 0.05).

In the 14d test, only the MP groups showed increased step-down latencies (P = 0.07 for control; P = 0.04 for MP -2h; P = 0.01 for MP +2h; P = 0.01 for MP +4h). The comparison of 14d test latencies show differences between groups ($H_{(4)}$ = 10.14; P = 0.01), and MP +2h group was different from control (P = 0.05).

In the 21d test, only the MP groups showed increased step-down latencies (P = 0.31 for control; P = 0.007 for MP -2h; P = 0.01 for MP +2h; P = 0.01 for MP +4h). The comparison of 21d test latencies show differences between groups ($H_{(4)}$ = 8.50; P = 0.03), and MP +2h group was different from control (P = 0.05).

Experiment 2 - Effects of a single dose of MP (5mg/ kg) on aversive memory extinction and persistence of extinction

Considering the results of the first experiment, in the second one the time of MP injection used was two hours after the extinction training. The results show that MP promotes the memory extinction (Fig 2B).

The results show that in the extinction sessions the rats from all groups remember the original memory (P < 0.05 for all groups, training *vs*. test, Wilcoxon test). In the retention tests the original memory persisted in control group, despite of the extinction sessions (P = 0.03 for 24h test; P = 0.03 for 7d test; P = 0.01 for 14d test; P = 0.01 for 21d test; training *vs*. test, Wilcoxon test); although, the rats from MP group were able to extinguish (P = 0.12 for 24h test; P = 0.06 for 7d test; P = 0.07 for 14d test; P = 0.15 for 21d test; training *vs*. test, Wilcoxon test).

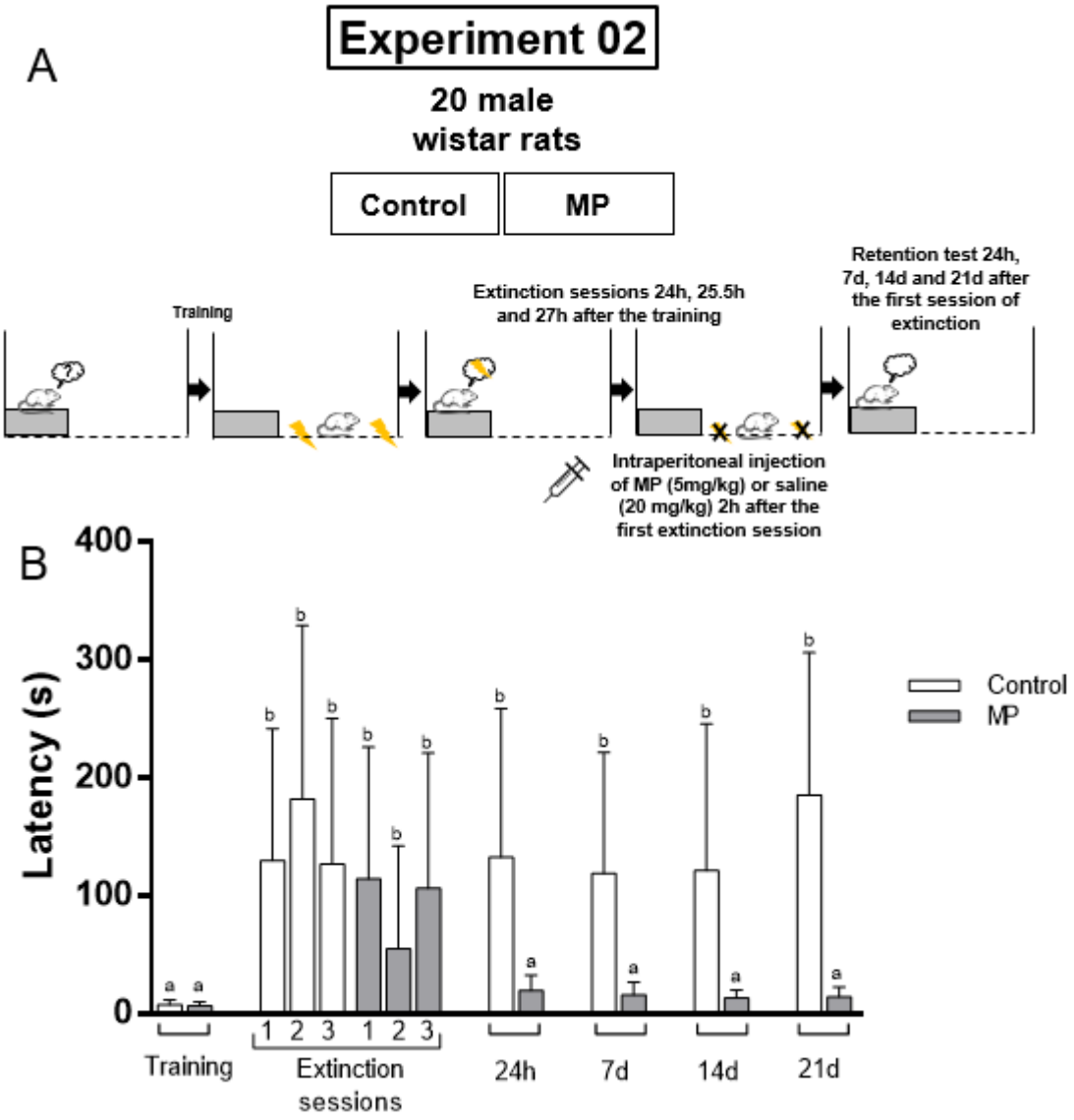

Figure 2. A – Experiment 2. The animals were divided into two groups (control received saline and MP received methylprednisolone 5 mg/kg, n = 10 per group), and were trained in the task of inhibitory avoidance, in the following day were submitted to three sessions extinction, and 24h, 7d, 14d and 21d after the first extinction session the retention and persistence of extinction memory were tested. **B - MP promotes the extinction of aversive memory and it stays for 21 days.** Different letters indicate difference vs training or between the groups (Kruskal-Wallis test followed by Dunn's post-hoc or Mann-Whitney test respectively, P <0.05).

Comparisons between groups in test days show differences in all tests (P = 0.03 on 24h test; P = 0.008 for 7d test; P = 0.0003 for 14d test; P = 0.0006 for 21d test).

Control behavioral tasks

As expected, no significant differences were observed in the behavioral control tasks, demonstrating that the MP effect is exclusively related to the action in the animals' memory (table 1).

Table 1. MP does not change locomotor and exploratory activity, anxiety and pain sensitivity of the animal (n = 8-10/rats/group; P > 0.05 ANOVA or t-test).

Behavioral task and parameter evaluated		Control	MP-2h	MP+2h	MP+4h
EPM	Total entries (n)	1.75 (1.38)	3.77 (2.27)	3.44 (3.24)	3.00 (2.61)
	Time in open arms (s)	90.75 (31.96)	92.89 (55.41)	124,9 (60.97)	86,88 (3268)
OF	Crossing (n)	41.63 (23.30)	52.89 (16.24)	50.00 (25.51)	51.63 (23,29)
	Rearing (n)	13.50 (8.12)	16.00 (6.36)	17.00 (7.03)	18.13 (8.91)
TF	Latency (s)	75,45 (5.58)	75.10 (7.09)	80.48 (7,80)	79.61 (7,90)

EPM = Elevated Plus Maze; OF = Open Field; TF = Tail Flick.

DISCUSSION

Our results demonstrated that a single dose of MP is able to improve the aversive memory consolidation when injected in a time near learning, and to promote aversive memory extinction when injected 2h after extinction training.

There are two main novelties in these results. The first one is the demonstration that MP is effective to promote memory improvement also in a single dose. Previously, our group show that chronic treatment with MP promoted the persistence of aversive memory with a low dose (de Vargas et al. 2017). Therefore, MP is effective as a memory enhancement tool, both acutely and chronically. A single dose of MP promoted the persistence of the aversive memory for 21 days, unlike the control group, which naturally passes through a process of forgetting.

Some studies associated the acute effect of the glucocorticoids with the beneficial to memory consolidation and the strength of the memory (Joels et al. 2011; Sandi 2011). In this senses, the use of a single low dose of MP shows a new possibility as cognitive enhancer, since the persistence of memory is related to a series of events related to synaptic neuroplasticity, which are directly related to cognition (Takeuchi et al. 2014). Among these events, as demonstrated previously by our group and other authors, long-term potentiation and the biochemical events associated with it are necessary for the formation of a strong memory (de Vargas et al. 2017; Izquierdo et al. 2008). Whitehead et al. (2013) verified that an acute stress

in rats can enhance the LTP when compared an unstressed rats, and Liston et al. (2013) demonstrated that, acutely, glucocorticoids can promote long-term memory in a mechanism of learning-dependent dendritic spines. In another way, some studies have demonstrated that glucocorticoids are capable to potentiate glutamatergic transmission, acting improving post-synaptic traffic of AMPA and NMDA receptors, which would increase the activation of intracellular proteins fundamental for memory persistence, such as MAPK and CaMKII (Popoli et al. 2011; Sandi 2011). Chen et al. (2012), showed that the consolidation of memory mediated by glucocorticoid receptors involves the CaMKIIα-BDNF-CREB pathway and culminates with increased expression of structural proteins such as Arc, fundamental for stabilization and synapses and consequently for long-term memory.

The second important novelty demonstrated in our research is that a single dose of MP facilitates the aversive memory extinction. In our study, only the animals that received MP were able to extinguish the aversive memory, and this effect persisted for 21 days. Considering strong aversive or fear memories, extinction is a difficult process, and, although it is used in clinic for PTSD treatment, it represents a challenge for therapists; thus, MP appears as a potential alternative to facilitate this process, since a single dose administrated after extinction training was able to promote extinction learning.

In a study with patients with acrophobia, Quervain et al. (2011) showed that a 20 mg dose of cortisol given orally 1 hour prior to exposure therapy was able to facilitate extinguishing and promote it for up to 1 month. This study demonstrates the real clinical applicability of the results presented here, since MP is already a marketed drug and it is a new possibility of use in the medical clinic.

Thus, this study suggests a new clinical applicability for MP both as a cognitive enhancer for memory-impairing diseases, promoting memory persistence, as well as a facilitator of memory extinction and an auxiliary tool in the treatment of disorders such as PTSD. Anyway, the mechanisms by which MP promotes this effects is still unclear, and new studies to elucidate them.

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ANEXOS

ANEXO I – Carta de Aprovação do CEUA

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DO PAMPA
(Lei nº 11.640, de 11 de janeiro de 2008)

Pró-Reitoria de Pesquisa

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

Fone: (55) 3413 4321, E-mail: ceua@unipampa.edu.br

CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

Número de protocolo da CEUA: **020/2016**

Título: **Efeito da Metilprednisolona na consolidação, extinção e persistência das memórias de medo**

Data da aprovação: **22.07.2016**

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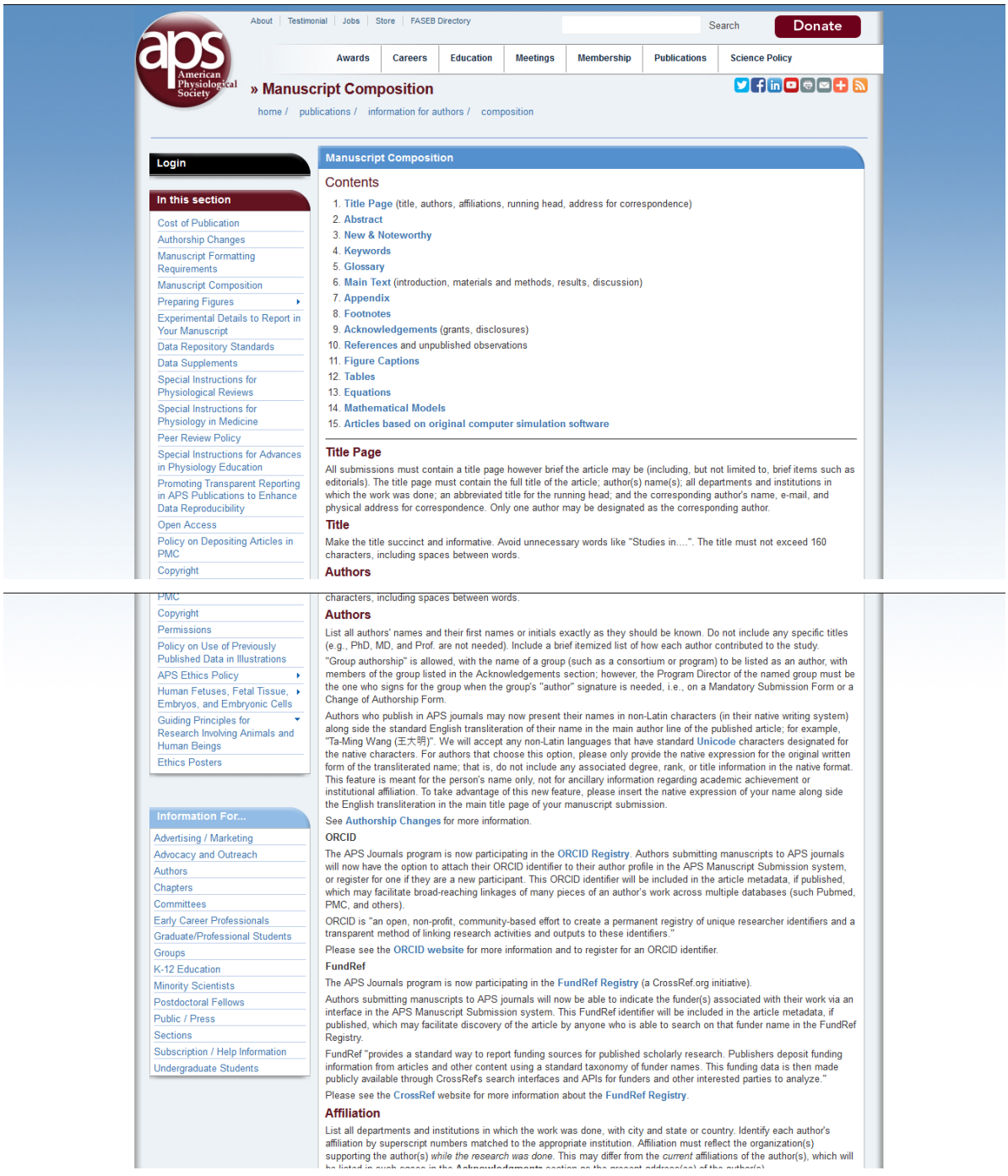
ANEXO II – Normas da revista Journal of Neurophysiology



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Manuscript Composition

Contents

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All submissions must contain a title page however brief the article may be (including, but not limited to, brief items such as editorials). The title page must contain the full title of the article; author(s) name(s); all departments and institutions in which the work was done; an abbreviated title for the running head; and the corresponding author's name, e-mail, and physical address for correspondence. Only one author may be designated as the corresponding author.

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Make the title succinct and informative. Avoid unnecessary words like "Studies in....". The title must not exceed 160 characters, including spaces between words.

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List all authors' names and their first names or initials exactly as they should be known. Do not include any specific titles (e.g., PhD, MD, and Prof. are not needed). Include a brief itemized list of how each author contributed to the study.

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affiliation by superscript numbers matched to the appropriate institution. Affiliation must reflect the organization(s) supporting the author(s) *while the research was done*. This may differ from the *current* affiliations of the author(s), which will be listed in such cases in the **Acknowledgments** section as the present address(es) of the author(s).

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A one-paragraph abstract of not more than 250 words must accompany each manuscript. Longer abstracts may be subject to editorial truncation, to conform to the conventional perceptions of brevity that characterize an "abstract". The abstract should state what was done and why (including species and state of anesthesia), what was found (in terms of data, if space allows), and what was concluded. Even for short editorial-style articles, a brief "abstract" should be provided, if only to identify the topic (e.g., "This is an editorial summarizing recent new developments in physiology.").

New & Noteworthy

Participating Journals: ***AJP-Heart*** *(AHeart),* ***Journal of Applied Physiology*** *(JAPPL),* ***Journal of Neurophysiology*** *(JN),* ***AJP-Gastrointestinal*** *(AGI),* and ***Advances*** *(ADV).*

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AHeart, JAPPL, JN, AGI

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Include three to five words or short phrases relevant to the article.

Include three to five words or short phrases relevant to the article.

Glossary

A glossary may be included (and is often helpful) in equation-laden articles with many different symbols (such as mathematical modeling or computational papers), specifying the units (and/or dimensions) as well as each definition. The glossary will usually precede the **Methods** section. See **this article** for an example.

Main Text

Introduction

Provide a brief overview of the scope and relevance of the study, especially with regard to previous advancements in related fields.

Materials and Methods

Describe techniques, cell/animal models used (including species, strain, and sex), and lists of reagents, chemicals, and equipment, as well as the names of manufacturers and suppliers, including URLS for those supplies obtained online, so that your study can be most easily replicated by others. For studies involving humans, the sex and/or gender of participants must be reported. Also in this section, describe the statistical methods that were used to evaluate the data. If clinical trials were used, a statement of registration is required; also, for all investigations involving humans or animals, a statement of protocol approval from an IRB or IACUC, or an equivalent statement, must be included (see **Guiding Principles for Research Involving Animals and Human Beings**). **All animal or human studies must contain an explicit statement that the protocols were submitted to, and approved by, an institutional review board or committee or that the protocols were performed under a license obtained from such a committee, board, or governing office.**

See **Experimental Details to Report in Your Manuscript** for more information.
See **Abbreviations, Symbols, and Terminology** for style information.

Results

Provide the experimental data and results as well as the particular statistical significance of the data. See **Promoting Transparent Reporting of Results** for more information.

APS has published an **editorial** on the use of statistics, and authors are encouraged to consult it.

Effective July 2017, *Advances in Physiology Education* will encourage two of the **2004 Guidelines for Reporting Statistics**:
Guideline 5. Report variability using a standard deviation. If the standard deviation is not a meaningful estimate of variability, as when the data are skewed, then the range or interquartile range provide more meaningful estimates. Report a standard deviation with notation of this form: 90 mmHg (SD 10).

Guideline 7. Report a precise P value. The **sequel to the 2004 Guidelines** (see Table 2) offers the appropriate precision for rounding P values.

Discussion

Sometimes combined with the results in a section called "Results and Discussion". Explain your interpretation of the data, especially compared with previously published material cited in the References.

Appendix

especially compared with previously published material cited in the References.

Appendix

An Appendix may be included (and is often helpful) in mathematical modeling or computational papers, e.g., to provide details of a solution strategy.

Acknowledgements

The acknowledgements section is where you may wish to thank people indirectly involved with the research (e.g., technical assistance; gifts of samples, reagents, or cell lines; loans of equipment or laboratory space; comments or suggestions during the creation of the manuscript). However, it is important that anyone listed here know in advance of your acknowledgement of their contribution, as documented during the submission process.

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List the grants, fellowships, and donations that funded (partially or completely) the research. However, industry-sponsored grants should be listed under **Disclosures**.

Disclosures

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The footnotes section is the place to list any additional items pertinent to your article, including but not limited to **links to non-peer-reviewed data** that may be available to readers from your institutional web site.

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The examples given below are shown with numbers because that is the style for most APS Journals, except for the *Journal of Neurophysiology* (see note, below, after these examples). The first is a standard book reference; the second is a standard reference to an "early view" or "prepress" reference, such as the APS "Articles in PresS" (note the use of the

of Neurophysiology (see note, below, after these examples). The first is a standard book reference; the second is a standard reference to an "early view" or "prepress" reference, such as the APS "Articles in PresS" (note the use of the "digital object identifier"—doi—in this citation); the third is a standard journal reference.

1. **Pollock DM.** Endothelin receptor subtypes and tissue distribution. In: *Endothelin Molecular Biology, Physiology, and Pathology*, edited by Highsmith RF. Totowa, NJ: Humana, 1998.
2. **Scarafia LE, Winter A, Swinney DC.** Quantitative expression analysis of the cellular specificity of HECT-domain ubiquitin E3 ligases. *Physiol Genomics* (April 26, 2001). doi:10.1152/physiolgenomics.00075.2001.
3. **Villalobos AR, Parmelee JT, Renfro JL.** Choline uptake across the ventricular membrane of neonate rat choroid plexus. *Am J Physiol Cell Physiol* 276: C1288-C1296, 1999.

MORE Example References

References for the *Journal of Neurophysiology* should be arranged alphabetically by author. The appropriate author name and year for each reference should be included in parentheses at the proper point in the text using the following style **(this is ONLY for the *Journal of Neurophysiology*, NOT for other APS Journals)**:

- one author (Brown 1982)
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Submitted papers still in preparation or in peer review and/or any other unpublished materials, observations, or personal communications cannot be included in the reference list, which may only list published work. However, such material can be cited in the text, but at submission, authors will be required to confirm that all individuals acknowledged in the manuscript are aware that they are being acknowledged and approve of the manner and the context of the acknowledgement. This includes, but is not limited to the following circumstances:

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figure, to place it in the proper context of the manuscript. Information reported in figures and legends should describe each individual experiment presented, including the number of samples or animals used per treatment. The statistics performed for each experiment should be reported as well. These textual figure captions must be listed in order in the manuscript, following the reference list.

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- Each table should have a brief title; explanatory notes should be in the legend, not in the title.
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- Statistical measures of variations, SD, SE, etc., must be identified. (Example: "Values are means ± SE.")
- Table footnotes should be listed in order of their appearance and identified by standard symbols: *, †, ‡, § for four or fewer; for five or more, consecutive superscript lowercase letters should be used (e.g., ^{a}, ^{b}, ^{c}, etc.).

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Manuscripts with many mathematical characters and equations should be prepared using **MathType** version 6.0 or higher (available from **Design Science, Inc.**). Manuscripts with a minimal amount of math may be prepared using word-processing tools such as Microsoft Word Equation Editor, or with features such as bold, italics, superscript and subscript together with characters in the Symbol or Greek fonts. Do not use the Wingdings or Webdings fonts. Please do not submit equations as images. Inline math text that can be regular text (e.g., $x^2[n - 2]$) should be provided as such. Please do not set these as MathType.

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1. Upon submission of a paper reporting computer simulation using original, author-generated code, authors are ***encouraged*** to deposit software and code via a public repository, such as GitHub, if not already deposited to a public repository. A link to the software deposit should be included in the cover letter. Alternatively, authors may submit a working version of their software and computer code as supporting data for access by the reviewers. ***It will be kept confidential during review, and in case of a negative decision (i.e., rejection).***

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